

## **Pulmonary evaluation of whole-body inhalation exposure of polycarbonate (PC) filament 3D printer emissions in rats**

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### **Introduction**

Additive manufacturing (AM) is a broad manufacturing term that encompasses a range of processes that create objects by adding material through a computer-aided design model. Three-dimensional (3D) printing is a form of AM, which builds objects layer-by-layer deposition of feedstock material using a 3D printer machine and computer software. Fused filament fabrication (FFF, also known as Filament Freeform Fabrication) is one 3D printing process in which filaments are melted and extruded from a heated nozzle to deposit material. FFF is an emerging technology and one of the most popular additive manufacturing processes, especially for consumers and small manufacturers. Polycarbonate (PC) is a versatile material and PC filaments are widely used for fused filament fabrication 3D printing. PC filaments are often loaded with additives to achieve different properties of the print objects. These additives range from dyes, organometallic compounds, carbon nanomaterials, nanometal oxides to micrometer-scale particles such as copper, bronze, steel, tungsten, gold, and aluminum nitride (Vance et al., 2017). Several engineered nanomaterials were infused into PC filaments, such as silicon dioxide nanoparticles, titanium nitride nanoparticles (Vidakis et al., 2021), titanium carbide nanopowder (Vidakis et al., 2022a), aluminum nitride nanoparticles (Vidakis et al., 2022b), and carbon nanotubes (Potter et al., 2021).

During heating, PC filament undergoes thermal degradation and releases fine particles (0.1 to 2.5  $\mu\text{m}$ ) and incidental nanoparticles ( $d < 100 \text{ nm}$ ) as well as numerous volatile, and semi-volatile organic compounds that are likely derived from PC polymer and additives in the polymer (Azimi et al., 2016; Byrley et al., 2020; Gu et al., 2019; Stefaniak et al., 2017; Stefaniak et al., 2019; Alijagic et al., 2022; Tedla et al., 2022). These emissions could pose a potential hazard to human health. Currently, the potential health hazard of PC filament printing emissions has not been determined.

A NIOSH research group used a condensation nuclei counter to study PC filament emission rates, and determined that the number-based particle emission rates from an industrial-scale material extrusion AM machine were around  $2.2 \times 10^{11}$  number/minute and the total volatile organic compound emission rates were around  $1.9 \times 10^4 \mu\text{g}/\text{minute}$  (Stefaniak et al., 2019). The same group also found low levels of acetone, benzene, toluene, and *m,p*-xylene during PC filament printing processes. Potter et al showed that PC filament emissions contained bisphenol A (BPA), phenol, chlorobenzene, DEHP, and di-tert-butylphenol (Potter et al., 2019). In our previous studies on PC filament printer emission-induced cell toxicity (Farcas et al., 2019), emissions from a commercial PC 3D printer were generated in a chamber using a 3D printer and collected in cell culture medium. The number-based size distribution of the particles inside the chamber was between 140-170 nm and the mean particle sizes in cell culture medium were  $201 \pm 18 \text{ nm}$ . Analysis of elemental composition of particles collected in the cell culture medium found C, O, Ca, Na, Si, Ni, Cr, Fe, S, Al, and Cl. The organic compounds in the emission collection cell culture medium were BPA, p-isopropenylphenol, and phenol. At 24 h post-exposure, PC emissions were internalized in human small airway epithelial cells (SAEC) and induced a dose-dependent cytotoxicity, oxidative stress, apoptosis, necrosis, and increases in pro-inflammatory cytokine and

chemokine production in SAEC (Farcas et al., 2019). The results demonstrated that PC filament 3D printing emissions induce a cellular toxicity in SAEC.

Although cell-based *in vitro* toxicity analysis is increasingly applied to screen and rank chemicals for prioritizing toxicity studies, as well as to study toxic mechanisms, the toxicological significance of *in vitro* study-generated data in hazard and risk assessment is limited. In comparison with animal-based *in vivo* studies, *in vitro* cell studies can lack tissue-specific differentiated functions, physiological context with other cells and tissues, and biological concordance in metabolism of xenobiotic chemicals (Blaauboer, 2008).

This study sought to evaluate PC filament printer emission-induced pulmonary and systemic toxicity in rats. A real-time 3D printer emission generation system was applied to simultaneously use three commercially available 3D PC filament printers to generate PC filament aerosols consisting of a mixture of particles and volatile organic compounds (VOCs) that were delivered to an animal exposure chamber. Male Sprague-Dawley rats were exposed to 3D PC filament emissions in a time-course via whole body inhalation. The rats were exposed to a single concentration (0.529 mg/m<sup>3</sup> average, 40 nm mean diameter) of the emissions for 1, 4, 8, 15, and 30 days (4 h/day, 4 days/week), and were sacrificed at 24 h after the last exposure. Bronchoalveolar lavage (BAL) samples were collected to analyze inflammatory, fibrotic, and oxidative stress responses. Lung and nasal tissues underwent histopathological analysis to evaluate the emission-induced tissue damages. Several blood biomarkers were tested for the emission-induced systemic toxicity in rats.

## Methods Collection

Experimental design:

- 60 male Sprague-Dawley rats were assigned to one of two treatment groups: air control or 3D printer emissions exposed.
- Rats were exposed to HEPA-filtered air or real-time emissions from FFF 3D printers printing with PC filament for 4 hours/day, 4 consecutive days/week, for 1, 4, 8, 15, or 30 days.
- Rats were sacrificed 24 hours post-exposure and blood, BALF, and lung tissues were collected for analysis.

Three-dimensional PC emissions inhalation exposure system:

- A custom-designed inhalation exposure system was used to deliver either HEPA-/carbon-filtered air or consumer-grade FFF 3D printer emissions to a whole-body rodent exposure chamber in real time.
- The system consisted of a vacuum and mass flow controller, a chamber housing three FFF 3D printers, and a temperature-controlled exposure chamber.
- Emissions and conditions inside the exposure chamber were continuously monitored and controlled in real-time using external equipment that was connected to sampling ports and custom software.

Three-dimensional PC emissions collection and characterization:

- Emissions were collected and characterized using a Data RAM, condensation particle counter, fast mobility particle sizer, field emission-scanning electron microscopy, and fused silica-lined, evacuated canisters.
- Average aerosol mass concentration was 0.529 mg/m<sup>3</sup> for 4 hours.
- The average concentration of specific VOCs was calculated for individual VOCs.

Three-dimensional PC filament emission particle deposition estimates in the nasal passages, tracheobronchial and alveolar regions:

- The Multiple-Path Particle Dosimetry (MPPD) model was used to estimate PC-emissions particle deposition mass for the head/nose, tracheobronchial, and alveolar regions.
- Particle deposition mass without clearance was estimated using FMPS data, animal breathing rate, tidal volume, exposure time, and aerosol mass concentration.
- Particle deposition mass with clearance was estimated using a previously described method (Farcas et al., 2020).

Bronchoalveolar lavage (BAL) analysis:

- BALF fluid was collected and centrifuged to separate the supernatant from the cell pellets.
- The supernatant was used to measure total protein levels, lactate dehydrogenase (LDH) activity, and surfactant and cytokine levels.
- The cell pellets were used for a total cell count and cell differential.
- Transmission electron microscopy (TEM) staining and scanning electron microscopy (SEM) images were used to visualize BAL cells and lung tissues, respectively.

Blood processing and analysis:

- Blood collected in EDTA-containing and clot-activator-/polymer gel-containing vacutainers was processed and analyzed using a ProCyte Dx Hematology Analyzer and a Catalyst One Chemistry Analyzer to measure blood cell counts and serum chemistry parameters, respectively.

Blood analysis:

- Complete blood count (CBC) was performed to measure blood cell counts and hematological parameters.
- Serum chemistry profile was performed to measure serum biochemical parameters.

Lung and nasal passage analysis:

- The unlavaged left lung was inflated with 10% neutral buffered formalin (NBF), embedded in paraffin, cut at 5 µm, and stained with Hematoxylin and Eosin (H&E) for histopathological evaluation.
- The nasal passages were collected and fixed in formalin for 1 week, then decalcified in 13% formic acid.

- Standard nasal sections (T1, T2, T3, and T4) were taken, embedded in paraffin, cut at 5 µm, and stained with H&E.

The histopathological evaluation of the lung and nasal passage tissues was performed by a veterinary pathologist and classified in the following manner:

- WNL = within normal limits
- 1 = minimal change (barely exceeds WNL)
- 2 = mild/slight change (lesion is identifiable but is of limited severity)
- 3 = moderate change (lesion is prominent with a potential for increased severity)
- 4 = severe change (lesion occupies the majority of the organ and is as severe as possible)

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