Exposure to emissions generated by 3-dimensional printing with polycarbonate affects vascular morphology and expression of markers of oxidative stress and vascular dysfunction in cardiac tissue_dataset

Introduction:

Three-dimensional (3D) printing is increasingly being used in manufacturing settings, homes and schools. Fused deposition modeling (FDM) 3D printers are the most widely used systems with standard thermoplastics such as acrylonitrile butadiene styrene (ABS), polylactic acid and polycarbonate (PC) commonly used in the manufacturing processes. Heating of the thermoplastic generates and releases particulates and fumes. Emission constituents frequently measured include aldehydes, benzene, toluene, ethylbenzene, and xylenes. Inhalation of the emitted particulates and/or the fumes, that contain bisphenol A (BPA) may pose health problems to users of these systems as well as bystanders.

The goal of the current study was to examine the effects of inhalation of PC-emissions generated during 3D-printing. PC-emissions can include bisphenol A (BPA). Bisphenols are known endocrine and metabolic disruptors (i.e., they interfere with actions of steroid and thyroid hormones) and have been shown to have significant effects on a number of physiological systems including the endocrine and cardiovascular systems. Because steroid hormones have major effects on cardiovascular function, it is possible that inhalation of PC particulate and/or BPA impact cardiovascular function.

To begin to understand how inhalation of PC-emissions generated during 3D printing might affect the cardiovascular system, the current study examined the effects of inhaling PC-emissions after 1, 4, 8, 15 and 30d of exposure, on peripheral vascular responses to vaso-

modulating agents, on cardiac morphology and on the expression of proteins and transcripts that are markers of inflammation, oxidative stress and cardiovascular dysfunction.

Methods:

- *Animals.* Male Sprague Dawley rats ([H1a: (SD) CVF, n = 6 rats/group; 6-7 weeks of age, 200 – 250 g) were obtained from Hilltop Lab Animals, Inc., Scottdale, PA. All animals were free of viral pathogens, parasites, *mycoplasms*, *Helicobacter* and cilia-associated respiratory (CAR) bacillus. All procedures were approved by the NIOSH Animal Care and Use Committee and are in compliance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals and the NIH Guide for the Care and Use of Laboratory Animals.
- *Exposure.* After 1 week of acclimation to the facility, animals were assigned to purified air (control) or PC-emissions. More specifically, three desktop 3D-printers (Manufacturer 1, NY) were placed in a stainless-steel chamber. Black polycarbonate filament was fed into each printer and the printers were operated continuously during a 4 h exposure period. Concentrations of measured PC-emissions were 0.6 mg/m^3.
- *Analysis of volatile organic compounds.* Volatile organic compounds were measured by gas chromatography/mass spectroscopy (GC/MS). TBPA and bisphenol A diglycidyl ether were also assessed in the PC-emissions. The average level of bisphenol in the samples was 5.3 ± 0.18 µg., The estimated cumulative deposition of BPA in the entire respiratory tract was 36 ng per day 1. , Bisphenol A diglycidyl ether was not detectable in any of the samples.

- *Tissue collection:* Groups of animals (6 air control and 6 treated) were euthanized 24 h after 1, 4, 8, 15 or 30d of exposure and cardiac tissue was collected and processed for histological and immunohistochemical analyses to assess morphological changes and for qRT-PCR to measure changes in transcript expression. Ventral tail arteries were also dissected and vasoconstriction and vasodilation in response to phenylephrine and acetylcholine were measured in a microvessel system (Scincta, Ontario).
- *Tissue preparation: Histology and Immunohistochemistry.* Cardiac tissue was fixed in formalin, paraffin embedded and sections (5 μm) were cut on a microtome. One set of sections was stained with Harris hematoxylin and eosin (H&E) for histological analyses. The other sections were stored in boxes until used for immunohistochemical identification of steroid receptors, oxidative stress and vascular remodeling.
- *Immunohistochemistry.* Slides used for immunohistochemistry were de-paraffinized and antigen retrieval was performed. The primary antibodies used were all from Santa Cruz Biotechnology (Dallas, TX) After incubation with the primary antibodies, sections were incubated in the appropriated secondary antibody ,cover slipped and stored at 4ºC.
- *Microscopy.* For immunostaining, multiple photomicrographs of arteries were taken at 20x magnification. All pictures for a specific antibody were taken at the same intensity so that the area labeled and the intensity of the labeling could be measured using Image J (NIH, Bethesda, MD). An average of the area that was immunolabeled in the endothelium and vascular smooth muscle of arteries was

calculated and this average was used for analyses. H&E staining was also performed on one set of sections. The internal and external perimeters of five randomly chosen arteries in the lateral atria and ventricles were chosen for analyses. The ratio of the internal to external diameter was calculated. In addition, four measures of arterial wall muscle thickness were collected from each artery and averaged.

- *Microvessel physiology:* Tails were dissected from rats after exsanguination and placed in cold Dulbecco's modified Eagle's medium with glucose (Invitrogen/Gibco; Carlsbad, CA). Ventral tail arteries were dissected, mounted on glass pipettes and pressurized to 60 mm/Hg. To assess the effects of treatment on sensitivity to α_1-adrenoreceptor-mediated vasoconstriction, PE was added to the chamber so that changes in the concentration occurred in half-log increments (-9.0 to -5.5 M) and the internal diameter of the artery was recorded. To assess sensitivity to ACh-induced re-dilation, arteries were rinsed and then pre-constricted to approximately 50% of their baseline diameters with PE. To assess the dilatory effects, ACh was added in half-log increments (-10.0 to -5.0) and changes in the internal diameter of the vessel were measured as described for PE.
- *Quantitative reverse transcriptase-polymerase chain reaction (qtRT-PCR).* qRT-PCR was performed to measure transcript levels in the heart. RNA was extracted from eight 5 μm, formalin fixed sections using the RNeasy FFPE Kit (Catalogue number 73504: Qiagen, Valencia, CA). Because only a limited amount of RNA could be isolated from paraffin-embedded sections, transcripts for factors that

changed in response to inhalation of other types of particulate or toxic fumes were chosen for analyses.

Citations:

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