

#### 4. 健康影響と管理基準のあり方に関する研究

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##### 研究要旨

電子写真方式の事務機器から稼働時に排出されるエミッションによる室内空気汚染の問題について、粒径が小さく吸入された場合に呼吸器の末梢部まで到達し得る微小粒子(FP)、超微小粒子(UFP)を中心に、Medlineを主たる対象とした文献検索を引き続き探索的に行い、昨年度以降の新たな報告として7編の論文を収集した。またこれまで検索から漏れていた重要論文2編を追加収集した。昨年度から生体影響に焦点を当てた研究報告が散見されるようになったことはすでに報告したが、今年度の新たな文献においても、ヒト由来培養細胞を用いた遺伝毒性を含む種々の評価項目を検討した報告や、印刷用トナー粒子を取り扱う作業者を対象とした大規模な前向きコホート研究の報告など、生体影響にかかわる研究が進展していることをうかがわせる報告が見られた。しかし今後の建築物環境衛生管理に役立てるためのリスク評価のためには、ハザード評価としての情報はいまだ十分とは言えず、更なる研究の促進が必要であると考えられる。

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##### A. 研究目的

本分担研究では、これまで建築物空気環境の重要な要素として、微小粒子(PM<sub>2.5</sub>、FP)などの空気中の粒径の小さな粒子状物質に着目し、中でも近年オフィスや家庭に急速に普及した電子写真方式の複写機/レーザープリンタからエミッションとして室内気中に放出されるFP及び超微小粒子(UFP)に焦点をあて、文献検索により情報の収集を行い、科学的知見の整理を行ってきた。

本年度は最終年度であることから、上記の観点からの文献検索による情報収集を継続するとともに、3年間で得られた情報の整理を行い、科学的知見のまとめを行った。とくに将来の建築物衛生管理に役立てる上で適切なリスク評価が必要であり、そのためには、ハザード評価が最も重要であることから、印刷用トナー粒子を含む、これら事務機器の使用に関連して生じる可能性がある粒子状物質曝露の生体影響についても文献の検索を行った。

##### B. 研究方法

過去2年度と同様に、データベースを利用した文献検索により文献を収集し、整理した。文献の範囲はレビューシステムを有する学術誌に掲載された原著論文を原則とすることとして、一般誌の解説記事的な文献や特定の対象に対する業務報告書、会議録、報道記録は除外した。国内(和文)文献は医学中央雑誌のデータベースを、海外を含む英文文献のデータベースにはMedlineを用いた。

昨年度収集した文献以降のものを原則とし、とくに下記の点を中心に、種々のキーワードを組み合わせて検索を行った。

- ・ 複写機/レーザープリンタからのFP、UFPの排出とそれに伴う空気汚染
- ・ 上記エミッションへの曝露に伴う生体反応、健康影響の可能性

また本年度が研究の最終年度であることから、昨年度までの検索で漏れていた重要文献や、事務機器の使用に関連して生じる可能性のある粒子状物質の空気汚染に関連する重要な文献についても収集を行った。

検索された文献については、内容を吟味して、文献リストを作成した。

### C. 研究結果

これまでの2年間に報告した文献以降の抽出文献として本年度は7件を収載し(2013年4件, 2012年3件), PDFファイルまたは印刷体のフルペーパーを入手した。昨年度から生体影響に関する研究報告が散見されるようになっていたが, 本年の検索でも, 培養細胞を用いて細胞毒性や遺伝毒性を検討した *in vitro* 実験研究の報告や, 複写機/レーザープリンタのトナー粒子曝露による生体影響を検討した疫学的前向きコホート研究の報告などがあった。

このほか, 培養細胞を用いて遺伝毒性を含む一連の毒性評価を行っているドイツの研究グループの報告, および複写機稼働時のUFP排出を初めて示したオーストラリアの研究グループの報告でこれまでの検索で抽出されなかった2008年の論文など, 重要論文2件を追加収集した。

以下に文献の書誌情報と著者抄録を文献リストとして示した。

1. Konczol M, Weis A, Gminski R, Merfort I, Mersch-Sundermann V. Oxidative stress and inflammatory response to printer toner particles in human epithelial A549 lung cells. *Toxicol Lett.* 2013 Feb 4;216(2-3):171-80.

#### Abstract :

Reports on adverse health effects related to occupational exposure to toner powder are still inconclusive. Therefore, we have previously conducted an *in vitro*-study to characterize the genotoxic potential of three commercially available black printer toner powders in A549 lung cells. In these cell-based assays it was clearly demonstrated that the tested toner powders damage DNA and induce micronucleus (MN) formation. Here, we have studied the cytotoxic and proinflammatory potential of these three types of printer toner particles and the influence of ROS and NF- $\kappa$ B induction in order to unravel the underlying mechanisms. A549 cells were exposed to various concentrations of printer toner particle suspensions for 24 h. The toner particles were observed to exert significant cytotoxic

effects in the WST-1 and neutral red (NR)-assays, although to a varying extent. Caspase 3/7 activity increased, while the mitochondrial membrane potential (MMP) was not affected. Particles of all three printer toner powders induced concentration-dependent formation of reactive oxygen species (ROS), as measured in the DCFH-DA assay. Furthermore, toner particle exposure enhanced interleukin-6 and interleukin-8 production, which is in agreement with activation of the transcription factor NF- $\kappa$ B in A549 cells shown by the electrophoretic mobility shift assay (EMSA). Therefore, it can be concluded that exposure of A549 lung cells to three selected printer toner powders caused oxidative stress through induction of ROS. Increased ROS formation may trigger genotoxic effects and activate proinflammatory pathways.

2. Bello D, Martin J, Santeufemio C, Sun Q, Lee Bunker K, Shafer M, Demokritou P. Physicochemical and morphological characterisation of nanoparticles from photocopiers: implications for environmental health. *Nanotoxicology.* 2013 Aug;7(5):989-1003.

#### Abstract:

Several reports link printing and photocopying with genotoxicity, immunologic and respiratory diseases. Photocopiers and printers emit nanoparticles, which may be involved in these diseases. The physicochemical and morphological composition of these emitted nanoparticles, which is poorly understood and is critical for toxicological evaluations, was assessed in this study using both real-time instrumentation and analytical methods. Tests included elemental composition (40 metals), semi-volatile organics (100 compounds) and single particle analysis, using multiple high-sensitivity/resolution techniques. Identical analyses were performed on the toners and dust collected from copier's exhaust filter. Engineered nanoparticles, including titanium dioxide, iron oxide and fumed silica, and several metals were found in toners and airborne nanoscale fraction. Chemical

composition of airborne nanoscale fraction was complex and reflected toner chemistry. These findings are important in understanding the origin and toxicology of such nanoparticles. Further investigation of their chemistry, larger scale exposure studies and thorough toxicological characterisation of emitted nanoparticles is needed.

3. Murase T, Kitamura H, Kochi T, Terunuma N, Kurosaki S, Hata K, Yanagi N, Uchino B, Kitahara K, Morimoto Y, Kasai H, Sasaki T, Ogami A, Higashi T. Distributions and ranges of values of blood and urinary biomarker of inflammation and oxidative stress in the workers engaged in office machine manufactures: evaluation of reference values. *Clin Chem Lab Med*. 2013 Feb;51(2):421-8.

Abstract:

**BACKGROUND:**

Interleukins, interferons and oxidative DNA products are important biomarkers assessing the inflammations and tissue damages caused by toxic materials in the body. We tried to evaluate distributions, reference values and age related changes of blood levels of inflammatory cytokines, C-reactive protein (CRP), IgE and urine levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) among workers in a cohort study evaluating the health influences of toner particles.

**METHODS:**

A total of 1366 male workers under age 50 years (age 19-49 years; 718 exposed and 648 not exposed to toner particles) in a cross sectional study of 1614 (categorized as 809 exposed and 805 not exposed, age 19-59 years) workers in a photocopier company has been followed prospectively as the cohort. Blood levels of interleukin (IL)-4, IL-6, IL-8, interferon- $\gamma$  (IFN- $\gamma$ ), CRP, IgE and urine 8-OHdG were measured annually for 5 years.

**RESULTS:**

Reference values of the biomarkers are; CRP: 0.01-0.63 $\times$ 10<sup>(-2)</sup> g/L, IgE: 6-1480 IU/mL, IL-4: 2.6-76.1 pg/mL, IL-6: 0.4-4.9 pg/mL and 8-OHdG: 1.5-8.2 ng/mgCr. We could not evaluate reference

values for IL-8 and IFN-  $\gamma$  because most of the values were below the sensitivity limits (2.0 pg/mL and 0.1 IU/mL, respectively). There were no differences of the biomarker levels between the toner exposed and the control workers. We observed a statistically significant age related decrease of serum IL-4 levels.

**CONCLUSIONS:**

This is the first report assessing the distributions and reference values of inflammatory biomarker levels in a large scaled cohort. We observed age related changes of some of the biomarkers. We could not detect any differences of the studied biomarker values between the toner exposed and the control workers.

4. Matsuda Y, Harada Y, Tanno Y. State of Toner Exposure of Workers Who Handle Toners. *J Occup Health*. 2013 May 23. [Epub ahead of print]

Abstract:

**Objectives:** The aim of this study was to ascertain the actual state of toner exposure of workers who handle toner. **Methods:** Personal exposure measurements were conducted on workers handling toner in which the respirable dust (RD) concentration by work type was determined. Targeted work types consisted of "machine recycling," "toner manufacturing," "toner research/development," "machine design/development" and "servicing." The implementation period lasted from April 2003 to March 2011, and measurements were conducted annually. The measurement method conformed to the Japanese Working Environment Measurement Standards (new standards adopted starting in 2005). **Results:** Comparing the RD concentrations for fiscal year 2003 by work, significant differences were found between machine recycling and the other four work types, as well as in toner manufacturing and the other four work types. Similarly, based on the new legislative standards applied in Japan from fiscal year 2005, significant differences were found between machine recycling and the other four work types, as well as in toner manufacturing and the other

four work types. Discussion: It is clear that workers engaged in machine recycling and toner manufacturing are exposed to toner, and that a certain level of exposure is continuing. Although it cannot be said that workers involved in toner research/development, machine design/development and servicing have no toner exposure, the concentration is of an extremely low level. Conclusion: At present, toner exposure levels by work type can be divided into two groups?one consisting of machine recycling and toner manufacturing, and the other consisting of toner research/development, machine design/development and servicing.

5. Castellano P, Canepari S, Ferrante R, L'Episcopo N. Multiparametric approach for an exemplary study of laser printer emissions. *J Environ Monit.* 2012 Feb;14(2):446-54.

Abstract :

The aim of this work was the study, by a multiparametric approach, of emissions from a laser printer in an experimental box-chamber, with particular attention to nanoparticles release. The experimental design included number concentration measurements by Fast Mobility Particle Sizer (FMPS) and chemical characterizations (elements) of size segregated samples collected by Nanomoudi cascade impactor. Volatile Organic Compounds (VOCs) were also sampled by charcoal sorbent tubes by personal sampling pumps. Monitoring of ozone, total volatile organic compounds concentrations and of temperature and humidity values inside the experimental box during the printing processes were also performed by automatic analyzers. The performed monitoring allowed to evidence different ways for particles emissions by laser printers, in particular showing that nanoparticles, characterised by high concentrations of Ba, Zn, B, K, Sr and Na, are set free at the beginning of the printing process. This emission seems to be directly ascribable to the use of toner powder, as all these elements are present in it. The emission of larger particles (ca. 100-320

nm) was observed in subsequent phases of the print process, probably due to the condensation of vapours released during the progressive heating of the fuser roller. This contribution was proved by both the FMPS measurements and the cascade impactor results. Also, a low emission of particles in higher size ranges was evidenced, mainly due to paper related particles. A very high concentration of VOCs was detected inside the chamber and the chemical speciation shows that the major contribution is associated to toner components, even if some species are released from other printer components. Although the formation of secondary species by reaction of VOCs with ozone cannot be excluded, these species were present inside the chamber at concentrations lower than the detection limit.

6. Shafer MM, Toner BM, Overdier JT, Schauer JJ, Fakra SC, Hu S, Herner JD, Ayala A. Chemical speciation of vanadium in particulate matter emitted from diesel vehicles and urban atmospheric aerosols. *Environ Sci Technol.* 2012 Jan 3;46(1):189-95.

Abstract:

We report on the development and application of an integrated set of analytical tools that enable accurate measurement of total, extractable, and, importantly, the oxidation state of vanadium in sub-milligram masses of environmental aerosols and solids. Through rigorous control of blanks, application of magnetic-sector-ICPMS, and miniaturization of the extraction/separation methods we have substantially improved upon published quantification limits. The study focused on the application of these methods to particulate matter (PM) emissions from diesel vehicles, both in baseline configuration without after-treatment and also equipped with advanced PM and NO(x) emission controls. Particle size-resolved vanadium speciation data were obtained from dynamometer samples containing total vanadium pools of only 0.2-2 ng and provide some of the first measurements of the oxidation state of vanadium in diesel vehicle PM emissions. The emission rates and the measured fraction of V(V) in PM from diesel

engines running without exhaust after-treatment were both low (2-3 ng/mile and 13-16%, respectively). The V(IV) species was measured as the dominant vanadium species in diesel PM emissions. A significantly greater fraction of V(V) (76%) was measured in PM from the engine fitted with a prototype vanadium-based selective catalytic reductors (V-SCR) retrofit. The emission rate of V(V) determined for the V-SCR equipped vehicle (103 ng/mile) was 40-fold greater than that from the baseline vehicle. A clear contrast between the PM size-distributions of V(V) and V(IV) emissions was apparent, with the V(V) distribution characterized by a major single mode in the ultrafine (<0.25  $\mu\text{m}$ ) size range and the V(IV) size distribution either flat or with a small maxima in the accumulation mode (0.5-2  $\mu\text{m}$ ). The V(V) content of the V-SCR PM (6.6  $\mu\text{g/g}$ ) was 400-fold greater than that in PM from baseline (0.016  $\mu\text{g/g}$ ) vehicles, and among the highest of all environmental samples examined. Synchrotron based V 1s XANES spectroscopy of vanadium-containing fine-particle PM from the V-SCR identified V(2)O(5) as the dominant vanadium species.

7. Wu XM, Apte MG, Bennett DH. Indoor particle levels in small- and medium-sized commercial buildings in California. *Environ Sci Technol*. 2012 Nov 20;46(22):12355-63.

Abstract:

This study monitored indoor and outdoor particle concentrations in 37 small and medium commercial buildings (SMCBs) in California with three buildings sampled on two occasions, resulting in 40 sampling days. Sampled buildings included offices, retail establishments, restaurants, dental offices, and hair salons, among others. Continuous measurements were made for both ultrafine and fine particulate matter as well as black carbon inside and outside of the building. Integrated PM(2.5), PM(2.5-10), and PM(10) samples were also collected inside and outside the building. The majority of the buildings had indoor/outdoor (I/O)

particle concentration ratios less than 1.0, indicating that contributions from indoor sources are less than removal of outdoor particles. However, some of the buildings had I/O ratios greater than 1, indicating significant indoor particle sources. This was particularly true of restaurants, hair salons, and dental offices. The infiltration factor was estimated from a regression analysis of indoor and outdoor concentrations for each particle size fraction, finding lower values for ultrafine and coarse particles than for submicrometer particles, as expected. The I/O ratio of black carbon was used as a relative measure of the infiltration factor of particles among buildings, with a geometric mean of 0.62. The contribution of indoor sources to indoor particle levels was estimated for each building.

8. Gminski R, Decker K, Heinz C, Seidel A, Konczol M, Goldenberg E, Grobety B, Ebner W, Giere R, Mersch-Sundermann V. Genotoxic effects of three selected black toner powders and their dimethyl sulfoxide extracts in cultured human epithelial A549 lung cells in vitro. *Environ Mol Mutagen*. 2011 May;52(4):296-309.

Abstract:

Until now, the adverse effects of toner powders on humans have been considered to be minimal. However, several recent reports have suggested possible significant adverse health effects from toner dust inhalation. The aim of this study was to evaluate the genotoxic potential of black toner powders in vitro. For the study of DNA damage, A549 cells were exposed to toner-powder suspensions and to their DMSO extracts, and then subjected to the comet assay and to the in-vitro cytokinesis block micronucleus test (CB-MNvit). Cytotoxic effects of the toner samples were assessed by the erythrosin B assay. Furthermore, size, shape, and composition of the toner powders were investigated. None of the three toner powders or their DMSO extracts reduced cell viability; however, they did induce DNA damage and formed micronuclei at concentrations from 80 to 400  $\mu\text{g cm}^{-2}$ , although to a varying

extent. All toner powders contain considerable amounts of the pigments carbon black and magnetite (Fe<sub>3</sub>O<sub>4</sub>) as well as small amounts of polycyclic aromatic hydrocarbons (PAHs). The overall results of our in-vitro study suggest that the investigated toner-powder samples are not cytotoxic but genotoxic. From the results of the physical and chemical characterization, we conclude that metals and metalloids as components of magnetite, or PAHs as components of the carbon-bearing material, are responsible for the genotoxic effects. Further research is necessary to determine the relevance of these in-vitro observations for private and occupational toner powder exposure.

9. Schripp T, Wensing M, Uhde E, Salthammer T, He C, Morawska L. Evaluation of ultrafine particle emissions from laser printers using emission test chambers. *Environ Sci Technol.* 2008 Jun 15;42(12):4338-43.

Abstract:

It has now been recognized that some hardcopy devices emit ultrafine particles ( $d(p) < 100$  nm) during their operation. As a consequence, the time-dependent characterization of particle release from laser printers is of high interest in order to evaluate the exposure of office workers to such emissions. The emission profiles of different printers can be compared in test chambers using a standardized test protocol and measuring devices with high time resolution. The extraction of meaningful and comparable data from the obtained data set is a complex procedure due to the different emission behavior patterns of the printers. The calculation of the unit specific emission rate (SER<sub>u</sub>) is of limited use because the emission profiles during the printing process ranged between short-term bursts and constant particle release. Therefore, other parameters such as the particle loss-rate coefficient, beta, which provides information about the testing conditions, and the area below the time vs concentration curve, F, which characterizes the particle release, allow for a comparison of the

different printer tests. Variations in the emission behavior could not be associated with specific manufacturers or product lines. In addition, when performing several print jobs on the same device, with only short pauses between jobs, the emission rate was reduced in some cases. This further complicates the ability to determine the influence of printer construction and consumables, such as toner and paper, on the concentration of particles emitted.

#### D.考察

これまで2年間に実施した文献検索の結果、複写機などの事務機器が稼働時にFP, UFPを排出することは多くの研究報告で明らかとなっており、さらにその排出に影響を及ぼす機器の種類や稼働の条件、また排出されるFP, UFPの粒径分布や成分組成などについてもいくつかの研究報告が行われていた。

一方建築物内における実際の使用状況下で生じる曝露の状況や、これらのFP, UFPが吸入されたときに起こる生体反応や健康影響に関する報告はごく限られていた。しかし実際のオフィスに市販の複写機等を持ち込んで稼働させ、室内空气中濃度の状況を検討した報告や、ヒトの志願者曝露実験により急性の生体反応を検討した報告、また培養細胞を用いて変異原性を検討した報告などが散見されるようになり、生体影響に関する研究が加速していることが推察された。

本年度の検索でも生体影響に関連する報告で興味深いものが見られた。文献1はドイツのSundermannらのグループによる培養細胞を用いた一連のin vitro実験研究の報告で、文献8として追加収集したように、複写機/レーザープリンタで使用されるトナー粒子が炎症反応や、小核試験で示唆される遺伝毒性を示すとし、そのメカニズムの可能性として、トナー粒子曝露により酸化敵ストレス負荷が生じることを指摘したものである。

また文献3および4は、いずれもわが国で行われた疫学研究の報告で、複写機やレーザープリンタ稼働時のエミッションを直接扱っているものではないが、これらの機器で使用される印

刷用トナー粒子の取扱いやそれに伴う曝露によって生じる可能性のある健康影響について報告したものである。前向きコホート研究の一部として報告されたもので、長期曝露による健康影響を評価する本格的な疫学研究であり、これまでのところでは有意な有害影響は示されていないが、今後のデータの集積による精密な解析結果が期待される。

また文献7はカリフォルニアの37の建築物について、その内外で粒子状物質の濃度を測定した研究報告で、室内の発生源や室内外差などについて検討されている。事務機器のエミッションだけを扱っている研究ではないが、実際の建築物における環境濃度が測定されており、曝露の推定などに貴重な報告である。

このほか、文献2、文献5はエミッション粒子の組成などの詳細に関する研究で、有害性のメカニズムの理解に重要であると考えられる。また文献6は自動車排ガス粒子の組成等に関する文献で本研究課題に直接関連する文献ではないが、環境中の人為的粒子状物質の代表で、生体影響についても比較的理解が進んでいるものであることから、事務機器エミッション粒子のリファレンスとして採用したものである。

#### E. 結論

複写機やレーザープリンタなどの事務機器からエミッションとして粒径の小さな粒子状物質（FP，UFP）が排出されることについては多くの研究報告から明らかであり、その発生に関連する稼働時の条件なども明らかにされつつあり、制御技術は進歩している。一方エミッションを吸入した際の生体影響については、種々の研究デザイン、種々のアウトカムを用いた報告が散見されるが、まだハザードとしての評価を行うだけの科学的知見は集積されておらず、今後の研究の進展が期待される。また実際の事務機器使用条件下における粒子状物質曝露に関する情報も限られており、今後のリスク評価のために、曝露評価に役立つデータの集積が必要であると考えられる。

F. 研究発表  
該当なし

