Effects of toner-handling work on respiratory function, chest X-ray findings, and biomarkers of inflammation, allergy, and oxidative stress: a prospective Japanese cohort study from 2003 to 2013

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Abstract

Background: Exposure to toner, a substance used in photocopiers and printers, has been associated with siderosilicosis and other adverse effects. However, these findings are limited, and there is insufficient evidence on the long-term effects of toner exposure. Using longitudinal analysis, this study aimed to examine the effects of work involving toner exposure on the respiratory system over time.

Methods: We conducted a prospective cohort study in a Japanese toner and copier manufacturing enterprise between 2003 and 2013. The cohort included a total of 1468 workers, which comprised 887 toner-handling workers and 581 non-toner-handling workers. We subdivided the toner-handling workers into two groups according to the toner exposure concentration, based on the baseline survey in 2003. We compared the chest X-ray results, respiratory function indicators, and serum and urinary biomarkers of inflammation, allergy, and oxidative stress among three groups: high-concentration toner exposure group, low-concentration toner exposure group, and non-toner-handling group. To consider the effects of individual differences on longitudinal data, we used a linear mixed model.

Results: The chest X-ray results and most of the biomarkers and respiratory function indicators were similar for the non-toner-handling and toner-handling groups. There were no significant yearly changes in the percentage of vital capacity (%VC) and peak expiratory flow rate (PEFR) in the high-concentration toner exposure group, while there was a significant yearly increase in %VC and PEFR in the low-concentration toner exposure group and non-toner-handling group. Regarding interleukin-8, we did not observe a significant yearly change in the toner-handling group but observed a significant yearly increase of 0.1 pg/ml in the non-toner-handling group.

Conclusions: Toner-handling work was not associated with the deterioration of respiratory function and an increase in biomarker values for inflammation, allergy, and oxidative stress. This finding suggests that toner-handling work is irrelevant to the onset of respiratory disease and has minimal adverse effects on the respiratory system under the well-managed work environment.

Background

Toner is a particulate substance, with a diameter of 5–10 μm, used in photocopiers and laser printers to form a printed image or text on paper. The inside of a toner resin particle contains colorants such as carbon black, whereas the surface of the particle contains nanoparticle additives such as titanium dioxide and amorphous silica. In 1994, Gallardo et al. reported the first case of siderosilicosis owing to toner exposure, and since then, there have been further case reports of sarcoidosis, allergic rhinitis, asthma, etc., being associated with toner exposure [1–4]. As the use of photocopiers, printers, and toners has increased, their respiratory effects have been highlighted. Recent studies have shown that office machines such as printers or photocopiers can emit particulate matter (PM) when in use, and PM may cause indoor air pollution [5–7]. However, the diameter of PM emitted from office machines is mostly in the submicron level [5–7], and it is unlikely that PM related to the printing process has exactly the same

properties as toner dust itself. Thus, it is necessary to assess the health effects of toner exposure and those of PM emitted from office machines separately.

Several previous studies have reported the health effects of toner exposure in toner-manufacturing workers and suggest that toner particle inhalation has potential adverse effects [8–11]. However, these studies were limited owing to statistical analysis methods, sample size, and other factors. Moreover, there is insufficient information on the long-term health effects of toner exposure.

We commenced a 10-year cohort study regarding the respiratory health effects of working in Japanese toner and copier manufacturing enterprise in 2003. In the results of this cohort study, the effects of toner-handling work on the incidence of lung diseases and changes in the prevalence of subjective respiratory symptoms have already been published [12]. The purpose of this paper is to report the effects of toner-handling work on the findings of chest X-ray, respiratory function tests, and serum or urinary biomarker tests using longitudinal analysis.

Methods

Study design and setting

This prospective cohort study was conducted across successive 10 years. We conducted a baseline survey in 2003 and implemented follow-up surveys yearly from 2004 (first survey) to 2013 (tenth survey). Each participant received a periodic health check and completed 1) a toner-handling work status survey, 2) a questionnaire-based survey on self-reported respiratory symptoms and diseases, 3) chest radiography, 4) respiratory function tests, and 5) serum and urinary biomarker tests. We particularly examined the effects of toner-handling work on chest X-ray findings, respiratory function, inflammation, allergy, and oxidative stress.

Participants

A total of 918 male toner handlers aged 19–50 years who worked in one toner and copier manufacturing enterprise (toner-handling group) participated in this study starting April 2003. Their toner-handling work included toner development, toner manufacturing, toner or copy machine development, toner or copy machine recycling, and customer service. Additionally, we recruited male non-toner-handling workers aged 19–50 years who also worked in the workplace where the toner handlers worked. A total of 586 non-toner handlers were enrolled as controls (non-toner-handling group). We confirmed that the control group mainly engaged in desk work not often involving copy printing and had never engaged in toner-handling work.

Chest X-ray examination

We performed a yearly chest X-ray examination on each subject following the standard examination method regulated by the Pneumoconiosis Law in Japan [13, 14]. The chest X-ray images were interpreted based on the international classification of pneumoconiosis (a 12-point scale from 0/- to 3/+) [15] and electronically stored using a film digitizer. To avoid differential misclassification, the readers of the X-ray images were not given information about the toner-handling status of the participants.

Respiratory function tests: spirometry and flow-volume curve

We conducted yearly respiratory function tests for each subject, including the following parameters: vital capacity (VC), percentage of VC to predicted VC value (%VC), forced expiratory volume in 1 s (FEV1), percentage of FEV1 to predicted FEV1 value (%FEV1), percentage of forced expiratory volume in 1 s to forced vital capacity (FEV1/FVC), percentage of FEV1/FVC to predicted FEV1/FVC value (%FEV1/FVC), maximal expiratory flow at 25% FVC (V25), percentage of V25 to predicted V25 value (%V25), and peak expiratory flow rate (PEFR). The respiratory function tests were performed using a pneumotach-type spirometry measuring unit, for example, Microspiro HI–701 and Microspiro HI–801 (CHEST Corporation, Tokyo, Japan), meeting the standards regulated by the American Thoracic Society [16]. We measured each parameter three times on the same day to obtain adequate values. To ensure consistent and valid measurement, a skilled examiner at the same medical institution conducted the respiratory function tests throughout each 1-year study period. We calculated the predicted values for the VC, FEV1, FEV1/FVC, and V25 of each participant using the formula based on sex, age, and height indicated by the Japanese Respiratory Society [17, 18].

Serum and urinary biomarker tests

Each participant underwent yearly biomarker tests for inflammation, allergy, and oxidative stress, such as those for C-reactive protein (CRP), immunoglobulin E (IgE), interleukin (IL)–4, IL–6, IL–8, and interferon-gamma (IFN-γ) in serum, and 8-hydroxy–2'-deoxyguanosine (8-OHdG) in urine. To maintain accuracy and precision throughout the whole survey, we requested OHG Institute Co., Ltd. (Kitakyushu, Japan), to perform the analysis of 8-OHdG, and SRL Inc. (Tokyo, Japan) to analyze other biomarkers. IL–4, IL–6, IL–8, and IFN-γ are well-known to be related to in vivo immunity and inflammation. However, we discontinued measuring these four parameters by 2008 (fifth follow-up) because the responses to these cytokines were not specific to the biological effects of toner exposure. We continued to measure the rest of the biomarkers until the final survey year.

We used latex immunoagglutination assays for analyzing CRP, fluorescent enzyme immunoassays for IgE, chemiluminescent enzyme immunoassays for IL–4 and IL–6, enzyme-linked immunosorbent assays for IL–8, enzyme immunoassays for IFN-γ, and high-performance liquid chromatography for 8-OHdG. Spot urinary 8-OHdG concentrations could be unstable due to the participants' physical activity intensity,

urine collection time, and other factors. Hence, creatinine-corrected 8-OHdG values were adopted in this study. The limits of detection (LODs) at SRL Inc. were 0.02 mg/dl for CRP, 5.00 IU/ml for IgE, 2.00 pg/ml for IL–4, 0.20 pg/ml for IL–6, 2.00 pg/ml for IL–8, and 0.10 IU/ml for IFN-γ. We allotted the values of LOD/2 to the undetectable values of each biomarker.

Toner particle

Convention toner (C toner) and emulsion aggregation toner (EA toner) were manufactured (C toner is produced by pulverizing row materials) in the toner- and copy- machine-manufacturing enterprise wherein this study was conducted. This factory produced less EA toner than C toner from 2004 to 2006. However, the proportion of production was reversed in 2007; the production of EA toner steadily continued to increase [19]. We conducted a survey on workers in this work environment who had to handle a mixture of C and EA toners.

The mean particle diameters of the C toner and EA toner manufactured by this enterprise were 6.5 μm and 5.8 μm, respectively. Black C toner is composed of 70–80% polyester resin, 10–20% ferrite powder (iron oxide and manganese oxide), <10% amorphous silica, <10% carbon black, and <1% titanium dioxide. Black EA toner is composed of 60–70% styrene-acrylate resin, 10–20% ferrite powder (iron oxide and manganese oxide), <10% polyethylene, <10% amorphous silica, <10% carbon black, and <1% titanium dioxide [20].

Toner exposure assessment

We have previously reported our findings from detailed assessments of toner exposure levels [19, 21–24]. In particular, Matsuda et al. described the details of the actual state of toner exposure in workers who handled toner in the same enterprise where this study was conducted.

In a previous study, subjects were randomly selected from among workers who engaged in five categories of work. Their toner exposures were measured using a personal dust sampler every year between 2003 and 2011. In fiscal years 2003 and 2004, we used a Roken-type Filter Holder for Personal Total and Respirable Dust Sampler (Model PS–43; Shibata Scientific Technology Ltd., Soka, Saitama, Japan) to measure the particles. These samplers were equipped with glass-fiber filters (PTFE binding and T60A20 type ϕ 25 mm; Tokyo Dylec Corp., Tokyo, Japan). An AirChek 2000 Sample Pump (SKC Inc., Pennsylvania, USA) or Gilian GilAir–5 Air Sampling Pumps (Sensidyne, St. Petersburg, Florida, USA) was used, with a flow rate of 1.5 L/min. These instruments collected particles with a size classification that was characteristically set at 5 μm (50% cutoff-point). In the fiscal years 2005 to 2010, we used a Model NWPS–254 Filter Holder for Personal Dust Sampler (Shibata Scientific Technology). This sampler was equipped with glass-fiber filters (PTFE binding and T60A20 type ϕ 25 mm; Tokyo Dylec.), and AirChek 2000 Sample Pumps or Gilian GilAir–5 Air Sampling Pumps were used, with a flow rate of 2.5 L/min.

These instruments collected particles with a size classification that was characteristically set of at 4 μm (50% cutoff- point).

We used electron microscopy and infrared spectroscopy to identify the toner origin of the dust collected on the personal dust sampler filters. The levels of personal exposure to toner particles were different for each type of toner-handling work, being significantly higher in machine-recycling work and toner-manufacturing work than in three other types. The mean 8-h time-weighted average (TWA–8h) (SD) of each worker according to the five types of toner-handling work at the baseline survey was 0.989 (0.786) mg/m^3 for toner and copy machine recycling (hereafter referred to as “recycling”), 0.203 (0.441) mg/m^3 for toner manufacturing, 0.034 (0.030) mg/m^3 for toner development, 0.019 (0.063) mg/m^3 for toner and copy machine development, and 0.020 (0.060) mg/m^3 for customer service. In all types of toner-handling work, the TWA–8h value was much lower than the 3.0 mg/m^3 maximum level allowed for unspecified particles, defined as the threshold limit value–time-weighted average (TLV-TWA), recommended by the American Conference of Governmental Industrial Hygienists (ACGIH) [25].

Subgrouping according to toner exposure assessment

We divided the toner-handling group into two groups based on the toner exposure assessment, namely the high-concentration toner exposure group, who engaged in recycling and toner manufacturing, and the low-concentration toner exposure group, who engaged in the other three types of toner-handling work, thereby creating three groups in total including the non-toner-handling group. We then evaluated the health effects of toner particle exposure among the three groups.

Statistical analysis

To analyze the panel data in each survey year, qualitative variables were compared using a chi-squared test or Fisher’s exact test, and quantitative variables were compared using a simple t-test and Welch’s t-test. We used a linear mixed model (LMM) [26] to analyze longitudinal data. Dependent variables consisted of the respiratory function test parameters and the biomarker values, and the following four models were analyzed. In model 1, we treated toner-handling work, the survey year, and the interaction between toner-handling work and survey year as fixed effects and treated only the individual differences at baseline as the random effects (random intercept model). In model 2, assuming that responses to toner exposure are also different among individuals during the observation period, we added both individual differences at baseline and responses to toner exposure as random effects (random intercept and slope models). Akaike’s Information Criterion (AIC) was used to determine the model with high fitness. In model 3, we used the higher goodness-of-fit model in models 1 and 2 and adjusted the model using age at baseline, body mass index, smoking, asthma, allergic rhinitis, pneumonia, sinusitis, exposure to dust other than toner at work, and organic solvent-handling work as confounding factors. Baseline surveys [21, 27] and interim reports [22–24] have suggested that these variables may influence

dependent variables. Additionally, in model 4, with regard to toner-handling work, analysis was performed using the three groups, that is, the high-concentration toner exposure group, low-concentration toner exposure group, and non-toner-handling group.

In all models, the health effects attributed to toner exposure were indicated as the differences in yearly changes in parameters between the toner-handling group (subgroups included) and non-toner handing group. The differences in yearly changes were calculated as estimated values of the coefficient of interaction between toner-handling work and survey year in the LMM. Any significant differences in yearly changes in some parameters were considered as indicating additional changes in the toner-handling group compared to the non-handling group. In other words, the differences indicate the presence of health effects caused by toner handling. Yearly changes in each parameter in the non-toner-handling group corresponded to the estimated values of the coefficient of the survey year in the LMM. If any significant effects of toner exposure on each parameter were observed, we also performed LMM analysis adjusted with the same confounding factors as models 3 and 4, respectively for each exposure concentration level group to determine the amount of change over time in each group. In all analyses, the threshold for significance was at $P<0.05$. SPSS23.0J analytical software (IBM) was used.

Results

Participants

Among 1504 subjects, 9 toner handlers and 2 non-toner handlers withdrew to participate in this study before the baseline survey. The reasons of the withdrawal were not related to the onset of respiratory disorder. The number of participants in the baseline survey was 909 for the toner-handling group and 584 for the non-toner-handling group. We excluded 25 participants (22 toner handlers and 3 non-toner handlers) who enrolled in the baseline survey from analysis owing to deficiency of work history data. Finally, we analyzed the data of 1468 participants (887 for the toner-handling group and 581 for the non-toner-handling group). None of them had a history of chronic granulomatous pneumonia, pneumoconiosis, or lung cancer at the baseline survey.

On average, the participants completed 8.8 out of 10 follow-up surveys. The average length of follow-up (from the baseline survey to the last follow-up survey) was 8.9 years. There were no significant differences in these parameters between the toner-handling group and non-toner-handling group. Baseline characteristics of participants are shown in Table 1. Of the 887 participants in the toner-handling group, 49 participants, who worked for recycling process and toner manufacturing process, were assigned to the high-concentration toner exposure group and the other 838 participants were assigned to low-concentration toner exposure group.

During the study period, a total of 370 participants (203 toner handlers and 167 non-toner handlers) withdrew from this study. We confirmed the reason for withdrawal for each participant who withdrew their consent. There was no withdrawal due to the onset of respiratory disease. Table 2 shows a comparison

of baseline data between subjects who completed follow-up and those who withdrew from the study. In the toner-handling group, the mean age of subjects who withdrew was significantly higher than that of who completed follow-up, and the VC, %VC, FEV1 and V25 values were significantly lower in the subjects who withdrew than in those who completed follow-up. These significant differences in respiratory function parameters disappeared after adjustment for age. No significant differences were observed in the non-toner-handling group.

Chest X-ray examination

In the baseline survey, all participants did not have lung fibrosis that was 1/1 or greater on a 12-point profusion scale using chest X-ray. A total of 11,563 chest X-ray examinations were conducted in the 10-year follow-up period (7368 chest X-ray photographs in the toner-handling group which consisted of 461 photographs of high-concentration toner exposure and 6925 photographs of low-concentration toner exposure, and 4177 chest X-ray photographs in the non-toner-handling group). One participant of the low-concentration toner exposure group scored 1/1 on the 12-point scale in the second follow-up survey, and one participant of the non-toner-handling group scored 1/2 in the seventh follow-up survey. However, these findings disappeared in the subsequent follow-up surveys.

Respiratory function and serum and urinary biomarkers

In the baseline survey, the data of 186 participants for serum and urinary biomarkers (toner-handling group, 169; non-toner-handling group, 17) could be unreliable due to inappropriate blood or urine sample collection procedures or damage of the samples during transport. Therefore, these data were excluded from this study. Four cytokines, namely IL−4, IL−6, IL−8, and IFN-γ, were assessed until the fifth follow-up survey. However, of 7664 measurements of IL−8 and IFN-γ conducted from the baseline survey until the fifth follow-up survey, 6746 measurements of IL−8 (88%) and 7128 measurements of IFN-γ (93%) were below the LOD.

Panel data analysis

The mean values of the respiratory function test indicators and biomarkers in each follow-up survey were compared between the toner-handling group and non-toner-handling group (Tables 3 and 4). We observed significant differences in some respiratory function test indicators between the two groups sporadically until the third follow-up survey, but no significant differences in the fourth and later follow-up surveys.

Regarding serum or urinary biomarkers, the CRP levels of the toner-handling group were significantly lower than those of the non-toner-handling group in the first follow-up survey, but there were no significant differences in the other follow-up surveys. Meanwhile, the IL−6 and IFN-γ values of the toner-handling group were significantly higher than those of the non-toner-handling group in the second survey,

but there were no significant differences in the other follow-up surveys. For 8-OHdG, we observed significant differences in values at the third, seventh, and eighth follow-up surveys. The 8-OHdG values of the toner-handling group were significantly higher than those of the non-toner-handling group in the third and seventh follow-up surveys but were significantly lower in the eighth follow-up survey. There were no significant differences in the other follow-up surveys.

Longitudinal data analysis

Table 5 shows the estimated health effects of toner exposure using model 1 and 2 represented by differences in yearly changes in parameters between the toner-handling group and non-toner-handling group, and also shows the AIC of models 1 and 2. The model 2 fitted better than the model 1 in all parameters except IL–8. Therefore, models 3 and 4 were analyzed using the random intercept model for IL–8 and the random intercept and random slope models for other parameters.

Table 6 shows the estimated health effects of toner exposure using model 3. Table 7 shows the differences in yearly changes in parameters among the high-concentration toner exposure group, low-concentration toner exposure group, and non-toner-handling group using model 4. Additionally, Tables 6 and 7 show the yearly changes in each parameters of the non-toner-handling group using models 3 and 4. We observed significant effects in some parameters of the respiratory function tests and biomarker tests.

As for %VC, the analysis of model 3 comparing the whole toner-handling group with the non-toner-handling group showed no significant difference in yearly changes. In model 4, analyzed using three levels of toner exposure, the difference in yearly changes between the low-concentration toner exposure group and non-toner-handling group was not significant, while a significant difference was observed between the high-concentration toner exposure group and non-toner-handling group. %VC showed a significant upward trend in the non-toner-handling group. When the analysis using the LMM adjusted with the same confounding factors as models 3 and 4 was performed respectively for each exposure concentration group, the yearly change in each group was as follows: high-concentration toner exposure group, –0.11% (95% confidence interval [CI], –0.29 to 0.08; P = 0.250); low-concentration toner exposure group, 0.13% (95% CI, 0.09–0.17; P<0.001); and non-toner-handling group, 0.15% (95% CI, 0.01–0.20; P<0.001).

For PEFR, the analysis of model 3 showed significant differences in yearly changes between the whole toner-handling group and the non-toner-handling group. In the analysis of model 4, significant differences in yearly changes were observed only between the low-concentration toner exposure group and non-toner-handling group. PEFR showed a significant yearly increase in the non-toner handling group. When the analysis using the LMM adjusted with the same confounding factors as models 3 and 4 was performed respectively for each exposure concentration group, the yearly change in each group was as follows: high-concentration toner exposure group, –0.006 l/s (95% CI, –0.04 to 0.02; P = 0.67); low-concentration

toner exposure group, 0.02 l/s (95% CI, 0.01–0.03; P<0.001); and non-toner-handling group, 0.019 l/s (95% CI, 0.01–0.03; P<0.001).

For IL–8, the analysis of model 3 found no significant difference in yearly changes between the whole toner-handling group and the non-toner-handling. In the analysis of model 4, however, a significant difference in yearly changes was found between the high-concentration toner exposure group and non-toner-handling group. When the analysis using the LMM adjusted with the same confounding factors as models 3 and 4 was performed respectively for each exposure concentration group, the yearly change in each group was as follows: high-concentration toner exposure group, –0.13 pg/ml (95% CI, –0.76 to 0.49; P = 0.670); low-concentration toner exposure group, 0.03 pg/ml (95% CI, –0.009 to 0.08; P = 0.12); and non-toner-handling group, 0.1 pg/ml (95% CI, 0.06–0.13; P<0.001).

With regard to IFN-γ, the difference in yearly changes was significant in all comparisons between the whole toner-handling group and the non-toner-handling group, between the high-concentration toner exposure group and non-toner-handling, and between the low-concentration toner exposure group and non-toner-handling group. In the non-toner-handling group, the significant yearly decrease of IFN-γ was observed. When the analysis using the LMM adjusted with the same confounding factors as models 3 and 4 was performed respectively for each exposure concentration group, the yearly change in each group was as follows: high-concentration toner exposure group, –0.008 IU/ml (95% CI, –0.01 to –0.003; P = 0.001); low-concentration toner exposure group, –0.009 IU/ml (95% CI, –0.01 to –0.007; P<0.001); and non-toner-handling group, –0.003 IU/ml (95% CI, –0.004 to –0.002; P<0.001).

Discussion

To clarify the health effects of toner exposure, we explored the differences in yearly changes in the parameters of chest X-ray examination, respiratory function indicators measured by spirometry and flow-volume curve, and biomarkers of inflammation, allergy, and oxidative stress, between tone-handling workers and non-toner-handling workers. We did not observe any increased rate of onset of lung fibrosis associated with toner-handling work in the chest X-ray examination. Furthermore, almost all yearly changes in respiratory function indicators and serum and urinary biomarkers were similar between the toner-handling group and non-toner-handling group. On the other hand, the yearly changes in %VC and PEFR for respiratory function and the yearly changes in IL–8 and IFN-γ for serum and urinary biomarkers differed depending on the presence or absence of toner-handling work.

Some cross-sectional studies have evaluated the health effects of toner-printing work at copy centers. In a survey conducted at a copy center in India, a significant increase in serum IL–8 was observed in toner-printing workers compared with non-toner-printing workers [28]. Another survey in the United States reported a transient increase in urinary 8-OHdG levels in healthy participants who spent time in copy centers for several days [29]. Moreover, a cross-sectional study of Iranian copy centers reported that FVC and FEV1 were significantly lower in toner-printing workers than in non-toner-printing groups [30]. These reports suggest that toner-printing work at copy centers may cause inflammatory reactions, oxidative

stress, and deterioration of respiratory function. In general, exposure to toner particles may occur in workers in copy centers only when the toner is not fused to the paper owing to printing failure or when toner particles leak during toner cartridge replacement. However, these exposures secondary to printing failure and copy center work likely occur at a low rate. The participants of the studies mentioned above might have very little direct exposure to toner particles. These copy center studies were designed primarily to investigate the health effects of PM emitted during printing. Therefore, in these studies, it is difficult to evaluate only the health effects of toner particle exposure excluding the effects of exposure to other PM related to the printing process. Furthermore, since cross-sectional studies are influenced by factors other than exposure, such as individual differences, the causal relationship between work at the copy center and changes in the levels of respiratory function and biomarkers cannot be clearly defined.

Several epidemiological cohort studies aimed at investigating the health effects of toner particle exposure have been conducted at toner manufacturing plants [8–11]. No significant differences were reported between the toner-handling and non-toner-handling groups in terms of the development of new-onset lung fibrosis on follow-up chest X-ray examination. Regarding respiratory function, yearly changes and the occurrence of outliers of PEFR, VC, %VC, FEV1, %FEV1, FVC, and FEV1/FVC have been investigated in these studies, but no clear differences have been reported between the toner-handling group and the non-toner-handling group. Regarding biomarkers, the relationship between serum CRP, serum IgE, urinary 8-OHdG, etc., and toner particle exposure has been also investigated. However, these studies reported that the number of occurrence of outliers and the yearly changes in the values in the toner-handling group had a close resemblance to those inthe non-toner-handling group.

Remarkable differences in changes in respiratory function among individuals over time have been reported [31–33]. Furthermore, lifestyle habits such as smoking and alcohol consumption have been reported to affect urinary 8-OHdG levels [34–36]. Therefore, the parameters evaluated in this study could be influenced not only by toner exposure but also by individual differences. However, the previous cohort studies were limited in terms of analysis that considered inter-individual differences. The strength of this study is that we performed analyses that modeled inter-individual differences as a random effect.

In this study, we hypothesized that each parameter of respiratory function in the toner-handling group decreased more than the usual decrease in respiratory function with aging if there were chronic health effects owing to toner exposure. We also hypothesized that the higher the toner exposure concentration, the greater the decline over time of each parameter, according to the dose-response relationship. However, this study showed that the %VC and PEFR of the non-toner-handling group and low-concentration toner exposure group increased over time, while those of the high-concentration toner exposure group did not change significantly.

The effect of toner exposure on %VC was significant in the high-concentration toner exposure group, with the range of yearly increase becoming smaller throughout the survey years, compared to the non-toner-handling group. This is consistent with the fact that only the %VC in the high-concentration toner exposure group did not increase over time, unlike the %VC in the low-concentration toner exposure group

and non-toner-handling group increased over time. In contrast, the measured values of VC decreased significantly in the non-toner-handling group, as shown in Table 7. In addition, since the difference in yearly changes between the low-concentration toner exposure group and non-toner-handling group was not significant, the decrease in VC over time in the low-concentration toner exposure group was considered to be similar to that in the non-toner-handling group. These findings suggest that the observed improvement in %VC in the low-concentration toner exposure group and non-toner-handling group was not actual improvement in respiratory function, but was an apparent change influenced by the reference value for VC calculated by prediction equation [17]. There was no significant change in %VC over time in the high-concentration toner exposure group. These results might have been due to the insufficient statistical power that originated from the small amount of change and the sample size. The analysis of PEFR, IL–8, and IFN-γ in the high-concentration toner exposure group could have also been affected by the small sample size and small amount of change.

The effect of toner exposure on PEFR was significant in the low-concentration toner exposure group, with the range of yearly increase becoming larger throughout the survey years, compared to the non-toner-handling group. Focusing on the amount of yearly change in each group, both groups' average PEFR increased over time. As shown in Tables 1 and 3, the mean values of PEFR for both the non-toner-handling group and toner-handling group were lowest at the baseline survey among all survey points, and the values for both groups improved after the first follow-up survey. This improvement in PEFR in both groups could be related to the training effect of participants on respiratory function tests after the first follow-up survey. Moreover, as shown in Table 7, the amount of difference in change over time between the low-concentration exposure group and non-toner-handling group was estimated to be 0.01 l/s. This effect of the low-concentration toner exposure is certainly statistically significant but may have less clinical significance. In any case, we found no significant causal relationship between toner exposure and deterioration of respiratory function.

As with respiratory function, when there was a chronic health effect due to toner exposure, each biomarker for inflammation, allergy, and oxidative stress in the toner-handling group was predicted to demonstrate different yearly changes from those in the non-toner-handling group. Furthermore, according to the dose-response relationship, we predicted that the differences of the yearly changes in each biomarker would proportionally increase with higher toner-exposure concentration.

Toner exposure significantly affected IL–8 levels in the high-concentration toner exposure group such that it decreased yearly, compared to that in the non-toner-handling group; however, no effect was observed in the low-concentration toner exposure group. With regard to IFN-γ, toner exposure had a significant effect in both the low-concentration and high-concentration exposure groups, with an increasing widening of the range of yearly decrease throughout the survey years when compared to that in the non-toner-handling group. Contrary to our hypothesis, IL–8 values increased only in the non-toner-handling group over time, whereas IFN-γ values decreased more in the low-concentration and high-concentration toner exposure groups than in the non-toner-handling group. However, as mentioned above, it must be considered that measurements of approximately 90% of both biomarkers were below the LOD,

and even the mean values of these biomarkers were below the LOD in many survey points. These findings indicate that observed yearly changes in biomarker values could be primarily due to values being assigned for the convenience of measurements that were below the LOD. Nevertheless, we found no significant causal relationship between toner exposure and increase in the values of biomarkers for inflammation, allergy, and oxidative stress.

In terms of low toxicity associated with toner exposure, the findings of the chest X-ray examination and the yearly changes in respiratory function indicators and serum and urinary biomarkers in this study are consistent with the trend indicated by the findings of inhalation exposure studies in animals [37–39].

Limitation and future direction

This study has several limitations. First, there was a problem with the sample size. We attempted to determine the dose-response relationship between exposure levels and health effects by comparing various parameters among the non-toner-handling group, low-concentration toner exposure group, and high-concentration toner exposure group. However, the sample size for the high-concentration toner exposure group was 49, which may have been small. While this study had reasonably sufficient power to detect distinct outcome discrepancy between the toner-handling group and non-toner-handling group, this may not have been the case for the three-group comparison. According to Cohen, assuming with 80% of power, a small effect size, and 5% significance, a total of 969 cases (323 cases per group) are needed for a three-group mean comparison [40]. Recently, several other epidemiological studies dealing with health effects related to toner exposure have been conducted [41, 42]. A pooled analysis using the data from these studies may be helpful in elucidating the dose-response relationship. Second, we may need to continue to observe these toner-handling workers, our observation period may have been short. In particular, it takes a long time for lung fibrosis or pulmonary obstructive disorder to develop. Third, the health effects of toner exposure could have been underestimated due to healthy worker bias [43]. As toner-handling work may involve higher physical load than non-toner-handling work, healthy workers might have been preferentially assigned to toner-handling work. Fourth, the actual level of toner exposure may have been lower than expected. At the study site, adequate ventilation was in operation to control dust scattering, and workers engaged in frequent toner-handling works wore appropriate respiratory masks. Although there could have been an overestimation of toner exposure, personal exposure measurements had to be performed outside the mask to protect the health of the toner-handling workers during measurement.

Conclusions

We explored the effects of toner-handling work on the findings of chest X-ray examination, respiratory function tests, and serum and urinary biomarkers by performing a longitudinal analysis of a cohort occupationally exposed to toner particle in a Japanese toner and copy machine manufacturing enterprise. We observed that toner-handling work was irrelevant to the onset of lung fibrosis, deterioration

of respiratory function, or increases in the values of biomarkers for inflammation, allergies, and oxidative stress. Our study shows that toner-handling work has minimal adverse effects on the respiratory system in work environments where dust aerosolization is sufficiently controlled by ventilation.

List Of Abbreviations

AIC: Akaike's Information Criterion, CI: confidence interval, CRP: C-reactive protein, FEV1: forced expiratory volume in 1 s, %FEV1: percentage of FEV1 to predicted FEV1 value, FEV1/FVC: percentage of forced expiratory volume in 1 s to forced vital capacity, %FEV1/FVC: percentage of FEV1/FVC to predicted FEV1/FVC value, FVC: forced vital capacity, IgE: immunoglobulin E, IFN-γ: interferon-gamma, IL: interleukin, LMM: linear mixed model, LOD: limit of detection, 8-OHdG: 8-hydroxy–2'-deoxyguanosine, PEFR: peak expiratory flow rate, PM: particulate matter, TLV-TWA: threshold limit value–time-weighted average, TWA–8h: mean 8-h time-weighted average at baseline survey, V25: maximal expiratory flow at 25% FVC, %V25: percentage of V25 to predicted V25 value, VC: vital capacity, %VC: percentage of VC to predicted VC value

Declarations

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Ethics approval and consent to participate

This study was approved by the Ethics Committee of Medical Research, University of Occupational and Environmental Health, Japan. We obtained written informed consent from each participant.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Authors' contributions

Methodology, TH. Validation, NT, HK, KI, and AO. Statistical analysis, NT. Investigation, NT, HK, HA, SK, MM, TK, NY, and KI. Data curation, NT and HK. Writing—original draft preparation, NT. Writing—review and editing, KI. Supervision, YF, AO, and TH. Project administration, AO and TH. Funding acquisition, AO and TH. All authors read and approved the final manuscript.

Competing interests

Fuji Xerox Co. funded this study but had no control over its design, interpretation of data, writing, or publication.

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