Toxicological Summary

PFOS

Dietary Acute Mallard Study

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-heptadecafluoro-, potassium salt, CAS# 2795-39-3

Remarks: The test substance is a white powder (3M lot #217). The sample was stored under ambient conditions and purity was originally determined to be 90.49% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques. In a subsequent analysis of the test substance, purity was determined to be 86.9%. The nominal and measured dietary PFOS concentrations in this summary are based on the 86.9% purity value.

The in-life phase report was originally completed in 2000, and reported values based on the 90.40% purity estimation. The report was revised in 2004 to reflect the subsequent revision in sample purity. This Toxicological Summary reflects these changes as well as including results from all of the associated reports of analyses of tissues collected during the in-life phase.

METHODS

Method: OPPTS 850.2200 (Draft), FIFRA Subdivision E, Section 71-2, OECD 205 and ASTM Standard E857-87 (1997)

Type: Dietary Acute

Year: 1999 in life test, 2000 for report, 2004 for amended report

Species: Mallard (*Anas platyrhynchos*)

Test Phases: Acclimation – 9 days
Exposure period – 5 days
Post-exposure recovery period – 3 or 17 days (Experimental Day 8 or 22, respectively)

Test Bird Age: 10 days old at test initiation

Number of Replicates: There were six replicates of the control treatment, and two replicates in each test substance treatment. There were five mallards per replicate (pen) for a total of 30 mallards in the control treatment and 10 mallards in each test substance treatment (which included eight dose groups).

The animals were all exposed for five days, and then held for a recovery period. At Day 8, half the replicates from each treatment group (3 of the 6 replicates from the control group and 1 replicate from each dose level) were sacrificed while the other half were continued in the study until Day 22.

Feed and Water: Food and water were provided ad libitum during acclimation and testing phases of the study. The mallards did not receive any form of antibiotic treatment during either the acclimation or test phase of the study.

Analytical Monitoring: The test substance concentration was determined in feed and mallard liver and serum by reverse-phase HPLC and mass spectrometry.

Statistical Methods: LC50 (Lethal concentration that results in 50% mortality of a population for a given exposure time) and LT50 (Lethal time that results in 50% mortality of a population for a given dose) values were calculated by probit analysis using the Statistical Analysis System (SAS/STAT: PROC PROBIT). Body weight data were compared by Dunnett's test using TOXSTAT software (Dunnett, 1955; Gulley, 1990). All other statistical evaluations were conducted with Excel worksheets including regressions with concentrations of PFOS in tissues.

Average Daily Intake (ADI) of PFOS for each treatment group was estimated on a pen basis and did not take into account potential differences between male and females. Food consumption and adult mallard body weight data were averaged over the duration of the exposure period and the ADI was calculated as follows (Equation 1):

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

Test Diet Preparation: The test substance was mixed directly into the feed ration by means of a Hobart mixer. No carrier was used. Nominal dietary concentrations of PFOS used in this study were 0 (control), 8.7, 17.6, 35.1, 70.3, 141, 281, 562, and 1125 parts per million (ppm).

RESULTS

Measured Diet Concentrations: Wildlife International Ltd. determined concentrations of PFOS in the test diet. The mean percent recovery of the matrix-spiked feed was 94.7%, while the test feed samples had measured concentrations of PFOS that ranged from 92 to 119% of the nominal value. Measured mean concentrations of PFOS were: <LOQ, 9.41, 18.7, 38.6, 71.5, 167, 279, 516, and 1148 ppm.

Mortalities and Clinical Observations: A summary of the percent mortality for each treatment group is given (Table 1).

Table 1. Cumulative percent treatment-related mortality of juvenile mallard exposed to PFOS via the diet.

Nominal Conc. (ppm)	Measured Conc. (ppm)	ADI [A] (mg/kg/day)	Days								
			Exposure period					Recovery Period			
			1	2	3	4	5	6	7	8	20[B]
Control	<LOQ [C]	<LOQ	0	0	0	0	0	0	0	0	0
8.7	9.41	2.71	0	0	0	0	0	0	0	0	0
17.6	18.7	5.74	0	0	0	0	0	0	0	0	0
35.1	38.6	12.0	0	0	0	0	0	0	0	0	0
70.3	71.5	20.4	0	0	0	0	0	0	0	0	0
141	167	61.3	0	0	0	0	0	0	0	0	0
281	279	74.2	0	0	0	0	0	0	10	20	20
562	516	149	0	0	0	0	10	20	30	30	30
1125	1148	229	0	0	0	20	60	80	90	90	90

[A] Average daily intake (ADI; mg PFOS/kg body weight/day) was calculated with body weight and feed consumption values from Days 0-5.
[B] No mortalities occurred in any treatment levels in the extended recovery period from Day 8 to Day 22.
[C] Limit of quantitation (LOQ) for PFOS in the diet was 1.15 ppm.

In the control group, no mortalities occurred at any phase of the study and all mallards were normal in appearance and behavior. There were no treatment-related mortalities or any overt signs of toxicity throughout the study at concentrations less than or equal to the 141 ppm nominal dose. However, mortality was noted for mallards treated with nominal doses of 281, 562 or 1125 ppm PFOS in the diet.

In the 281 ppm treatment, signs of toxicity were observed prior to death starting at Day 4 and continued until the afternoon of Day 8. These signs included ruffled appearance, reduced reaction to stimuli (sound and motion), lethargy, and lower limb weakness. Mortality of mallards occurred on Days 7 and 8, and by Day 8 a cumulative mortality of 20% was observed. Following Day 9, the remaining mallards in the 281 ppm treatment had returned to a normal appearance and behavior for the duration of the study.

In the 562 ppm treatment group, signs of toxicity were initially observed on Day 3 and continued until termination for those birds sacrificed on Day 8 and until the afternoon of Day 8 for those birds sacrificed on Day 22. Signs of toxicity included a ruffled appearance, reduced reaction to stimuli, lethargy, and lower limb weakness. Single mortalities were noted on Days 5, 6 and 7 for a total cumulative mortality of 30% by Day 7. Remaining mallards appeared to have recovered and were normal in appearance and behavior from Day 9 of the study.

In the 1125 ppm treatment group, signs of toxicity were first observed on Day 2, and included ruffled appearance, reduced reaction to stimuli, lethargy, depression, loss of coordination, prostrate posture, depression, convulsions, and lower limb weakness. By Day 7, a cumulative mortality of 90% was observed. Following Day 7, no additional mortality was observed. The one mallard that survived to Day 8 recovered, and its appearance and behavior was normal throughout the remainder of the recovery period.

Both LC50s and LT50s were determined by probit analysis using the mortality data (Table 2). The dietary 8-day LC50 for PFOS was 603 ppm with a 95% confidence interval of 431 to 938 ppm. The slope of the concentration-response curve was 3.655. Body weight and food consumption data were then used to estimate average daily dose of PFOS and probit analyses was conducted with the mortality data. The 8-day LD50 based on average daily intake (ADI) was 150 mg PFOS/kg body weight/day with a 95% confidence interval of 117 to 201 mg PFOS/kg body weight/day. The slope of the dose-response curve was 5.479. The least LT50, estimated from the 1125 ppm dose group data, was 4.97 days. This result agreed with cumulative mortality data where 20% mortality was observed at day 4 and 90 % mortality was observed at day 7.

Table 2. Estimates of lethal concentration (LC_{50}) and lethal time (LT_{50}) of a population of juvenile mallards in a dietary acute toxicity study with PFOS. [A]

Effect metric	**Day**	**LC50 (ppm)**	**95% CI**	**Slope**
LC50	5	1002	743-2039	5.244
	6	799	595-1126	5.832
	8	603	431-938	3.655
	Nominal Dose	**LT50 (days)**	**95% CI**	**Slope**
LT50	281 ppm	9.23	NA	12.39
	562 ppm	9.18	7.38-55.5	5.433
	1125 ppm	4.97	4.32-5.55	8.403

[A] All statistical measures estimated by Probit analysis (SAS/STAT:PROC PROBIT)
NA indicates not applicable. No confidence interval was calculated.

Body Weight: Treatment-related effects on body weight and body weight gain were observed throughout the study (Table 3). Statistically significant treatment-related reductions in body weight were observed in birds exposed to PFOS at 281 mg/kg (-42% of control) or greater concentrations starting at Day 5 and continuing on through Day 15. On Day 22, body weight was also significantly reduced in the 146 ppm and higher treatment levels. While there was a statistically significant reduction in body weight in mallards from the 8.7 ppm treatment for Days 5 and 8, these effects were not considered treatment related. This conclusion was based on the fact that the mallards in the 8.7 ppm dose group weighed less than the controls and other treatment groups and the differences observed at this dose may have been due to this initial difference and not to PFOS exposure. In addition, there were no dose-related reductions in body weight in mallards from the 17.6, 35.1 and 70.3 ppm groups that would be consistent with a dose-response relationship.

Table 3. Average body weight (g) of juvenile mallards exposed to various dietary concentrations of PFOS.

Treatment (ppm)	Body Weight Day 0 [A]	Body Weight Day 5	Body Weight Day 8	Body Weight Day 15	Body Weight Day 22
Control	135 ± 24	279 ± 42	410 ± 30	640 ± 56	823 ± 49
8.7	119 ± 14	226* ± 36	317* ± 66	575 ± 32	773 ± 38
17.6	146 ± 14	277 ± 26	377 ± 32	625 ± 42	811 ± 81
35.1	147 ± 16	275 ± 32	375 ± 37	620 ± 44	828 ± 73
70.3	143 ± 30	260 ± 55	343 ± 90	579 ± 97	782 ± 135
141	143 ± 19	242 ± 37	331 ± 48	564 ± 56	688* ± 86
281	129 ± 17	161* ± 28	220* ± 55	467* ± 51	701* ± 41
562	144 ± 23	137* ± 27	175* ± 34	394* ± 82	613* ± 91
1125	147 ± 25	112* ± 28	185[B]	373[B]	634[B]

[A] Body weight is reported as means and standard deviations on a wet weight basis.
[B] Since n = 1, data could not be evaluated statistically with Dunnett's t-test.
* Statistically different from control group at $p < 0.05$ (Dunnett's t-test).

Treatment-related effects on body weight gain were noted at lesser concentrations than that observed for mortality. By Day 5 (the end of the exposure phase) treatment related effects on the change in body weight from Day 0 were noted in treatment groups equal to or greater than the 70.3 ppm dose. This trend continued into the observation period in that by Day 8, there was a statistically significant difference in the rate of body weight gain ($p < 0.05$) in mallards from the 70.3 (-12%), 141 (-17%), 281 (-60%) and 562 (-87%) ppm treatment groups when compared to control mallards. However, while body weight remained significantly reduced at treatment concentrations greater than or equal to 70.3 ppm, recovery in terms of weight gain was apparent in mallards that survived the exposure period. By study termination (Day 22), there were no significant or treatment-related effects noted on the total change of body weight (Day 8-15, Day 15-22 or Day 8-22) for any of the treatment groups.

To examine the impact of PFOS on growth of juvenile mallards, the growth rates for each treatment group were calculated for various time spans within the study (0-8 days, 8-22 days, and 0-22 days) (Table 4). (Data from the 562 and 1125 ppm treatments were not included due to greater than 25% cumulative mortality prior to day 8). Growth rate (1/day) was estimated with a general growth model (Equation 2) that was linearized and then fitted to the body weight data collected during the study.

$$W_A = W_0 \exp^{gt} \qquad \text{(Equation 2)}$$

Where: W_A is the body weight (g) of a mallard at time (t) of the study
W_0 is the body weight (g) of a mallard at the onset of the study (t=0)
g is the overall growth rate (1/day)
t it time (days)

The regression results of the growth rate data are in agreement with the body weight data. There was a marked reduction in the growth rate for the 281 ppm treatment as compared to the control group Day 0-8. The overall growth rate for mallards in the 17.6,

35.1, 70.3, and 141 ppm treatments did not differ from the growth rate of mallards in the control group.

Table 4. Growth rates (1/day) for juvenile mallards exposed to PFOS via the diet for 5 days and observed for 17 days post-exposure. [A]

	Treatment group (ppm PFOS)						
Days	0	8.7	17.6	35.1	70.3	141	281
0 to 8	0.125	0.123	0.120	0.118	0.110	0.105	0.065
8 to 22	0.059	0.064	0.055	0.056	0.059	0.052	0.083
0 to 22	0.080	0.085	0.076	0.077	0.076	0.072	0.083

[A] Growth rates estimated by linear regression: (Ln Body weight (g)= growth rate (time) + y-intercept).

Feed Consumption: When compared to controls, there was a reduction in feed consumption during the exposure period in the treatment groups equal to and greater than the 281 ppm treatment (Table 5). Furthermore, the reduction in feed consumption at these concentrations continued to be observed through Day 15. However, by Day 22, feed consumption rates returned to control levels indicating that the effect of PFOS was not permanent and that the mallards in the remaining treatment groups had recovered.

Table 5. Average feed consumption (FC) of juvenile mallards exposed to various dietary concentrations of PFOS [A]

Treatment (ppm)	FC 0-5 days	FC 6-8 days	FC 8-22 days
Control	0.335	0.329	0.243
8.7	0.311	0.352	0.278
17.6	0.326	0.349	0.275
35.1	0.342	0.332	0.241
70.3	0.290	0.287	0.238
141	0.432	0.481	0.260
281	0.264	0.279	0.211
562	0.265	0.314	0.261
1125	0.204	0.141	0.260

[A] Average feed consumption measured as g feed/g body weight/day on a pen basis.

Gross Necropsy: All mallards that died during the course of the study, all of the surviving mallards of half the pens at day 8, and all remaining mallards at Day 22 were subjected to a gross necropsy. For mallards found dead during the study, the necropsy findings included thin condition, loss of muscle mass, altered spleen color, empty crops and empty gastrointestinal tracts (Table 6). These finding were considered to be treatment related. Necropsy results for most mallards that survived to Day 8 and Day 22 were unremarkable as compared to control results.

Table 6. Number of gross pathological anomalies in mallards that died in response to dietary PFOS exposure during the study period.

	Nominal Treatment Dose (ppm)		
Finding	281 (N=2)	562 (N=3)	1125 (N=9)
Crop, empty	0	2	7
Emaciated	1	1	4
G.I. tract empty	1	1	1
Gizzard contents bile stained	0	2	4
Gizzard, empty	0	0	1
Intestinal contents black and tar-like	0	0	1
Keel, prominent	0	0	2
Kidneys, pale	0	0	1
Loss of muscle mass	2	2	5
Spleen, gray	0	0	1
Spleen, small and pale	1	1	2
Spleen, pale	0	2	3
Thin	1	1	4
Not Remarkable			

Concentrations of PFOS in Tissues: Liver concentration data are presented separately for mallards that died during the study and for those sacrificed at study termination. Serum data were also collected from mallards at study termination. Liver samples collected from mallards that died during the first seven days of the study were analyzed for PFOS content, and the data are presented in Table 7. During the first seven days of the study, mallard mortality was associated with PFOS concentrations in livers that exceeded 115 µg/g. This concentration was attained in the liver by Day 3, Day 5 and Day 7 in the 1125 ppm, 562 ppm and 281 ppm treatments, respectively. Since mallards were not sampled in a systematic manner, a pharmacokinetic analysis of the data could not be conducted to evaluate the uptake and elimination kinetics of PFOS in these mallards.

Table 7. Average concentrations of PFOS (µg/g) in the liver of select mallards with treatment-related mortality during the acute dietary study. [A]

	Nominal PFOS Dose (ppm)		
Day	281	562	1125
4	No mortality	No mortality	119 (N=2)
5	No mortality	216 (N=1)	160 (N=4)
6	No mortality	197 (N=1)	126 (N=2)
7	148 (N=1)	180 (N=1)	178 (N=1)

[A] All liver PFOS concentrations reported in units of µg/g,, wet weight. The data represent an average for all mallards from a dose that died on the specified day. Number in parenthesis is the number of samples analyzed, not the number of birds that were subjected to analysis.

Samples of liver and serum collected from surviving mallards on Days 8 and 22 indicate that PFOS was retained in the exposed mallards following the five day exposure period (Table 8).

Table 8. Measured concentrations of PFOS in the liver and serum collected from mallards on Days 8 and 22 of the study[A]

	Day 8		Day 22	
Treatment (ppm)	Liver (µg/g)	Serum (µg/ml)	Liver (µg/g)	Serum (µg/ml)
Control	<LOQ [B]	<LOQ	<LOQ	<LOQ
8.7	4.67 ± 1.50	7.38 ± 1.96	1.03 ± 0.32	1.49 ± 0.70
17.6	6.65 ± 2.90	13.8 ± 6.86	2.04 ± 0.42	4.32 ± 0.52
35.1	15.3 ± 3.78	30.5 ± 9.75	3.36 ± 1.35	6.82 ± 1.99
70.3	29.7 ± 14.8	48.1 ± 8.19	9.21 ± 1.27	11.3 ± 7.49
141	38.8 ± 10.9	53.9 ± 18.8	12.7 ± 4.86	18.9 ± 7.94
281	55.6 ± 4.61	65.8 ± 6.29	9.20 ± 3.21	13.4 ± 5.51
562	59.5 ± 23.5	107 ± 46.5	20.2 ± 7.81	34.0 ± 15.4

[A] All concentrations reported as means and standard deviations on a wet weight basis.
[B] Samples were less than the limit of quantitation. LOQ= 0.005 µg/ml (serum), LOQ = 0.03 µg/g (liver)

The average ratio of concentrations of PFOS for all dose groups (control not included) in the blood serum to that in the liver (S:L) for all mallards was 1.73 on Day 8 and 1.64 on Day 22. There were no significant differences in the S:L ratio between male and female mallards, therefore these ratios were not reported separately. The overall relationship between treatment dose and serum and liver concentrations of PFOS were not linear over the entire dose range, rather the relationships were logarithmic. The relationships between PFOS treatment dose and liver and serum PFOS concentrations measured on Day 8 are given:

$$\text{Liver PFOS (µg/g)} = 14.49*\text{Ln(Dose, mg/kg)} - 32.51 \qquad R^2 = 0.7967$$

$$\text{Serum PFOS (µg/ml)} = 21.39*\text{Ln(Dose, mg/kg)} - 45.49 \qquad R^2 = 0.7461$$

As with the S:L ratios, there were no sex specific differences in serum and liver concentrations on Day 8. The relationship between PFOS dose and tissue concentrations on Day 22 were not analyzed since growth and depuration may have significantly altered the relationships, and the information gained would not be applicable to environmental situations.

Post exposure, PFOS concentrations in liver decreased throughout the recovery period. This decrease was most likely due to two factors, growth dilution and excretion. Assuming the biological mechanism for elimination was the same at lower body burden levels, a simple one-compartment model was employed to estimate the loss of PFOS from liver due to depuration from the mallards.(Equation 3). The following model evaluates the rate of loss of PFOS from liver:

$$C_A = C_0 * \exp^{-k't} \qquad \text{(Equation 3)}$$

Where: C_A is the concentration at time (t) during elimination phase
C_0 is the concentration at the onset of elimination (µg/g)
k' is the overall elimination rate constant (day^{-1})
t is time (days)

The overall elimination rate constant (k') was estimated by determining the slope of the concentration in liver plotted as a function of time during the elimination phase of the study (Days 8-22). However, it is important to remember that the estimated elimination rate constant takes into account losses from two concurrent processes, loss due to growth dilution and excretion. In addition, these two processes may differ in importance relative to the elimination of PFOS from mallards based on age, sex and season. For this analysis, it was assumed that both processes acted in an independent but additive manner such that the overall elimination or loss could be represented as follows:

$$k' = k_2 + g$$

In this equation, g is the growth rate that was estimated from body weight measurements recorded during the study, while k' was estimated as the overall rate of decrease in measured liver PFOS concentrations. Once k' and g were calculated, the elimination rate was estimated (k_2). Based on the measured concentrations of PFOS in the liver and growth rates estimated from body weights measured between Days 8 to 22, elimination rate constants were estimated for the 8.7, 17.6, 35.1, 70.3, 141 and 281 ppm treatments (Table 9). Based on these results, the average elimination rate of PFOS (based on liver concentrations) was 0.0397 day^{-1} with a half-life in the liver estimated to be approximately 17.5 days.

Table 9. Growth rates, liver and serum elimination constants, and overall liver excretion rate constants for PFOS in juvenile mallards. [A]

Treatment (mg PFOS/kg)	Whole Body Growth Rate (day^{-1})	Liver		Serum
		k' (day^{-1})	k_2 (day^{-1})	k_2 (day^{-1})
Control	0.0586	NA	NA	NA
8.7	0.0636	0.1077	0.0441	0.1171
17.6	0.0547	0.0811	0.0264	0.0776
35.1	0.0565	0.1111	0.0546	0.1065
70.3	0.0590	0.0767	0.0177	0.1117
141	0.0523	0.0821	0.0298	0.0765
281	0.0828	0.1481	0.0653	0.1181

[A] All rate constants based on data derived from measurement made during the recovery period (Days 8 to 22).
NA not applicable

Concentrations of PFOS in the blood serum decreased in a similar manner as that observed for liver PFOS concentrations. For the post exposure period, Day 8 through Day 22, the average elimination rate constant (k_2) for PFOS from serum was 0.101 day^{-1} with an estimated half- life of 6.86 days. When compared to liver concentrations over the same experimental period, serum concentrations decreased at a greater rate. The half-life of PFOS in the serum was approximately 3-fold less than the estimated half-life in the liver. These results indicate that while PFOS is rapidly eliminated from serum, the losses from liver and other tissues are slower and are more likely to contribute to a significant body burden after exposures have ceased. In addition, it is assumed that the biological mechanism for elimination is the same at lower body burden levels. Since typically 20% or more of the initial body burden remained at day 22, it is indeterminate if the rate of elimination can be extrapolated to lower body burden levels.

Conclusions:

The dietary 8-day LC50 for PFOS was 603 ppm (CI = 431-938 ppm) while the 8-day LD50 based on ADI was 150 mg PFOS/kg body weight/day (CI=117-201 mg PFOS/kg body weight/day). The no mortality concentration was 141 ppm PFOS (or 61.3 mg PFOS/kg body weight/day) based on 5 days of exposure. Based on the reduction of body weight at Day 8, the No Observable Adverse Effect Concentration (NOAEC) was determined to be 35.1 ppm (or 12.0 mg PFOS/kg body weight/day) while the Lowest Observable Adverse Effect Concentration (LOAEC) was 70.3 ppm (or 20.4 mg PFOS/kg body weight/day). The 8-day No Observable Adverse Effect Level (NOAEL) and Lowest Observable Adverse Effect Level (LOAEL) values based on liver concentrations were 15.4 and 29.7 µg/g PFOS, respectively. The 8-day NOAEL and LOAEL values based on serum concentrations were 30.5 and 48.1 µg/ml PFOS, respectively. However, the NOAEL and LOAEL values based on either liver and serum concentrations are conservative estimates of threshold values. This is because these data were collected on Day 8, three days post-exposure and does not take into account potential elimination of PFOS between days 5 and 8. The NOAEL, LOAEL, and threshold doses (TD) (the geometric mean of NOAEL and LOAEL) are summarized (Table 10).

Table 10. NOAEL, LOAEL and threshold doses (TD) of PFOS derived from a mallard acute feeding study. [A]

Endpoint	NOAEL [B]	LOAEL [B]	TD
Dietary (ppm)	35.1	70.3	49.7
Average Daily Dose (mg/kg/day)	12.0	20.4	15.6
Serum (µg/ml)	30.5	48.1	38.3
Liver (µg/g)	15.4	29.7	21.4

[A] All values based on the reduction of body weight in treated mallards. Liver and serum PFOS concentrations measured from Day 8 samples.

[B] NOAEL and LOAEL values for ADI and diet are based on dietary concentrations. Serum and liver effect values are measured values.

DATA QUALITY:

Reliability: Klimish Ranking = 1

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