

Pharmacokinetics and Acute Lethality of Perfluorooctanesulfonate (PFOS) to Juvenile Mallard and Northern Bobwhite

John L. Newsted,¹ Susan A. Beach,² Sean P. Gallagher,³ John P. Giesy^{4,5}

¹ ENTRIX, Inc., 4295 Okemos Rd., Okemos, Michigan 48864, USA

² 3M Company, Environmental Laboratory, St. Paul, Minnesota 55144, USA

³ Wildlife International, Ltd, Easton, Maryland 21601, USA

⁴ Department of Zoology, National Food Safety and Toxicology Center, Center for Integrative Toxicology, Michigan State University, East Lansing, Michigan 48824, USA

⁵ Biology and Chemistry Department, City University of Hong Kong, Kowloon, Hong Kong, SAR, China

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Abstract. Ten-day-old mallards (*Anas platyrhynchos*) and northern bobwhite quail (*Colinus virginianus*) were fed perfluorooctanesulfonate (PFOS) in their diet for 5 days. The birds were then observed for 3 days while being given uncontaminated feed, and half of the birds were sacrificed on Day 8 of the trial. The remaining birds were maintained for an additional two weeks prior to being euthanized on Day 22 of the trial. Birds were assessed for growth, rate of feed consumption, behavior, physical injury, mortality, and gross abnormalities. Liver weight and concentrations of PFOS in blood serum and liver were also assessed. Based on the average daily intake (ADI) of PFOS calculated over the 5-day exposure period, the LD₅₀ for juvenile mallards was determined to be 150 mg PFOS/kg body weight (bw)/day, equivalent to a total cumulative dose of 750 mg PFOS/kg bw calculated over a 5-day period. For juvenile quail, the LD₅₀ based on the ADI was 61 mg PFOS/kg bw/day, equivalent to a total cumulative dose of 305 mg PFOS/kg bw. Reductions in weight gain and body weight were observed in quail from the 141 mg PFOS/kg treatment, but these measures returned to control levels by Day 22. The no-mortality dietary treatments were 70.3 and 141 mg PFOS/kg feed for quail and mallards, respectively. Both mallards and quail accumulated PFOS in blood serum and liver in a dose-dependent manner. The half-lives of PFOS in mallard blood serum and liver were estimated to be 6.86 and 17.5 days, respectively. In quail, the half-life of PFOS in liver was estimated to be 12.8 days, while the half-life of PFOS in quail blood serum could not be estimated. Concentrations of PFOS in juvenile mallard and quail liver associated with mortality are at least 50-fold greater than the single maximum PFOS concentration that has been measured in livers of avian wildlife.

Perfluorinated alkyl acids (PFAAs) are synthetic, fully fluorinated, straight chain, or branched fatty acid analogues with terminal sulfonate or carboxylate groups, which can result from degradation of materials containing amine, amide, or alcohol groups (Giesy and Kannan 2001; Kissa 2001). One PFAA of particular interest is perfluorooctanesulfonate (PFOS). PFOS is a fluorine-saturated, eight-carbon acid with a terminal sulfonate that has been found to be resistant to hydrolysis, photolysis, microbial degradation, and metabolism by animals (Giesy and Kannan 2002). PFOS has unique properties such that it is both oleophobic and hydrophobic, and, as a result, does not mix well with either water or oil. PFOS or its precursors have been used in numerous products and applications including stain-resistant coatings for fabrics and carpet, oil-resistant coatings for paper products, fire-fighting foams, mining and oil well surfactants, floor polishes, and insecticide formulations (Giesy and Kannan 2002). PFOS and related compounds that can degrade to PFOS have been released into the environment from product manufacturing processes, supply chains, product use, and disposal.

In global monitoring studies, PFOS and related chemicals have been observed in both human and wildlife populations (Giesy and Kannan 2001; Kannan *et al.* 2002a, 2004; Inoue *et al.* 2004; Olsen *et al.* 2003, 2004). In samples collected from remote oceanic locations, the blood sera of Laysan and Black-footed albatrosses contained 3–26 ng PFOS/ml (Kannan *et al.* 2001). PFOS concentrations in the blood of cormorants and herring gulls taken from the North American Great Lakes were approximately 10-fold greater than those found in albatrosses collected from Midway Atoll. In juvenile bald eagles from the Midwestern United States, blood plasma contained PFOS concentrations up to 2570 ng PFOS/ml, while detectable PFOS concentrations greater than 100 ng PFOS/ml have been found in other fish-eating birds, such as the common loon and brown pelican (Kannan *et al.* 2001). Overall, the results of these studies show that the presence of PFOS in wildlife is widespread. Concentrations in wildlife from more populated and

industrialized regions tend to be greater than those measured in wildlife from remote areas, but overall the concentrations tend to be low compared to other compounds such as polychlorinated biphenyls (PCBs), dioxins, or DDT. Predatory animals such as polar bears, mink, and eagles tend to contain greater concentrations of PFOS than other wildlife (Martin *et al.* 2004), although at generally low concentrations except in instances of potential localized sources.

The toxicity of PFOS to mammals has been investigated in rats, mice, rabbits, and monkeys (3M 2003; Case *et al.* 2001; Lau *et al.* 2003, 2004; Seacat *et al.* 2002, 2003; Thibodeaux *et al.* 2003). Exposure to PFOS has resulted in reduced body weight, hepatocellular hypertrophy, and a decrease in serum cholesterol and triglycerides in rats (Seacat *et al.* 2002, 2003). In an oral gavage two-generation study, the no observed adverse effect level (NOAEL) and lowest observed adverse effect level (LOAEL) based on alteration in body weight gain and feed consumption of adult rats were 0.1 and 0.4 mg PFOS/kg bw/day, respectively (Christian *et al.* 1999). The reproductive NOAEL based on pup viability and growth was 0.4 mg PFOS/kg bw/day, while exposure of dams to 1.6 mg PFOS/kg bw/day resulted in statistically significant pup mortality. In addition, exposure of pregnant rats and mice to PFOS resulted in significant physiologic alterations including maternal, developmental, and postnatal toxicities (Lau *et al.* 2003; Thibodeaux *et al.* 2003). While the mode of action of PFOS is still under investigation, it has been suggested to interfere with mitochondrial bioenergetics, gap junctional intercellular communication, and fatty acid protein binding in the liver (Berthiaume and Wallace 2002; Hu *et al.* 2002; Luebker *et al.* 2002; Sohlenius *et al.* 1993; Starkov and Wallace 2002; Shipley *et al.* 2004).

To conduct ecological risk assessments, information is needed on the effects of both acute exposures and chronic exposures in birds. We have evaluated the acute and chronic effects of PFOS on two avian species, the mallard (*Anas platyrhynchos*) and the northern bobwhite quail (*Colinus virginianus*). The chronic effects on survival, growth, reproduction, and changes in histology are reported elsewhere (Newsted *et al.* 2005). Here we report the short-term accumulation kinetics, disposition, and acute lethality of PFOS to quail and mallards. This information is necessary to assess the potential effects of short-term exposures. In addition, we have examined the rates of clearance and recovery so that a complete assessment of such acute exposures can be made.

Materials and Methods

Test Material

A production lot of potassium perfluorooctanesulfonate (PFOS) was obtained from 3M Company, Specialty Materials Manufacturing Division (St. Paul, MN). The white powder was identified as FC-95 (Lot No. 217). Purity was determined to be 86.9% by liquid chromatography/mass spectrometry and other elemental analysis techniques. All PFOS concentrations in the feed and standards were adjusted to reflect purity.

Quail and Ducks

Fertilized mallard and northern bobwhite quail eggs were obtained from Whistling Wings, Inc. (Hanover, IL) and Wildlife International Ltd. (Easton, MD), respectively. Individuals from each species were from the same hatch and were phenotypically indistinguishable from wild mallards or bobwhites. Once hatched, individuals from both species were acclimated to the holding facilities. Throughout the acclimation, exposure, and recovery phases of the study, birds were fed a game bird diet formulated to Wildlife International, Ltd. specifications (Gallagher *et al.* 2004a). Feed specifications included a minimum of 27% protein and 2.5% crude fat, a maximum of 5% crude fat, and a vitamin mix. To ensure the health of the birds, all housing and husbandry practices were conducted as established by the National Research Council (NRC 1996).

Following acclimation, 10-day-old bobwhite chicks and mallard ducklings were sorted by weight and randomly assigned from each weight class to either the control (30 birds) or treatment (10 birds/treatment) group. The groups were housed in brooding pens containing five birds each. Bobwhite quail chick pens (72 cm × 90 cm × 23 cm) were located in a thermostatically controlled room having a mean (± SD) daily temperature and relative humidity of 27.3°C (± 1.2°C) and 31% (± 14%), respectively. Mallard duckling pens (62 cm × 90 cm × 25.5 cm) were located in a room with mean (± SD) daily temperature and relative humidity of 25.2°C (± 0.7°C) and 53% (± 18%), respectively. Each group of birds was identifiable by pen number and test concentration and leg bands identified individual birds. A 16-h light and 8-h dark photoperiod was maintained for both acclimation and test phases. Both water and feed were provided *ad libitum* during acclimation and test phases.

Dietary Dosing

Each dose was prepared independently by mixing PFOS as a solid directly into the ration in a Hobart mixer (Model no. AS200T). Target nominal diet concentrations for mallards were 8.7, 17.6, 35.1, 70.3, 141, 281, 562, and 1125 mg PFOS/kg feed. For quail, nominal concentrations were 17.6, 35.1, 70.3, 141, 281, 562, and 1125 mg PFOS/kg feed. To evaluate homogeneity, stability, and verification of nominal concentration, dose preparations were extracted using a methanol extraction method and analyzed by high-pressure liquid chromatography/mass spectrometry (HPLC/MS) (Gallagher *et al.* 2004a, b). The limit of quantitation (LOQ) for these analyses was set at 1.15 parts per million (ppm, mg/kg feed). Dose preparations were found to be homogeneous, stable, and on average all dose mixtures for each dose group were found to be within 10% of the nominal dose (data not shown).

Analytical Monitoring

Analysis of PFOS concentrations in serum and liver was by HPLC-MS/MS according to published methods (Hansen *et al.* 2001). Quantitation of PFOS was based on comparisons of a single ion peak area to the response of a standard curve with midlevel calibration checks. Due to small amounts of sera and liver available for some birds, standard curves were prepared in methanol instead of the specific matrix. Additionally, when serum samples were too small, rabbit serum was used as a surrogate for matrix spikes. The results from these analyses were considered quantitative to ± 30% based on the precision and accuracy of the standard curves.

Procedures

Ten-day-old juvenile mallards and quail were fed the appropriate PFOS-treated or control feed for five days. Following the exposure period, all groups were given untreated basal diet for three days. On Day 8, one-half of the treatment and control birds were euthanized and tissue, liver, and blood samples were collected for analysis. The remaining birds were fed basal ration until Day 22 when the surviving birds were euthanized. Birds were observed daily for abnormal behavior, physical injury, or mortality. Body weights were measured at test initiation and on Days 5 and 8 for all birds, and the remaining birds were also weighed at Days 15 and 22. Feed consumption was measured as the difference in the amount of feed given to each pen at the start and end of each study phase. The amount of the feed wasted by the birds was not measured; therefore, the measured feed consumption values are presented as an estimate of total feed consumption. All birds that died during the test were subjected to gross necropsy. In addition, liver weight and tissue, as well as blood, were collected from birds euthanized on Days 8 and 22 and, when possible, from birds that died during the study.

Statistics

LC₅₀ and LD₅₀ (the concentration or dose that results in 50% mortality of a population for a given exposure time) and LT₅₀ (the exposure 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 1999). For LC₅₀s, values were based on PFOS feed concentrations (mg PFOS/kg feed), while LD₅₀s were based on ADI (mg PFOS/kg bw/d). The sample units for statistical analyses were the individual pens within each treatment group for feed consumption, while for body and liver weight, the sample unit was the individual bird. Body weight and feed consumption data were analyzed by Dunnett's test using TOXSTAT software (Gulley 1990). Average Daily Intake (ADI) of PFOS for each treatment group was estimated on a pen basis. Feed consumption and bird body weight data were averaged over the duration of the exposure period and the ADI was calculated as follows:

$$\text{ADI} = \frac{\text{Average Feed Consumption}}{\text{Average Body Weight}} \times \text{Feed Concentration} \quad (1)$$

where: ADI is in units of mg PFOS/kg body weight/day, feed consumption is in g feed/bird/day, body weight is in g/bird, and feed concentration is in mg PFOS/kg feed.

Results

Dietary PFOS Characterization

PFOS concentrations measured in feed samples collected during the study ranged from 92 to 119% of nominal values. Mean PFOS concentrations in the feed were 9.41, 18.7, 38.6, 71.5, 167, 279, 516, and 1148 mg PFOS/kg feed for nominal test concentrations of 8.7, 17.6, 35.1, 70.3, 141, 281, 562, and 1125 mg PFOS/kg feed, respectively. Analyses of the control feeds did not indicate the presence of PFOS or other co-eluting compounds. For mallards, the estimated ADI values associated with the nominal exposures were 2.71, 5.74, 12.0, 61.3, 74.2,

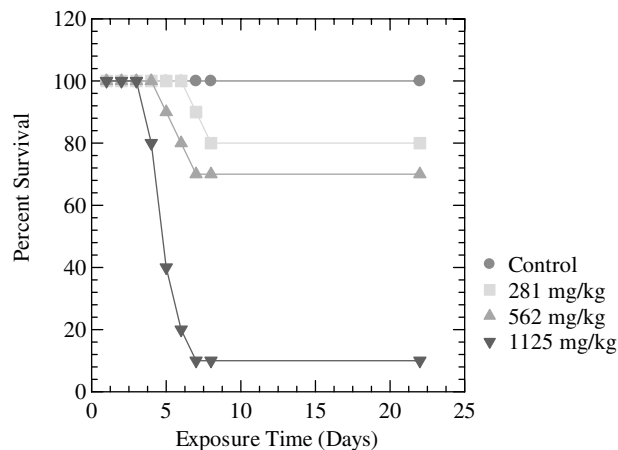


Fig. 1. Survival of juvenile mallards exposed to the three greatest doses of PFOS in the diet

149, and 229 mg PFOS/kg bw/day. For quail, estimated ADI values associated with the nominal exposures were 4.83, 8.52, 23.8, 44.7, 76.4, 193, and 225 mg PFOS/kg bw/day.

Toxicity to Mallard

In the acute dietary study, treatment-related mortalities were observed in juvenile mallards from the 281, 562, and 1125 mg PFOS/kg feed treatments starting at Days 7, 5, and 4 of dosing, respectively (Figure 1). Overt signs of toxicity in mallards from the 281, 562, and 1125 mg PFOS/kg feed treatment were observed starting at Days 4, 3, and 2 of dosing, respectively. Signs of toxicity included ruffled appearance, reduced reaction to stimuli, lethargy, loss of coordination, prostrate posture, convulsions, and lower limb weakness. After Day 8, no further mortality was observed in the study. Mortality and signs of toxicity were not observed at doses of 141 mg PFOS/kg feed or below at any phase in the study.

LC₅₀ and LT₅₀ values were a function of cumulative dose (concentration and duration). The dietary, 8-day LC₅₀ was 603 mg PFOS/kg feed with a 95% confidence interval of 431–938 mg PFOS/kg (Table 1). The 8-day LD₅₀ based on average daily intake (ADI) was 150 mg PFOS/kg bw/day with a 95% confidence interval of 117–201 mg PFOS/kg bw/day. This represents a cumulative dose over five days of 750 mg PFOS/kg bw. The least LT₅₀, estimated from the 1125 mg PFOS/kg dietary group data, was 4.97 days. This result agreed with the data from the 1125 mg PFOS/kg dietary group where the cumulative mortality was 20% and 90% at Day 4 and Day 7, respectively.

PFOS treatment-related effects on body weight and body weight gain of juvenile mallards were observed throughout the study (Table 2). At Day 22, statistically significant reductions in total body weight ($p < 0.05$) were observed in birds exposed to dietary concentrations of 141 mg PFOS/kg feed or greater when compared to controls. On Day 5, statistically significant effects on body weight change were noted among mallards exposed to dietary concentrations of 70.3 mg PFOS/kg feed or greater ($p < 0.05$). However, by Day 22 no treatment-related effects on body weight gain were observed in mallards exposed to PFOS in the feed at any treatment level. When compared to controls, there was a statistically significant re-

Table 1. Estimates of lethal concentration (LC₅₀) and lethal time (LT₅₀) of a population of juvenile mallards in a dietary acute toxicity study with PFOS

Effect metric	Day	LC ₅₀ (ppm)	95% CI	Slope
LC ₅₀	5	1002	743–2039	5.244
	6	799	595–1126	5.832
	8	603	431–938	3.655
	Nominal dose	LT ₅₀ (d)	95% CI	Slope
LT ₅₀	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

All statistical measures estimated by Probit analysis (SAS/STAT:PROC PROBIT). na: not applicable. No confidence interval was calculated.

Table 2. Average body weight and body weight change (g) of juvenile mallards exposed to PFOS in the diet

Treatment (ppm)	Body weight (g) ^a					Body weight change (g)			
	Day 0	Day 5	Day 8	Day 15	Day 22	0–5	5–8	8–15	D 8–22
Control	135 ± 24	279 ± 42	380 ± 30	640 ± 56	823 ± 49	144 ± 22	101 ± 16	230 ± 47	413 ± 42
8.7	119 ± 14	226* ± 36	317* ± 66	575 ± 32	773 ± 38	108* ± 23	91 ± 33	230 ± 20	427 ± 34
17.6	146 ± 14	277 ± 26	377 ± 32	625 ± 42	811 ± 81	131 ± 18	100 ± 11	241 ± 22	427 ± 62
35.1	147 ± 16	275 ± 32	375 ± 37	620 ± 44	828 ± 73	128 ± 22	100 ± 12	243 ± 40	451 ± 71
70.3	143 ± 30	260 ± 55	343 ± 90	579 ± 97	782 ± 135	117 ± 33	82 ± 42	216 ± 21	418 ± 56
141	143 ± 19	242 ± 37	331 ± 48	564 ± 56	688* ± 86	100* ± 21	89 ± 16	232 ± 22	356 ± 106
281	129 ± 17	161* ± 28	220* ± 55	467* ± 51	701* ± 41	32* ± 33	57 ± 44	256 ± 35	490 ± 54
562	144 ± 23	137* ± 27	175* ± 34	394* ± 82	613* ± 91	–6* ± 26	36 ± 41	221 ± 37	439 ± 47
1125	147 ± 25	112* ± 28	185 ^b	373 ^b	634 ^b	–37* ± 13	31 ^b	198 ^b	449 ^b

^a Body weight and body weight change are reported as means and standard deviations. Body weight gain is reported as the difference between body weights measured on the indicated days and are based on individual bird measurements.

^b Since n = 1, data could not be evaluated statistically.

* Statistically different from control group at $p < 0.05$ (Dunnett's t -test).

duction in feed consumption in mallards from 281 mg/PFOS kg feed or greater dietary treatments through Day 8 of the study (Table 3). This reduction in feed consumption was observed through Day 15 but returned to approximately 80% of control levels by Day 22.

Necropsy results for mallards that survived to Days 8 and 22 were unremarkable when compared to control birds. However, for mallards that died during the study necropsy, findings included thin condition, loss of muscle mass, altered spleen color, empty crops, and empty gastrointestinal tracts. These findings were considered to be treatment-related.

Mallard Tissue PFOS Analyses

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 exposure period (Figure 2). The average ratio of PFOS concentrations in the blood serum to that in the liver was 1.7 on Day 8 and 1.6 on Day 22. There were no significant differences in the serum-to-liver ratio between male and female mallards. Liver samples collected from birds that died during the study indicated a potential relationship between PFOS concentrations and mortality (Table 4). In these samples, mallard mortality was associated with liver PFOS concentrations that

exceeded 119 µg PFOS/g wet weight (ww). In contrast, the Day 8 average liver concentration associated with the no mortality treatment (141 mg PFOS/kg feed) was 38.8 µg PFOS/g ww. However, because some birds were sampled on Day 8 (3 days after the end of dosing) and others were sampled when they died prior to Day 8, the comparison could be affected by time of sampling and possible elimination of PFOS during the nondosing period.

Postexposure, PFOS concentrations in liver and serum decreased throughout the recovery period. This decrease was most likely due to two factors, growth dilution and excretion (Wagner 1979). 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 2). The following model evaluates the rate of loss of PFOS from liver:

$$C_A = C_0 * \exp^{-k_2 t} \quad (2)$$

where: C_A is the concentration at time (t) during elimination phase, C_0 is the concentration at the onset of elimination (µg PFOS/g ww), k_2 is the overall elimination rate constant (day^{-1}), and t is time (day).

Using the measured liver or serum PFOS concentrations and growth rates estimated from postexposure body weights,

Table 3. Average feed consumption (g feed/bird/d) of juvenile mallards exposed to PFOS in the diet

Treatment (ppm)	Feed consumption (g feed/bird/day)			
	0–5 Days ^a	6–8 Days ^a	8–15 Days ^b	15–22 Days ^b
Control	92 ± 10	125 ± 17	171 ± 13	180 ± 20
8.7	73	117	172	198
17.6	91	132	186	204
35.1	94	125	165	179
70.3	77	101	148	173
141	105	159	159	164
281	36*	63*	109	132
562	36*	55*	114	143
1125	22*	25*	106	154

Feed consumption is reported as means and standard deviations on a pen basis. Measures are estimates due to unavoidable and variable wastage by the birds.

^a Number of pens in the control and treatments were 6 and 2, respectively.

^b Number of pens in control and treatments were 3 and 1, respectively.

* Statistically different from control group at $p < 0.05$ (Dunnett's *t*-test).

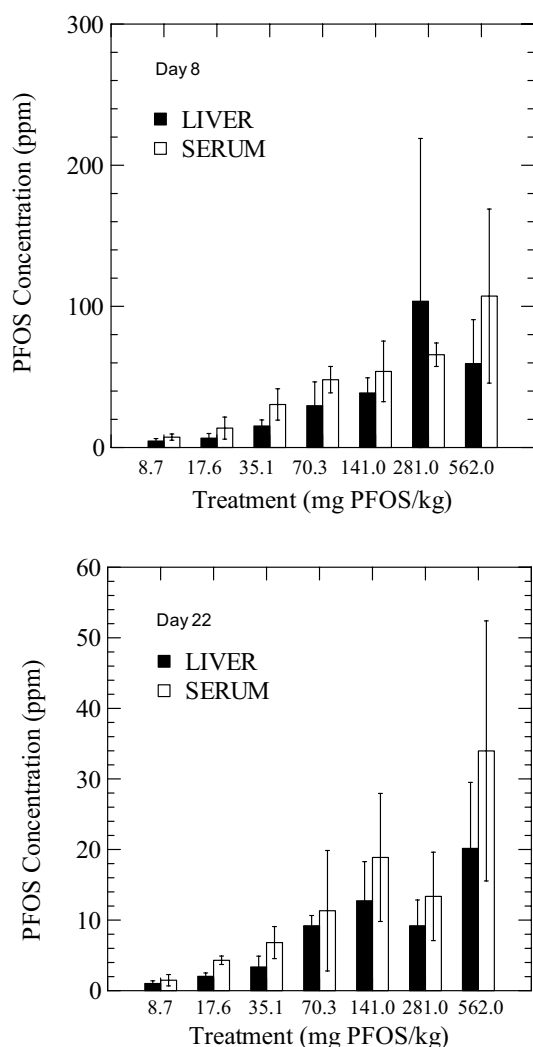


Fig. 2. Serum and liver PFOS concentrations collected from juvenile mallard on Day 8 and Day 22 (the recovery phase) of a dietary acute study

elimination rate constants were estimated for the 8.7, 17.6, 35.1, 70.3, and 141 mg PFOS/kg feed treatments. The average elimination rate constant (k_2) from liver was 0.0397 day^{-1} with an estimated half-life of approximately 17.5 days. Concentrations of PFOS in the blood serum decreased in a similar manner as that observed for liver PFOS concentrations. For these data, 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.

Toxicity to Quail

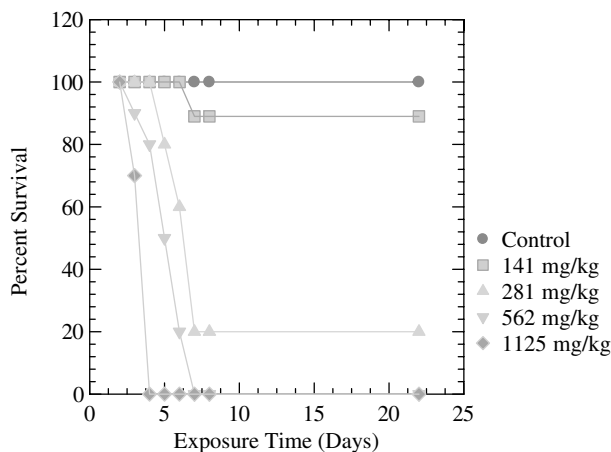
No treatment-related mortalities or overt signs of toxicity were observed in juvenile quail that were exposed to concentrations less than or equal to 70.3 mg PFOS/kg feed (Figure 3). In the 141 mg PFOS/kg treatment group, a single treatment-related mortality was noted at Day 7 while two other quail displayed clinical signs of toxicity (wing droop). In the 281, 562, or 1125 mg PFOS/kg feed treatments, mortality was first observed at Days 5, 3, and 3 of dosing, respectively. Signs of toxicity observed prior to death included ruffled appearance, reduced reaction to stimuli (sound and motion), lethargy, wing droop, loss of coordination, lower limb weakness, and convulsions. However, after Day 9 all surviving quail from the PFOS treatment groups had recovered and their appearance and behavior returned to normal. The dietary Day 8 LC_{50} was 212 mg PFOS/kg feed with a 95% confidence interval of 158–278 mg PFOS/kg (Table 5). The LD_{50} based on the ADI was 61 mg PFOS/kg bw/day with a 95% confidence interval of 48–77 mg PFOS/kg bw/day. This represents a cumulative dose over five days of 305 mg PFOS/kg bw. The LT_{50} that was estimated from the 1125 mg PFOS/kg dietary treatment data was 3.06 days. This result agreed with cumulative mortality data where 30% mortality was observed at Day 3 and 100% mortality was observed at Day 4 at this dose (Figure 3).

When compared to controls, there were no apparent PFOS-related effects on body weight for quail in the 17.6,

Table 4. Average concentrations of PFOS ($\mu\text{g/g}$ ww) in the liver of mallards with treatment-related mortality during the acute dietary study

Day	Nominal PFOS dose (ppm)		
	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)

All PFOS concentrations reported in units of $\mu\text{g/g}$ wet weight. The data represent an average for all mallards that died in a treatment group on a specified day. The number in parentheses is the number of samples analyzed and not the number of birds that were subjected to analysis. Some samples were composites.

**Fig. 3.** Survival of juvenile northern bobwhites exposed to the four greatest doses of PFOS in the diet

35.1, or 70.3 mg PFOS/kg feed treatments (Table 6). There was a statistically significant reduction in body weight in the 141, 281, and 562 mg PFOS/kg feed treatments during the exposure period when compared to controls. However, by Day 15 this reduction in body weight was no longer apparent in the 141 mg PFOS/kg feed treatment group. While body weight was slightly reduced at greater dietary concentrations, statistical analysis could not be conducted due to lack of replicates. Statistically significant treatment-related effects on body weight change were also observed during the exposure period at concentrations greater than 70.3 mg PFOS/kg feed. Again, treatment-related effects on body weight change were no longer apparent by Day 15. There were statistically significant reductions in feed consumption in quail exposed to 281 mg PFOS/kg feed or greater through Day 5 (Table 7). However, by Day 8 no PFOS-related effects on food consumption were observed in quail. By Day 22, feed consumption approximated control values in quail from the 141 mg PFOS/kg feed treatment while in the 281 mg PFOS/kg treatment feed consumption recovered to approximately 70% of the control levels.

Necropsy findings in quail that died during the study included thin condition, loss of muscle mass, altered spleen color, autolysis of tissues, and pale organs. These findings were considered to be PFOS treatment related. In birds sacrificed on Day 8, only a single bird from the 293 mg PFOS/kg feed treatment had treatment-related findings while the results for all other birds were unremarkable. Similarly,

necropsy findings were unremarkable for all birds sampled on Day 22.

Quail Tissue PFOS Analyses

Results from liver and serum samples collected at Days 8 and 22 showed that PFOS persisted in quail well past the exposure period (Figure 4). While liver PFOS concentrations decreased during the postexposure period, the serum PFOS concentration data either remained unchanged or increased over the recovery period. Based on the results of the PFOS concentrations, the average serum to liver PFOS concentration ratio was 0.515 and 2.42 for Days 8 and 22, respectively. The difference in the serum to liver PFOS ratio estimated from Day 8 and Day 22 data was due in part to the fact that while liver concentrations tended to decrease during the recovery period, serum concentration either remained constant or actually increased over the duration of the recovery period. No differences were observed between the serum to liver ratios measured in male and female quail at either day. Analysis of livers taken from quail that died during the study indicated that mortality was associated with liver PFOS concentrations greater than 111 μg PFOS/g ww (Table 8). In contrast, the liver concentration in the no mortality treatment at Day 8 averaged 44 μg PFOS/g ww. Again, due to differences in sampling time and the potential role of depuration, a direct comparison of liver PFOS concentrations in birds sampled during the exposure phase and recovery phase cannot be made with these data.

Based on the same analysis and models used for the mallard data, elimination rate constants were determined for liver concentrations in quail. The average elimination rate (k_2) of PFOS from liver was 0.0542 day^{-1} with the half-life in liver estimated to be approximately 12.8 days. However, the trend in serum PFOS concentrations in quail did not behave in a manner that was consistent with that observed for liver PFOS concentrations. For instance, during the recovery period the mean serum PFOS concentrations in quail from the 17.6 and 35.1 mg PFOS/kg feed treatments increased from less than LOQ to 5.89 μg PFOS/ml and from 3.04 to 7.27 μg PFOS/ml, respectively. In contrast, serum PFOS concentrations in quail from the 70.3 mg PFOS/kg dietary treatment decreased from 41.2 to 26.2 μg PFOS/ml over the same time period. Due to these inconsistencies and the relatively great variability in the measured serum PFOS concentrations (coefficient of variation (CV) greater than 100%) for all treatment groups, kinetic analyses were not conducted with the serum data.

Table 5. Estimates of the lethal concentration of PFOS (LC₅₀) and the lethal time (LT₅₀) of a population of juvenile northern bobwhite quails in a dietary toxicity study with PFOS

Effect metric	Time	LC ₅₀ (ppm)	95% CI	Slope
LC ₅₀	Day 3	1,593	na	3.174
	Day 5	482	356–672	4.722
	Day 6	319	228–448	3.923
	Day 8	212	158–278	7.036
	Dose	LT ₅₀ (d)	95% CI	Slope
LT ₅₀	281 ppm	6.41	5.69–7.61	9.54
	562 ppm	4.78	4.17–5.48	8.29
	1125 ppm	3.06	na	60.5

All statistical measures estimated by Probit analysis (SAS/STAT: PROC PROBIT).
na, not applicable. No confidence interval was calculated.

Table 6. Average body weight and body weight change (g) of juvenile northern bobwhites exposed to PFOS in the diet

Treatment (ppm)	Body weight (g) ^a					Body weight change (g) ^a			
	Day 0	Day 5	Day 8	Day 15	Day 22	0–5	5–8	8–15	8–22
Control	20 ± 1	30 ± 4	38 ± 5	59 ± 10	82 ± 13	10 ± 3	8 ± 2	23 ± 5	45 ± 8
17.6	21 ± 1	31 ± 4	40 ± 5	68 ± 8	87 ± 7	11 ± 3	9 ± 2	24 ± 3	47 ± 3
35.1	20 ± 1	31 ± 3	39 ± 3	65 ± 5	89 ± 7	11 ± 2	8 ± 1	26 ± 2	50 ± 4
70.3	20 ± 1	30 ± 2	37 ± 3	60 ± 4	79 ± 4	9 ± 1	7 ± 1	24 ± 2	44 ± 2
141	20 ± 1	27* ± 3	33* ± 3	58 ± 3	79 ± 2	7* ± 3	6* ± 2	24 ± 1	45 ± 1
281	20 ± 1	18* ± 2	18* ± 4	35 ^b	55 ^b	-2* ± 2	-2* ± 4	14 ^b	34 ^b
562	20 ± 1	16* ± 2	—	—	—	-4* ± 2	—	—	—
1125	20 ± 1	—	—	—	—	—	—	—	—

^a Body weight and body weight change are reported as means and standard deviations. Body weight change is reported as the difference in body weight between the indicated days and are based on individual bird measures. (—) = No data available due to mortality.

^b Since n = 1, data could not be evaluated statistically.

* Statistically different from control group at *p* < 0.05 (Dunnett’s *t*-test).

Table 7. Average feed consumption (g feed/bird/d) of juvenile quail exposed to PFOS in the diet

Treatment (ppm)	Feed consumption (g feed/bird/day)			
	0–5 Days ^a	6–8 Days ^a	8–15 Days ^b	15–22 Days ^b
Control	9 ± 2	10 ± 2	9 ± 2	13 ± 1
17.6	9	11	10	12
35.1	8	12	14	15
70.3	10	13	13	15
141	9	10	11	14
281	5*	9	8	9
562	6*	19	—	—
1125	4*	—	—	—

Feed consumption is reported as means and standard deviations on a pen basis. Measures are estimates due to unavoidable and variable wastage by the birds. (—) = No data available due to mortality.

^a Number of pens in the control and treatments were 6 and 2, respectively.

^b Number of pens in control and treatments were 3 and 1, respectively.

* Statistically different from control group at *p* < 0.05 (Dunnett’s *t*-test).

Discussion

Species-specific differences in toxicity were observed between juvenile mallards and bobwhite quail exposed to PFOS in the feed. The LC₅₀ for bobwhite quail was approximately 3 times less than that determined for mallards. In addition, the slope of

the dose-mortality curve for quail was approximately 2-fold greater than that determined for mallards (Tables 1 and 5). The lethality of PFOS toward quail occurred over a narrower concentration range when compared to mallards and clinical signs of toxicity were also observed earlier and were more pronounced at lesser concentrations in quail than in mallards at

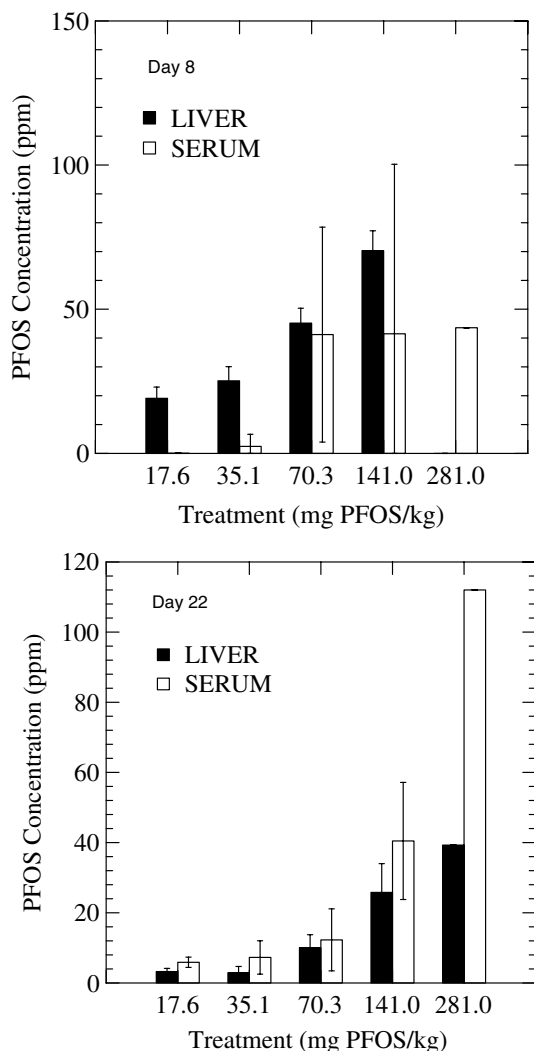


Fig. 4. Serum and liver PFOS concentrations collected from juvenile northern bobwhite on Day 8 and Day 22 of the recovery phase of a dietary acute study

equivalent treatment levels. Therefore, based on the acute effects, quail were slightly more sensitive to PFOS exposure than mallards. However, when comparing or extrapolating toxicity data between species, it is necessary to take into account differences in size and other physiological variables (Davidson *et al.* 1986). When the 8-day LC_{50} for mallards was adjusted for body weight using a physiological scaling model, the difference between the mallard (adjusted LC_{50} of 253 mg PFOS/kg feed) and quail (LC_{50} of 212 mg PFOS/kg feed) was not significant (Mineau *et al.* 1996). When reductions in body weight were evaluated, the NOAEL for mallards and bobwhite quail were 35.1 mg PFOS/kg feed (12 mg PFOS/kg bw/day) and 70.3 mg PFOS/kg feed (23.4 mg PFOS/kg bw/day), respectively. Similar responses were also observed when changes in body weight gain for both species were evaluated. Overall, the exposure threshold for PFOS-related effects in quail and mallard were within a factor of 2, which indicates that the differences in species-specific sensitivity to PFOS exposure were not great.

To date, no other laboratory studies have been published on the toxicity of PFOS to avian species. As a result, the only comparisons that can be made are to mammalian species exposed orally to PFOS. In an acute study with rats given a single oral dose, the LD_{50} and 95% confidence limits were 251 (199–318) mg PFOS/kg bw (Dean *et al.* 1978). In our study, the average 8-day dietary acute LD_{50} values for mallards and quail, respectively, were 150 and 61 mg/kg/d, or 750 and 305 mg PFOS/kg bw cumulative dose over five days. This narrow range seems to indicate an equivalent response between rodents and birds exposed to PFOS.

The total cumulative dose associated with no mortality was 180 mg PFOS/kg bw in a 90-day subchronic rat study (Goldenthal *et al.* 1978a), and 135 mg PFOS/kg bw in a 90-day study of rhesus monkeys (Goldenthal *et al.* 1978b). In a 6-month study in cynomolgus monkeys (Seacat *et al.* 2002), there was no mortality at 27 mg PFOS/kg bw, and mortality began to occur at a cumulative dose of 116 mg PFOS/kg bw (first of 2 mortalities out of 12 monkeys). In quail and mallards, the no mortality cumulative dose was calculated to be 119 mg PFOS/kg bw and 306 mg PFOS/kg bw, respectively. Likewise, the cumulative PFOS dose associated with effects on body weight gain and other signs of toxicity was 180 mg PFOS/kg bw for rats (Goldenthal *et al.* 1978a) while for quail and mallards it was 224 and 102 mg PFOS/kg bw, respectively. Thus, while there are differences in dosing interval between the rat and avian studies and differences in physiology and clearance rates of rodents and birds, there is a remarkable similarity in the total cumulated dose that is associated with adverse effects across species.

PFOS was accumulated in serum and liver of mallards and quail in a dose-dependent manner. The distribution of PFOS between serum and liver appeared to differ slightly between mallards and quails. However, given the relatively great variability observed in the quail serum (average CV = 88%) and liver (average CV = 25%) as determined at Days 8 and 22 of the study, these differences in mean values may not be meaningful. The species differences in measured serum and liver PFOS concentrations were not statistically significant.

The relationship between serum and liver PFOS concentrations in mallards differed from that observed in mammals. In rodents, PFOS concentrations in the liver were greater than in serum (Seacat *et al.* 2003; Thibodeaux *et al.* 2003), whereas in mallards, concentrations of PFOS were slightly greater in blood serum as compared to the liver. Reasons for these differences have not yet been investigated but may be related to several factors. One factor is that the accumulation of PFOS by tissues is not a lipid partitioning process but rather is based on protein binding (Jones *et al.* 2003). If differences in the protein profiles in liver and serum exist between species, the magnitude of PFOS accumulation into these tissues may be a reflection of these differences. In addition, the levels and duration of exposure may also influence the overall distribution of PFOS between liver and blood serum. In mice exposed to 10 mg PFOS/kg bw via daily gavage, serum concentrations appeared to reach saturation at 250 μ g PFOS/ml, while for liver saturation occurred at 560 μ g PFOS/g ww (Thibodeaux *et al.* 2003). Thus, the time to reach saturation may be dependent on the protein profile of target tissues and differences in the rate of accumulation by these tissues. Finally, the

Table 8. Measured concentrations of PFOS ($\mu\text{g/g}$) in the liver of quail with treatment-related mortality during the acute dietary study

Day	Nominal PFOS concentrations (ppm)			
	141	281	562	1125
3	No mortality	No mortality	Not analyzed	134 (N = 1)
4	No mortality	No mortality	Not analyzed	126 (N = 3)
5	No mortality	249 (N = 1)	126 (N = 1)	—
6	No mortality	202 (N = 1)	235 (N = 1)	—
7	111 (N = 1)	139 (N = 2)	111 (N = 1)	—

All PFOS concentrations reported in units of $\mu\text{g/g}$ wet weight. The data represent an average for all quail that died in a treatment group on a specified day. The number in parentheses is the number of samples analyzed and not the number of birds that were subjected to analysis. Some samples were composites.

role of enterohepatic circulation of PFOS, known to occur in mammals (Johnson *et al.* 1984), has not been characterized in birds.

Unlike birds, the elimination of PFOS from mammals appears to be a slow process, likely in part due to enterohepatic circulation (Johnson *et al.* 1984; Lau *et al.* 2004). The elimination half-life of PFOS has been estimated to be greater than 90 days for male rats and approximately 100–200 days for male and female cynomolgus monkeys (Seacat *et al.* 2002; 2003; Noker and Gorman 2003). In contrast, the estimated half-life of PFOS in mallard and quail livers was 17.5 and 12.8 days, respectively, while the half-life in serum was estimated to be 6.86 days in mallards. Thus, the overall accumulation of PFOS over the lifetime or reproductive phase of a bird may not be as great as that predicted based on mammalian kinetic values. Therefore, the potential or risk for accumulation to toxic levels of PFOS in birds may not be as great as that predicted from mammalian data.

Concentrations of PFOS have been measured in livers of water birds from various locations across the globe. In Japan, liver concentrations ranged from less than 19 to 650 ng PFOS/g ww with the greatest concentrations being measured in black-headed gulls (mean 294 ng PFOS/g ww). In Korea, liver concentrations ranged from less than 10 to 500 ng PFOS/g ww with the greatest concentrations being measured in common cormorants (mean 387 ng/g ww) (Kannan *et al.* 2002a). Birds from various locations in the United States had PFOS concentrations in their livers ranging from 8.6 to 1780 ng/g with an average concentration of 242 ng/g ww (Kannan *et al.* 2001). In cormorants from the Italian coast of the Mediterranean Sea, liver concentrations averaged 61 ng PFOS/g ww (range of 32 to 150 ng PFOS/g). Liver concentrations in white-tailed sea eagles from the Baltic Sea averaged 38 ng/g ww (range of less than 3.9 to 127 ng/g ww) (Kannan *et al.* 2002b). In the present study, concentrations of PFOS in the livers of mallards and quail associated with mortality (Tables 4, 8; 111 or 119 $\mu\text{g/g}$ ww) were more than 50-fold greater than the greatest PFOS concentration measured in avian wildlife (1.8 $\mu\text{g/g}$ ww in bald eagles). Thus, the environmental concentrations of PFOS measured in birds are at levels that are less than the threshold for PFOS-related effects on avian mortality following acute exposure.

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References

- Berthiaume J, Wallace KB (2002) Perfluorooctonate, perfluorooctane sulfonate, and N-ethyl perfluorooctanesulfonamido ethanol, peroxisome proliferation and mitochondrial biogenesis. *Toxicol Lett* 129:23–32
- Case MT, York RG, Christian MS (2001) Rat and rabbit oral developmental toxicology study with two perfluorinated compounds. *Int J Toxicol* 20:101–109
- Christian MS, Hyberman AM, York RG (1999) Combined oral (gavage) fertility, developmental and perinatal/postnatal reproduction toxicity of PFOS in rats. Argus Res. Laboratory, Inc., Horsham, PA. Available at USEPA Docket 8EHQ-0200-00374
- Davidson IWF, Parker JC, Beliles RP (1986) Biological basis for extrapolation across mammalian species. *Reg Toxicol Pharmacol* 6:211–237
- Dean WP, Jessup DC, Thompson G, Romig G, Powell D (1978) Fluorad fluorochemical surfactant FC-95 acute oral (LD_{50}) study in rats. Study No. 137-083, IRDC. Available at USEPA Docket 8EHQ-0200-00374
- Gallagher SP, Casey CS, Beavers J.B, Van Hoven RL (2004a) PFOS: A dietary LC50 study with the mallard. Amended Report, Wildlife International Ltd., Project No. 454-102. Available at USEPA Docket AR-226-1735
- Gallagher SP, Casey CS, Beavers JB, Van Hoven RL (2004b) PFOS: A dietary LC50 study with the Northern Bobwhite. Amended report, Wildlife International Ltd., Project No. 454-103. Available at USEPA Docket AR-226-1825
- Giesy JP, Kannan K (2001) Global distribution of perfluorooctane sulfonate in wildlife. *Environ Sci Technol* 35:1339–1342
- Giesy JP, Kannan K (2002) Perfluorochemical surfactants in the environment. *Environ Sci Technol* 36:146A–152A
- Goldenthal EI, Jessup DC, Geil RG, Jefferson ND, Areco RJ, Ruecker FA (1978a) 90-day subacute rat study. Study No. 137-085, International Research and Development Corp., Mattawan, MI. Available at USEPA Docket AR-226-0139
- Goldenthal EI, Jessup DC, Geil RG, Mehring JS (1978b) Ninety-day subacute Rhesus monkey toxicity study. Study No. 137-085, International Research and Development Corp., Mattawan, MI. Available at USEPA Docket AR-226-0137
- Gulley DD (1990) TOXSTAT Release 3.2. The University of Wyoming
- Hansen KJ, Clemen LA, Ellefsen ME, Johnson HO (2001) Compound-specific, quantitative characterization of organic fluorochemicals in biological matrices. *Environ Sci Technol* 35:766–770
- Hu W, Jones PD, Upham BC, Trosko JE, Lau C, Giesy JP (2002) Inhibition of gap junctional intercellular communication by perfluorinated compounds in rat liver and dolphin kidney epithelial cell lines in vitro and Sprague-Dawley rats in vivo. *Toxicol Sci* 68:429–436

- Inoue K, Okada F, Ito R, Kato S, Sasaki S, Nakajima S, Uno A, Saijo Y, Sato F, Yoshimura Y, Kishi R, Nakazawa H (2004) Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: assessment of PFOS exposure in a susceptible population during pregnancy. *Environ Health Perspect* 112:1204–1207
- Johnson JD, Gibson SJ, Ober RE (1984) Cholestyramine-enhanced fecal elimination of carbon-14 in rats after administration of ammonium [¹⁴C]perfluorooctanoate or potassium [¹⁴C] perfluorooctane sulfonate. *Fund Appl Toxicol* 4:972–976
- Jones PD, Hu W, DeCoen W, Newsted J, Giesy JP (2003) Binding of perfluorinated fatty acids to serum protein. *Environ Toxicol Chem* 22:2639–2649
- Kannan K, Franson JC, Bowerman WW, Hansen KJ, Jones PD, Giesy JP (2001) Perfluorooctane sulfonate in fish eating water birds including bald eagles and albatrosses. *Environ Sci Technol* 35:3065–3070
- Kannan K, Choi J, Iseki N, Senthilkumar K, Kim DH, Masunaga S, Giesy JP (2002a) Concentrations of perfluorinated acids in livers of birds from Japan and Korea. *Chemosphere* 49:225–231
- Kannan K, Corsolini S, Falandysz J, Oehme G, Focardi S, Giesy JP (2002b) Perfluorooctane sulfonate and related fluorinated hydrocarbons in marine mammals, fishes, and birds from coasts of the Baltic and the Mediterranean Seas. *Environ Sci Technol* 36:3210–3216
- Kannan K, Corsolini S, Falandysz J, Fillmann G, Kumar KS, Loganathan BG (2004) Perfluorooctane sulfonate and related fluorochemicals in human blood from several countries. *Environ Sci Technol* 38:4489–4495
- Kissa E (2001) Fluorinated surfactants and repellents, 2nd ed. Marcel Dekker, New York
- Lau C, Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Stanton ME, Butenhoff JL, Stevenson LA (2003) Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II. Post-natal evaluation. *Toxicol Sci* 74:382–392
- Lau C, Butenhoff JL, Rogers JM (2004) The developmental toxicity of perfluoroalkyl acids and their derivatives. *Toxicol Appl Pharmacol* 15:231–241
- Luebker DJ, Hansen KJ, Bass NM, Butenhoff JL, Seacat AM (2002) Interactions of fluorochemicals with rat liver fatty acid-binding protein. *Toxicology* 176:175–185
- Martin JW, Smithwick MM, Braune BM, Hoekstra PE, Muir DCG, Mabury SA (2004) Identification of long-chain perfluorinated acids in biota from the Canadian Arctic. *Environ Sci Technol* 38:373–380
- Mineau P, Collins BT, Baril A (1996) On the use of scaling factors to improve interspecies extrapolation of acute toxicity in birds. *Reg Toxicol Pharmacol* 24:24–29
- National Research Council (1996) Guide for care and use of laboratory animals. National Academy Press, Washington DC, 125 pp
- Newsted JL, Coady KC, Beach SA, Butenhoff JL, Gallagher S, Geisy JP (2005) Effects of perfluorooctanesulfonate on mallard (*Anas platyrhynchos*) and Northern bobwhite quail (*Colinus virginianus*) when chronically exposed via the diet. *Environ Toxicol Pharmacol* (in press)
- Noker PE, Gorman GS (2003) A pharmacokinetic study of potassium perfluorooctane sulfonate in the cynomolgus monkey. Southern Research Institute, Research Triangle Park, NC, Unpublished report. Available on USEPA Docket AR-226-1228
- Olsen GW, Hansen KJ, Stevenson LA, Burris JM, Mandel JH (2003) Human donor liver and serum concentrations of perfluorooctane sulfonate and other perfluorochemicals. *Environ Sci Technol* 37:888–891
- Olsen GW, Church TR, Larson EB, van Belle G, Lundberg JK, Hansen KJ, Burris JM, Mandel JH, Zobel LR (2004) Serum concentrations of perfluorooctane sulfonate and other fluorochemicals in an elderly population from Seattle, Washington. *Chemosphere* 54:1599–15611
- SAS Institute (1999) SASSTAT User's Guide, Release 8.02 Edition, SAS Institute, Cary, NC
- Seacat AM, Thomford PJ, Hansen KJ, Olsen GW, Case MT, Butenhoff JL (2002) Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys. *Toxicol Sci* 68:249–264
- Seacat AM, Thomford PJ, Hansen KJ, Clemen LA, Eldridge SR, Elcombe CR, Butenhoff JL (2003) Sub-chronic dietary toxicity of potassium perfluorooctane sulfonate in rats. *Toxicology* 183:117–131
- Shibley JM, Hurst CH, Tanaka SS, DeRoos FL, Butenhoff JL, Seacat AM, Waxman DJ (2004) Trans-activation of PPAR α and induction of PPAR α target genes by perfluorooctane-based chemicals. *Toxicol Sci* 80:151–160
- Sohlenius AK, Andersson K, DePierre JW (1993) Perfluorooctane sulfonic acid is a potent inducer of peroxisomal fatty acid β -oxidation and other activities known to be affected by peroxisome proliferators in mouse liver. *Pharmacol Toxicol* 72:90–93
- Starkov AA, Wallace KB (2002) Structural determinants of fluorochemical-induced mitochondrial dysfunction. *Toxicol Sci* 66:244–252
- Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Barbee BD, Richards JH, Butenhoff JL, Stevenson LA, Lau C (2003) Exposure to perfluorooctanesulfonate during pregnancy in rat and mouse. I: maternal and prenatal evaluations. *Reprod Dev Toxicol* 74:369–381
- 3M (2003) Environmental and health assessment of perfluorooctane sulfonate and its salts. Available on USEPA Docket AR-226-1486
- Wagner JG (1979) Fundamentals of clinical pharmacokinetics. Drug Intelligence Publications Inc, Hamilton, IL, 461