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When Present in Caenorhabditis Elegans at Quantities Comparable to Those in Contaminated Areas, Perfluorooctanoic Acid (PFOA) Causes Toxicological Effects on Behaviour, Reproduction, and Development

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Abstract

The persistent organic pollutant (POP) perfluorooctanoic acid (PFOA) is widely distributed in the environment. Globally, there is growing concern over the possible hazardous effects of PFOA on both human and ecological health. In this work, we examined the acute toxicity of PFOA on Caenorhabditis elegans (C. elegans), a species of free-living, ecologically significant animal model. We looked at growth, mortality, lifespan, neurobehavior, fertility, and livelihood. 4.42 M (1.83 mg/L) was discovered to be the acute toxicity (LC50) of PFOA. The locomotor behaviour of C. elegans was altered by PFOA exposure. Additionally, after being exposed to 0.1 M (41.4 g/L) PFOA, C. elegans' ability to reproduce was diminished. Likewise, the modification in chemotaxis plasticity and lifespan reduction above 1 M (414 g/L) PFOA were both evidence of developmental toxicity.

Keywords: Perfluorooctanoic acid; Caenorhabditis elegans; PFOA and reproductive toxicity

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic chemicals used in a variety of products, including surfactants in firefighting foams, grease-resistant paper for food packaging, non-stick coating for cookware, and stain- and grease-resistant coatings for carpets, fabrics, and upholstery. Heat resistance is provided by the distinctive physicochemical features of PFAS-based polymers, which have been employed in industrial manufacture since 1950 [1]. Because of its longevity in the environment and toxicity to biota, perfluorooctanoic acid (PFOA) is one of the PFAS chemicals that has drawn significant attention from the general public and researchers. In addition to being employed as a surfactant in many industrial items, PFOA is commonly used in the production of non-stick (Teflon^{*} coated) cookware. As a result, PFOA has been discovered in a number of environmental matrices, such as soil, water.

Human unfavourable health consequences from PFOA have been found by epidemiological investigations. Serum PFOA levels have been linked to changes in low-density lipoprotein (LDL), total cholesterol, and high-density lipoprotein (HDL) in human investigations. Exposure to PFOA has been linked to obesity, according to research. Additionally, through the placental bypass of the exposed mother, PFOA can impact foetal development and the risk of obesity in the offspring. PFOA may change liver enzymes, lipids, and size, according to animal studies, which enhance the development of rodent liver peroxisomes. Bartell and co. (2009). Caenorhabditis elegans, an aquatic and terrestrial freeliving invertebrate that belongs to the phylum Nematoda, has been proven to be sensitive to a wide range of pollutants. Due to their high brood size (300+) and brief lifespan, C. elegans is simple to cultivate in the laboratory. They can also generate a big number of animals in a short amount of time. Additionally, the genes of C. elegans are identical to human genes by 60-80%, and the majority of human disease pathways are also present in them. For its capacity to anticipate outcomes in higher eukaryotes (such as humans and rats), C. elegans has been regarded as a superb toxicity model animal [2,3].

Materials and Method

Reagents

The reagents, cadmium nitrate (as a positive control), and perfluorooctanoic acid (PFOA) of analytical grade (96% purity) were purchased from Sigma-Aldrich (St. Louis, MO, USA). K-medium (KCl 2.36 g, NaCl 3 g, up to 1 L H2O, cholesterol (5 mg mL1), 1 mL 1 M CaCl2, and 1 mL 1 M MgSO4) was used to generate the PFOA stock solution. PFOA concentrations ranging from 0.25 M (103.5 g/L) to 500 M (207 mg/L) were employed for the acute lethality assay. The following concentrations were employed for various assays: 0.001 M (0.414 g/L), 0.01 M (4.14 g/L), 0.1 M (41.4 g/L), 0.5 M (207 g/L), 1 M (414 g/L), and 2 M (828 g/L). Liquid chromatography-mass spectrometry (LC-MS) was used to assess the PFOA concentrations in the medium; the measured amounts were 99 [4].

PFOA bioaccumulation

Synchronized L4 nematodes were subjected to PFOA at concentrations of 0.001 (0.414 g/L), 0.1 M (41.4 g/L), and 2 M (828 g/L) for 48 hours in order to assess the bioaccumulation. Centrifugation was performed to clean and flush away the bacteria from the worms' intestines using M9 buffer (1 ml 1M MgSO4, 5 g NaCl, 3 g KH2PO4, 6 g Na2HPO4, H20 to 1 L). In Concentrator Plus, samples were dried (Eppendorf). Few modifications were made to Surowice et alpreviously .'s disclosed procedure for sample extraction (2011). Samples were resuspended in 1 ml of LC-MS grade methanol after being weighed, and

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they were then homogenised for 5 min using a Branson digital sonifier (Model: 102 C - CE) at 15% amplitude. As previously mentioned, the frequency of C. elegans' head thrashing and body bending was investigated (Tsalik and Hobert, 2003). 48 hours were spent exposing C. elegans to concentrations of 0.001, 0.01, 0.1, 0.5, 1 and 2 M PFOA. Animals that had been exposed to PFOA were carefully moved into a brand-new NGM plate. For the duration of the recuperation period, head thrashes were recorded minute by minute [5, 6]. C. elegans exposed to PFOA were placed on the NGM plate, and the frequency of body bending was measured every 20 s. After 48 hours of exposure to PFOA (0.001, 0.01, 0.1, 0.5, 1, and 2 M), L4 nematodes' generation time and brood size, which are regarded indicators of reproductive potential, were assessed. One individual nematode was used in each well of the 24 well plates used for the experiment. The interval from the P0 egg to the F1 egg served as the measurement for generation time. Animals were examined and moved to a new NGM plate every second day for the brood size assay. Every stage of the egg and larval life cycle was recorded.

Discussion

Research focuses on persistent organic pollutants (POPs) because of their prodigious ability to migrate through a variety of environmental matrices and growing accumulation in flora and fauna. Even at ecologically relevant low quantities, it has been demonstrated that these compounds are hazardous (El-Shahawi et al., 2010). PFOA is still found in the environment, people, and animals despite the fact that large businesses stopped producing and using it a few years ago. This is because of PFOA's extremely persistent nature. So, study on the PFOA's toxicity to organisms that are useful to the environment is a top priority. In the short-term (acute) toxicity testing for numerous substances, the LC50 is a frequently used toxicity metric. As a result of exposure to PFOA, feeding C. elegans in this study, the LC50 value for PFOA was calculated. Additionally supporting this is the greater bioaccumulation of PFOA in exposed C. elegans from this study. The lower and upper limits of an organism's toxicity are often indicated by the LC20 and LC90 values. The LC20 in this investigation was determined to be 0.96 M (0.4 mg/L), and the LC90 was found to be 53.55 M (22.17 mg/L) PFOA (Table S1). These findings are rather higher than the PFOA concentrations in the environment that have been reported. According to Haug et al. (2009), PFOA is more likely to bioaccumulate in mammals than its shorter-chain counterparts. The BAF of PFOA discovered in this investigation was consistent with earlier studies, which showed that BAF was higher at lower exposure amounts. Blood albumin protein is the main binding site for PFOA and other contaminants in biota [7, 8].

Conclusion

This study shows that even at stated polluted area concentrations

(such as 0.1 M), PFOA exposure impacts C. elegans' locomotor behaviours, development, fertility, and lifespan. The findings showed that, at higher concentrations, PFOA had altered the development and chemotaxis learning behaviour of nematodes. Given that PFOA is widely present in the environment and that C. elegans is an important ecological organism in both soil and aquatic systems, the toxicological data collected on C. elegans in this study are crucial for ecological risk assessment of PFOA. There is a need for more research on the longterm and transgenerational impacts of PFOA on C. elegans [9, 10].

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Potential Conflicts of Interest

The publication of this material is free of any conflicts or rival interests.

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