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Hazard/Risk Assessment

DISTURBANCE OF PERFLUOROOCTANOIC ACID ON DEVELOPMENT AND BEHAVIOR IN *DROSOPHILA* LARVAE

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Abstract—Perfluorooctanoic acid (PFOA) is a well-known perfluorinated compound (PFC), and its toxicological impact is currently of worldwide concern. In this study, we sought to evaluate the potential biological effects and modes of action of PFOA in a range of physiologically and developmentally related phenotypes in the fruit fly *Drosophila melanogaster*. The results clearly indicated that the toxic effects of PFOA at the organismal level were associated with the developmental status of the organism, with larvae being most sensitive to this chemical. Except for the decreased weight of both sexes and the reduced longevity of male adults, PFOA had a robust effect on larval development, as determined by reduced body volume, aberrant foraging behavior, molting arrest, and polyphasic lethality. Remarkably, nutrient supplementation of the diet efficiently rescued the lethal effect of high PFOA concentrations on larval development. This result indicated that PFOA probably competed with nutritional components, leading to a disruption of the metabolic pathways responsible for larval development. Environ. Toxicol. Chem. 2010;29:2117–2122. © 2010 SETAC

Keywords—Perfluorooctanoic acid Fruit fly Developmental toxicity Longevity Nutrient supplementation

INTRODUCTION

Perfluorooctanoic acid (PFOA) is a well known perfluorinated compound (PFC). Perfluorinated compounds are being used in an increasing variety of consumer and industrial products, including food packaging, fire-retardant foams, cosmetics, upholstery, surfactants, and surface protectors [1–3]. The carbon-fluorine covalent bonds in PFCs render this class of compounds stable and persistent in the environment, as well as in the blood of humans and wildlife [4–9]. The effects of PFOA in vertebrates have been studied extensively. High doses of PFOA have been reported to cause loss of body weight, liver toxicity, tumorigenicity, physical development delays, endocrine disruption, and other adverse effects [10–14].

One prevailing hypothesis suggests that PFOA acts as a ligand for peroxisome proliferator activated receptors (PPARs), especially PPARα. In support of this, adverse effects of PFOA have been shown to be mediated by the PPAR signaling pathway in laboratory animals [11]. Although strong evidence supports the idea that PFOA induces developmental toxicity, liver toxicity, and adenomas via a PPAR-agonistic mode in rodents [15,16], treatment with PFOA may result in changes mediated by other pathways [17]. For example, in both wild-type and PPARα-null mice, PFOA exposure led to marked changes in the expression of a set of genes involved in xenobiotic metabolism, including phase I and phase II genes [18]. Although a number of species, mainly vertebrates, have been used to assess the biological effects of PFOA, its exact mode of action remains unclear to environmental scientists. In addition, little is known about its toxic effects on untested species. In this study, we used a relatively simple model organism, the fruit fly *Drosophila melanogaster*, to evaluate the potential biological effects and modes of action of PFOA in various physiologically and developmentally related phenotypes.

MATERIALS AND METHODS

Chemicals and groups

Perfluorooctanoic acid ammonium salt was purchased from Sigma Aldrich (CAS: 3825-26-1). Based on our preliminary experiment, the exposure concentrations of PFOA were 0, 100 μM, and 500 μM. PFOA was added to the media with constant stirring after the media was boiled and cooled to 60°C. They were then aliquoted vials (or plates), stored at 4°C, and used within one week.

Fly husbandry

The *Drosophila melanogaster W1118* stock used in all experiments was raised on a 12:12 h light:dark cycle at 25°C and at a relative humidity of approximately 60%. Experimental adult flies were raised on standard medium (850 ml water, 15 g autolysed yeast powder, 75 g sucrose, 95 g maize meal, 9 g agar, and 25 ml Nipagin M in 95% ethanol). Adults were collected over a 12-h period, transferred to vials with fresh food, and allowed to mate.

Weight gain analysis

Cohorts of 20 one-day-old flies for each sex were anesthetized under CO_2. The weight of all 20 flies together was measured immediately using a SHIMASZU analytical scale to obtain the initial body weight. They were assigned randomly into vials in which the medium in the tube contained 0, 100 μM, and 500 μM PFOA. After 5 days of exposure, each vial of flies was weighed again and the weight gain was calculated.

Emergence of adults

Cohorts of 20 one-day-old female flies were assigned randomly into vials with three food regimens (standard food

All Supplemental Data may be found in the online version of this article.

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with 0, 100 μM, and 500 μM PFOA). Flies were then cleared from the vials after 5 days. The eggs laid during this period were allowed to develop. The emerging progeny of adult flies were removed and scored shortly after eclosion (within 8 h) until no more emerged (approximately 23 days after the exposure of maternal adults).

Fecundity assay

At 24 h post-eclosion, six replicate vials of 10 females and 10 males were used for the fecundity assay. They were placed on medium containing the different PFOA concentrations listed above. Food was changed daily and the number of eggs laid per vial of females was counted. Data are reported as the mean number of cumulative eggs laid per female over an 8-day period.

Lifespan assays

For the longevity experiments, one-day-old flies were collected under light CO_2 anesthesia and placed into single-sex vials containing the experimental food formulations (standard medium with 0, 100 μM, and 500 μM PFOA). For each group, at least 10 independent vial replicates at a density of about 20 females or males were performed. Vials were placed on their sides to allow flies access to both food and non-food surfaces. Vials of fresh food were supplied every four days and the number of dead flies was recorded daily.

Larval growth volume analysis

Cohorts of 20 one-day-old female flies were assigned to food vials with or without 100 μM and 500 μM PFOA; they were removed from the vials after 2 h. Eggs laid within the 2-hr period were allowed to hatch and develop on the media. At 30 48, 72, 96, and 110 h after egg laying (AEL), larvae were collected from the culture media and washed with PBS (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO4, 1.8 mM KH_2PO_4, pH 7.3). Larval dimensions were measured, and the formula $4/3\pi(L/2)2(D/2)$ was used to determine the larval volume (L = length, D = diameter) [19].

Larval lethality and molting analysis

Flies were allowed to lay eggs on petri dishes with 5 $g \cdot dl^{-1}$ yeast/10 $g \cdot dl^{-1}$ sucrose diet (with 0, 100 μM, and 500 μM PFOA) for 2 h. Embryos were then allowed to hatch and develop on these petri dishes. Survival and death were measured 60 and 90 h AEL. At these two time points, the larvae should have finished their first and second molts, respectively. Surviving larvae were then transferred to fresh petri dishes under the same conditions. The survival rate was determined using the equation: percent survival = $S/T \times 100$, where S is the number of living larvae at the indicated time points and T is the total number of larvae (the sum of living individuals and corpses) 60 h AEL. To evaluate the effect of the nutrient supplementation on larvae lethality, a more nutrient-rich diet of 10 $g \cdot dl^{-1}$ yeast/10 $g \cdot dl^{-1}$ sucrose medium was used. In all cases, three independent cohorts of 60 to 160 larvae each were monitored over time. These data were combined for Chi-square analysis. The nutritional compositions and calorie content of these two diets were estimated and are shown in Supplemental Data, Table S1.

Behavioral assay and morphological observation

The location of living larvae in the petri dish (immersed within the media, on the surface of media, or on the side and lid of the dishes) after the first-stage larvae/second-stage larvae (L1/L2) transition was also recorded. Morphological changes of larvae raised on the three different food formulations were observed under a stereomicroscope. For finer analysis, the larvae and mouth-hooks were observed in PBS solution on a standard microscopic slide with a coverslip under a microscope. For the locomotion assay, the 2nd instar larvae were washed from the plates by floatation in 3 M sucrose, washed with PBS, and transferred onto a petri dish with 1% agar. After a 1-min adaptation period, the body-wall contractions of larvae from the three groups were scored over 30 s.

Statistical analyses

All statistical analyses were performed using SPSS 16.0 statistical software (SPSS, Chicago, IL, USA). One-way analysis of variance (ANOVA) was used for weight gain, larval volume, locomotion, progeny adult emergence, and fecundity analysis. The unpaired Student's t test was used to analyze data from the feeding assays. Statistical analysis of lifespan data was performed using Kaplan-Meier survival functions and log-rank tests; the median lifespan was based on the age, in days, required to reach 50% survival. The chi-square test was used to analyze larval location, molting, and lethality.

RESULTS

PFOA treatment

Decreased weight gain in adults. The weight of the one-day-old female flies in the control group increased by approximately 1.57×10^{-4} g in 5 days. However, weight gain decreased depending on the levels of PFOA in the diets, compared with the control group (Figure 1). Adult weight gain in female flies decreased by 22% and 28% in the 100 μM ($n = 9$, $p = 0.02$) and 500 μM PFOA groups ($n = 9$, $p = 0.007$), respectively, compared with controls. In male flies, although there seemed to be no reduction in weight gain in the 100 μM PFOA treatment group compared with the control group, flies on the 500 μM PFOA diet showed a significant reduction in weight gain compared with those reared on the standard medium ($n = 9$, $p = 0.02$). To exclude the possibility that PFOA, by altering the characteristics of the diet, caused gustatory changes that elicited repellent behavior, leading to the decreased weight gain, we performed the two-dye preference test and the proboscis-extension assay (Supplemental Data, Figure S1A, S1B). The results showed that 500 μM PFOA did not elicit a repellent

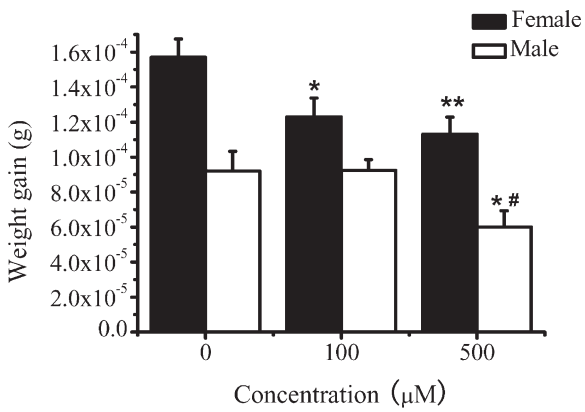

Fig. 1. Weight gain in flies treated with perfluorooctanoic acid (PFOA) for 5 days. The results are presented as means ± SEM ($n = 9$). $^{*}p < 0.05$; $^{**}p < 0.01$ versus control, # $p < 0.05$ versus 100 μM.

reaction in adult flies. To exclude this possibility completely, feeding rates were measured by adding blue dye to the food. Feeding data indicated that *Drosophila* reared on the 500 μM PFOA diet consumed a similar volume of food (calorie intake) to those on the control diet (Supplemental Data and Figure S1C-S1E). These results confirmed that PFOA in the diet did not alter feeding behavior and rates in adult flies, and the difference in calorie intake did not contribute to weight gain reduction in adults of both sexes.

Retarded larval development. Larvae treated with PFOA appeared abnormally small. For example, 72 h AEL, larvae in the control group measured 2.32 mm in length and averaged 0.42 mm in width (diameter), while animals on the high concentration of PFOA generally averaged 1.98 mm in length and 0.36 mm in width. Larvae in the 100 μM PFOA group were intermediate in size, averaging 2.18 mm in length and 0.38 mm in width. To confirm the effect of PFOA on larval development, the body volumes of larvae reared on the three diet types were calculated (Figure 2A). The average body volume in larvae exposed to 500 μM PFOA was significantly smaller than the controls at all times. Body weights of the white pupae reared on the 500 μM PFOA diet were also significantly lower than controls ($p = 0.0002$, Figure 2B).

More sluggish larvae. To assess whether PFOA was involved in the disruption of larval activity, we quantified the locomotive activity by measuring the number of body-wall

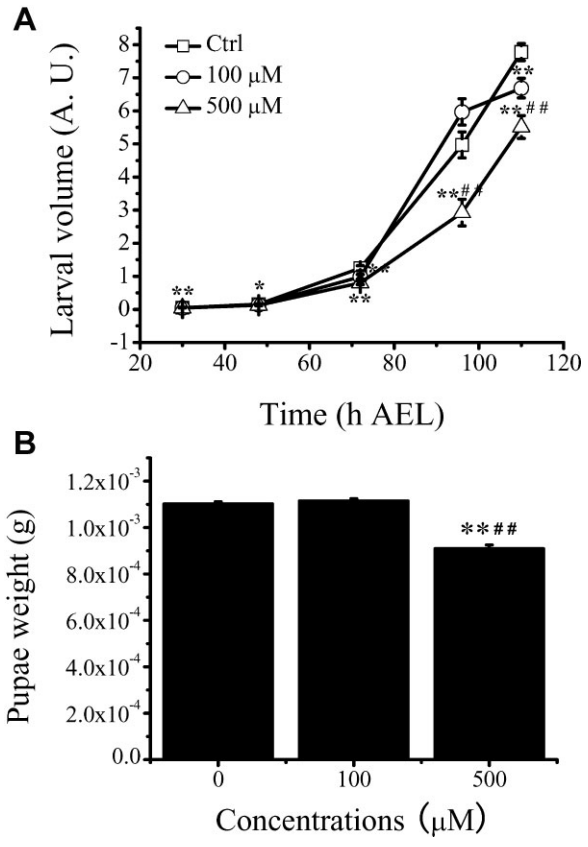

Fig. 2. PFOA-treated larvae exhibited growth defects. (A) Graph of the average volume of larvae at different times after egg laying (AEL). Larvae raised in media containing PFOA grew more slowly than controls. More than 20 larvae were scored per time point for each group. A. U., arbitrary units. (B) Measurement of the weight indicated a significant reduction in white pupae exposed to diets containing PFOA. One-way ANOVA was used for the statistical analysis, and the results are presented as means ± SEM. * $p < 0.05$, ** $p < 0.01$ versus control, ## $p < 0.01$ versus 100 μM.

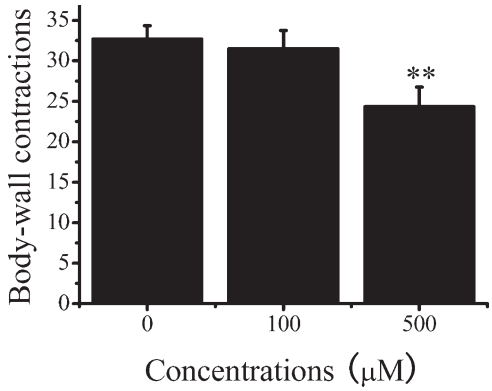

Fig. 3. Locomotion assay for 2nd-instar larvae reared on standard medium with 0, 100 μM, and 500 μM PFOA. Body-wall contractions were counted within a 30-s period. The number of animals tested in each group was 20-40. For the statistical analysis, one-way ANOVA was used, and the results are presented as means ± SEM. ** $p < 0.01$ versus control.

contractions. Body-wall contractions of early 2nd instar larvae were calculated for 30 s. The larvae in the 500 μM PFOA treatment group had a slower mean rate of body-wall contractions than the control animals ($n = 20$–40, $p = 0.004$, Figure 3).

Decreased lifespan of male Drosophila *populations.* To characterize the impact of PFOA on fly longevity, we measured the lifespan of single-sex populations of *W1118* flies maintained on each of the three food types throughout their adult lives. In our trials, survival was moderately reduced when male adults were maintained on 500 μM PFOA, relative to the controls (Log-rank test, $p = 3.60 \times 10^{-7}$) (Figure 4A). The median longevity for males fed control, 100 μM, and 500 μM PFOA diets was 45.00 ± 1.53, 43.00 ± 0.98, and 34.00 ± 2.44 days (median ± SEM, $n = 233$, 212, 215, respectively), respectively. However, PFOA had no obvious effect on longevity in female adults (Log-rank test, $p = 0.12$) (Figure 4B). The median longevity was similar in control, 100 μM, and 500 μM PFOA-treated animals (27.00 ± 0.77, 26.00 ± 0.97, and 27.00 ± 1.05 days, and $n = 239$, 252, 281, respectively).

Decreased numbers of emerging progeny. Following exposure to diets containing 100 μM and 500 μM PFOA for 5 days, the emergence of progeny was reduced compared with the control group (Figure 5A). The final number of progeny adults in the control group was 33 per vial, while in the 100 μM and 500 μM PFOA treatment groups, the average number was 25 and 9 per vial, respectively (Figure 5B). We then measured egg production during the first 8 days of life. The results showed that cumulative egg deposition was similar between control and PFOA treatment conditions (Figure 5C). This result demonstrated that the decrease in progeny was not due to fecundity reduction in females exposed to the chemical.

Multiple-stage lethality in larvae. Adults were exposed to standard medium with 0, 100 μM, and 500 μM PFOA for 5 days and then discarded. The laid eggs were allowed to develop on the media. In the 500 μM PFOA exposure group, progeny larvae frequently moved away from the medium before the wandering stage. These larvae eventually died on the sides of vials, exhibiting different body sizes and polyphasic larval lethalities with the high concentration of PFOA. More than 60% progeny died on the side of the vials as larvae in the 500 μM PFOA group. However, in the control and 100 μM groups, larvae stayed on the food source until the wandering stage, and almost no larvae died on the side of the vials (Chi-square test, $p = 2.02 \times 1^{-85}$). In addition, most of the

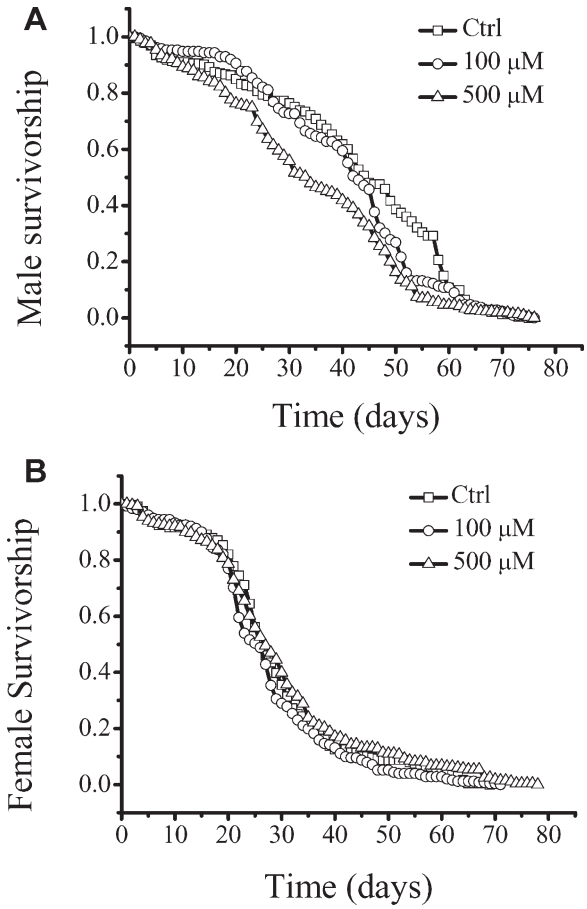

Fig. 4. PFOA treatment reduced the lifespan of male fruit flies. Results are expressed as a percentage of living flies each day. A decreased lifespan was observed in male (**A**). However, no effect on longevity was observed in female adults (**B**). Sample sizes in the control, 100 μM, and 500 μM PFOA-treated groups were 233, 212, and 215 for males; and 239, 252, and 281 for females, respectively.

animals in the 500 μM PFOA group that prematurely moved away from the food source exhibited an aberrant morphological phenotype, with tan stripes or spots on their cuticles (Supplementary Figure S2).

Arrested larval development. To assess larval survival of larvae, the 5 $g \cdot dl^{-1}$ yeast/10 $g \cdot dl^{-1}$ sucrose medium was used. The survival rate for larvae 60 h AEL in the 0, 100 μM, and 500 μM PFOA groups was 97.94%, 97.14%, and 81.03%, respectively. Survival rates at 90 h AEL were 79.82%, 59.48%, and 18.75% in the 0, 100 μM, and 500 μM PFOA-treated groups, respectively (Supplemental Data, Table S2). These results indicated that the mortality values were significantly increased in the treatment groups compared to those maintained on the control diet (Chi-square test). Mouth-hook analysis of larvae was performed by examining the structures of the mouthparts and anterior spiracles. The results revealed that at 60 h AEL, most of the animals in the 500 μM PFOA-treated group could not successfully perform the L1/L2 transition and remained in the 1st-instar stage, while larvae in the control groups had more highly serrated mouth-hooks, indicating that they had entered the 2nd-instar stage. We roughly estimated the number of molting individuals by counting the number of shed cuticles. Only 20.04% of the larvae had successfully molted in the 500 μM PFOA group 60 h AEL, compared to 75.21% in the control group (Chi-square test, $p = 9.35 \times 10^{-62}$). Most of the larvae (98.06%) died before their transition to the 3rd instar

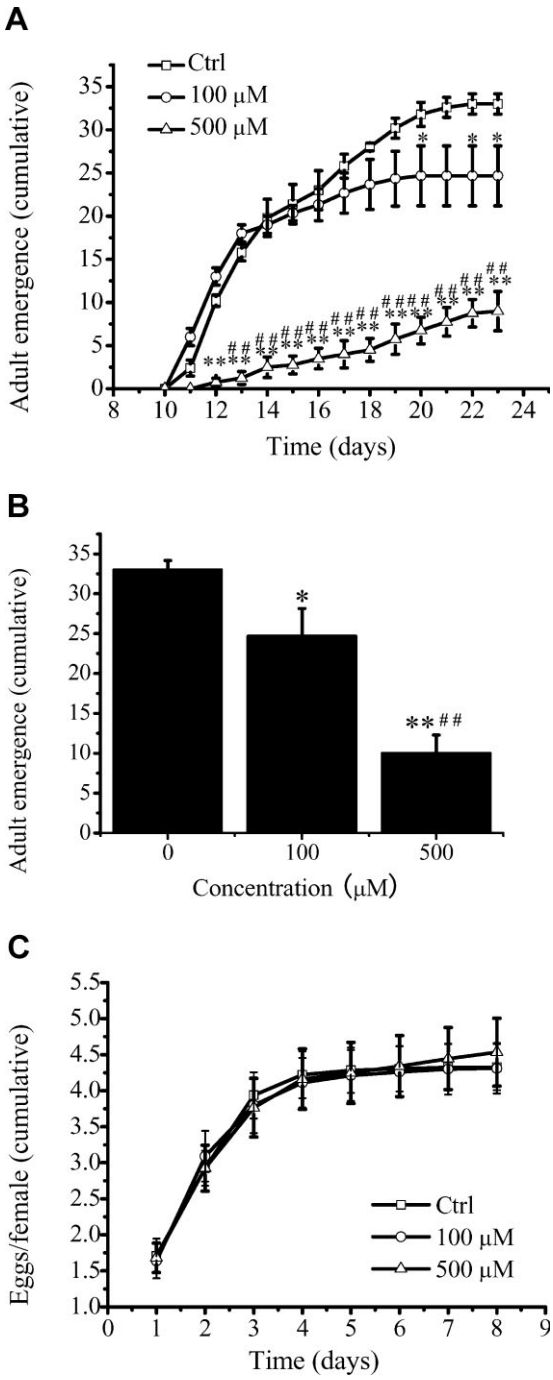

Fig. 5. PFOA treatment led to reduced progeny. Twenty adults were exposed to diets containing 0, 100 μM, and 500 μM PFOA for 5 days. The adults were then discarded, and the eggs were allowed to hatch and develop on the media. (**A**) Cumulative emergence curve for adults. (**B**) Cumulative numbers of adults in the three groups until day 23 are shown separately. (**C**) Cumulative numbers of eggs laid during the first 8 days by maternal adults were not different among animals fed the standard diet containing 0, 100 μM, and 500 μM PFOA ($p > 0.05$). Six to ten vials were tested in each group, and the experiment was repeated twice. All of the results are presented as means ± SEM. One-way ANOVA was used for the statistical analysis. $^{*}p < 0.05$, $^{**}p < 0.01$ versus control, ## $p < 0.01$ versus 100 μM.

in the 500 μM/L PFOA group. The few animals (1.94%) that did progress to the 3rd instar (as determined by anterior spiracle morphology) were abnormally small. These results clearly indicated that exposure to the high concentration of PFOA retarded growth and was lethal to larvae.

Defective burrowing. After the L1/L2 transition, most of the larvae were found buried under the food in the control groups (93.89% buried). However, the larvae in the 500 μM PFOA group became increasingly sluggish, and most of them failed to dig into the medium after the L1/L2 transition (6.90% buried; Chi-square test, $p = 1.48 \times 10^{-42}$). They were located mainly on the surface of the medium, while some of them had moved away from food source to the side and lid of the dishes. The larvae that retreated from the food source typically did not return and retained their larval identity, with tan stripes and spots on the cuticles, until death (Figure S2). This aberrant behavior was not an escape strategy from noxious stimuli, since larvae reared on the control medium did not immediately show food aversion upon being transferred to a diet containing 500 μM PFOA (data not shown).

Effects of nutrient supplementation

Female flies were allowed to lay eggs on petri dishes containing the nutrient-rich diet for 2 h. The numbers of dead and living larvae were recorded 60 and 90 h AEL. The results showed that nutrient supplementation of the diet attenuated the lethality and extended the lifespan of larvae raised on PFOA. In the control groups, larvae reared on both diets showed similar survival; however, the survival rate of larvae in the 500 μM PFOA treatment group significantly increased when they were raised on the more versus the less nutritional diet (Chi-square test, Supplemental Data, Table S1). For example, when reared on the more nutrient-rich diet, 59.02% of the 500 μM PFOA treatment animals survived to 90 h AEL, while on the 5 $g \cdot dl^{-1}$ yeast/10 $g \cdot dl^{-1}$ sucrose diet, the values were only 18.75% ($p = 2.91 \times 10^{-16}$). These data suggested that PFOA probably interfered with the ability of larvae to utilize nutritional ingredients that are critical for growth and development.

DISCUSSION

Effect of PFOA on weight gain and lifespan in adult flies

Although a large amount of information is available on the effects of PFOA on mammals, little is known about its effects on insects. In mammals, the nuclear receptor PPAR has been proposed to play an essential role in producing the toxicological effects of this chemical. Peroxisome proliferator activated receptors are an important ligand-regulated transcription factor that controls key metabolic pathways in vertebrates [20,21]. One of the effects of PFOA on vertebrates is weight reduction, which is thought to be mediated by the PPAR signaling pathway [18]. An interesting feature in the fruit fly is that it possesses only 21 nuclear receptor genes, which is far fewer than vertebrates; however, none of them are homologous to mammalian PPARs [22,23]. Although no homolog of PPAR nuclear factor in flies, a similar effect on weight was observed in adult *Drosophila* with that in mammals. Adult flies exposed to 500 μM PFOA for 5 days demonstrated a significant reduction of their weight gain, compared with that of controls. In addition to underweight flies, PFOA treatment resulted in a moderate reduction of longevity in adult males. Using a two-dye food preference test, the proboscis-extension assay, and the feeding rate assay, we confirmed that these physiological alterations, such as weight gain and longevity decrease in adult flies did not result from under-feeding, but were due to the toxic effect of this reagent on flies.

Toxic features of PFOA on adult and larval flies

Although negative effects on weight gain were detected in both sexes, and a small decrease in lifespan was found at the highest PFOA concentration in males, PFOA was not lethal to adult flies as to larvae. Not only were the larval volumes in the treatment groups smaller than those of the controls, but also the numbers of adult progenies reared on the PFOA diets were also dramatically reduced, compared with the control group. These results indicated that the toxic effects of PFOA were associated with the developmental status of the organism, and larvae were more sensitive to this chemical. Therefore, we investigated the toxic effects of PFOA on the development of larvae in detail.

Effects of PFOA on the behavior and morphology of larvae

Drosophila develops through three larval instars separated by molting events that allow continued growth, and a final molt that initiates pupation [24]. These events require coordination between gene expression, behavioral changes, and environmental conditions. During most of the larval phase, animals remain immersed within the food source and feed constantly, displaying foraging behavior [25]. At mid-third instar, the essential transition from foraging to wandering behavior occurs. The wandering stage is characterized by cessation of eating, purging of the gut, and moving away from the food to search for a suitable pupation site [26]. In our experiment, after the L1/L2 transition, larvae exposed to 500 μM PFOA displayed abnormalities in their foraging behavior, with more than 90% of the individuals located on the surface of the food source. However, at these stages in the control group the majority of animals had burrowed into the food. Many larvae on PFOA, with different body sizes, crawled up the sides of the vials and died at that position, before the normal wandering stage. In addition, many of these larvae exhibited tan stripes and spots on their cuticles. The pigmentation of the cuticle was reminiscent of that in the pupariation stage, which requires 3, 4-dihydroxyphenylalanine produced by the hydroxylation of tyrosine [27]. Additionally, PFOA treatment led to sluggish animals with reduced body-wall contractions. Taken together, the results indicated that *Drosophila* larvae exhibited pronounced behavioral differences under PFOA treatment. However, it was difficult to discern whether these abnormalities were primary effects due to the chemical or secondary ones due to the generally ill health of larvae exposed to PFOA.

Lethality of PFOA may be efficiently rescued

In many metazoans, final body size depends on the growth rate and the duration of the growth period, two parameters that are influenced by nutritional cues [28]. In *Drosophila*, nutrition controls the duration of larval development by acting on the prothoracic gland (PG), which secretes the molting hormone ecdysone [29]. The physiology of growth control in insects differs, of course, from that in mammals, but the genes and signaling pathways involved are similar [30]. For example, two major nutrient-sensing systems, the insulin and target of rapamycin (TOR) pathways, are highly conserved between *Drosophila* and mammals [31,32]. Growth rates during larval development are affected by nutritional availability in their rearing environment. *Drosophila* larval development is complete after 4 days on rich food at 25°C. However, when food is limited, a TOR-dependent nutrition sensor in the fat body downregulates the general insulin/insulin-like growth factor signaling system, reducing the animal's growth rate [28]. Notably, PFOA causes global growth retardation, similar to that observed

in *Drosophila* reared under relatively poor nutritional conditions. Larvae raised on a diet containing PFOA grew at a much slower rate compared with larvae in the control group. The deleterious effects on larval development were also reflected by the inhibition of molting in the PFOA treatment group, compared with that in control group; for instance, 80% of the larvae reared on 500 μM PFOA failed to perform the L1/L2 transition 60 h AEL. To characterize the effects of PFOA further, we performed the developmental effect studies using a more nutrient-rich diet. The results showed that with the same concentration of PFOA, the survival rate of larvae raised on the $10\,g \cdot dl^{-1}$ yeast/$10\,g \cdot dl^{-1}$ sucrose diet increased compared with those on the $5\,g \cdot dl^{-1}$ yeast/$10\,g \cdot dl^{-1}$ sucrose diet. For example, 60 h AEL, survival on 500 μM PFOA increased from 81.03% on the $5\,g \cdot dl^{-1}$ yeast/$10\,g \cdot dl^{-1}$ sucrose diet to 91.48% on the $10\,g \cdot dl^{-1}$ yeast/$10\,g \cdot dl^{-1}$ sucrose diet (Chi-square test, $p = 6.72 \times 10^{-5}$). This effect was in accordance with a significant reduction in the death rate in the 500 μM PFOA treatment group 90 h AEL (Chi-square test, $p = 2.91 \times 10^{-16}$).

In summary, PFOA showed deleterious effects, especially on the larval stages of *Drosophila*, and the developmental slowing observed in larvae exposed to PFOA were similar to the effects caused by restricting dietary protein. The administration of a nutrient-rich diet efficiently rescued the lethality of PFOA. Given that most essential metabolic functions have been conserved through evolution, and *Drosophila* shares most of the same basic metabolic functions as vertebrates [33] and (2) the structure of PFOA is similar to fatty acids, our results imply that PFOA affected the ability of the larvae to utilize nutritional components critical for growth and development. Importantly, the fact that nutrient supplementation efficiently rescued the lethal effect of PFOA on *Drosophila* larvae encourages the development of possible strategies that may antagonize the deleterious effects of PFOA in vertebrates; however, further research is needed to realize this goal.

SUPPLEMENTAL DATA

Two-dye food preference test, proboscis extension response assay, feeding rate assay, fecundity assay, larval lethality, molting analysis details, and statistical analyses are provided in the Supplemental Data.

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