

EFFECTS OF AIR CELL INJECTION OF PERFLUOROOCTANE SULFONATE BEFORE INCUBATION ON DEVELOPMENT OF THE WHITE LEGHORN CHICKEN (*GALLUS DOMESTICUS*) EMBRYO

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(Received 18 August 2004; Accepted 8 July 2005)

Abstract—Fifty white leghorn chicken (*Gallus domesticus*) eggs per group were injected with 0.1, 1.0, 10.0, or 20.0 µg perfluorooctane sulfonate (PFOS)/g egg before incubation to investigate the effects of PFOS on the developing embryo. Hatchlings were weighed, examined for gross developmental abnormalities, and transferred to a battery brooder, where they were raised for 7 d. Chicks were then weighed, and 20 birds per treatment were randomly chosen for necropsy. The brain, heart, kidneys, and liver were removed and weighed. Livers were processed further for determination of PFOS concentrations and histological assessment. Hatchability was reduced significantly in all treatment groups in a dose-dependent manner. The calculated median lethal dose was 4.9 µg PFOS/g egg. Perfluorooctane sulfonate did not affect posthatch body or organ weights. Exposure to PFOS caused pathological changes in the liver characterized by bile duct hyperplasia, periportal inflammation, and hepatic cell necrosis at doses as low as 1.0 µg PFOS/g egg. Perfluorooctane sulfonate concentrations in the liver increased in a dose-dependent manner. Based on reduced hatchability, the lowest-observed-adverse-effect level was 0.1 µg PFOS/g egg.

Keywords—Perfluorooctane sulfonate Fluorochemicals Chicken embryo Hatchability Egg injection

INTRODUCTION

Sulfonated fluorochemicals were first manufactured by Minnesota Mining and Manufacturing Company (3M; St. Paul, MN, USA) beginning in 1961. They have been widely used in a variety of products as surfactants; as carpet, textile, and paper protectants; and as fire-fighting foams [1]. The basic building block of sulfonated fluorochemicals is perfluorooctane sulfonyl fluoride (Fig. 1). The sulfonated fluorochemicals derived from perfluorooctane sulfonyl fluoride can degrade to perfluorooctane sulfonate (PFOS) (Fig. 1), which cannot be metabolized further [1].

Perfluorooctane sulfonate is widespread in the environment, being detected in water, soil, fish, birds, and mammals, including mink (*Mustela vison*), river otters (*Lutra canadensis*), polar bears (*Ursus maritimus*), and humans [2–13]. Its stability, persistence, ubiquitous presence, and potential for bioaccumulation make PFOS a chemical of concern. As a result, the U.S. Environmental Protection Agency mandated that PFOS and some related perfluorinated precursor compounds be phased out of the market within a three-year period beginning in 2000, and the major manufacturer (3M) agreed [1].

Perfluorooctane sulfonate has not been studied as thoroughly as other persistent organic pollutants, such as organobromines and organochlorines. A limited number of studies assessing the effects of PFOS in avian species have been conducted. These include dietary acute and chronic reproductive studies using northern bobwhites (*Colinus virginianus*) and

mallards (*Anas platyrhynchos*) [14–17] (http://www.epa.gov/opptintr/tsca8e/doc/8esub/2004/8e0907_091704.htm). Results from the reproductive trials indicated that the female bird transferred a portion of the PFOS into the egg, based on a significant decline in PFOS concentrations in the blood at the time egg-laying commenced [16,17]. A preliminary screening activity conducted in our laboratory demonstrated the presence of PFOS in chicken (*Gallus domesticus*) eggs collected at the Michigan State University Poultry Science Research and Teaching Center and in those purchased from a local supermarket, which also suggested maternal transfer of PFOS into the egg. Additionally, the occurrence of PFOS in eggs of fish-eating waterbirds has been reported [2–5].

The purpose of the present study was to assess the effects of PFOS on development of the avian embryo using an egg injection technique. Specific objectives were to evaluate hatchability, survival, and growth of chicks as well as pathological changes in tissues and liver concentrations of PFOS.

MATERIALS AND METHODS

White leghorn chicken (*G. domesticus*) eggs ($n = 250$) were obtained from the Michigan State University Poultry Science Research and Teaching Center and randomly assigned to each of five treatment groups: Vehicle (dimethyl sulfoxide) control, and 0.1, 1.0, 10.0, and 20.0 µg PFOS/g egg. The injection volume was 0.1 µl/g egg. Perfluorooctane sulfonate was injected into the air cell of the egg following the procedure of Powell et al. [18]. The air cell is a pocket of air that is formed at the blunt side of the egg as a result of cooling-

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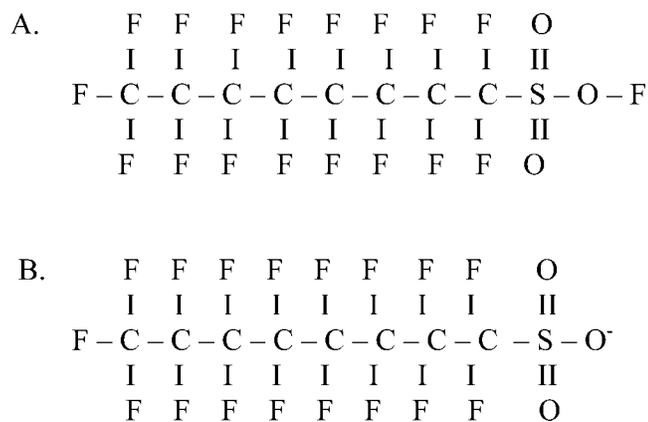


Fig. 1. Structures of perfluorinated chemicals. (A) The structure of perfluorooctane sulfonyl fluoride (POSF), which is the basic building block of perfluorooctyl sulfonates. (B) The structure of perfluorooctane sulfonate (PFOS), which is the ultimate degradation product of POSF-based compounds.

induced contraction of egg contents after laying. Doses of PFOS were chosen to bracket concentrations detected in the blood plasma and eggs of birds collected from the Great Lakes region, USA [7].

After injection, eggs were placed in an incubator (Petersime, Gettysburg, OH, USA) with their blunt end up. The temperature of the incubator was maintained at 37.5 to 37.7°C; the relative humidity was maintained at 60% (wet bulb temperature, 29.4–30.6°C). Eggs were candled weekly to assess embryo viability and to estimate the stage of development at which the embryo died. Eggs that did not develop were considered to be infertile and excluded from the analysis.

On day 18 of incubation, eggs were placed in pedigree baskets and transferred to a hatcher (Natureform, Jacksonville, FL, USA). Temperature and humidity of the hatcher were maintained at 37.5 to 37.7°C and 65% (wet bulb temperature, 31.1–37.7°C), respectively.

Hatching began on day 21 of incubation and was allowed to continue until day 24. Hatchlings were identified with a Swiftak identification tag (Heartland Animal Health, Fairplay, MO, USA), weighed and examined for gross deformations within 24 h of hatching, and then transferred to a Petersime battery brooder. Eggs that did not hatch by day 24 were opened to assess the stage of embryo death and the incidence of gross developmental abnormalities. Chicks were provided Purina Chick Starter (Purina Mills, St. Louis, MO, USA) and water ad libitum for 7 d.

At 7 d of age, 20 chicks per treatment were randomly chosen, weighed, and killed by cervical dislocation. The brain, heart, kidneys, and liver were removed and weighed. Five livers per treatment were frozen on dry ice before placement in an ultracold freezer (–78°C) for subsequent determination of PFOS concentration according to the method described by Kannan et al. [7], and five livers per treatment were placed in 10% neutral-buffered formalin for subsequent histological examination.

Liver tissue (1 g) was homogenized in Milli-Q® (Millipore, Bedford, MA, USA) water, and 1 ml of 0.5 M tetrabutyl ammonium hydrogen sulfate solution (adjusted to pH 10) and 2 ml of 0.25 M sodium carbonate buffer were added to an aliquot of the homogenate. After thorough mixing, 5 ml of methyl-tert-butyl ether (MTBE) was added to the solution, and the mixture was shaken for 20 min. The organic and aqueous layers

were separated by centrifugation, and an exact volume of MTBE (4 ml) was removed from the solution. The aqueous mixture was rinsed with MTBE and separated twice; all rinses were combined in a second polypropylene tube. The solvent was allowed to evaporate under nitrogen before being reconstituted in 0.5 to 1 ml of methanol. The sample was vortex-mixed for 30 s and passed through a 0.2- μ m nylon mesh filter into an autosampler vial.

Analyte separation was performed using a Hewlett-Packard HP1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA) interfaced to a Micromass® (Beverly, MA, USA) Quattro II atmospheric pressure ionization tandem mass spectrometer operated in the electrospray-negative mode. Instrumental parameters were optimized to transmit the [M-K][–] ion for PFOS before fragmentation to one or more product ions. At least two transitions were monitored and showed quantitative agreement to within $\pm 30\%$. Data quality-assurance and quality-control protocols included matrix spike, surrogate spike, laboratory blank, and continuing calibration verification. Recoveries of PFOS spiked into rabbit plasma and liver and passed through the analytical procedure ranged from 85 to 101%. The reported concentrations were not corrected for the matrix spike recoveries.

All slides from liver samples were read by a diplomat of the American College of Veterinary Pathologists (Madison, WI, USA) without knowledge of the treatment group. After lesions were identified and scored for severity, the slides for the control group were identified and re-evaluated for normality. All slides were re-examined in comparison with a normal slide to ensure accurate recognition and grading of lesions.

All liver slides were examined microscopically for histological evidence of degeneration, inflammation, hyperplasia, pigments, or neoplasia. Discrete lesions (e.g., inflammation or necrosis) were evaluated for number and size, whereas diffuse lesions (e.g., hepatocellular degeneration or biliary hyperplasia) were evaluated for severity. Severity scores were based on a scale of 0 to 3, which corresponded to normal, mild, moderate, and severe, respectively. Hepatic lipidosis was scored as follows: 0 = no detectable cytoplasmic vacuolation; 1 = scattered individual vacuoles or low numbers of vacuoles within the cytoplasm of some hepatocytes; 2 = clusters of vacuoles within the cytoplasm of many hepatocytes; 3 = clearing of the cytoplasm because of advanced vacuolation in nearly all hepatocytes. Biliary hyperplasia was scored as follows: Portal areas in 5 to 10 high-power fields were examined and the mean number of bile ducts per portal triad calculated (scoring: 0 = one to two bile ducts; 1 = three to four bile ducts; 2 = five to six bile ducts; 3 = greater than six bile ducts). The control samples were used as a guide for the normal histological appearance and natural rate of lesion occurrence.

All statistical comparisons were made with respect to the vehicle control. Effects of PFOS on hatchability were evaluated using logistic regression. Body weights, organ weights, and liver histopathology were analyzed by one-way analysis of variance followed by Dunnett's test for comparisons with the vehicle control using SAS Software [19]. The median lethal dose (LD50) was obtained by U.S. Environmental Protection Agency probit analysis program (Ver 1.5; Cincinnati, OH, USA; <http://www.epa.gov/nerleerd/stat2.htm>). The level of statistical significance was $p < 0.05$ unless otherwise specified.

RESULTS

Hatchability was significantly ($p < 0.0001$) reduced in all dose groups compared to the control group (Table 1). In ad-

Table 1. Effect of perfluorooctane sulfonate injected into the air cell of white leghorn chicken embryos before incubation on hatchability^a

Dose ($\mu\text{g/g}$ egg)	No. hatchlings/ no. fertile eggs	Hatchability (%)
Vehicle ^b	42/49	85.7 A
0.1	27/44	61.4 B
1.0	23/48	47.9 B
10.0	20/50	40.0 C
20.0	17/46	37.0 C

^a Values followed by different uppercase letters are significantly different at $p < 0.05$.

^b The vehicle was dimethyl sulfoxide.

dition, hatchability in the 10.0 and 20.0 μg PFOS/g egg groups was significantly lower compared to that in the 0.1 and 1.0 μg PFOS/g egg groups. The LD₅₀ was 4.9 μg PFOS/g egg (95% confidence limit, 0.28–297.12 μg PFOS/g egg). The incidence of gross abnormalities was similar across dose groups. Posthatch mortality through 7 d of age was not affected by treatment.

No significant differences were found in chick body weights among dose groups at hatch and at 7 d of age. Similarly, absolute and relative organ weights (brain, heart, kidneys, and liver) were not affected by injection of PFOS.

On gross examination, all livers, including those in the control group, were pale. Lesions observed during histological examination included hepatocellular vacuolation, bile duct hyperplasia, periportal inflammation, multifocal hepatocellular necrosis, multifocal subcapsular hepatocellular degeneration, and one case of fatty cysts in the 1.0 $\mu\text{g/g}$ dose group (Table

Table 2. Histopathology of chicken livers from 7-d-old white leghorn chickens exposed to perfluorooctane sulfonate in ovo via air cell injection before incubation^a

Treatment ($\mu\text{g/g}$ egg)	Chick ID	Hepatocellular vacuolation	Bile duct hyperplasia	Other
Vehicle	B854	1	0	
Vehicle	B857	1	0	
Vehicle	B861	1	0	
Vehicle	B882	1	0	
Vehicle	P831	1	0	
0.1	P840	1	0	
0.1	P845	1	0	
0.1	P864	1	0	
0.1	P869	1	0	
0.1	P871	1	0	A
1.0	PC399	1	0	A
1.0	PC872	2	1	B
1.0	PC873	2	1	B
1.0	PC877	1	1	B, C
1.0	PC888	1	0	
10.0	Y397	1	0	
10.0	Y403	2	1	
10.0	Y404	1	2	C
10.0	Y406	1	1	
20.0	YC411	1	1	
20.0	YC412	1	1	
20.0	YC415	2	1	
20.0	YC416	2	1	B
20.0	YC421	1	1	

^a The vehicle was dimethyl sulfoxide. Severity of lesion was graded using the following scale: 0 = normal; 1 = mild; 2 = moderate; 3 = severe. Other lesions: A = mild periportal inflammation; B = multifocal hepatocellular necrosis; C = fatty cyst; D = hepatocellular necrosis.

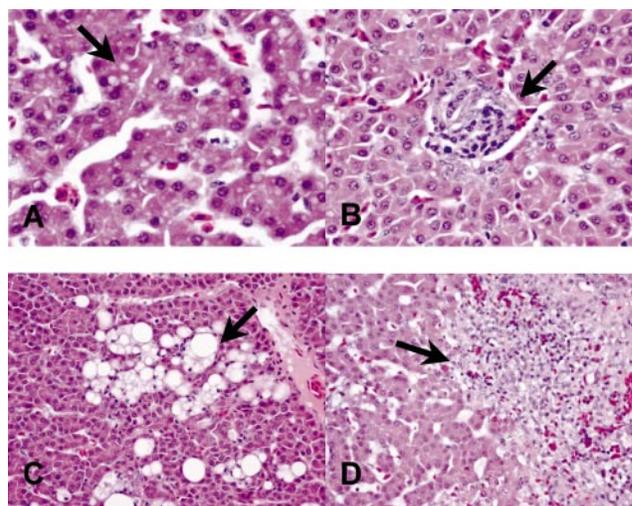


Fig. 2. Histopathology of livers from 7-d-old white leghorn chickens exposed to perfluorooctane sulfonate (PFOS) in ovo via air cell injection before incubation. (A) Mild hepatocellular vacuolation in a control chick. (B) Mild inflammation in a chick exposed to 0.1 μg PFOS/g egg. (C) Fatty cysts in a chick exposed to 1.0 μg PFOS/g egg. (D) Moderate bile duct cell hyperplasia, hepatocellular necrosis, proliferating bile, and inflammatory cells (Kupffer cells) in a chick exposed to 10.0 μg PFOS/g egg.

2 and Fig. 2). Hepatocellular vacuolation was observed in all dose groups but tended to be more prevalent in the 1.0, 10.0, and 20.0 $\mu\text{g/g}$ egg groups compared to the vehicle control and the 0.1 $\mu\text{g/g}$ egg groups. Bile duct hyperplasia was significantly prevalent in the 10.0 and 20.0 $\mu\text{g/g}$ egg groups compared to the vehicle control. Multifocal hepatocellular degeneration and necrosis were observed in a total of five liver samples: Three in the 1.0 $\mu\text{g/g}$ egg group, and one each in the 10.0 and 20.0 μg PFOS/g egg groups.

Perfluorooctane sulfonate concentrations in livers significantly increased in proportion to dose (Table 3). Concentrations in the 10.0 and 20.0 μg PFOS/g egg groups were significantly greater compared to concentrations in the vehicle control and 0.1 and 1.0 μg PFOS/g egg groups.

DISCUSSION

A PFOS injection dose as low as 0.1 $\mu\text{g/g}$ egg resulted in a significant decrease in hatchability compared to controls (Table 1). The LD₅₀ for chicken eggs injected with PFOS via the air cell before incubation was 4.9 μg PFOS/g egg, although it should be pointed out that the confidence interval around this mean ranged over four orders of magnitude. Perfluorooct-

Table 3. Hepatic perfluorooctane sulfonate concentrations of 7-d-old white leghorn chicks exposed to perfluorooctane sulfonate in ovo via injection into the air cell^a

Dose ($\mu\text{g/g}$ egg)	Concentration ($\mu\text{g/g}$ wet wt)
Vehicle ^b	1.1 \pm 0.54 A
0.1	1.4 \pm 0.54 A
1.0	1.8 \pm 0.54 A
10.0	3.2 \pm 0.54 B
20.0	4.8 \pm 0.54 B

^a Data are presented as the mean \pm standard error of the mean. Sample size was five for each dose group. Values followed by different uppercase letters are significantly different at $p < 0.05$.

^b The vehicle was dimethyl sulfoxide.

tanene sulfonate concentrations causing embryo mortality in the present study were comparable to PFOS concentrations found in eggs collected from wild bird species near urbanized areas. Double-crested cormorants (*Phalacrocorax auritus*) from the Great Lakes area contained PFOS concentrations from 0.57 to 1.8 $\mu\text{g/g}$ egg. Caspian tern (*Sterna caspia*) eggs from the same region contained PFOS at concentrations from 1.9 to 3.4 $\mu\text{g/g}$ egg (unpublished data). Double-crested cormorant eggs from Lake Winnipeg, Manitoba, Canada, had slightly lower PFOS concentrations, ranging from 0.02 to 0.32 $\mu\text{g/g}$ egg [2]. Thus, concentrations of PFOS in wild bird eggs were within an order of magnitude of the LD50 value reported in the present study. It is possible that hatching success of eggs in the wild may be compromised by PFOS if the sensitivity for these species in terms of mortality is similar to the chicken and if air cell injection accurately represents whole-egg concentrations achieved by dietary exposure of adults.

In two avian acute dietary studies, 10-d-old northern bobwhites and mallards were fed diets containing 0 to 1,148 μg PFOS/g feed for a period of 5 d and observed for an additional 3 d. The dietary 8-d median lethal concentration derived for the northern bobwhite was 212 μg PFOS/g feed, and the LD50 based on average daily intake of PFOS was 61 μg PFOS/g body weight/d [14]. The dietary 8-d median lethal concentration for the mallard was 603 μg PFOS/g feed, and the 8-d LD50 was 150 μg PFOS/g body weight/d [15]. These data suggest that the chicken is more sensitive to PFOS than the northern bobwhite and mallard, although the route of exposure and age at exposure were different compared to those of the present study. Possibly, the detoxification mechanisms of an avian embryo may not be as fully developed compared to those of a hatchling. However, two dietary chronic reproductive studies were conducted with northern bobwhites and mallards that assessed the effects of maternal deposition of PFOS into eggs on hatchability. In mallards, maternal dietary concentrations of up to 10 μg PFOS/g feed, which resulted in an average PFOS concentration of 53 μg PFOS/ml in the yolk, had no effect on hatchability [17]. In northern bobwhites, a slight, but not statistically significant, decrease was found in hatchability of eggs laid by hens fed 10 μg PFOS/g feed, which contained an average of 62 μg PFOS/ml yolk [16]. Assuming that PFOS is deposited only in the yolk, that the yolk accounts for 33% of the egg's weight [20], and that the density of yolk is 1 g/ml, the concentration of PFOS in the mallard egg was 18 and 21 $\mu\text{g/g}$ egg in the northern bobwhite egg. Both of these values are considerably greater than the LD50 of 4.9 $\mu\text{g/g}$ egg in the present study.

Posthatch survival was not affected by PFOS during the 7-d period that the chicks remained in the brooder battery. Similarly, survival of mallard hatchlings derived from hens fed 10 μg PFOS/g feed was not affected over 14 d [17], but a slight, yet significant, reduction was found in the number of 14-d-old northern bobwhite survivors as a percentage of the number of eggs set at the parental feed concentration of 10 μg PFOS/g feed [16].

Perfluorooctane sulfonate did not induce a significant number of abnormalities in chicken embryos compared to the vehicle. The few abnormalities observed in the present study fell within the normal range (6–7%) of spontaneous malformations for chicken embryos [21]. Occasionally, malformed embryos have more than one malformation, as was observed in this trial. Three embryos had multiple abnormalities, including deformed extremities (0.6%), anophthalmia (absence of one or

both eyes; 0.3%), exencephalia (exposed brain; 0.3%), celosomia (exposed viscera; 0.3%), and mandibular/maxillary malformation (0.3%). None of the embryos with abnormalities hatched, with the exception of two chicks. One had curled toes and died before the end of trial, and the other had a protruding eye and survived to the end of the trial.

Perfluorooctane sulfonate did not affect body weights or absolute and relative organ weights of 7-d-old chickens exposed in ovo. These results are similar to those reported for 14-d-old northern bobwhite chicks [16] and mallard ducklings [17] with mothers that were fed 10 μg PFOS/g feed, resulting in approximate egg concentrations of 21 and 18 μg PFOS/g egg, respectively. However, liver weights were significantly greater in adult female northern bobwhites fed a diet containing 10 μg PFOS/g feed for 21 weeks compared to controls [16].

Perfluorooctane sulfonate caused morphological changes in the livers of chicken embryos at concentrations as low as 1.0 $\mu\text{g/g}$ egg (Table 2 and Fig. 2). Mild hepatocellular vacuolation was observed among all dose groups; however, moderate vacuolation was observed more frequently at doses of 1.0 $\mu\text{g/g}$ egg or greater. Vacuolation is the result of accumulation of lipids in hepatocytes, which occurs because of the absorption of the lipid-rich yolk sac that continues after hatching. Although it is not always injurious to the liver, vacuolation can be indicative of impairment, such as blockage of triglyceride secretion into plasma or reduced synthesis of carrier lipoprotein [22]. Previous studies have reported that PFOS may interfere with fatty acid binding to lipoprotein, contributing to lipid accumulation in the liver [23]. Mild to moderate bile duct hyperplasia was observed in the highest-dose groups (1.0, 10.0, and 20.0 μg PFOS/g egg). This lesion consists of an abnormal number of bile ducts occurring in the portal area accompanied by periportal inflammation. Bile duct hyperplasia is considered to be a common lesion, and whether this can progress into benign or malignant tumors is unclear [24]. Bile duct hyperplasia may be induced by chemicals or by obstruction of the common bile duct. Chemical proliferation of bile ducts often is initiated by necrosis of the bile duct epithelium, leading to simple bile duct proliferation as a reparative process. This proliferation may remain static, regress over time, or progress to a more proliferative state with surrounding fibrous connective tissue (cholangiofibrosis). Neither active bile duct necrosis nor cholangiofibrosis were found in any livers of the present study.

Multifocal hepatocellular degeneration and necrosis were observed in five liver samples. Multifocal hepatocellular necrosis is observed infrequently in toxicological studies; generally, a specific pattern of necrotic lesions (centrilobular, midzonal, periportal, or massive) is produced. In livers used during the present study, the foci of necrosis were randomly distributed, low in number, and affected relatively low numbers of adjacent hepatocytes, leading us to believe that it was not a direct toxic effect of the PFOS. Inflammatory foci also tended to be randomly distributed throughout the liver, sometimes in association with bile duct hyperplasia. The inflammatory infiltrates were mild in severity and consisted of mixed inflammatory cells, including lymphocytes, plasma cells, and occasionally, heterophils. Inflammation most commonly is associated with infectious agents, immune responses to foreign antigens, or response to tissue necrosis. In the present study, the limited and mild inflammation may have been secondary to earlier bile duct necrosis.

Because three of the samples were from the 1.0 $\mu\text{g/g}$ dose

group, it was not clear if this effect was related to treatment. In contrast to the results of the present study, no lesions of the liver were reported in adult and hatchling mallards and northern bobwhites in the 10 μg PFOS/g feed treatment groups [16,17].

Mean PFOS concentrations in the livers of vehicle control chicks and those exposed in ovo to 0.1, 1.0, 10.0, or 20.0 μg PFOS/g egg ranged from 1.0 to 4.8 $\mu\text{g}/\text{g}$ (Table 2). The greatest concentration of PFOS measured in chick livers was 7.2 μg PFOS/g, from a bird exposed to the maximum dose (20.0 μg PFOS/g egg). The lowest concentrations were detected in the vehicle control group, yet some individual liver samples in the vehicle control group had PFOS concentrations as great as 1.7 μg PFOS/g egg. The presence of PFOS in control chick livers could have been the results of maternal transfer or consumption of PFOS originating from the paper bags in which the chicken feed was packaged. However, subsequent analysis of layer and chick starter feeds indicated no detectable concentrations of PFOS.

The average PFOS concentrations in the livers of 14-d-old mallard ducklings from hens that were fed 10 μg PFOS/g feed was 3.4 $\mu\text{g}/\text{g}$ [17], whereas the average hepatic PFOS concentration of 14-d-old northern bobwhites in the same treatment group was 5.6 $\mu\text{g}/\text{g}$ [16]. The estimated PFOS concentrations in the eggs were 18 $\mu\text{g}/\text{g}$ for the mallard and 21 $\mu\text{g}/\text{g}$ for the northern bobwhite. Concentrations of PFOS in the livers of mallard and northern bobwhite hatchlings were similar to concentrations of PFOS in the livers of chicken hatchlings in the 10.0 and 20.0 μg PFOS/g egg groups. Perfluorooctane sulfonate concentrations in the livers of wild avian species, mostly from urbanized areas [2,5,7,8], were similar to those found in liver samples of embryos from the vehicle control and 0.1 and 1.0 μg PFOS/g egg treatment groups (Table 2). For example, Brandt's cormorants (*Phalacrocorax penicillatus*) collected from San Diego, California, USA, had hepatic PFOS concentrations ranging from 0.046 to 1.78 $\mu\text{g}/\text{g}$ [7]. The PFOS concentrations in the livers of common cormorants (*Phalacrocorax* sp.) collected from Tokyo Bay, Japan, ranged from 0.17 to 0.65 $\mu\text{g}/\text{g}$, and livers of mallards and pintails contained an average concentration of 0.50 μg PFOS/g [5].

CONCLUSION

The results of the present study indicate that PFOS caused significant mortality of the chicken embryo after a single exposure before incubation at a dose as low as 0.1 $\mu\text{g}/\text{g}$ egg. The LD50 for the chicken embryo was 4.9 μg PFOS/g egg. Perfluorooctane sulfonate did not affect posthatch body weights or organ weights. A single exposure to PFOS caused pathological changes in the liver, including bile duct hyperplasia, periportal inflammation, and necrosis at doses as low as 1.0 $\mu\text{g}/\text{g}$ wet weight egg. Hepatic PFOS concentrations increased in a dose-dependent manner. The lowest-observed-adverse-effect level based on reduced hatchability was 0.1 μg PFOS/g wet weight egg.

The similarity between the lowest-observed-adverse-effect level in the present study and PFOS concentrations detected in eggs of wild avian species as well as the similarity between hepatic concentrations of PFOS in 7-d-old chicken hatchlings and wild avian species suggest that if wild avian species are as sensitive to PFOS as the chicken embryo, then PFOS may have a detrimental effect on birds inhabiting contaminated areas. Results of chronic reproductive studies with mallard and northern bobwhite [16,17] suggest that the chicken embryo is considerably more sensitive to PFOS compared to those spe-

cies more likely to be exposed naturally to the contaminant. Thus, toxicity reference values derived for the chicken embryo may be protective of some wild avian species. Based on the similarity of hepatic concentrations of PFOS in chicks exposed via injection of PFOS into the egg, and in northern bobwhites and mallards exposed via maternal deposition of PFOS into the egg, it appears that the egg injection technique can be used to approximate natural exposure of the avian embryo to PFOS and related compounds. Because the egg injection route of exposure resulted in effects thresholds similar to those observed for mallards and bobwhite quail, this method could be used as a rapid method to screen for the effects of other, similar perfluorinated compounds.

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