Attention: Docket Number EPA-HQ-OW-2014-0138
April 28, 2014

We appreciate the opportunity to comment on the draft "Health Effects Document for Perfluorooctane Sulfonate (PFOS)". Our comments relate to the critical effect chosen by the United States Environmental Protection Agency (USEPA) as a basis for derivation of the Reference Dose (RfD).

As authors of the developmental neurotoxicity study of potassium PFOS in rats from which USEPA chose their critical effect for derivation of the proposed RfD (Butenhoff et al. 2009), we are disturbed by the choice of a single, transient observation in one sex from that study without full consideration of the lack of evidence for developmental neurological effects observed in the study as well as corroborating studies. USEPA chose the observation of reduced habituation based on consecutive time-period locomotor-activity measurements made on postnatal day (PND) 17 in 1.0 mg/kg/d maternal dose group male pups. The use of this single, transient observation as a critical endpoint to derive a RfD for regulatory purposes when more significant data are available from the Butenhoff et al. study, as well as other studies mentioned below, that demonstrate normal neurological development is at odds with guidance for data interpretation in developmental neurotoxicity studies outlined in Francis et al. (1990), OECD Test Method 426 (OECD 2007), and USEPA's own guidelines (USEPA 1991, 1998). These guidelines state that a weight of evidence approach and expert judgment should be used. It is evident that this has not been the case in derivation of the proposed RfD for PFOS.

Locomotor activity was one of many developmental neurotoxicological endpoints evaluated in the study using sets of 20 pups per sex per maternal dose group, and this endpoint was assessed on PND days 13, 17, 21, and 61. The characteristic inverted "U" pattern in cumulative locomotor activity over a 60-minute period was observed in all groups between PND 13 and PND 21, with higher activity on PND 17 than on PNDs 13 and 21, was observed in all groups (Figure 2 in Butenhoff et al., *vide infra*). Male pups in the 0.3 and 1.0 mg/kg/d maternal dose groups had statistically-significantly higher cumulative locomotor activity as compared to controls on PND 17.

Habituation, a primitive form of learning, was evaluated from data obtained in the locomotor activity assay as well as from data obtained in the acoustic startle assay. When PND 17 locomotor activity counts were evaluated in sequential 15-minute time periods, the 1.0 mg/kg/d maternal dose group male pups did not show the normal diminution in motor activity over the 60-minute period (habituation) that typically can be observed on PND 17 (Figure 3 in Butenhoff et al., *vide infra*). However, the motor activity pattern of these males, including habituation, was not different than that of the control males on PNDs 21 and 61, nor was it different on PND 13. Therefore, the observed PND 17 reduced habituation response in 1.0 mg/kg/d dose group males likely represents a slight developmental delay. These male pup body weights were not affected relative to control male pups; therefore, pup body weight alone does not explain the apparent delay. However, body weights of the 1.0 mg/kg/d dose-group dams, tracked lower than controls, but without statistical significance, throughout most of the gestation period, and, as a result, mean body weights of these dams was lower with statistical significance from PND 4 through PND 21 (weaning) (Figure 1 of Butenhoff et al., *vide infra*). Thus, the maternal influence on a slight delay in habituation cannot be ruled out. Gene transcriptional profiling of liver tissue obtained from dams and fetuses on gestation day (GD) 20, and from male pups at weaning on PND 21 indicated increased CYP2B2 expression in GD 20 dams and increased Acox, Cyp2B2

and Cyp7A1 expression in PND 21 male pups (Chang et al. 2009). These data provide evidence for adaptive metabolic responses to which rodents are known to be particularly sensitive relative to humans (Cheema and Agellon 2000; Corton et al. 2014; Elcombe et al. 2014). The potential contribution of these metabolic adaptations to the observed locomotor habituation response on PND 17 also deserves further consideration. Regardless of cause, the fact remains that the locomotor activity and habituation responses of 1.0 mg/kg/d dose-group male pups were not statistically significantly different than male controls on PNDs 13, 21, and 61.

As noted above, acoustic startle response habituation was also evaluated in the same sets of male pups on PNDs 20 and 60. Habituation to acoustic startle response was unaffected by maternal treatment with potassium PFOS on either PND (Figures 4 and 5 for males and females, respectively, of Butenhoff et al., *vide infra*). Thus, this measure of habituation also showed normal attainment.

In addition to habituation as a measure of primitive learning, higher learning and memory were evaluated in three phases of the Biel maze swimming assessment on PNDs 22 through 28. In these phases, rats were placed in a water-filled maze and required to swim through the maze to find a submerged platform from which they could escape. Phase 1, conducted on PND 22, measured swimming ability and motivation to escape. Rats were placed in a straight channel opposite the submerged platform, and time to reach the platform was recorded in four consecutive trials. There were no statistically significant differences between groups in time to reach the escape platform during the four trials, with all rats showing similar decreases in time during the four trials (Figures 6 and 7 for males and females, respectively, of Butenhoff et al., *vide infra*). Phase 2 evaluated sequential learning over PNDs 23 through 27. On PND 23 and 24, rats were allowed two trials to solve path A of the maze. This was followed on PNDs 25 through 27 with two trials per day to solve path B of the maze, which was the reverse of path A. Rats were given 180 seconds to solve the path B maze. If it was not solved, they were placed on the escape platform for 20 seconds and then removed from the maze. The two daily trials were separated by a minimum of one hour. As shown in Figures 6 and 7 of Butenhoff et al., there was no effect of maternal treatment on sequential learning. Memory was tested in phase 3, conducted on PND 28. The rats were allowed two trials to solve path A of the maze. Again, no effect of maternal treatment with PFOS was observed (Figures 6 and 7 of Butenhoff et al.). Therefore, the tri-phasic Biel maze swimming trial test paradigm to evaluate learning and memory did not reveal an effect of PFOS on the studied parameters.

The lack of an effect on learning and memory is supported by the results of Lau et al. (2003) and Luebker et al. (2005). In Lau et al., PND 22 rat pups from dams given 3.0 mg/kg/d throughout gestation did not differ from controls when tested using a T-maze with alternation (Figure 4 of Lau et al., *vide infra*). In Luebker et al., F1-generation pups were tested for learning, short-term retention, and memory in a passive avoidance paradigm beginning on PND 24, and, beginning on approximately PND 70, were evaluated in a water-filled M-maze for neuromuscular coordination, swimming ability, learning, and memory. No effects of treatment were observed.

In the developmental neurotoxicology study reported by Butenhoff et al., there were no other observations among the many recorded that were suggestive of a neurotoxicological effect of PFOS on development through the PND 66 observation period. A functional observation battery

(FOB) was performed with the same sets of 20 rats per sex per group on PNDs 4, 11, 21, 35, 45, and 60 and included, stage of development permitting: ease of cage removal; ease of handling in hand; lacrimation/chromodacryorrhea; salivation; piloerection; appearance of fur; palpebral closure; respiratory rate/character; red, crusty deposits; mucous membranes/skin color; eye prominence; eye color; mobility; muscle tone; convulsions/tremors; hindlimb extension; grooming; arousal; bizarre/stereotypic behavior; urination/defecation; papillary response; backing; forelimb/hindlimb grip strength; tail pinch response; gait; and air righting. None of these FOB endpoints was affected by treatment with PFOS.

Based on the weight of evidence, it is unreasonable for USEPA to choose the PND 17 habituation endpoint in light of attainment of a normal response by PND 21 and maintenance of that normal response when evaluated on PND 61, along with the lack of a PFOS-treatment effect on acoustic startle response habituation, as well as all of the many other key neurodevelopmental endpoints evaluated in the study. In their report of the USEPA Workshop on the Qualitative and Quantitative Comparability of Human and Animal Developmental Neurotoxicity, Francis et al. state:

> *Work Group III (15) also addressed interpretation of developmental neurotoxicity end points by indicating that a significant change in one parameter may be sufficient to be considered an adverse effect in certain cases, but that a statistically significant effect for a single end point in one test procedure does not necessarily reflect developmental neurotoxicity. The data must be evaluated in the context of other information (e.g., dose-response, correlation with other findings, degree of consistency in terms of other known effects of the agent) in order to provide a measure of the confidence in the data and in the interpretation of the findings. All of the known developmental neurotoxicants discussed at the Workshop produce a variety of behavioral, neuropathologic and other types of neurotoxic effects, but it is not known to what degree this sample of agents is representative of all agents. EPA's protocol contains a variety of observations and measures that generally would be expected to be highly intercorrelated. In the end, scientific judgement, which must take into account all the data available on an agent, will play a role in interpreting the toxicological significance of statistically significant findings.*

The weight of evidence suggests that PFOS should not be viewed as a specific developmental neurotoxin, and USEPA's choice of critical effect for the RfD is at odds with the guidance originally provided by the workshop. We respectfully request that USEPA reconsider its choice of critical endpoint.

Respectfully submitted,

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Figures cited in the text:

Butenhoff et al. (2009) Figure 1

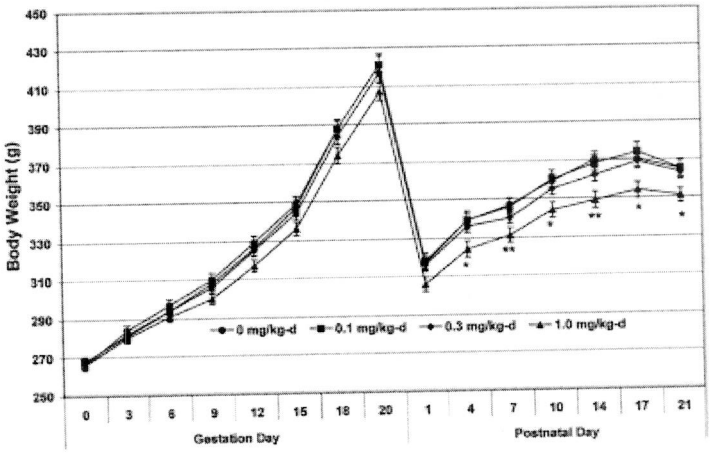

Fig. 1. Maternal weights during gestation and lactation. Data points represent means and error bars represent standard errors of the mean. Statistically significant (*$p < 0.05$, **$p < 0.01$) decreased maternal body weights were evident in the 1.0 mg/kg-d dose-group dams from postnatal day (PND) 4 through PND 21. During gestation days (GD) 9 through 20, these dams showed somewhat decreased body weights but without statistical significance.

Butenhoff et al. (2009) Figure 2

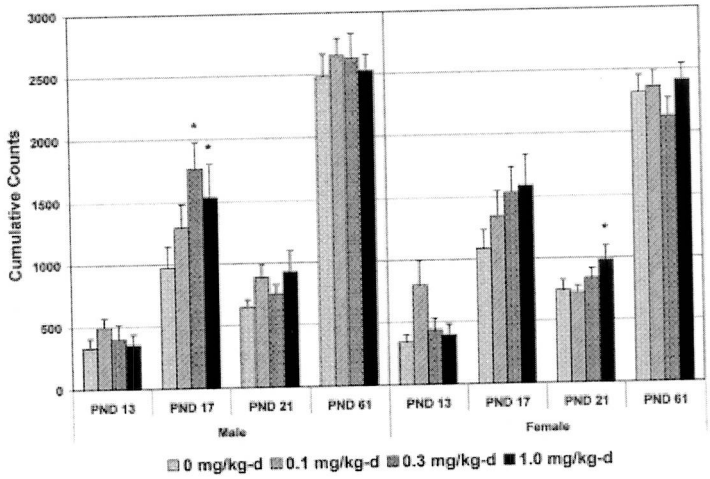

Fig. 2. Motor activity cumulative counts by postnatal day (PND) for males and females. Males in the 1.0 mg/kg-d maternal dose-group showed increased motor activity and lack of habituation on PND 17. Increased activity was also noted on PND 17 in males from the 0.3 mg/kg-d maternal dose-group and was largely driven by increased activity in the 16–30 min test session (see Fig. 3); however, these males displayed expected habituation response. Females in the 1.0 mg/kg-d maternal dose-group had statistically significant higher total activity on PND 21; however, habituation to the test environment in these females was similar to the concurrent control group on PND 21 and there were no differences between the controls and the 1.0 mg/kg-d maternal dose-group females for the individual evaluation time periods (data not shown).

Butenhoff et al. (2009) Figure 3

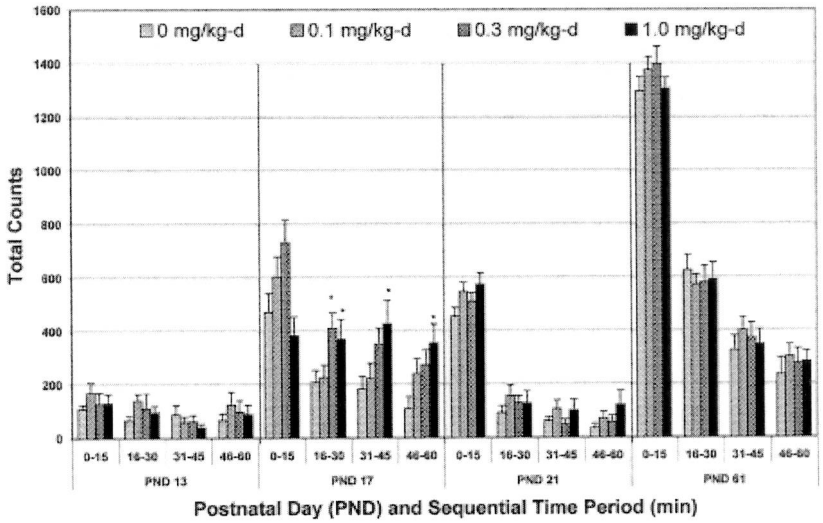

Fig. 3. Male motor activity counts by time period and postnatal day (PND). Rats from the 1.0 mg/kg-d maternal dose-group displayed increased motor activity compared to controls on PND 17 and failed to show habituation. Rats in the 0.3 mg/kg-d maternal dose-group showed increased activity in the 16–30 min period, and total activity was increased (see Fig. 2); however, these rats showed a normal habituation pattern.

Butenhoff et al. (2009) Figure 4

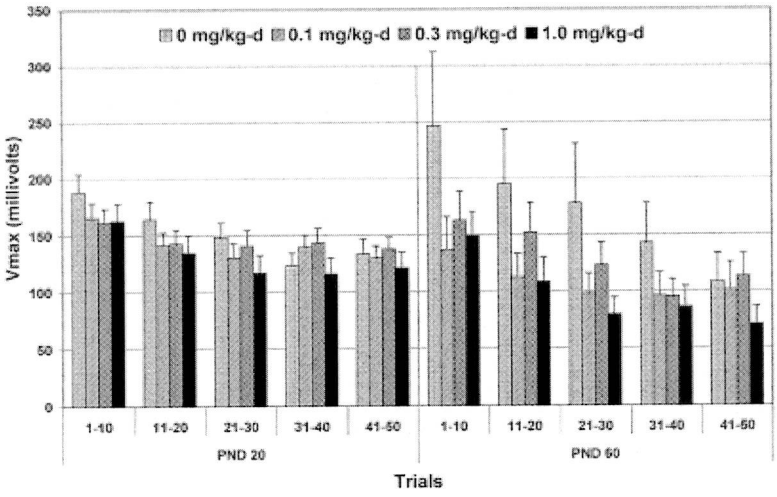

Fig. 4. Male offspring acoustic startle V_{max} on postnatal day (PND) 20 and 60. No statistically significant differences from control rats were noted.

Butenhoff et al. (2009) Figure 5

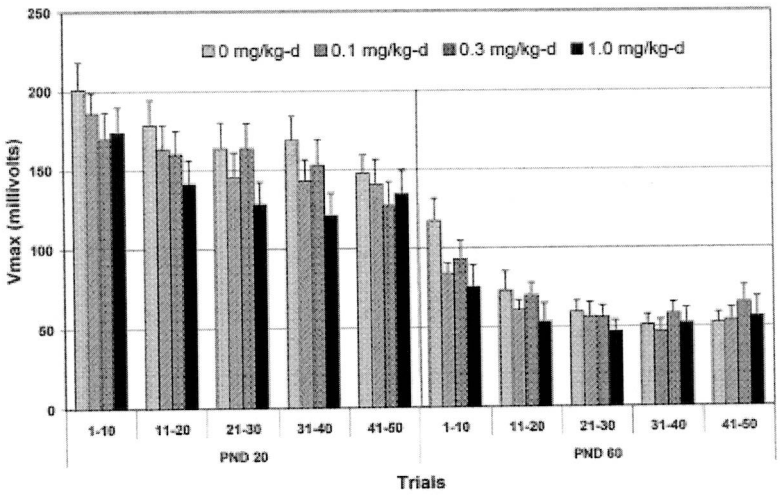

Fig. 5. Female offspring acoustic startle V_{max} on postnatal day (PND) 20 and 60. No statistically significant differences from control rats were noted.

Butenhoff et al. (2009) Figure 6

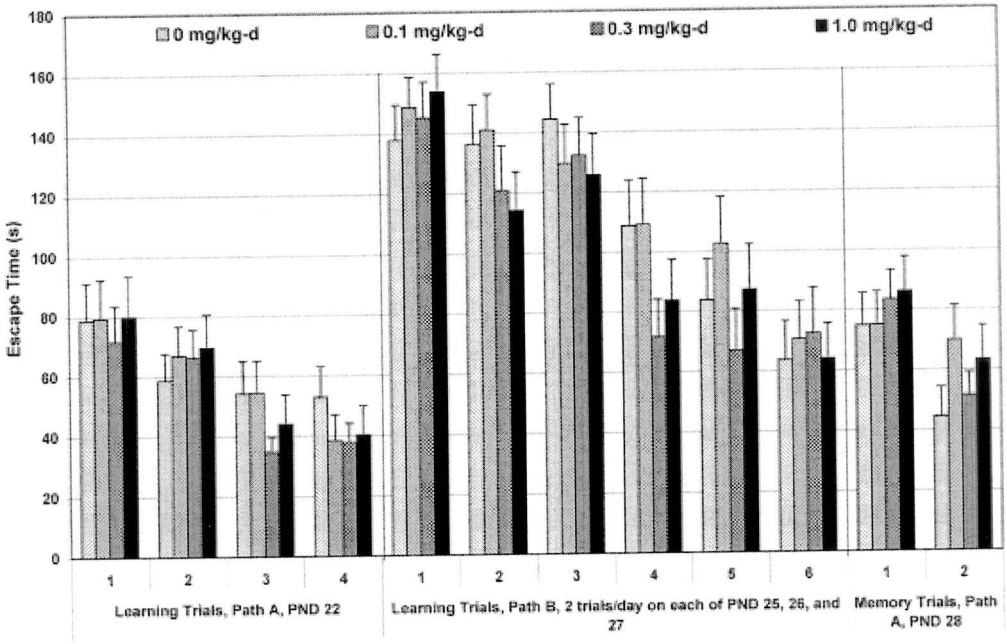

Fig. 6. Male offspring Biel maze trial data. No statistically significant differences from control rats were noted.

Butenhoff et al. (2009) Figure 7

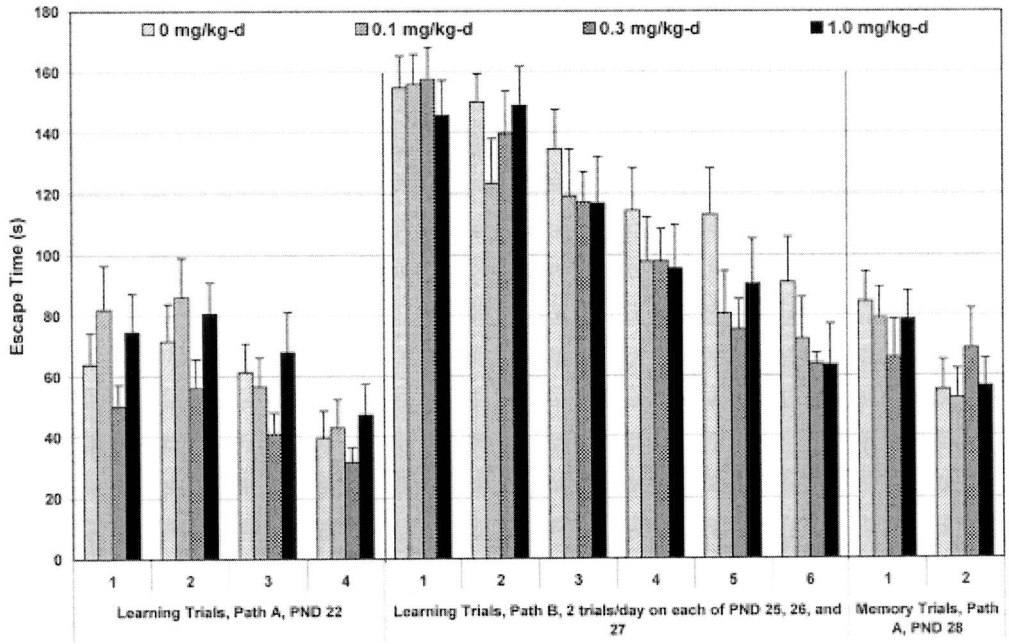

Fig. 7. Female offspring Biel maze trial data. No statistically significant differences from control rats were noted.

Lau et al. (2003) Figure 4

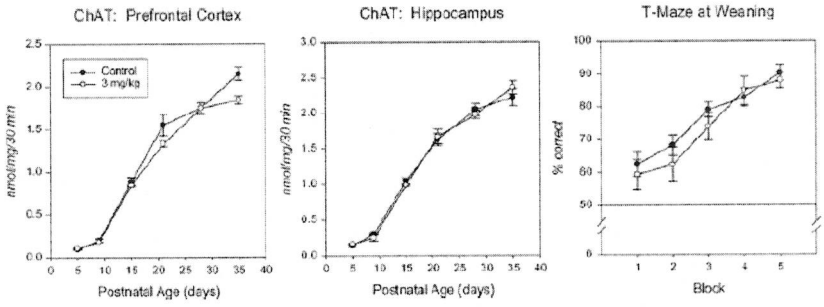

FIG. 4. Effects of prenatal PFOS exposure on cortical and hippocampal choline acetyltransferase activities and T-maze performance of developing rats. For ChAT activity, each data point represents mean ± SE of determination from six rats, except for PDs 9 and 15, where $n = 2$ and 3, respectively, for the 3 mg/kg dose group. For T-maze, each data point represents mean ± SE of repeated measure from eight male and eight female pups. For cortical ChAT activity, two-way ANOVA indicates a significant main effect for treatment ($p < 0.02$). For hippocampal ChAT activity and T-maze, two-way ANOVA did not indicate any significant main treatment effect or interaction.

Citations in the text:

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