Summary - APFO reproductive effects, 8/23/2002

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DuPont Summary – August 23, 2002

Oral (Gavage) Two-Generation (One Litter Per Generation) Reproduction Study of Ammonium Perfluorooctanoate (APFO) in Rats

3M study conducted at Argus (Argus Study #T-6889.6)

The multigeneration reproduction study with APFO has been reviewed and DuPont's position on the study is reported below, in addition to some comments on potential endocrine effects of APFO. In general, we agree with the conclusions stated in the study report with the 2 exceptions noted below. After reviewing the report, Haskell contacted the Argus Study Director on May 31, 2002 to discuss our differences in data interpretation. The study director was in agreement with the recommended changes stated below. Overall, the data from the multigeneration reproduction study illustrate that the parental animals are more severely affected than the offspring, and hence, APFO is not a reproductive toxin. However, there are effects (i.e., decreased pup weights, increased pup mortality, altered estrous cyclicity, delayed puberty) that could be suggestive of reproductive effects, and 30 mg/kg/day could represent the beginning of the dose-response curve based on the effects observed in the reproduction study. In particular, the effects on pup viability, estrous cyclicity, and attainment of puberty (vaginal opening and preputial separation) are suggestive, although not indicative, of reproductive effects. As a result of the small alterations in these endpoints, which were only observed at 30 mg/kg/day, the biological significance of the effects is unclear. Clearly, these effects do not compromise the reproductive success (i.e., mating and fertility) of the animals at dosages of ≤ 30 mg/kg/day under the conditions of this study. Due to the toxicity of APFO to the males, testing for reproductive effects at dosages higher than 30 mg/kg/day may be problematic.

The summary of the findings of the study are summarized below:

P1 Generation:

- The paternal no-observable-adverse-effect-level (NOAEL) for APFO is less than 1 mg/kg/day based on the significant increases in organ weights (liver and kidney) that were observed at 1 mg/kg/day and above.

- The maternal NOAEL is 10 mg/kg/day based on the significant decreases in kidney weights that were observed at 30 mg/kg/day.

- The reproductive NOAEL in males is greater than 30 mg/kg/day since there were no affects on mating or fertility at 30 mg/kg/day.

- The reproductive NOAEL in females is 10 mg/kg/day based on the decreased pup weights and pup survival at 30 mg/kg/day (see discussion below).

F1 Generation:

- The paternal no-observable-adverse-effect-level (NOAEL) for APFO is less than 1 mg/kg/day based on the significant alterations in body and organ weights that were observed at 1 mg/kg/day and above.

- The maternal NOAEL is 1 mg/kg/day based on the significant decreases in pituitary weights that were observed at 3 mg/kg/day and above.

- The reproductive NOAEL in males and females is greater than 30 mg/kg/day since there were no affects on mating, fertility, or maternal delivery parameters at 30 mg/kg/day.

Potential endocrine-related effects were as follows:

- In both male and female F1 weanlings, there were alterations in attainment of puberty [delayed age of preputial separation (PPS; androgen-dependent process) in males and delayed age at vaginal opening (VO; estrogen-dependent process) in females]. While these alterations can be attributed to perturbations in endocrine homeostasis, the pattern of the effects is inconsistent with know endocrine mechanisms. Typically, compounds that will attenuate androgen levels will delay PPS while not affecting VO, whereas compounds that attenuate estrogen levels will delay VO while not affecting PPS. A general delay in both can occur in cases of broad steroid depletion where both estrogens and androgens are attenuated. While there is evidence that APFO has the potential to induce aromatase in rodents (the enzyme that converts testosterone to estradiol), there is no evidence that APFO possesses broad steroid biosynthesis inhibition properties that could account for the effects that were observed. General delays in PPS and VO can also be attributed to non-specific effects on body weight. In the current study, it was concluded that the delays in sexual maturation in male and female rats may be due to the decreased gestational age in the animals at puberty. For this reason, it is not suggestive of an endocrine-mediated effect.

- In F1 females there was a slight increase in the number of estrous cycles per 21 days (4.7 versus 5.4 stages), which is outside of the historical control for the testing laboratory. While this may represent a compound-related effect, it does not manifest in altered reproductive success.

The following modifications to the original report were discussed with Argus and the Study Director was in agreement with Haskell's recommendations:

1. *The P1 generation Maternal Reproductive NOAEL:* The study report states that the P1 generation female's offspring have decreased pup body weights on lactation days 1, 5 and 8 in the 30 mg/kg/day dosage group. Yet the report states the P1 generation Maternal Reproductive NOAEL is greater than 30 mg/kg/day. In addition, the significantly increased number of pup deaths in the 30 mg/kg/day was considered by the Study Director to be unrelated to the test article because the deaths did not affect the indices of pup viability, although the lactation index value in the 30 mg/kg/day was significantly decreased (and considered by the Study Director to be unrelated to the test article because the value was within historical control limits). We agree that while no other measures of pup viability were affected, the decreased pup body weights and the failure to thrive that was noted as a cause of death for several of these animals would lead us to conclude that this was possibly a treatment-related effect.

   All factors taken together, including the slightly higher number of F1 pup deaths during lactation, the small reduction in mean pup weight on days 1, 5, and 8 (approx 90% of control), and the post-weaning F1 pup deaths (due to failure to thrive) at 30 mg/kg/day suggest that there was a compound related effect on F1 pup growth and survival. The significant reduction in lactation index is also probably compound-related since it is consistent with the increased overall pup mortality in the F1 generation at 30 mg/kg/day.

This strengthens the determination to revise down the P1 generation Maternal Reproductive NOAEL from greater than 30 mg/kg/day to 10 mg/kg/day. A comprehensive evaluation of pup mortality is not made in the report, which is why this conclusion may have been overlooked. The data for the F1 pups are summarized below.

Dose (mg/kg/day APFO)	0	1	3	10	30
Dead pups day 1	2	2	3	0	3
Dead pups days 2-5	7(6)[a]	6(5)	7(6)	5(5)	11(9)*
Dead pups days 6-22	1(1)	3(3)	7(3)	5(3)	12(7)*
Dead pups days 2-22	8(7)	9(8)	14(8)	10(6)	23(11)*
Dead pups post-weaning (male & female)	3	5	4	3	13
Dead pups post-weaning (males)	3	3	3	2	7*
No of above attributed to failure to thrive	1	1	2	0	4
Dead pups post-weaning (females)	0	2	1	1	6*
No of above attributed to failure to thrive	0	1	1	1	4
Total dead pups pre- and postweaning	13	16	21	12	39
Mean pup weights:					
Day 1	6.3	6.0	6.2	6.2	5.7*
Day 5	9.4	8.8	9.5	9.3	8.5*
Day 8	13.3	12.7	13.5	13.0	11.9*
Day 15	25.0	24.2	26.4	24.8	22.9
Day 22	37.4	36.7	39.7	38.8	35.7

[a] Number of pups (litters).

2. *The F1 generation Maternal NOAEL:* The study report further states that the F1 generation females have decreased absolute and relative pituitary weights at 3 mg/kg/day and higher. The Study Director may have overlooked this finding when setting the F1 generation Maternal NOAEL at 3 mg/kg/day. The F1 generation Maternal NOAEL should be revised to 1 mg/kg/day [the 3 mg/kg/day and higher doses caused decreased absolute and relative pituitary weight (3 mg/kg/day and higher), postweaning mortality (30 mg/kg/day), decreased preweaning body weight and gain (30 mg/kg/day), decreased feed consumption (30 mg/kg/day), increased number of estrous cycles per 21 days (30 mg/kg/day), and delayed sexual maturation possibly due to decreased gestational age at acquisition (30 mg/kg/day)].

The following tables summarize the statistically significant results of this study.

P1 Generation Male Rats	Mg/Kg/day (mkd) APFO			
Parameter	1	3	10	30
Mortality (Treatment Related)	0	0	0	1
Clinical Observations	-	-	-	⇔
Body Weight/Gains	-	⇐	⇐	⇐
Feed Consumption (Absolute)	-	-	-	⇐
Feed Consumption (Relative)	-	⇔	⇔	⇔
Mating	-	-	-	-
Fertility	-	-	-	-
Necropsy Observations	-	-	-	-
Terminal Body Weight	-	⇐	⇐	⇐
Organ Weights (Absolute)				
Epididymides, seminal vesicles, prostate, pituitary, adrenals, spleen, thymus	-	-	-	⇔
Liver	⇔	⇔	⇔	⇔
Kidney	⇔	⇔	⇔	⇐
Organ Weights (Relative to Body)				
Epididymides, testes, seminal vesicles	-	-	-	⇔
Brain		⇔	⇔	⇔
Kidneys	⇔	⇔	⇔	⇔
Adrenal	-	-	-	⇔
Organ Weights (Relative to Brain)				
Epididymides, prostate, pituitary, spleen, thymus	-	-	-	⇐
Liver	⇔	⇔	⇔	⇔
Kidney	⇔	⇔	⇔	⇐
Sperm Quality	-	-	-	-
Histopathology (Reproductive Organs)	-	-	-	-
Histopathology (Other Organs)	-	-	Adrenal	Adrenal
General Toxicity (NOAEL <1 mkd)	LOAEL			
Reproductive Toxicity				NOAEL

P1 Generation Female Rats	Mg/Kg/day APFO			
Parameter	1	3	10	30
Mortality (Treatment Related)	0	0	0	0
Clinical Observations	-	-	-	-
Body Weight/Gains	-	-	-	-
Feed Consumption (Absolute)	-	-	-	-
Feed Consumption (Relative)	-	-	-	-
Mating	-	-	-	-
Fertility	-	-	-	-
Necropsy Observations	-	-	-	-
Pregnancy	-	-	-	-
Terminal Body Weight	-	-	-	-
Organ Weights (Absolute kidney)	-	-	-	⇐
Organ Weights (Relative kidney)	-	-	-	⇐
Estrous Cyclicity	-	-	-	-
Histopathology (Reproductive Organs)	-	-	-	-
Histopathology (Other Organs)	-	-	-	-
General Toxicity			NOAEL	
Reproductive Toxicity			NOAEL	

F1 Generation Male Rats	Mg/Kg/day APFO			
Parameter	1	3	10	30
Mortality (post lactation; control=3)	3	3	2	7
Clinical Observations	⇔	⇔	⇔	⇔
Body Weight/Gains	⇐	⇐	⇐	⇐
Feed Consumption (Absolute)	-	-	⇐	⇐
Feed Consumption (Relative)	-	-	⇔	⇔
Preputial Separation	-	-	-	Delayed
Mating	-	-	-	-
Fertility	-	-	-	-
Necropsy Observations	-	⇔	⇔	⇔
Terminal Body Weight	⇐	⇐	⇐	⇐
Organ Weights (Absolute)				
Liver	⇔	⇔	⇔	⇔
Spleen	⇐	⇐	⇐	⇐
Kidney	⇔	⇔	-	⇐
Thymus	-	⇐	⇐	-
Prostate, brain, adrenals	-	-	-	⇐
Organ Weights (Relative to Body)				
Seminal vesicles, liver, kidneys	⇔	⇔	⇔	⇔
Testes	-	⇔	⇔	⇔
Epididymides, brain	-	-	⇔	⇔
Organ Weights (Relative to Brain)				
Seminal vesicles, liver	⇔	⇔	⇔	⇔
Kidney	⇔	⇔	⇔	-
Testes	-	⇔	⇔	⇔
Spleen	⇐	⇐	⇐	⇐
Thymus	-	-	⇐	⇐
Sperm Quality	-	-	-	-
Histopathology (Reproductive Organs)	-	-	-	-
Histopathology (Other Organs)	-	Liver	Liver	Liver/Adrenal
General Toxicity (NOAEL <1 mkd)	LOAEL			
Reproductive Toxicity				NOAEL

F1 Generation Female Rats	Mg/Kg/day APFO			
Parameter	1	3	10	30
Mortality (post lactation; control=0)	2	1	1	6
Clinical Observations	-	-	-	-
Body Weight/Gains	-	-	-	⇐
Feed Consumption (Absolute)	-	-	-	⇐
Feed Consumption (Relative)	-	-	-	-
Vaginal Patency	-	-	-	Delayed
Mating	-	-	-	-
Fertility	-	-	-	-
Necropsy Observations	-	-	-	-
Pregnancy	-	-	-	-
Terminal Body Weight	-	-	-	-
Organ Weights (Absolute pituitary)	-	⇐	⇐	⇐
Organ Weights (Relative pituitary)	-	⇐	⇐	⇐
Estrous Cyclicity	-	-	-	⇔
Histopathology (Reproductive Organs)	-	-	-	-
Histopathology (Other Organs)	-	-	-	-
General Toxicity	NOAEL			
Reproductive Toxicity				NOAEL

F2 Generation Rats (pups)	Mg/Kg/day APFO			
Parameter	1	3	10	30
Viability/mortality	-	-	-	-
Litter size	-	-	-	-
Clinical Observations	-	-	-	-
Body Weight	-	-	-	-
Terminal body weight	-	-	-	-
Necropsy Observations	-	-	-	-
Organ Weights (brain, spleen, thymus)	-	-	-	-
Anogenital distance	-	-	-	-

Summary of F2 pup mortality

Dose (mg/kg/day APFO)	0	1	3	10	30
Dead pups day 1	0	0	5*	4*	1
Dead pups days 2-5	2	5	5	3	3
Dead pups days 6-22	5	4	5	3	5
Dead pups days 2-22	7	9	10	6	8

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