Diesel Exhaust Particle Induced Obstruction of Rotifer Digestion as a Biosensor Characterizing Pollutant Impact on Human Health

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Introduction

Air pollution in the United States has been an important issue due to the millions of emissions of diesel exhaust particulate (DEP) that is released yearly. "In 2013, about 94 million tons of pollution were emitted into the atmosphere in the United States" (1). Among pollutants, diesel exhaust characterized as particulate matter, is a widely waste. Particulate matter is created by the incomplete combustion of diesel, it is then released into the environment and can be found on roads, ponds, and can spread. Half all nitrogen oxides and two thirds of particulate are emitted from transportation sources in the United States (2). The emissions are released into the air can remain and be inhaled by humans (3). Several studies have concluded a high hospital admission rate for respiratory diseases, cardiovascular diseases, chronic obstructive pulmonary diseases, and heart failure in association with four common pollutants, like particulate matter 10 (PM 10) during hot, humid summers and mild, dry winters (4). Further increase in novel pollutants are yet uncharacterized on their impact on human health. Whole cell biosensors, such as microscopic animals, can be used to detect changes in human health. In this study, *Philodina* rotifers are subjected to different concentrations of diesel exhaust particles at room temperature and at an increased temperature, similar to a hot summer day, in order to determine reaction to pollution, such as alimentary abnormalities.

Objective

To test the feasibility of using rotifers as a organism-based biosensor to assess air pollution-induced changes.

References

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Results

Figure 1

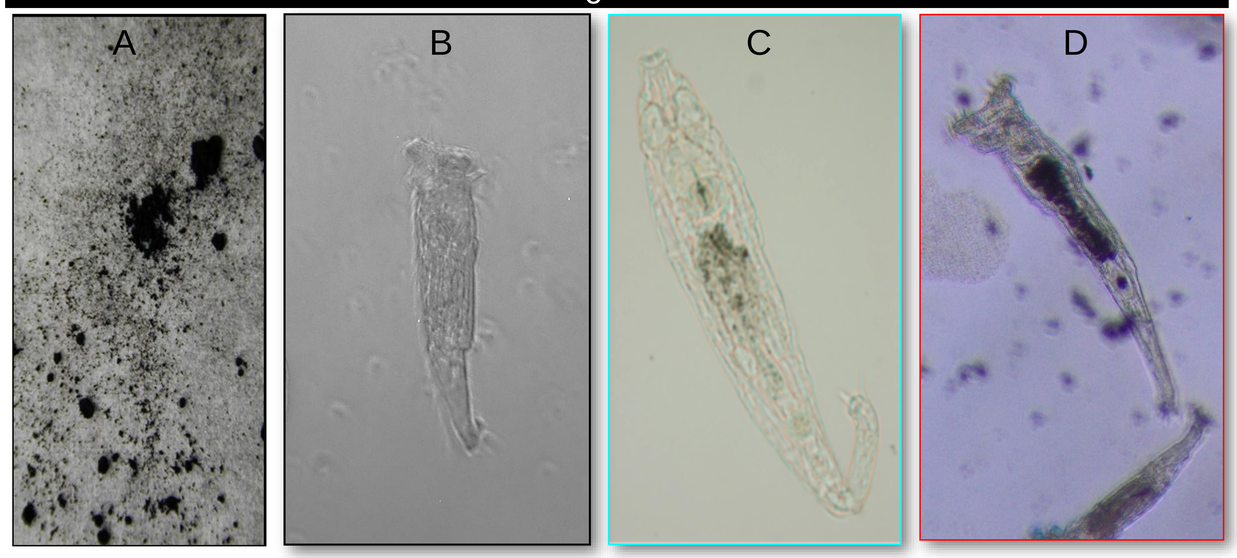

Figure 1: Diesel exhaust particles (DEP) obstruct rotifer alimentary canal. *(A) Picture of diesel exhaust particles prior to solubilization; (B) rotifer without DEP; (C) rotifer 1 day after exposure to 0.1 mg/mL DEP at 20°C and (D) at 34°C.*

Figure 2

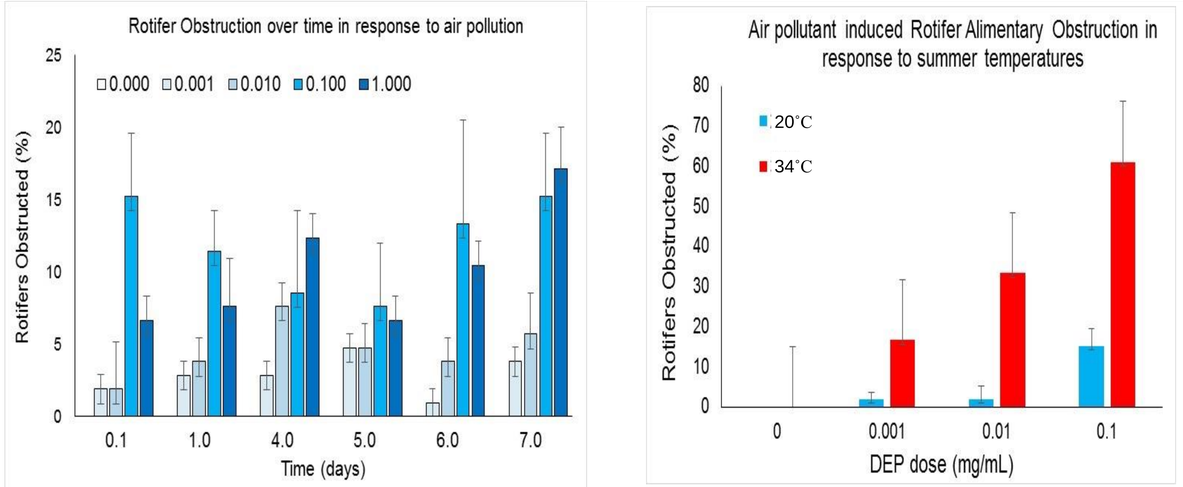

Figure 2: Diesel exhaust particle (DEP) dose, time and environmental temperature impacts percentage of rotifers with alimentary canal obstruction. (A) Percentage rotifers with alimentary obstruction (%) exposed to a single dose of 0, 0.001, 0.01, 0.1 or 1.0 mg/mL DEP (clear, white, light blue, blue, dark blue, respectively) for two hours (0.1) or 1, 4, 5, 6 or 7 days. (B) Percentage rotifers with alimentary obstruction (%) exposed to a single dose of 0, 0.001, 0.01 or 0.1 mg/mL DEP for two hours (0.1 day) at 20˚C (blue bar) [spring temperature, 68˚F] or 34˚C (red bar) [summer temperature, 95˚F]. Dosage at 1.0 mg/mL at high temperature is not reported due to visual obstruction of rotifer by DEP at 34°C. Bars indicate average of triplicate counts with standard deviation error bars. Representative experiment of two performed.

Figure 3

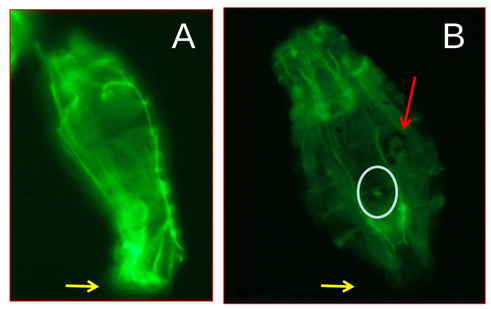

Figure 3: Actin filament dynamics are involved in DEP-induced alimentary obstruction in rotifers. *Actin filament staining of rotifer without DEP exposure (A, lateral view) or 2 hours (0.1 day) after exposure to a single dose of 0.1 mg/mL DEP at 20°C (B, anterior view). Red arrow: DEP obstruction. White circle: actin filament aggregate surrounding DEP. Yellow arrow: caudal end.*

Conclusions

The data presented here showed that rotifers consume Diesel Exhaust Particles (DEP) leading to an accumulation in their alimentary canal and an observable obstruction.

Rotifer alimentary obstruction was induced by DEP at a range from 0.001 - 0.1 mg/mL. The effect was exacerbated by increased temperatures similar to the heat of summer indicated by a higher percentage of rotifers obstructed at 34°C in comparison to rotifers at 20°C.

It is feasible that this organism-based model could be used as a biosensor to detect pollution levels as the central bolus of DEP in the rotifer alimentary canal is colorimetric and could be used as a detection system. Dosing of DEP needs adjusting as an average total diesel exhaust exposure is 2 µg/m3 (6), which is substantially lower than the doses assessed here.

The data presented here supports the next phase of this project focusing on the hypothesis that alternations of actin filament dynamics lead to DEP-induced rotifer obstruction.

Acknowledgments

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Materials and Methods

Rotifers: *Philodina* rotifers are Class I. Rotifera, Order 2. Bdelloidea multi-cellular, freshwater organisms that are characterized with two ciliary aooaratus, corona, differentiated pharynx, and an alimentary canal that the study will focus upon (5).These rotifers can be of subjected to desiccation and remain in a dormant stage up to three years under stable conditions, making them a good choice for a biosensor. Living *Philodin*a rotifers were obtained from Carolina Biological Supply Company and maintained in aerated, spring water containing wheat grains. To use, rotifers were concentrated by centrifugation, counted on a hemacytometer and aliquoted to a final concentration of 85,000 rotifer per well or 250 x 10^3 rotifer per cm^2.

DEP Preparation: *Philodina* rotifers were exposed to particles from a diesel powered automobile (see picture) [standard reference material 2975, NIST, Gaithursburg, MD]. Just prior to rotifer exposure, particle stock suspensions (2 mg/ml) were prepared in dH_2O and vortexed thrice for 30 seconds at maximum speed. The particles were diluted to achieve exposures between 0.001 and 1 mg/ml, or a maximum of 300 µg/cm^2.

Microfilament stain: After exposure, *Philodina* rotifers were fixed with methanol, permeabilized with 0.1% triton X-100 and stained with 200 nM phalloidin prepared in methanol. Once staining was complete, cells were viewed under an Olympus CKX41 with phase and fluorescence microscope.

Experimental Design: *Philodina* rotifers were mixed in triplicate with or without ten-fold dilutions of DEP in 96-well plate to a final volume of 200 µL. Plates were maintained at 20˚C (room temperature) or 34˚C (using a humidified incubator) up to 7 days. At each time point (0.1 (2hrs) and 1, 4, 5, 6 or 7 days), rotifers were treated with ProtoSlo® and thirty were counted for obstruction use of an Olympus inverted CKX41microscopeat at a total magnification of 100. The experiment was repeated.