Perfluorooctane Sulfonic Acid Contamination Reduced Fitness in *Drosophila hydei* (Diptera: Drosophilidae)

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ABSTRACT Studies on the effects of perfluorooctane sulfonic acid (PFOS) exposure on terrestrial invertebrates are very scarce. Here, we quantified the effects of PFOS on the number of offspring for two generations of *Drosophila hydei* Sturtevant 1921 (Diptera: Drosophilidae) reared in the laboratory. The first generation was exposed to a range of PFOS concentrations from 5 to 5,000 ng/ml and a control through their diet. For the second generation, offspring of the 50 ng/ml PFOS treatment and the control were further reared in a PFOS-free environment. For the first generation, the number of offspring decreased with increasing levels of PFOS contamination. Although the second generation was not exposed to PFOS, we still observed a reduction in the number of offspring when the parental flies were exposed to 50 ng/ml PFOS. The results of the current study indicate that the sensitivity to PFOS of *D. hydei* is several magnitudes higher than of the other terrestrial invertebrate species tested so far. This sensitivity falls within the concentration range detected in its natural food.

KEY WORDS perfluorooctane sulfonic acid, *Drosophila*, offspring number, fitness, terrestrial

Perfluorooctane sulfonic acid (PFOS; $C_8F_{17}SO_{3-}$) has useful physical and chemical properties in that it repels water and fat. This makes PFOS commercially attractive for producing a variety of goods. PFOS has been manufactured in products as diverse as cosmetics, flame-retardants, insecticides, refrigerants, and surfactants. Several of these applications involve long-lived goods which, combined with PFOS' resistance to breakdown processes, results in potential sources of environmental contamination for many years to come. PFOS has been found in animal tissues all over the globe. Indeed, although PFOS is a artificial product, it has not only been detected in wildlife from industrialized areas but also in vertebrates in the arctic region far from urban sources of contamination (for reviews, see Giesy and Kannan 2001, Beach et al. 2006). In addition, several other perfluorinated compounds degrade to PFOS that is a stable end product and the predominant perfluorinated compound found in our environment. The high persistence and biomagnification potential made the United Nations Environmental Program and the Stockholm Convention on persistent organic pollutants (http://www.pops.int/) call for research on the possible dangers of PFOS.

In nature, PFOS has been detected in various animal species, including humans (Giesy and Kannan 2001, Kannan et al. 2004, Yeung et al. 2006). Previous re-search on the effects of PFOS mainly focused on vertebrate species (Beach et al. 2006, Newsted et al. 2007, Hagenaars et al. 2008). Invertebrate species have been far less studied. Information is available for some marine species such as *Artemia*, shrimps and oysters (Drottar and Krueger 2000a,b; Beach et al. 2006) and several freshwater species such as zooplankton, shrimps, snails, chironomids, and damselflies (Boudreau et al. 2003, MacDonald et al. 2004, Sanderson et al. 2004, Li 2009, Van Gossum et al. 2009). For terrestrial invertebrates, we only found two species: the earthworm *Eisenia fetida* (Sindermann et al. 2002) and the honey bee, *Apis mellifera* (L.) (Wilkins 2001).

The difficulty with studies in the wild is that the sites usually also contain other contaminants, such that it is not straightforward to link animal health to PFOS pollution. Various in vivo experiments on a diversity of animals also showed sometimes dramatic effects on animal survival, metabolic function and condition (e.g., Beach et al. 2006). However, the experimental exposure to PFOS was often short-term, from only a few hours to a few days, and mostly not for the entire life span. Results of such experiments may therefore not reflect the long-term impact of pollutant stress as it is experienced in the natural world. Indeed, previous research with pollutants other than PFOS has shown differences in sensitivity, individual recovery and acclimatization for different ontogenetic stages (Beyers et al. 1999, Stuijfzand et al. 2000). Also, chronic toxicity tests with PFOS (long-term, lifetime, or even over generations) indicate that acute exposures may not provide sufficiently accurate information to predict the ecotoxicological consequences for the natural environment of organisms (Ji et al. 2008, Du et al. 2009).

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Here, we evaluate the fitness response of two generations of *Drosophila hydei* Sturtevant 1921 (Diptera: Drosophilidae) to lifetime exposure (egg to adult fly) to PFOS contamination under experimental laboratory conditions.

Materials and Methods

Study Species and Treatments. *D. hydei,* is a very common species that occurs in a variety of natural habitats and is widely associated with humans worldwide. The fly breeds in various decaying plant and fungal material, including fruit, slime fluxes, flowers, and mushrooms. It has sexual reproduction and four developmental stages: egg, larvae, pupae, and winged fly. Generation time is ≈2 wk. In the reproductive stage (winged flies), male and female *D. hydei* can be identified based on body size, shape of the abdomen, and genital structure (e.g., Markow and O'Grady 2006).

To start our experiments, *D. hydei* adults were collected with an insect net in a large compost bin in the garden of L.D.B. The flies were reared on a diet of a 50:50 mixture of dechlorinated tap water and food (oatmeal, fruit, water, yeast and Nipagin). PFOS was dissolved in the water before being mixed in the diet. PFOS (tetraethyl ammonium salt, purity 98%) was obtained from Sigma-Aldrich (St. Louis, MO). Test solutions were prepared (taking purity into account) with dechlorinated tap water. This tap water contained a negligible concentration of PFOS (2.64 ng/liter; D'Hollander et al. 2009). Diet experimental treatments were 0 (control), 5, 50, 500, and 5,000 ng/ml PFOS. For comparison, in nature, PFOS concentrations have been measured up to 8.6 ng PFOS/ml water for a pond near to the same fluorochemical plant in Antwerp, Belgium, for which high concentrations were reported in rodents (Hoff et al. 2004, ARCADIS Gedas NV 2006). For the water channels adjacent to this pond measures ranged from 93.15 to 207.5 ng PFOS/ml water. For sediment, values have been reported up to two 610 ng PFOS/g dry weight sediment (Higgins et al. 2005). PFOS concentrations reported in this study are all nominal concentrations. Solution and tissue concentrations could not be measured. However, studies showed that PFOS has such low potential for degradation (Boudreau et al. 2003) that the nominal concentrations will not have changed over the generational exposure time (<2 wk).

Experiments. *Generation 1.* Ten male and ten female wild-caught flies were placed in closed recipients with the different PFOS concentrations. Each recipient consisted of two bottle-shaped jars connected by the necks so the flies could freely move between the bottles. The bottom bottle (1-liter volume) contained the food and oviposition solution. The top bottle (1.5-liter volume) served to collect the newly emerged flies. The bottom of the top bottle was removed and replaced by a fine mesh for ventilation. All recipients were placed at room temperature while providing a photoperiod of 16:8 (L:D) h.

Each experimental treatment was replicated three times (i.e., three recipients per treatment). The gender of the flies was identified after cooling individuals to 5–7°C and using a microscope. All recipients contained three sticks to provide a roosting site for the flies. Flies were observed to feed and lay eggs on the PFOS contaminated substrates. After 4 d, all flies were removed from the recipient. Subsequently, the number of emerged flies was monitored daily by darkening the bottom jar. The newly emerged flies crawl toward the light and end up in the top bottle. The top bottle with the flies was cooled to 5–7°C so the flies could easily be counted and sexed under a microscope. The experiment was stopped after three subsequent days without newly emerged flies. We thus did not monitor survival of offspring, but we removed all emerged flies daily. We did not examine the recipients at the end of the exposure to determine whether some larvae were still present. In this manner, we obtained data on offspring number for a first generation of flies reared under different PFOS treatments, i.e., number of offspring refers to the number of adult flies that emerged successfully.

Generation 2. Ten male and ten female flies of the first generation reared in the control and in the 50 ng/ml PFOS treatment were transferred to recipients containing PFOS-free substrates, on which they could feed and oviposit for 4 d (again using three replicates). Using a similar methodology as described above, the number of offspring was assessed (i.e., second generation). The second generation flies were not sexed because gender differences in response to PFOS exposure were absent for first generation flies (see Results).

Statistical Analyses. Data for the first generation were analyzed with mixed model analysis of variance (ANOVA). Explanatory variables were PFOS concentration, gender and their interaction. Gender and PFOS concentration were treated as a class variable. The dependent variable was the number of offspring. Because each experimental treatment was replicated three times, the recipient was included as a random effect to account for the fact that flies within a recipient were not statistically independent. The correct degrees of freedom for the fixed effects were assessed with Kenward–Roger method. A *t*-test was applied to analyze differences in number of offspring of the second generation between the control and 50 ng/ml PFOS. Analyses were performed using SAS 9.1 (SAS Institute, Cary, NC). Results are presented as means ± SE.

Results

All parental flies survived the four days in the recipient, except in the 5,000 ng/ml PFOS treatment (see below). Overall significantly more females than males emerged successfully (gender: $F_{1, 11} = 6.36$; $P = 0.028$). The total number of offspring emerging was significantly influenced by the PFOS treatment (PFOS concentration: $F_{3, 8} = 18.28$; $P = 0.028$). The highest number of offspring emerging was observed in
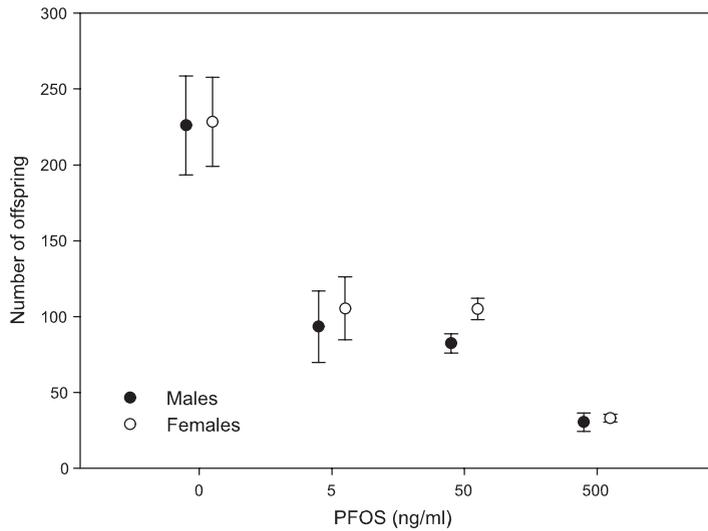
Fig. 1. Number of male and female offspring produced by *D. hydei* exposed to different PFOS treatments (means ± 1 SE).

the control treatment. The 5 ng/ml PFOS treatment already produced significantly less offspring ($P = 0.007$; Tukey test). This number further decreased with increasing concentration of PFOS, and ultimately no offspring emerged in the 5,000 ng/ml PFOS treatment (Fig. 1). In this context, it is noteworthy that for the 5,000 ng/ml PFOS treatment parental flies only survived a single day. Nevertheless, flies succeeded in laying eggs and larvae emerged shortly after. These larvae, however, did not survive long. They did not make it to the pupal stage. There was no significant gender × PFOS interaction ($F_{3, 8} = 1.83$; $P = 0.220$).

Although not exposed directly to PFOS contamination, the second generation produced significantly more offspring when the parental flies had been exposed to a control versus 50 ng/ml PFOS treatment ($t = 2.285$, df $= 18$, $P = 0.035$) (Fig. 2). All parental flies survived in both treatments.

Discussion

D. hydei development was clearly affected by experimental PFOS pollution, with flies only successfully surviving and reproducing in environments with <5,000 ng/ml PFOS. Studies on other terrestrial invertebrate organisms to compare with are very scarce. We only found two examples. The earthworm *Eisenia fetida*, exposed for 14 d to a PFOS-spiked artificial soil substrate, produced a no observed effect concentration (NOEC) mortality value of 7,700 ng/g dry weight ($\approx$5200 ng/g wet weight), and the 14-d LC_{50} value was 37 300 ng/g dry weight (Sindermann et al. 2002). A closer example is a study where honey bee workers were fed for 4 h with PFOS-spiked sucrose solutions (Wilkins 2001). The resulting mortality NOEC was 1,050 ng/g sugar solution and the 72-h LD_{50} value was 2000 ng/g. In our study on *D. hydei,* the lowest concentration of 5 ng/ml PFOS already resulted in a 56% reduction in offspring, which implies that the NOEC value is much lower. Indeed, this makes *D. hydei* >200 times more sensitive to PFOS than the honey bee. However, we have to bear in mind that the honey bee was exposed for 4 h, whereas *D. hydei* was exposed during its entire lifetime ($\approx$2 wk). In comparison, the most sensitive aquatic invertebrates studied until now are the damselfly *Enallagma cyathigerum* (Charpentier) (behavioral NOEC, 10 ng/ml; Van Gossum et al. 2009; metamorphosis NOEC, <10 ng/ml; unpublished data) and the chironomid *Chironomus*
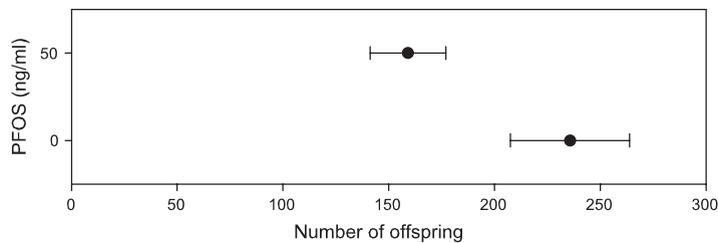

Fig. 2. Offspring number of *D. hydei* when parents were exposed to different PFOS treatments, but offspring were reared in PFOS-free environments (means ± 1 SE).

tentans (F.) (metamorphosis NOEC, < 2.3 ng/ml; MacDonald et al. 2004).

Very limited information is available on the presence of PFOS in the terrestrial environment. We only found one study where PFOS was measured in soils (range, 59.75–98.60 ng/g$_{ww}$) (D'Hollander 2007). It is however not clear how representative this study is because samples were taken in an area where extreme high PFOS levels were measured in water samples (up to 93.15–207.5 ng/ml, ARCADIS Gedas NV 2006) and in the livers of wood mouse, *Apodemus sylvaticus* (470–178 550 ng/g, Hoff et al. 2004). In sediment and sludge, values have been reported up to 85 and 2,610 ng/g PFOS dry weight, respectively, but most concentrations are <1 ng/g (Houde et al. 2006, Nakata et al. 2006). Because *D. hydei* lives in decaying vegetable matter it is relevant to use PFOS loads in plant material. Unfortunately, we only found one study, for which PFOS was measured in fruits of *Sambucus niger* (range, 13.14–74.10 ng/g$_{ww}$) and *Rubus* sp. (range, 0–20.34 ng/g$_{ww}$), but in the same natural environment where extremely high PFOS concentrations were measured in water and rodents (D'Hollander 2007). An alternative source of data that can be used was collected in the scope of risk assessment of human exposure to PFOS. Various types of commercially available food were screened for the presence of PFOS (Food Standards Agency 2006, Ericson et al. 2008, Trudel et al. 2008). Highest concentrations for vegetable foodstuff were obtained for potatoes and derived products (10 ng/g wet weight). The majority of reported concentrations were in the sub- to low nanograms per gram range (≤ 0.84 ng/g$_{ww}$), and many samples contained no PFOS at all (Fromme et al. 2009). Although most concentrations of vegetable foods are lower, the NOEC value of <5 ng/ml in *D. hydei* observed during the current study lies within the range of observed PFOS concentrations in its natural food.

Reduced offspring number with increasing PFOS concentration may have several, not mutually exclusive, causes. First, parental flies feeding on a PFOS polluted diet may lay lower numbers of eggs. For example, in *C. tentans* a concentration-dependent reduction was observed in the number of egg masses laid, although the number of eggs laid per mass did not vary across PFOS treatments (MacDonald et al. 2004). The flies in our 5,000 ng/liter treatment died after 1 d but still produced some eggs. If the flies in the other treatments also experienced reduced vigor (although they did not die within the 4-d oviposition period) the lower number of offspring in the PFOS treatments might be explained by a reduced fecundity. An alternative situation is that females from contaminated environments may produce the same number, or even more eggs in comparison with females from less-polluted sites but that these eggs are less likely to develop (Lagisz and Laskowski 2008). Second, flies may be capable of recognizing contaminated environments (e.g., Scheirs et al. 2006). Thus, flies may detect PFOS pollution in their oviposition substrate and decide to lay fewer eggs retaining eggs for when a more suitable

environment is reached (i.e., use a bet-hedging strategy) as was observed in *Drosophila melanogaster* (Meigen) when exposed to deltamethrin in their oviposition substrate (Renou et al. 1997). Third, after hatching from eggs, larvae feeding and living in the PFOS-contaminated environments may experience reduced survival. Although our results do not provide information on the first two possibilities, the observation that larvae in the 5,000 ng/ml PFOS environment were initially present but died before reaching the pupal stage, indicates that larval survival may be dramatically reduced, at least for high levels of PFOS contamination.

All flies of the second generation were reared in a PFOS-free environment. Still, parental flies that were exposed to PFOS contamination produced less offspring. Possibly, parental condition was reduced resulting in a lower fecundity as in *C. tentans* (MacDonald et al. 2004). This implies that PFOS effects are carried over from the larval stage to the adult stage. However, it also might be that the eggs laid by the first generation were of lower quality, resulting in a lower hatching success and/or reduced larval development in the second generation. In the latter case, the effects of PFOS are cross-generational. To disentangle both hypotheses, more research is needed.

Acknowledgments

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