Toxicological investigation of laser printer emissions – Effects on human cells

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Introduction

Laser printer emissions have recently received much interest due to increasing health-related effects at the office workplace. The aim of the present study was to establish an in vitro test system to reveal the potential risk of laser printer emissions to human health. For this purpose, human lung epithelial A549 cells or peripheral mononuclear blood cells (PMBC) were exposed to laser printer emissions on transwell inserts in a Vitrocell® air-liquid interface exposure system. Physical properities such as temperature, humidity, particulate matter (PM1.0, PM2.5, PM10), ultrafine particles (diameter range between 10 – 1000 nm), ozone and chemical properties such as total organic carbons (TOC), volatile organic compounds (VOC), total volatile organic compounds (TVOC) of laser printer emissions were studied. Furthermore, biological effects including cytotoxicity and genotoxicity of laser printer emissions on human lung cells (A549) and release of cytokines of peripheral mononuclear blood cells (PMBC) had also been studied.

Fig 1:The emission test chamber with an investigated laser printer.

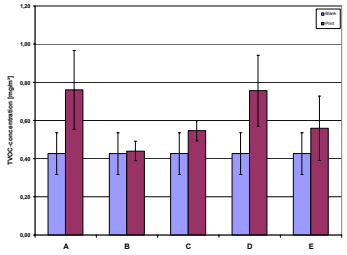

Fig 2. TVOC concentration of laser printers A - E during printing compared to chamber blank; results are mean ± SD (two independent experiments)
total volatile organic compounds = sum of all identified VOCs (quantified by their own response factors) and all unidentified VOCs (quantified by toluol response factor, TE)

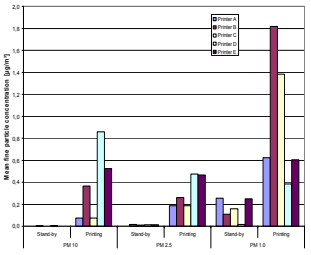

Fig 3. Mean fine particle concentrations of PM10, PM2.5 and PM1.0 of five laser printers during stand-by and printing process.

Material & Methods

The atmosphere was generated in a conditioned emissions test chamber (volume 1 m³, temp. 21°C, rel. humidity 50%, air exchange 1^{-h}) where the laser printer were placed. TOC was measured by a photoacoustic detector using propane as reference. Ozone was measured by a chemiluminescent analyzer. PM1.0, PM2.5 and PM10 were measured by an aerosol spectrometer. Ultrafine particles were measured by a condensation particle counter (CPC). VOC and TVOC were analysed according to ISO 16000-9 using sorbent tube thermal desorption (TD) and gas chromatography (GC) coupled to mass spectrometry (MS). After exposure experiments of 1 h at a constant flow of 5 mL/min cell cultures were tested in a Vitrocell® air–liquid interface exposure system for different biological responses like cell viability (WST-1 assay), release of cytokines as a marker of pro-inflammatory changes and for induction of micronuclei (CB-MNvit test) as a marker of genotoxicity.

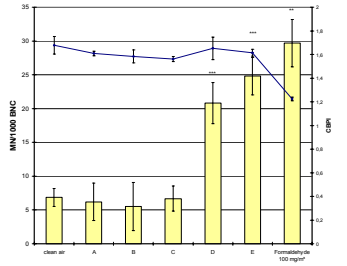

Fig 4. Micronuclei induction (column) and Cytochalasin-Block-Proliferation Index CBPI (line) in human A549 lung epithelial cells after 1h exposure to laser printer emissions in an Vitrocell®-Exposure module. Results are mean±SD of two independant experiments with N = 5-6 cell cultures. ** p < 0.01, *** p < 0.001; student´s t-test

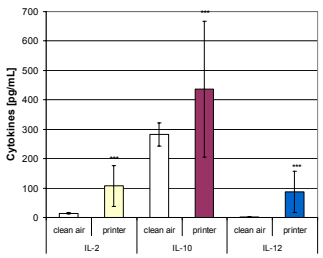

Fig 5. Induction of cytokines IL-2, IL-10 and IL-12 by laser printer emission (printer D, 1-h exposure, Vitrocell® Exposure module) in human epithelial A549 lung cells compared to clean air. Results of 3 independent experiments.

Printer	VOC- and particle emission					Toxicological effects	
	TVOCa	Max. conc. fine/ultra fine particles (CPC, 10 nm -1000 nm)	Mean PM 10	Mean PM 2.5	Mean PM 1.0	Micronuclei induction (1 h Exp.)	Cell-Viability (WST-1)
	[mg/m³]	[P/cm³]	[µg/m³]	[µg/m³]	[µg/m³]	MN/1000 BNC	Cell viability [%]
Clean air		14	0.03	0.06	0.20	6.83 ± 1.33	103.4± 6.3
A	0.334	47	0.06	0.12	0.62	6.20 ± 2.77	98.8 ± 4.5
B	0.014	32	1.84	1.04	1.09	5.00 ± 3.56	93.7 ± 2.8
C	0.120	368232	0.59	0.29	0.54	6.67 ± 1.86	94.4 ± 9.9
D	0.330	6091	4.27	0.43	0.64	20.80 ± 3.03	96.6 ± 3.6
E	0.134	50587	1.41	0.34	0.35	24.80 ± 2.77	104.0 ± 2.6

Tab 1: Overview of emissions of TVOC, fine and ultra fine particles as well as cell viability and micronuclei induction in A549 lung epithelial cells after 1h exposure to different laser printer emissions.

Results & Conclusions

The physical and chemical results showed that each tested printer had its own specific emission profile (Tab 1). Mean PM10, PM2.5, PM1.0 and ultra fine particle emissions were 0.06 – 4.27, 0.12 – 1.04, 0.35 – 1.09 µg/m³ and 14 – 368232 Particles/cm³, respectively. The major detected volatile compounds were 2-butanone, hexanal, hexamethylcyclotrisiloxane, styrene, o-xylene, ethylbenzene, m,p-xylene, benzaldehyde and ethylacetate. Exposure of A549 cells for 1 hour to laser printer emissions an increased induction of micronuclei was detected for two out of the five tested laser printers compared to cells exposed to filtered, clean air, whereas cell viability (WST-1-assay) and cell proliferation (CBPI) were not affected. The micronuclei number of the two positive printers were 20.8 ± 3.03 (n = 5) and 24.8 ± 2.77 (n = 5), respectively. Compared with clean air 6.83 ± 1.33, the two printers showed significant increased micronuclei frequencies (*p < 0.001, student`s t-test*). Furthermore, exposure of A549 cells for 1 h to the emissions of laser printer D resulted in enhanced IL-2, IL-10 and IL-12 levels after 24 h of incubation. We were able to show that our emissions test chamber/in-vitro exposure system is suitable for assessment of toxic effects of laser printer emissions on primary or cultured human cells. Our results indicate that laser printer emissions can cause pro-inflammatory and genotoxic effects in human cells in vitro. Further experiments have to be performed to analyse various types of laser printers and to evaluate the agents which are responsible for the observed toxic effects.

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