Environ. Sci. Technol. **2005**, *39,* 9357−9362

Avian Toxicity Reference Values for Perfluorooctane Sulfonate

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Toxicity reference values (TRVs) and predicted no effect concentrations (PNECs) were derived for perfluorooctane sulfonate (PFOS) based on the characteristics of a top avian predator. On the basis of the protective assumptions used in this assessment, the benchmarks are protective of avian populations and were based on acute and chronic dietary exposures of northern bobwhite quail and mallard. Toxicological endpoints included mortality, growth, feed consumption, and histopathology. Reproductive endpoints included egg production, fertility, hatchability and survival, and growth of offspring. On the basis of the U. S. Environmental Protection Agency Great Lakes Initiative methodology, and a lowest observable adverse effect concentration (LOAEC) of 10 mg PFOS kg^{-1} feed, an uncertainty factor of 36 was derived. The TRV based on PFOS dietary intake was 0.021 mg PFOS kg^{-1} body weight day^{-1}, while for serum, liver, and egg, TRVs were 1.7 μg PFOS mL^{-1}, 0.6 μg PFOS g^{-1} wet weight, and 1.7 μg PFOS mL^{-1}, respectively. On the basis of the European Commission methodology, a correction factor of 2 (for lowest observed effect level to no observable effect level) and an assessment factor of 30, for a total adjustment of 60, were used to derive PNECs. PNECs based on dietary, mean serum, liver, and egg PFOS concentrations were 0.013 mg PFOS kg^{-1} body weight day^{-1}, 1.0 μg PFOS mL^{-1}, 0.35μg PFOS g^{-1} wet weight, and 1.0 μg PFOS mL^{-1}, respectively.

Introduction

Perfluorinated compounds (PFCs) have been manufactured for over 50 years and, due to their unique properties of repelling both water and oil, have been used as surfactants and surface protectors in carpets, leather, paper, food containers, fabric, and upholstery. They have also been used as application performance chemicals in products such as fire-fighting foams, floor polishes, and shampoos (*1*). Since the 1970s, there has been a steady increase in the use of PFCs for a variety of industrial applications, but due to the lack of suitable analytical methods and authentic standards, it is only recently that these compounds have been found to be widespread in the environment (*2*, *3*).

Perfluorooctane sulfonate (PFOS) is the predominant PFC found in the tissues of wildlife that have been studied and is considered to be the terminal degradation product of many of the commercially used sulfonated perfluorinated products (*1*, *3*). PFOS is found in many environmental media even in regions such as the Arctic, Antarctic, and remote oceanic environments that are far from sources. PFC residues have been found in marine mammals (*5*, *6*), oysters (*7*), mink and otters (*2*, *8*), and birds even in very remote areas of the open ocean (*4*, *9*, *10*) and the Arctic (*2*, *4*). In Europe, PFOS has been measured in ringed and gray seals from the Baltic Sea (*2*), polar bears in the Arctic (*12*−*13*), and birds from the Mediterranean Sea (*2*, *9*).

Various studies have demonstrated the toxicity of PFOS in rats, mice, rabbits, and monkeys (*13*−*19*). 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 (*20*−*25*).

While there has been extensive testing of the effects of PFOS on mammals, there have been few studies conducted with wildlife, and only recently has information been published for birds (*26*, *27*). In this report, the results from acute and chronic toxicity tests with birds have been used to estimate toxicity reference values (TRVs) and predicted no effect concentrations (PNECs). These TRVs and PNECs are based on threshold concentrations of PFOS in the diet, average daily consumption of PFOS, and tissue PFOS concentrations that would be associated with the no observable adverse effect level (NOAEL) and the lowest observable adverse effect level (LOAEL).

Materials and Methods

Review of Avian Toxicity Studies with PFOS. Dietary acute and chronic laboratory studies have been conducted to evaluate the toxicity of PFOS on two bird species, the mallard (*Anas platyrhynchos*) and the northern bobwhite quail (*Colinus virginianus*). Detailed methods and results of these studies are given elsewhere (*26*, *27*). Endpoints evaluated in the acute toxicity studies with juvenile mallard and quail included mortality, growth, behavior, and feed consumption (*26*). Pilot and definitive reproductive studies have also been conducted with quail and mallards exposed to dietary concentrations of PFOS for up to 21 weeks (*27*). In the pilot studies, adult birds were exposed to nominal PFOS concentrations of 1.8, 6.2, or 17.6 mg PFOS kg^{-1} feed (*28*, *29*). To initiate laying of eggs, the adult birds were photostimulated 1 week prior to the start of the study. At week 6, the 1.8 and 6.2 mg PFOS kg^{-1} feed treatments were terminated as given in the study protocol, while the control and 17.6 mg PFOS kg^{-1} feed treatments were continued for up to 20 weeks.

In the definitive reproductive studies, adult birds were exposed to nominal dietary PFOS concentrations of 0, 10, 50, or 150 mg PFOS kg^{-1} feed for up to 20 weeks and sacrificed at week 21. Adult birds were given a treated diet for up to 10 weeks prior to photostimulation and the onset of egg-laying. Endpoints monitored in the study included growth, behavior, and histopathology of adult and offspring. Reproductive endpoints included egg production, fertility, hatchability, and hatching survival and growth. Concentrations of PFOS were measured in the diet, liver, and serum of adult and juvenile birds and in eggs during the study.

Results

Acute Dietary Exposures. The 8 day LC_{50} for juvenile quail was 212 mg PFOS kg^{-1} feed with a 95% confidence interval of 158−278 mg PFOS kg^{-1} feed (*26*). The 8 day LD_{50} was 61

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10.1021/es050989v CCC: $30.25
Published on Web 10/14/2005

TABLE 1. NOAEL and LOAEL Values Based on Nonlethal Endpoints for Juvenile Northern Bobwhites and Mallards from an Acute Dietary Study with PFOS

	northern bobwhite			mallard		
	NOAEL	LOAEL	TD[a]	NOAEL	LOAEL	TD
dietary (mg PFOS kg^{-1} wet weight)[b]	70	140	99	35	70	50
ADI (mg PFOS kg^{-1} body weight day^{-1})[c]	24	48	34	12	20	16
serum (μg PFOS mL^{-1})	41	42	41	30	48	38
liver (μg PFOS g^{-1} wet weight)	44	70	55	15	30	21

[a] Threshold dose calculated as the geometric mean of the NOAEL and LOAEL liver and serum data based on day 8 values. [b] Dietary concentration given as mg PFOS kg^{-1} feed. [c] Average daily intake (ADI; mg PFOS kg^{-1} body weight day^{-1}) values are based on average feed consumption per pen.

mg PFOS kg^{-1} body weight day^{-1} with a 95% confidence interval of 48−77 mg PFOS kg^{-1} body weight day^{-1} based on estimates of average daily intake (ADI) of PFOS. The NOAEL based on lethality was 70.3 mg PFOS kg^{-1} feed (or 24 mg PFOS kg^{-1} body weight day^{-1}). For juvenile mallards, the 8 day dietary LC_{50} was 603 mg PFOS kg^{-1} feed with a 95% confidence interval of 431−938 mg PFOS kg^{-1} feed. The 8 day LD_{50} was 150 mg PFOS kg^{-1} body weight day^{-1} with a 95% confidence interval of 117−201 mg PFOS kg^{-1} body weight day^{-1}. The NOAEL based on lethality for mallards was 141 mg PFOS kg^{-1} feed (or 61 mg PFOS kg^{-1} body weight day^{-1}). In contrast, the NOAEL based on reductions in body weight and feed consumption was 35 mg PFOS kg^{-1} feed (or 12 mg PFOS kg^{-1} body weight day^{-1}) (Table 1).

Mallard Reproduction. In the pilot study, no treatment-related mortalities, overt signs of toxicity, or effects on feed consumption were observed in adult mallards exposed to 1.8, 6.2, or 17.6 mg PFOS kg^{-1} feed (*28*). In adult females, no treatment-related effects were noted on body weight, body weight gain, and liver weights, nor was there any evidence of pathological lesions in females. In addition, no treatment-related effects were observed on any reproductive endpoint including measures of egg production, embryo viability, hatching success, or offspring survival and health. In adult males, there was a statistically significant reduction in body weight and an increase in the incidence of small testes (length) in birds fed 17.6 mg PFOS kg^{-1} feed (3 of 5 males) as compared to controls (0 of 5 males). However, the incidence of small testes was not related to any alteration of spermatogenesis or egg production and/or fertility. The NOAEL and LOAEL for adult birds based on dietary, serum, liver, and egg PFOS concentrations are given (Table 2).

In the definitive study, treatment-related mortalities were observed by week 7 in birds exposed to 50 or 150 mg PFOS kg^{-1} feed (*27*). As a result, mallards from these treatments were euthanized while the control and 10 mg PFOS kg^{-1} feed treatments were continued to the study termination. No treatment-related mortalities were observed in adults exposed to 10 mg PFOS kg^{-1} feed, nor were there any significant effects on body weight, body weight gain, or feed consumption. No treatment-related effects on any reproductive parameter or on offspring survival and growth were observed in the study. Males from the 10 mg PFOS kg^{-1} feed treatment had a greater incidence of small testes (length) when compared to controls. In the males with small testes, one control (1 of 2) and four treated (4 of 7) birds had altered spermatogenesis that was characterized by fewer or no maturing/mature spermatozoa in the seminiferous tubules. In the other birds with reduced testis size, spermatogenesis appeared to be normal. These effects were consistent with post-reproductive regression of testes. Because no effects were noted on egg production or fertilization, small testes did not appear to affect reproductive performance in males exposed to PFOS in the diet. On the basis of lethality, the LOAEL for female and male mallards was 50 mg PFOS kg^{-1} feed (6.4 mg PFOS kg^{-1} body weight day^{-1}) while the NOAEL was 10 mg PFOS kg^{-1} feed (1.5 mg PFOS kg^{-1} body weight day^{-1}). The NOAEL based on nonlethal endpoints was 10 mg PFOS kg^{-1} feed (1.5 mg PFOS kg^{-1} body weight day^{-1}). NOAEL values based on liver, serum, and egg PFOS concentrations are given (Table 2).

TABLE 2. NOAEL and LOAEL Values from Reproductive Studies Conducted with Mallards Exposed to PFOS in the Diet

	pilot study		definitive study	
	NOAEL	LOAEL	NOAEL	LOAEL
Diet (mg PFOS kg^{-1} wet weight)[a]				
male	6.2	17.6	10	50
female	17.6		10	50
ADI (mg PFOS kg^{-1} body weight day^{-1})[b]				
male	0.99	3.6	1.5	6.4
female	3.6		1.5	6.4
Liver (μg PFOS g^{-1} wet weight)				
male	1.2	9.6	61	
female	9.7		11	
Serum (μg PFOS mL)				
male	35	160	87	
female	140		17	
egg yolk (μg /mL)	51		53	

[a] Diet concentration in units of mg PFOS kg^{-1} feed. [b] Average daily intake (ADI; mg PFOS kg^{-1} body weight day^{-1}) values are based on average feed consumption per pen.

Bobwhite Quail Reproduction. In the pilot study, no treatment-related mortality or overt signs of toxicity were observed in quail exposed to 1.8, 6.2, or 17.6 mg PFOS kg^{-1} feed (*29*). For adult females, no PFOS-related effects were observed on growth, body weight, or any reproductive endpoints monitored in the study. In adult males exposed to 17.6 mg PFOS kg^{-1} feed, there was a statistically significant reduction in body and liver weight and a slight increase in the incidence of small testis (length) (2 of 5 males) when compared to controls (0 of 5 males). However, morphological analysis of birds with small testes indicated that spermatogenesis was not affected. On the basis of the week 6 data, the dietary NOAEL was 6.2 mg PFOS kg^{-1} feed (0.58 mg PFOS kg^{-1} body weight day^{-1}) while the dietary LOAEL was 17.6 mg PFOS kg^{-1} feed (2.01 mg PFOS kg^{-1} body weight day^{-1}) (Table 3).

In the definitive study, treatment-related mortalities were observed in quail exposed to 50 mg PFOS kg^{-1} feed (2.64 mg PFOS kg^{-1} body weight day^{-1}) and 150 mg PFOS kg^{-1} feed (7.32 mg PFOS kg^{-1} body weight day^{-1}) (*26*). As a result, these treatments were terminated by week 7. No treatment-related effects were noted on survival, body weight, body weight gain, or feed consumption in adult quail fed 10 mg PFOS kg^{-1} feed. While no treatment-related effects were noted on adult male liver weights, there was a statistically significant increase in liver weight in females fed 10 mg PFOS kg^{-1} feed. When the female liver weights were normalized to body weight, the differences were still statistically significant, indicating that the increase in liver weight was PFOS-related.

TABLE 3. NOAEL and LOAEL Values from Reproductive Studies Conducted with Northern Bobwhites Exposed to PFOS in the Diet

	pilot study		definitive study	
	NOAEL	LOAEL	NOAEL	LOAEL
Diet (mg PFOS kg^{-1} wet weight)[a]				
male	6.2	17.6	10	50
female	17.6			10
ADI (mg PFOS kg^{-1} body weight day^{-1})[b]				
male	0.58	2.0	0.77	2.6
female	2.0			0.77
Liver (μg PFOS g^{-1} wet weight)				
male	1.3	2.9	88	
female	1.4			4.9
Serum (μg PFOS mL^{-1})				
male	77	200	140	
female	44			8.7
egg yolk (μg /mL)	33			62

[a] Dietary concentration in units of mg PFOS kg^{-1} feed. [b] Average daily intake (mg PFOS kg^{-1} body weight day^{-1}) values are based on average feed consumption per pen.

However, the increase in liver weight was not accompanied by any other pathological findings. Gross pathological and histopathological evaluations indicated no treatment-related lesions in adult females. However, in males from the 10 mg PFOS kg^{-1} feed treatment there was a slight increase in the incidence of reduced testes size (length) (6 of 16 males) as compared to the control (1 of 16 males). The decrease in testis size was not associated with any decrease in spermatogenesis and was consistent with post-reproductive phase regression, a normal physiological phenomenon that has been observed in many avian species (*30*, *31*). No PFOS-related effects on egg production or fertility were observed in the study, while there were slight but not statistically significant reductions in the embryo viability, hatching success, and 14-day-old offspring survivors. When reproductive endpoints were expressed as percentages, there were slight reductions in several reproductive parameters and a statistically significant reduction in the number of 14-day-old survivors normalized to eggs set. No treatment-related effects were observed on offspring body or liver weights at termination. On the basis of the statistically significant effect observed on reproduction, the LOAEL was 10 mg PFOS kg^{-1} feed. Dietary, serum, liver, and egg PFOS concentrations associated with the LOAEL are given (Table 3).

PFOS TRVs and PNECs

Avian Receptors of Concern. On the basis of the results of the laboratory studies presented above, the quail was selected as the surrogate species to derive TRV values. This was based on treatment-related effects observed on several reproductive endpoints monitored in the definitive study. While effects were noted on testes size in the 10 mg PFOS kg^{-1} dietary group, the effect was slight and did not affect reproduction; hence, this effect does not appear to have toxicological or ecological significance. In contrast, the PFOS-related effects on reproduction are both toxicologically and ecologically important and were used as the basis for selecting toxic threshold values.

In a several monitoring studies, PFOS residues in fish-eating water birds were found to have some of the greatest liver and serum PFOS concentrations (*2*, *4*, *6*, *9*). In particular, top avian predators such as eagles and cormorants accumulated the greatest PFOS concentrations when compared to other lower trophic level bird species. To be protective of all avian species, avian TRVs were derived based on the characteristics of trophic level IV fish-eating birds such as eagles and ospreys. Many of these bird species have been shown to be sensitive to other classes of organic compounds and thus provide an early warning system for the presence and effects of contaminants within aquatic ecosystems (*32*–*34*). In addition, through the use of the characteristics of predatory birds, contributions of PFOS from both aquatic and terrestrial exposure pathways can be incorporated into the derivation of avian TRVs (*35*, *36*).

Derivation of TRVs for a Level IV Avian Predator. Uncertainty factor (UF) assignment was conducted using the U. S. Environmental Protection Agency (USEPA) Great Lakes Initiative (GLI) methodology (*37*). In this approach, three categorical uncertainties were delineated and included: (1) uncertainty with LOAEL-to-NOAEL extrapolation (UF_L), (2) uncertainty related to duration of exposure (UF_S), and (3) uncertainty related to intertaxon extrapolations (UF_A). In this approach, uncertainty factors for each category are assigned values between 1 and 10 that are based on available scientific findings and best professional judgment (*38*, *39*). On the basis of the data from the quail reproduction study and the characteristics of a level IV avian predator, a final uncertainty factor of 36 was assigned to account for data gaps and extrapolations in the analysis (Table 4).

TRVs based on dietary, average daily intake (ADI), egg PFOS concentrations were 0.28 mg PFOS kg^{-1} feed, 0.021 mg PFOS kg^{-1} body weight day^{-1} and 1.7 μg PFOS mL^{-1} egg yolk, respectively (Table 5). Since sex-specific differences in adult serum and liver PFOS concentrations were observed in the toxicity studies, TRVs based on these endpoints represent a range of values that encompass all reproductive conditions of birds (Table 5). The sex-specific differences in serum and liver PFOS concentrations at the study termination were likely a result of PFOS being transferred to eggs from adult females during egg-laying. This is substantiated by the fact that during the pre-reproductive phase of the study, serum concentrations in females were similar to those observed in males (*27*). Thus, the reproductive condition of the bird affects the relevant serum and liver values.

Because it may not always be possible to ascertain the reproductive condition of birds in the field or from available biomonitoring samples, it is reasonable to derive TRVs that incorporate the end-of-reproduction values along with the pre-reproduction values of both sexes that are applicable at other life stages and would not be an overly conservative measure of risk. These TRVs can then be used on a practical basis to evaluate avian biomonitoring samples regardless of their sex or reproductive condition. This was accomplished by first calculating the geometric mean for pre- and post-reproductive female serum PFOS concentrations and then a geometric mean between the latter mean and that for males. For liver, the geometric mean of male and female liver PFOS concentrations was used. On the basis of this approach, the TRVs for serum and liver were 1.7 μg PFOS mL^{-1} and 0.6 μg PFOS g^{-1} wet weight, respectively.

PNECs were determined using guidance outlined in the European Commission Technical Guidance Document (TGD) for risk assessment of existing substances (*40*). For PFOS, chronic reproductive studies with two avian species were conducted, and ecologically relevant endpoints including survival, growth, and reproduction were evaluated. Since the TGD indicates that NOAELs from mortality, reproduction, or growth are preferred over other endpoints that reflect generalized toxicity in ecological risk assessments, the effects of PFOS on offspring survival effects in quail were used to establish PNECs. Since the quail definitive study produced only a LOAEL and not a NOAEL, the TGD specifies a correction factor of 2 be applied to the LOAEL. The TGD also prescribes empirically derived assessment factors that are based on the quantity and quality of toxicity data available for derivation

TABLE 4. Assignment of Uncertainty Factors for the Calculation of a Generic Trophic Level IV Avian Predator Toxicity Reference Value for PFOS[a]

uncertainty factors	notes
intertaxon extrapolation (UF_A)	The laboratory study used to determine a threshold dose was from northern bobwhite quail. Since this species belongs to the same taxonomic class but different order, $UF_A = 6$.
toxicological endpoint (UF_L)	The quail study determined a LOAEL but not a NOAEL based on multiple endpoints that included reproduction. Furthermore, the difference between the LOAEL and control was less than 20% for the effected reproductive endpoints. Taken together with other study data, the $UF_L = 2$.
exposure duration (UF_S)	The quail reproductive study was conducted for 20 weeks and evaluated several important life stages including embryonic development and offspring growth and survival, so $UF_S = 3$.
overall UF for TRV	$UF = 6 \times 2 \times 3 = 36$

[a] The selection of uncertainty factors was based on the USEPA Great Lakes Initiative (*37*).

TABLE 5. PFOS Toxicity Reference Values and Predicted No Effect Concentrations for a Generic Trophic Level IV Predator Based on Dietary, Liver, and Serum Toxic Doses[a]

	male			female		
	LOAEL	TRV[b]	PNEC[c]	LOAEL	TRV[b]	PNEC[c]
ADI (mg PFOS kg^{-1} body weight day^{-1})[d]	0.77	0.021	0.013	0.77	0.021	0.013
liver (μg PFOS g^{-1} wet weight)	88	2.4	1.5	4.9	0.14	0.08
serum (μg PFOS mL^{-1})	141	3.9	2.4	8.7	0.24	0.15
egg yolk (μg PFOS mL^{-1})				62	1.7	1.0

[a] The LOAEL values were based on the bobwhite quail definitive study. [b] The TRVs were estimated with a total uncertainty factor of 36 derived using the USEPA GLI protocol. [c] The PNECs were estimated with a total assessment factor of 60. [d] Average daily intake (ADI; mg PFOS kg^{-1} body weight day^{-1}) estimates were based on average feed consumption per pen.

of a PNEC. Since the avian studies were chronic and evaluated reproductive endpoints, the TGD assigns an assessment factor of 30 to account for interspecies variation, endpoint extrapolation, and laboratory-to-field extrapolation. On the basis of the quail LOAEL of 10 mg PFOS kg^{-1} feed and using an overall assessment factor of 60, dietary- and tissue-based PNECs were calculated (Table 5).

Discussion

Derivation of TRVs. To assess the possible risks that PFOS poses to avian wildlife, benchmark values for PFOS were derived from existing toxicological data (*26*, *27*). These benchmarks provide points of reference for the concentrations of PFOS measured in the environment and can be used in ecological risk assessments. Species-specific effect levels based on dietary measures of exposure were defined based on measures of health (i.e., growth and gross pathology) and reproductive success and were used to derive TRVs (Figure 1). However, due to feed consumption being measured on a pen basis, sex-specific dietary effect TRVs could not be determined. The significance of any sex-related difference in feed consumption in quail is difficult to ascertain in that much of the published data does not make a distinction between the consumption rates of males and females (*41*). If feed consumption is assumed to be proportional to body weight, then less than a 1.5-fold difference between male and female feed consumption rates would exist. This difference is small relative to the variability observed for body weights measured during the study for each sex. As a result, the use of average feed consumption rates for both sexes was determined to be adequate to estimate dietary exposures in the study.

In contrast to the dietary exposure metrics, sex-specific mechanisms were important in moderating the accumulation and retention of PFOS. Male serum and liver concentrations were approximately 17-fold greater than those observed in females at study termination. In part, these differences were due to additional loss mechanisms in females where PFOS was eliminated at a greater rate than that of adult male birds during the egg-laying phase of the studies. Data that examines the elimination of PFOS from birds have been measured in

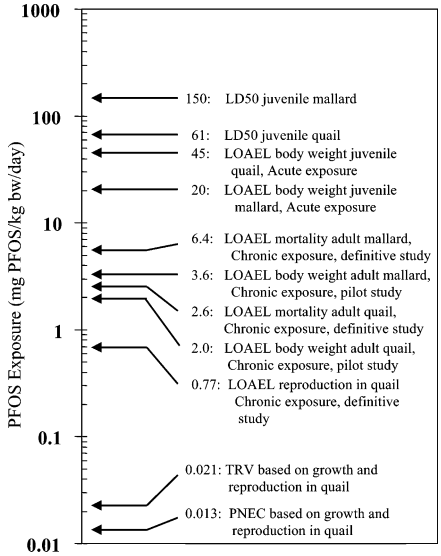

FIGURE 1. Toxicity thresholds for avian species exposed to PFOS in feed expressed as allowable daily intake.

both juvenile and adult quail and mallards. For instance, when juvenile birds were removed from treated diets during the recovery phase of the acute studies, PFOS concentrations in serum and liver samples decreased with time such that for liver there was an approximately 22% reduction in PFOS concentrations between samples collected 3 and 17 days post-exposure (*26*). This loss resulted in an estimated half-life in liver of less than 13 days for both species. Kinetic analysis of PFOS concentrations in the serum of adult male birds had estimated half-lives of 20.7 and 13.6 days for quail and mallards, respectively. In addition to these losses, in adult females there was also a transfer of PFOS to eggs that occurred during the egg-laying phase of the reproduction studies (*27*). In comparison to mammalian species, there was a more rapid loss of PFOS from both juvenile and adult mallard and quail, indicating species-specific differences in the disposition of PFOS. In male rats, the elimination half-

life from serum was estimated to be >90 days (*18*) and ranged from approximately 100−200 days in male and female cynomologus monkeys (*17*). Thus, while PFOS appears to be readily absorbed and distributed in serum and liver of birds and mammals, birds much more quickly eliminate PFOS with a more rapid recovery than that observed in mammals.

Evaluation of Uncertainties. An uncertainty analysis in risk assessments typically identify the presence of data gaps related to the interpretation and extrapolation of toxicity data among different species, the use of different laboratory endpoints to evaluate toxicity, and differences in experimental design. For example, the PFOS toxicity data used to derive TRVs for avian species came from controlled laboratory studies with only two species and not with species that may have the greatest exposure in the environment. In these instances, it is necessary to derive TRVs through the use of toxicological data from surrogate species that may not share similar life histories or feeding strategies. As a result, uncertainty factors, sometimes referred to as safety factors, are estimated and applied to the toxicity data such that a conservative and species-specific TRV is derived (*42*). Thus, as additional toxicological data become available, the magnitude of the uncertainty factors decreases, and a more accurate estimate of potential risk to species of concern is achieved.

When the USEPA GLI methodology was used, the single greatest source of uncertainty was associated with interspecies extrapolation where a factor of 6 was assigned. On the basis of the mallard and quail toxicity data, the overall difference between these two species was less than 2-fold when compared on a dietary basis. This result indicates that the interspecies differences in sensitivity to PFOS were not great. However, because laboratory data are available from only two bird species, an uncertainty value of 6 was used in the analysis to protect other avian species (*38*, *39*).

The second greatest source of uncertainty was associated with exposure duration. In the quail reproduction study, two generations were evaluated but only the adults were was exposed to PFOS. As a result, the cumulative effect of maternally transferred PFOS along with dietary exposure of hatchlings to PFOS in the diet was not examined. While effects of PFOS on juvenile and adult birds have been studied separately, a multigenerational exposure study has not been conducted to date. However, mammalian data have shown the consistency of PFOS-related effects thresholds (NOELs) for one- and two-generation studies as well as in a chronic lifetime study (*13*). In these studies, benchmark doses (BMDs) for rat pup weight gain, litter size, and mortality in a two-generation study, incidences of liver tumors in a lifetime rat study, and liver toxicity values in rats and monkey are all within a factor of 2. Thus, a value of 3 was assigned to account for this extrapolation.

The third source of uncertainty was associated with LOAEL-to-NOAEL extrapolation. On the basis of the pilot and definitive reproduction studies, a value of 2 was assigned to account for this extrapolation. The selection of this value was based on several factors including data that showed that while there were treatment-related effects on liver weights and reproductive parameters in quail fed 10 mg PFOS kg^{-1} feed, these differences were not great and typically were less than 15% of control values. In addition, the NOAEL in the quail pilot study was 6.2 mg PFOS kg^{-1} feed, indicating that the effects observed at 10 mg PFOS kg^{-1} feed in the definitive study were most likely near the threshold for adverse effects in quail. Thus, an UF of 2 was a reasonable value in the extrapolation of the LOAEL to NOAEL. Overall, while there were gaps in the toxicological data needed to derive TRVs, these gaps were accounted for in a scientifically defensible manner, and benchmarks were established that are protective of avian species. In addition, the studies used in this analysis represent the only rigorously conducted exposure and effect studies in avian species and are the most appropriate studies for use in the derivation of avian TRVs.

A comparison of effect levels in avian toxicity studies to those observed in mammalian species show some similarities. In a chronic lifetime dietary study, adverse effects were noted in male CD rats exposed to 5 mg PFOS kg^{-1} body weight day for 14 weeks and included an increased incidence of hepatic lesions (*43*). In addition, adverse effects including reduced body weight gain and an increase in liver weight were noted in both males and females exposed to approximately 1.5 mg PFOS kg^{-1} body weight day^{-1}. In Rhesus monkeys, the NOAEL based on body weight in males was 0.5 mg PFOS kg^{-1} body weight day^{-1} while the LOAEL was 1.5 mg PFOS kg^{-1} body weight day^{-1} (*13*). This result was in agreement with that observed in an oral gavage study with Cynomolgus monkeys where the NOAEL was 0.15 mg PFOS kg^{-1} body weight day^{-1} and the LOAEL was 0.75 mg PFOS kg^{-1} body weight day^{-1} (*17*). Taking into account differences in the exposure matrices and species-specific pharmacokinetics, the values observed in several mammalian species are within a factor of 3 of that observed in quail where the NOAEL was 0.77 mg PFOS kg^{-1} body weight day^{-1}. Overall, the distribution of exposure concentration associated with NOAELs and LOAELs is remarkably narrow and supports the concept that the effects of PFOS are related to a critical body burden that is similar regardless of species.

Acknowledgments

The authors thank 3M for financial support for this project. We also thank Sue Beach and John Butenhoff (3M) for their insightful comments and helpful advice on this manuscript.

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Received for review May 26, 2005. Revised manuscript received August 31, 2005. Accepted September 14, 2005.

ES050989V