

Toxicological Summary

PFOS

Dietary Chronic Pilot Reproductive Study: Mallard

- Test Substance:** Perfluorooctanesulfonate (PFOS)
- Structure:** 1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoropotassium salt, CAS# 2795-39-3
- Test Remarks:** The test substance is a white powder (3M Lot #217). The sample was stored under ambient conditions, and purity was determined to be 86.9% by LC/MS, ¹H-NMR, and elemental analysis techniques.

METHODS

Method: ASTM Standard E1062-86 and FIFRA Subdivision E., Section 71-4.

Type: Dietary Reproduction

Year: 2000-2001 in-life phase, 2000-2001 analytical phase, Final Report in 2003

Species: Mallard (*Anas platyrhynchos*)

Experimental design: Adult mallards were exposed to PFOS in the diet at nominal concentrations of 1.8, 6.2, or 17.6 ppm for a period of six weeks. Concurrently, a control group was maintained on a non-treated feed. After six weeks of exposure, blood was collected from mallards from all treatment groups and the 1.8 and 6.2 ppm treatments were euthanized and subjected to gross necropsy. The control and 17.6 ppm treatment groups were maintained on their respective exposure regimes until the end of Week 19. At the beginning of Week 20 the remaining mallards were euthanized and subjected to a gross necropsy. Effects on adult health, body weight and feed consumption were evaluated weekly. Because the two lower doses were terminated at 6 weeks, the evaluation of the data was divided into two separate analyses. The first analysis evaluated all data collected on each treatment group during the first six weeks of the study. The second analysis examined data from the control and 17.6 ppm treatments collected at Week 20 (Table 1).

Adult mallards were brought into their reproductive phase prior to the onset of the study by photostimulation. Consequently, egg laying was initiated approximately one week after the onset of the exposure. Egg production was evaluated on a weekly basis for the duration of the study. Eggs laid during Week 1 and 6 of the test were collected and analyzed for PFOS. Eggs laid during Week 5 of the study, were collected and set for incubation. Reproductive endpoints, including egg production, embryo viability, hatchability, health, and survival, were evaluated in this egg cohort. These offspring were maintained on non-treated feed for approximately 12 weeks. Necropsies were performed on offspring from each maternal treatment dose group. Samples were taken of liver, brain, kidney, gonad, proventriculus, gall bladder, adipose tissue, and Bursa of Fabricius for histopathological examination from both

adults and offspring. Liver weights were recorded for all necropsied mallards, and the livers were then analyzed for concentrations of PFOS.

At the end of Week 6, blood samples were collected from all surviving adult birds, when possible. Following collection of blood samples, birds in the 1.8 and 6.2 ppm treatment groups were euthanized and necropsied. At the time of necropsy, livers were collected and stored for chemical analysis. Additional blood samples were also collected from adult birds in the control and 17.6 ppm treatment groups prior to euthanasia and necropsy at the beginning of Week 20. At necropsy, liver samples were also collected from adult birds for chemical analysis. Prior to euthanasia of the offspring, blood samples were collected from 10 offspring from each treatment group. Additionally, tissues from 10 offspring were collected from each treatment group for analysis. All blood collected from adult and offspring was separated into serum and hemacyte/platelet fractions.

Table 1. Experimental design for the dietary chronic pilot reproductive study with mallards

Treatment (ppm)	Exposure Period	Endpoints Examined
Control	20 week	Health, body weight, feed consumption, gross morphology and histopathology of body organs, egg production, embryo viability, hatchability, offspring health and survival, PFOS concentrations in liver and blood serum of adult and offspring mallards, PFOS concentrations in egg components (membrane, albumen, and yolk)
1.8	6 week	
6.2	6 week	
17.6	20 week	

Test Bird Age: Adult mallards (approximately 27 weeks)

Number of Replicates: Five replicates (pens) per treatment group

Birds per Replicate: One male and female mallard per pen

Feed and Water: Food and water were provided ad libitum during all phases of the study to both adult mallards and offspring. Feed consumption was measured on a per pen basis, once a week.

Analytical Monitoring: Test substance concentration in feed, liver and serum samples were determined by reverse-phase HPLC and mass spectrometry.

Statistical Methods: Upon completion of the test, an analysis of variance (ANOVA) was performed to evaluate significant differences between treatment groups. Dunnett's multiple comparison procedure was used to compare the treatment effects with control. The student's T-test was used to make statistical comparisons in those instances where only the control and the 17.6 ppm treatment groups were compared

Average Daily Intake (ADI) of PFOS for each treatment group was estimated using data from each pen in that treatment group without taking into account potential differences between the male and female paired within a pen. Food consumption and adult mallard body weight data were averaged over the exposure duration of the study and the ADI was calculated as follows:

$$\text{ADI (mg/kg/day)} = \frac{\text{Average Feed Consumption (g/bird/day)}}{\text{Average Body weight (g/bird)}} \times \text{Feed Conc. (ppm)} \quad (\text{Equation 1})$$

Test Diet Preparation: Test diets were prepared by mixing PFOS into a premix that was used for weekly preparation of the test diet.

RESULTS

Measured Diet Concentrations: To confirm the concentrations of PFOS in the diet, chemical analyses were conducted by Wildlife International Ltd. The limit of quantitation (LOQ) for PFOS during Week 1 of the study was 0.879 ppm. During Week 6 the LOQ for PFOS in the feed was 1.41 ppm. The percent recoveries of PFOS in the matrix spiked-diets ranged from 97.0 to 113% throughout the study period. Analyses of control diets showed no presence of the test substance or other co-eluting compounds. PFOS concentrations in treated feed samples ranged from 95 to 111 % of the nominal target doses. The mean measured PFOS concentrations for diets determined in the study were: <LOQ, 1.8, 6.0 and 17.6 ppm in feed

Mortalities and Clinical Observations: No adult mortalities occurred in the control or PFOS treatment groups during the study. While several mallards had head or foot lesions and feather loss, these signs were considered a result of captivity stress and/or pen-mate aggression. In the 17.6 ppm treatment, one hen arched her neck and became rigid when captured at test termination. This hen also exhibited body tremors, rapid blinking, and rapid respiration during the bleeding process. These signs were most likely in response to handling stress, since the mallard appeared normal prior to test termination. All other mallards were normal in appearance and behavior throughout the duration of the study. Based on these results, the dietary No observable Adverse Effect Concentration (NOAEC) for adults was 17.6 ppm.

Adult Body Weight: At both Week 6 and 20, there were no treatment-related reductions in body weight in adult females from any of the treatment groups when compared to controls (Table 2). Compared to controls, there was a significant reduction in female body weight from Week 2 to Week 4 in the 1.8 mg PFOS/kg treatment. However, this reduction was not consistent or dose-responsive, therefore it was not considered to be treatment-related. In addition, there were mean body weight losses among females in the 17.6 mg PFOS/kg treatment that may have been related to treatment. However, due to small sample sizes (N=5) and data variability, these differences were not statistically significant. Compared to controls, there were no statistically significant reductions in male body weight at Week 6. By Week 10, 11 and 20, body weights of male mallards in the 17.6 mg PFOS/kg treatment were statistically significantly less than that of the controls. At Week 20, the mean male body weight in the 17.6 mg PFOS/kg treatment was 13% less than the mean male body weight in the control group. Based on body weight reduction, the dietary Lowest Observable Adverse Effect Concentration (LOAEC) for males was 17.6 mg PFOS/kg. Since only the 17.6 mg PFOS/kg treatment was conducted for the full 20 weeks of the study, the dietary NOAEC for males could not be determined. The dietary NOAEC for adult female mallards was 17.6 mg PFOS/kg.

Table 2. Daily exposure, feed consumption, body and liver weight for adult mallards exposed to PFOS in the diet. ^A

Treatment (ppm)	ADI ^B (mg/kg/d)	FC ^C (g/bird/d)	Sex	Body Weight (g)		Liver wt. (g)
				Week 6	Week 20	
Control	NA	212 ± 40	M	1124 ± 71	1204 ± 97	21.3 ± 4.79
			F	1052 ± 75	1005 ± 23	21.2 ± 5.38
1.8	0.269 ± 0.062	177 ± 50	M	1136 ± 78		25.3 ± 1.56
			F	1114 ± 91		39.8* ± 8.83
6.2	0.985 ± 0.253	178 ± 56	M	1118 ± 63		29.2 ± 4.90
			F	1038 ± 95		31.9* ± 4.5
17.6	3.601 ± 0.599	234 ± 84	M	1087 ± 51	1042* ± 94	23.4 ± 8.02
			F	978 ± 149	897 ± 139	20.6 ± 2.95

^A Control and 17.6 ppm treatments were sacrificed at Week 20 while 1.8 and 6.2 ppm treatments were sacrificed at Week 6. All values are given as means and standard deviations on a wet weight basis.

^B Average Daily Intake (ADI) (mg PFOS/kg body weight/day) for 1.8 and 6.2 ppm groups was based on average feed consumption measured through Week 6. For 17.6 ppm treatment group, feed consumption was averaged through Week 20.

^C Feed consumption data is taken at Week 6.

Asterisk indicates a statistical difference (p<0.05) from controls.

NA: No applicable, concentrations in diet less than the LOQ.

Feed Consumption: On Weeks 14, 16, 17, 18, and 19, mallards in the 17.6 ppm treatment had feed consumption that was significantly greater than that of mallards in the control treatment. However, mallards in the 17.6 ppm treatment did not have a corresponding increase in body weight, which suggests that the increase was the result of feed wastage in this treatment group. Based on these results, the dietary NOAEC was determined to be 17.6 ppm.

Liver Weight: When compared to the control group, there were no apparent treatment-related effects on adult liver weight in any treatment group. While there was a statistically significant increase in liver weight in females from the 1.8 and 6.2 ppm treatments, these differences were most likely due to the timing of the termination of these groups. Both groups were terminated at the end of Week 6, which was a period of high egg production. Therefore, the mallards, being in full reproductive condition, would be expected to have greater fat reserves in the liver. Based on these results, the dietary NOAEC for liver weight in male and female mallards was 17.6 ppm.

Gross Pathology: Adult mallards in the 1.8 and 6.2 ppm treatments were sacrificed at Week 6, while mallards in the control and 17.6 ppm treatment groups were sampled at Week 20. All mallards were subjected to gross necropsy. All findings were considered to be incidental and not related to treatment. Incidence of small testes was noted during gross necropsy, but was considered incidental to treatment.

Ten mallard offspring (approximately 12 weeks old) per treatment group were sampled and subjected to gross necropsy. Results from the necropsy showed that several mallards from each treatment group had bumble foot. In addition, a single mallard from the 17.6 ppm treatment had broken feathers, had an unkempt appearance and retained its yolk sac. For

males, one male offspring from the 17.6 ppm treatment lacked the posterior portion of the left kidney. However, these findings were considered incidental and unrelated to treatment.

Histopathology: Various tissues, including the liver, brain, kidney, gonad, proventriculus, gall bladder, adipose, and Bursa of Fabricius, were sampled from adult (control and 17.6 ppm treatments) and juvenile (all treatments) mallards and examined under a light microscope. There were no lesions in the liver, kidney, proventriculus, gall bladder, ovary, brain, or Bursa Fabricius in either adult male and female mallards or offspring at any of the PFOS concentrations tested. There were also no treatment-related lesions in the adipose tissues of adult females and offspring of either sex. However, the incidence of adipose microvesiculation in adult males was greater in the 17.6 ppm treatment. In addition, the testes of adult males exhibited several features including aspermia (complete absence of mature spermatozoa), decreased spermatogenesis and/or decreased seminiferous tubule diameter. The incidence of small testis size and the reduced diameter of the seminiferous tubules were more pronounced in males from the 17.6 ppm treatment as compared to controls. While these findings are part of the normal physiological phenomenon consistent with post-reproductive phase regression, the overall findings suggest that testicular regression may have been accelerated in the 17.6 ppm treatment as compared to the controls. However, a definitive statistical analysis of these data to evaluate the significance of these findings could not be conducted due to the small sample size (N=5). Based on the effects observed in males, the dietary LOAEC was determined to be 17.6 ppm PFOS.

Reproductive Results: There were no treatment-related effects on egg production at any PFOS concentration tested in the study. While there was a slight reduction in egg production in the 6.2 ppm treatment, this reduction was not considered treatment related, as egg production at 1.8 and 17.6 ppm exceeded control levels. When compared to mallards in the control treatment, there were no effects on embryo viability, hatchability, hatchling health, and survivability in mallards from the PFOS treatments. There were fewer 14-day old survivors per hen produced in the 1.8 and 6.2 ppm treatments when compared to controls. These reductions were the result of fewer eggs being set for incubation in these treatments. Yet, the significance of these effects is difficult to interpret since the number of 14-day old surviving offspring per hen from the 17.6 ppm treatment were similar to that of the controls. When compared to the control treatment, there were minor reductions in several normalized reproductive endpoints in the 17.6 ppm treatment that were not statistically significant. These endpoints included a reduction in the number of hatchling/3-wk embryos, number of hatchlings/eggs set, number of hatchlings/hen/day, and number of surviving offspring/hen/day. Finally, there were no apparent treatment-related effects on the body weight of hatchling or juvenile mallards when compared to control values. Based on these reproductive results, the dietary NOAEC was determined to be 17.6 ppm.

Hatchling and Juvenile Mallard Results:

There were no apparent treatment-related effects on the body weight of hatchlings and juvenile (12 week old) mallards in any of the PFOS treatments. When compared to controls, there were no treatment-related effects on juvenile liver weight at any concentration tested. While the mean liver weight of the 1.8 ppm treatment group was statistically greater than that

of controls, the effect was not dose-dependent and may have been a function of the greater mean body weight that was observed in juveniles from this group. The dietary NOAEC for hatching and juvenile mallards was 17.6 ppm.

Table 3. Mean liver and body weights of hatchling and juvenile mallards from various PFOS treatment groups. ^A

Treatment (ppm)	Body Weight (g)		Juvenile Liver ^B Weight (g)
	Hatching	Juvenile ^B	
Control	28.4 ± 2.0	1012 ± 31	20.89 ± 3.15
1.8	32.8 ± 1.4	1065 ± 92	27.66* ± 5.00
6.2	33.3 ± 0.4	1036 ± 71	23.22 ± 4.22
17.6	35.0 ± 1.6	1030 ± 35	24.72 ± 5.49

^A All data are presented as means and standard deviations.

^B Juvenile body and liver weights were measured approximately 12 weeks post-hatch.

Asterisk indicates a statistically significant difference from the control treatment at $p < 0.05$

Concentrations of PFOS in Blood and Liver:

Adults

Adult mallards accumulated PFOS in a dose-dependent manner (Table 4). To examine the relationship between exposure and tissue concentrations, the estimated average daily intake (ADI) of PFOS by adult mallards was compared to liver and serum concentrations collected from each PFOS treatment group at termination. For the 1.8 and 6.2 ppm treatments, serum and liver samples were collected at Week 6 while for the control and 17.6 ppm treatments, the serum and liver were collected at week 20. This analysis was based on the assumption that by Week 6, the concentrations in serum and liver in mallards would be approaching steady state. An ADI was used in this analysis because this parameter is commonly used as a measure of exposure that adult birds may encounter in the environment. The results of the regression analysis for PFOS concentrations in both serum and liver and ADI of PFOS are given below:

Male:

$$\begin{aligned} \text{Serum PFOS } (\mu\text{g/ml}) &= 41.62 (\text{ADI, mg/kg-day}) + 0.4595 & R^2 &= 0.7177 \\ \text{Liver PFOS } (\mu\text{g/g}) &= 2.717 (\text{ADI, mg/kg-day}) - 0.4814 & R^2 &= 0.6676 \end{aligned}$$

Female:

$$\begin{aligned} \text{Serum PFOS } (\mu\text{g/ml}) &= 42.80 (\text{ADI, mg/kg-day}) - 12.47 & R^2 &= 0.7827 \\ \text{Liver PFOS } (\mu\text{g/g}) &= 3.012 (\text{ADI, mg/kg-day}) - 1.124 & R^2 &= 0.6048 \end{aligned}$$

Thus, there was a moderate correlation between increasing dose and serum and liver levels.

Table 4. Mean concentrations of PFOS in liver and serum from adult mallards

Treatment (ppm)	Sex	Liver Conc. ^A (µg/g)	Serum Conc. ^B (µg/ml)	
			Week 6	Week 20
Control	M	0.157 ± 0.292	< LOQ	1.39 ± 2.64
	F	0.120 ± 0.165	< LOQ	1.05 ± 1.65
1.80	M	0.836 ± 0.738	17.2 ± 5.41	
	F	0.237 ± 0.105	3.65 ± 3.71	
6.20	M	1.19 ± 0.422	34.7 ± 11.8	
	F	0.650 ± 0.654	17.7 ± 25.0	
17.6	M	9.59 ± 5.96	101 ± 66.2	158 ± 70.0
	F	9.68 ± 8.21	30.9 ± 20.5	144 ± 67.8

^A Control and 17.6 ppm treatment groups were sacrificed at Week 20 while 1.8 and 6.2 ppm treatments were sacrificed at Week 6. All values are given as means and standard deviations on a wet weight basis.

^B LOQ for serum was 0.01 µg/ml.

Sex-specific differences were observed for both liver and serum PFOS concentrations as measured at study termination. Within the same treatment groups, PFOS concentrations in livers of males were 1.1 to 4 times greater than those in the livers of females. PFOS concentrations in male serum were approximately 1.1 to 5 times greater than PFOS concentrations observed in the serum of females sampled from the same treatment groups.

A closer evaluation of the relationship between PFOS exposure and tissue concentrations reveal sex and time dependent differences. These differences make it difficult to develop predictive quantitative relationships between concentrations in environmental matrices and tissue concentrations. In part, this variability is due to the experimental design where mallards in the control and 17.6 ppm treatments were exposed for 20 weeks while mallards in the 1.8 and 6.2 ppm treatment groups were exposed for only 6 weeks. Variability in the PFOS concentration data was also due to temporal differences in PFOS accumulation by both sexes throughout the study. For instance, a comparison of Week 6 and Week 20 serum data for control and 17.6 ppm treatments show that PFOS was accumulated in a time dependent manner but that there were differences in the pattern of accumulation between male and female mallards. In male mallards from the 17.6 ppm treatment, there was approximately a 1.6-fold increase in serum PFOS concentrations over this time period; however, this difference was not statistically significant. Over the same time period, female mallards from the 17.6 ppm treatment exhibited an approximate 5-fold increase in serum PFOS concentrations. This statistically significant increase in serum PFOS concentration in females is indicative of the presence of additional processes that differentially regulate the disposition of PFOS in male and female mallards. A comparison of the temporal trends of PFOS concentrations in blood serum to other information collected during the study suggests that egg-laying in females may be an important process in the disposition of PFOS. For instance, peak egg production in this dose group occurred during the first 10 weeks of the study, declined during the next 6 weeks, and totally ceased over the last 4 weeks of the study. Changes in egg production were related to changes in serum PFOS concentration. At Week 6, during peak egg production, serum PFOS concentrations in females were approximately 3.3-times less than those observed in males. At Week 20, when egg production had ceased, concentrations of PFOS in blood serum of female mallards were only 1.1 times less than those measured in males. These data indicate

that as egg production decreased in females, there was an increase in serum PFOS concentrations to levels that were equivalent to male values.

As a result of these sex-specific differences in PFOS accumulation in mallards, it is important to take into consideration the reproductive condition of females when interpreting the toxicological significance of blood serum or liver PFOS concentrations that may be collected as a part of environmental monitoring programs.

PFOS Concentrations in Juveniles

Analysis of 12-week old juvenile mallard serum and liver indicated that PFOS concentrations persist in juveniles post-hatch (Table 5). However, the serum and liver PFOS concentrations in juvenile mallards represent only a fraction of the total PFOS originally deposited into the egg from an exposed female mallard. Potential losses from the juvenile mallards include such processes as growth dilution, depuration and deposition in tissues other than liver or sera. To assess the quantitative relationship between juvenile liver and serum PFOS concentrations and egg yolk concentrations, a regression analysis was conducted. This analysis included all the data, including data that was above the limit of detection (LOD) but less than the LOQ, from each PFOS treatment group. The relationship between egg yolk and juvenile serum and liver PFOS concentrations are

$$\begin{aligned} \text{Liver PFOS } (\mu\text{g/g}) &= 0.0131 (\text{Egg yolk, } \mu\text{g/ml}) - 0.0157 & R^2 &= 0.8804 \\ \text{Serum PFOS } (\mu\text{g/ml}) &= 0.0247(\text{Egg yolk, } \mu\text{g/ml}) - 0.2928 & R^2 &= 0.9007 \end{aligned}$$

Thus, liver and serum concentrations in 12-week old mallards are highly correlated with levels in the egg yolk.

PFOS concentrations in the liver and blood serum of juvenile mallards increased in a dose-dependent manner, with the greatest concentrations occurring in mallards from the 17.6 ppm exposure. The average ratio of PFOS concentrations in serum to that in liver for juvenile mallards was 23.1 and ranged from 3.32 to 39.1. This average serum to liver ratio was similar to that observed for adult mallards (27 with a range of 2.33 to 50.7).

Table 5. Concentrations of PFOS in liver and serum of 12-week old juvenile mallards. ^A

Treatment (ppm)	Liver Conc. ($\mu\text{g/g}$)	Serum Conc. ($\mu\text{g/ml}$)
Control	<LOQ ^B	<LOQ
1.8	<LOQ	0.0507 ± 0.0701
6.2	<LOQ	0.0734 ± 0.0466
17.6	0.0262 ± 0.0168	0.496 ± 0.184

^A All concentrations are presented as means with standard deviations. (Mean based on duplicate analyses of ten tissue samples). Data given on a wet weight basis.

^B LOQ for liver was 0.01 $\mu\text{g/g}$ while serum was 0.01 $\mu\text{g/ml}$.

Concentrations of PFOS in Mallard Eggs: Eggs were sampled from the control, 6.2 and 17.6 ppm treatments during the first and sixth week of egg production. Eggs were separated

into three components, egg membrane, albumen and yolk, and each component was analyzed for PFOS (Table 6).

Table 6. PFOS concentrations (µg/ml) in mallard egg components ^A

Treatment (ppm)	<u>Egg Membrane</u>		<u>Albumen</u>		<u>Yolk</u>	
	1 week	6 week	1 week	6 week	1 week	6 week
Control	<LOQ ^B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
6.2	0.040 (0.011)	0.077 (0.018)	<LOQ	<LOQ	8.34 (4.72)	16.1 (6.71)
17.6	0.052 (0.026)	0.342 (0.065)	0.023 (0.002)	0.020 (0.004)	48.5 (127)	50.7 (9.37)

^A All concentrations of egg components were reported on a wet weight basis. Data is presented as means and standard deviations.

^B LOQ is the limit of quantitation (0.01 µg/ml)

For eggs sampled at both Week 1 and 6, a positive relationship was observed between adult hen dietary exposure concentrations and PFOS concentrations in eggs. Furthermore, there were differences between the concentrations of PFOS measured in different egg components at each treatment level, with yolk having the greatest concentrations while albumen had the least. When PFOS concentrations for each egg component were compared between Week 1 and Week 6 by treatment level, the concentrations in egg components for Week 6 was generally greater than those measured at Week 1. The exception to this was observed for albumen where PFOS concentrations slightly reduced at Week 6 when compared to Week 1. For the 6.2 ppm treatment, there was approximately a 1.9-fold increase in PFOS concentrations between Week 1 and 6 for egg membrane and yolk. However, for the 17.6 ppm treatment, there was approximately a 6.6-fold increase in membrane concentrations while for yolk there was approximately a 1.04-fold increase in concentrations over this time period. The implications of these observed differences are difficult to interpret but seem to indicate that the adult female mallards had yet not achieved steady state with respect to serum, liver or egg component PFOS concentrations by the end of Week 1 for either dose group. Instead, PFOS concentrations in both egg membranes and yolk were still increasing up until Week 6. In part, these trends are most likely a result of the fact that the adult females had only been on the treated diet for approximately one week prior to egg production and had not yet reached steady state.

A further evaluation of the egg PFOS data showed that there was a relatively constant relationship between egg yolk PFOS concentrations and PFOS exposure as measured by average daily intake (ADI). For week 6, the average yolk PFOS concentration to ADI ratio was approximately 15 for both the 6.2 and 17.6 ppm treatments. This ratio was similar to that ratio derived from a regression analysis of the yolk PFOS concentrations and ADI where the slope of regression model was approximately 13.1:

$$\text{Yolk } (\mu\text{g/ml}) = 13.082 (\text{ADI, mg/kg-day}) + 2.259$$

$$R^2 = 0.8568$$

However the relationships between exposure and egg membrane and albumen PFOS concentrations was slightly more variable. For instance, the egg membrane:ADI ratio for the 6.2 ppm treatment was 0.078 while for the 17.6 ppm treatment it was 0.095. A ratio

comparison could not be calculated for albumen due to the lack of quantifiable PFOS concentrations. Based on this preliminary analysis, it can be concluded that yolk PFOS concentrations were the best indicator of environmental exposure.

Since no treatment-related adverse effects were observed for any reproductive parameter measured in this study, quantitative relationships between egg concentrations and effects in hatchlings or juvenile survivors are not given in this report.

CONCLUSIONS:

Male and female mallards were exposed to PFOS at dietary concentrations of 0, 1.8, 6.2 and 17.6 ppm. The control and 17.6 ppm treatment mallards were exposed for 19 weeks, while the 1.8 and 6.2 ppm treatments were exposed for only 6 weeks prior to termination. No treatment-related mortalities or overt signs of toxicity were observed at any PFOS concentration tested. When compared to the control treatment, there were no PFOS-related effects on feed consumption at either Week 6 or Week 20 of the study. No treatment-related effects were observed in females at any dose evaluated in the study. At Week 20, males in the 17.6 ppm treatment had reduced body weights when compared to controls. In addition, male mallards in the 17.6 ppm treatment had an increased incidence of small testes when compared to controls. While testicular regression is a normal physiological process, the biological significance of the increased incidence in the 17.6 ppm treatment group over that observed in the control group could not be fully evaluated due to the small sample size. In addition, the significance of this finding is difficult to interpret in that no treatment-related effects or male-related effects were observed on reproduction in this study. Based on these results, the NOAEC and LOAEC for adult and juvenile endpoints evaluated in the study are given (Tables 7). The No Observable Adverse Effect Level (NOAEL) and Lowest Observable Adverse Effect Level (LOAEL) values for PFOS measured in various tissue matrices are also reported (Table 8).

Table 7. Measurement endpoints and associated dietary NOAEC and LOAEC values for PFOS in a chronic pilot study with mallards and their offspring.

Endpoint ^A	Dietary NOAEC (ppm)	Dietary LOAEC ^B (ppm)
ADULT		
Mortality	≥17.6	
Body weight	Females ≥ 17.6 Males = 6.2	Males = 17.6
Feed consumption	≥17.6	
Liver weight	≥17.6	
Gross pathology	≥17.6	
Histopathology	Females ≥ 17.6 Males = 6.2	Males = 17.6
Reproductive	≥17.6	
OFFSPRING		
14-day survivability	≥17.6	
Hatchling/juvenile body weight	≥17.6	
Juvenile liver weight	≥17.6	

^A Control and 17.6 mg PFOS/kg results are based on Week 20 data, while the 6.2 ppm results are based on Week 6 data.

All concentrations reported on a wet weight basis.

^B LOAEC for males based on a statistically significant reduction in body weight.

Table 8. NOAEL and LOAEL values in various matrices in adult and offspring mallards in a chronic pilot study with PFOS.

Measures of PFOS Exposure ^A	NOAEL ^B	LOAEL ^C
ADULT MALES		
Dose (ppm) ^A	6.2	17.6
ADI (mg PFOS/kg body wt./day)	0.99	3.60
Serum (µg/ml)	34.7	158
Liver (mg PFOS/kg)	1.19	9.6
ADULT FEMALES		
Dose (ppm)	17.6	
ADI (mg/kg body wt. /day)	3.60	
Serum (µg/ml)	144	
Liver (µg/g)	9.7	
OFFSPRING		
Yolk (µg/ml)	50.7	
Liver (µg/g)	0.0262	
Serum (µg/ml)	0.496	

^A Control and 17.6 mg PFOS/kg results are based on Week 20, while the 6.2 ppm results are based on Week 6 data. All concentrations reported on a wet weight basis.

^B No and Low effect values for diet and ADI are based on concentration. Serum and liver effect values are measured tissue values.

^C LOAEC for males based on a statistically significant reduction in body weight.

DATA QUALITY:

Reliability: Klimish ranking = 1

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OTHER:

Last changed: 05/05/04