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DIETARY EXPOSURE OF MINK (*MUSTELA VISON*) TO FISH FROM THE UPPER HUDSON RIVER, NEW YORK, USA: EFFECTS ON REPRODUCTION AND OFFSPRING GROWTH AND MORTALITY

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Running title: Effects of Hudson River fish on mink reproduction

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Abstract—The effects of feeding farm-raised mink (*Mustela vison*) diets containing polychlorinated biphenyl (PCB)–contaminated fish from the upper Hudson River (New York, USA) on adult reproductive performance and kit growth and mortality were evaluated. Diets contained 2.5 to 20% Hudson River fish, providing 0.72 to 6.1 µg ∑PCBs/g feed (4.8–38 pg toxic equivalents [$TEQ_{WHO\ 2005}$]/g feed). The percentage of stillborn kits per litter was significantly increased by dietary concentrations of 4.5 µg ∑PCBs/g feed (28 pg $TEQ_{WHO\ 2005}$/g feed) and greater. All offspring exposed to dietary concentrations of 4.5 and 6.1 µg ∑PCBs/g feed (28 and 38 pg $TEQ_{WHO\ 2005}$/g feed) died by 10 weeks of age, and all offspring exposed to 1.5 and 2.8 µg ∑PCBs/g feed (10 and 18 pg $TEQ_{WHO\ 2005}$/g feed) died by 31 weeks of age, leaving juveniles in the control and 0.72-µg ∑PCBs/g feed (0.41- and 4.8-pg $TEQ_{WHO\ 2005}$/g feed) groups only. The dietary concentration predicted to result in 20% kit mortality (LC20) at six weeks of age was 0.34 µg ∑PCBs/g feed (2.6 pg $TEQ_{WHO\ 2005}$/g feed). The corresponding maternal hepatic concentration was 0.80 µg ∑PCBs/g liver, wet weight (13 pg $TEQ_{WHO\ 2005}$/g liver, wet wt). Mink residing in the upper Hudson River would be expected to consume species of fish that contain an average of 4.0 µg ∑PCBs/g tissue. Thus, a daily diet composed of less than 10% Hudson River fish could provide a dietary concentration of ∑PCBs that resulted in 20% kit mortality in the present study.

Keywords—Hudson River, Mink, Polychlorinated biphenyl, Reproduction, Mortality

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INTRODUCTION

The Hudson River is contaminated with polychlorinated biphenyls (PCBs) from Fort Edward, NY, USA, to New York City. Capacitor manufacturing facilities at Fort Edward and Hudson Falls, NY, USA, are considered to be the major sources of PCBs in the upper Hudson River, with discharges beginning in 1947. Between 1966 and 1974, the Fort Edward and Hudson Falls facilities purchased 35,000 metric tons of PCBs or 15% of domestic sales in the United States. It is estimated that approximately 600 metric tons of PCBs were released into the Hudson River between the 1940s and 1977 [1,2].

Concentrations of PCBs measured in the livers of mink (*Mustela vison*) collected in the vicinity of the Hudson River are above thresholds associated with effects and have not decreased over time. Mink collected over 25 years ago had hepatic concentrations of PCBs [3] that were equivalent to concentrations associated with reproductive impairment in ranch mink experimentally exposed to PCBs [4,5]. More recently, mink collected in the vicinity of the Hudson River 16 years after the initial study [3] had no apparent decrease in hepatic PCB concentrations [6], which were above the criteria for impairment of mink health and reproduction [7,8].

Mink are among the most sensitive species to PCBs [9–18]. Because mink are fish-eating mammals that satisfy criteria, including chemical sensitivity, for a sentinel wildlife species [19], regulatory agencies such as the U.S. Environmental Protection Agency (U.S. EPA), U.S. Fish and Wildlife Service, Environment Canada, and the Swedish Environmental Protection Agency recognize the mink as such, making it one of the most commonly selected receptors in ecological risk assessments for sites involving aquatic habitats with elevated concentrations of PCBs and

related compounds [20].

Thus, the objective of the present study was to evaluate the health effects of feeding farm-raised mink diets containing PCB-contaminated fish from the Hudson River. In the present report, the effects on adult reproductive performance (percentage of females whelping, gestation length, percentage of stillbirths, and live kits whelped) and offspring growth and mortality through 31 weeks of age are discussed in terms of dietary and hepatic concentrations of ∑PCBs and World Health Organization (WHO) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxic equivalents ($TEQs_{WHO\ 2005}$). A companion paper presents the effects on organ mass and pathology, including the mandible and maxilla, of adult mink and their offspring [21].

METHODS

Fish preparation

Common carp (*Cyprinus carpio*) and Atlantic herring (*Clupea harengus*) were used as fish components of the experimental diets. Carp were collected from the upper Hudson River from Northumberland Pool (114 kg), from the vicinity of Lock 2 (879 kg), and from the first 610 m of Moses Kill (528 kg; Fig. 1) by the New York Department of Environmental Conservation and transported frozen to the Michigan State University Experimental Fur Farm. Fish were ground, blended, sampled for contaminant and nutrient analyses, and frozen for subsequent incorporation into mink feed. Whole Atlantic herring, purchased from Finicky Pet Food, was chosen as the control fish because they, like carp, contain the enzyme thiaminase [22]. The herring was ground, blended, and sampled as described above.

Dietary treatments

Treatment diets were based on the Michigan State University Experimental Fur Farm ranch diet formulated to meet the nutrient requirements of mink [22]. Diets contained 25% whole

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ground chicken, 24% water, 20% fish, 15% wheat middlings (Akey), 4% spray-dried eggs (Van Eldren), 3.5% spray-dried liver (Van Eldren), 3.5% soybean oil (North American Nutrition), 3% spray-dried blood (California Spray Dry), 1% phosphoric acid (food grade, 75%; Astaris), 0.48% vitamin premix (Akey), 0.48% mineral premix (Akey), and 0.04% biotin (Archer Daniels Midland). The proportion of fish incorporated into the feed (20%) is in the range of fish consumed by mink in the wild [12]. The control diet contained 20% ocean herring, and the remaining five treatment diets contained a mixture of the herring and Hudson River (HR) fish in the following proportions: 2.5% HR fish/17.5% herring; 5% HR fish/15.0% herring; 10% HR fish/10% herring; 15% HR fish/5% herring; 20% HR fish. The decision to incorporate no more than 20% HR fish into the diet was based on the high mean ∑PCB concentration in the collected fish (36 µg ∑PCBs/g wet wt) and the desire to not cause complete reproductive failure and/or adult mortality. Targeted dietary concentrations of ∑PCBs were 0, 0.90, 1.8, 3.6, 5.4, and 7.2 µg/g feed based on 36 µg ∑PCBs/g wet weight in the HR fish.

Analysis

Dietary ∑PCBs. Dietary and tissue samples were analyzed for a number of chemicals. Samples of each treatment diet were shipped to Alpha Woods Hole Laboratory for analysis of ∑PCBs, PCB homolog groups, and potentially toxic and bioaccumulative metals and minerals. For the PCB analysis (Alpha Woods Hole Lab SOP O-015 Rev. 02: Determination of PCB Homologs, Individual Congeners and Pesticides by GCMS-SIM, 6/19/06 and SOP O-015 Addendum for Hudson River NRDA, 11/6/06), samples were homogenized in diatomaceous earth containing 1:1 dichloromethane:acetone and exchanged to hexane. The extracts were cleaned with sulfuric acid. The extract was then analyzed for PCB congeners and homolog groups by low-resolution gas chromatography–mass spectrometry (GC–MS) in selective ion

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monitoring (SIM) mode. Blanks, duplicates, and spikes were analyzed at a frequency of one per analytical batch of up to 20 samples. In addition, to evaluate accurate and consistent extraction and analysis, a standard reference material (SRM), SRM 1946 (National Institute of Standards and Technology, USA, Lake Superior Fish Tissue), was extracted and analyzed with each analytical batch. This SRM has certified values for 30 PCB congeners. Results for the congener analyses were to be within 20 of the 95% confidence interval (CI) for certified concentrations. Overall, greater than 93% of the reference material results were within the control limits for the certified concentrations.

Dietary metals. Individual metals (Supplemental Data, Table S1) were analyzed by U.S. EPA Method 6020A (Alpha Woods Hole Lab SOP M-001 Rev. 3.0: Inductively Coupled Plasma-Mass Spectrometry, Method 6020A, 5/31/05) using inductively coupled plasma-mass spectrometry. Mercury was analyzed by U.S. EPA Method 7471A (Alpha Woods Hole Lab SOP M-006 Rev. 3.0: Mercury Determination by Cold Vapor Atomic Absorption Spectroscopy, Method 7471A, 4/15/07) using cold vapor atomic absorption spectroscopy.

Dietary and tissue PCB congeners. Diet samples were also sent to Axys Analytical Services for analysis of PCB congeners, polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), chlorinated pesticides, and polybrominated diphenyl ethers (PBDEs). In addition, liver samples from adults, six-week-old kits, and 31-week-old juveniles were submitted for analysis of PCB congeners. For chlorinated pesticides (Supplemental Data, Table S1) and PCB congeners, the sample was homogenized with anhydrous powdered sodium sulfate and extracted with dichloromethane. The extracts were cleaned with a Biobeads SX-3 (Bio-Rad) gel permeation column, followed by a Florisil (US Silica) column cleanup (Axys SOP #MLA-010, Rev. 08: Analytical Method for the

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Determination of 209 PCB Congeners by U.S. EPA Method 1688A of U.S. EPA Method CBC01.0, 4/12/06). The extract was then split, and one split was analyzed for pesticides (Axys SOP #MLA-007, Rev. 09: Analytical Method for the Determination of Aroclors, Total PCBs, Chlorinated Pesticides, PCB Congeners, Coplanar PCBs, Toxaphene, and Chlorobenzenes, 8/3/06) and most PCB congeners (Axys SOP #MLA-010, Rev. 08: Analytical Method for the Determination of 209 PCB Congeners by U.S. EPA Method 1688A of U.S. EPA Method CBC01.0, 4/12/06) using low-resolution GC–MS. The other split was analyzed for coplanar PCBs (Axys SOP #MLA-010, Rev. 08: Analytical Method for the Determination of 209 PCB Congeners by U.S. EPA Method 1688A of U.S. EPA Method CBC01.0, 4/12/06) with high-resolution GC–MS. The split for coplanar PCBs was further cleaned using an acid/base-layered silica column, a 4.5% carbon AX-21 (Anderson Development)/celite 545 (Sigma-Aldrich) mixture, and finally an alumina column.

Dietary and tissue PCDD, PCDF, and PBDE congeners. For PCDD/PCDF and PBDE analyses, the procedure was the same through the Biobeads SX-3 gel permeation column. After that, a fluid-management system was used with the following order of columns: jumbo acid silica, layered acid/base silica, alumina, and a carbon column. Individual PCDD/PCDF congeners (Axys SOP #MLA-017, Rev. 12: Analytical Method for the Determination of Polychlorinated Dibenzodioxins and Furans by U.S. EPA Method 1613B, 4/13/06) and individual PBDE congeners (Axys SOP #MLA-033, Rev 03: Analytical Method for Determination of Brominated Diphenyl Ethers (BDE) by U.S. EPA Method 1614, 6/26/06) were analyzed using high-resolution GC–MS. Percentage of lipids were determined gravimetrically for all samples by the weight of the total residue contained in an aliquot of the sample extract.

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Dietary and hepatic concentrations of analytes are presented on a wet-weight basis unless otherwise noted.

Data validation. Data validation was based on the quality-assurance/quality-control criteria documented in the Analytical Quality Assurance Plan: Hudson River Natural Resource Damage Assessment (version 2.0, September 1, 2005), U.S. EPA National Functional Guidelines for Organic Data Review (1999), U.S. EPA National Functional Guidelines for Inorganic Data Review (1994), and the individual laboratory standard operating procedures cited above.

Animals

The Michigan State University Institutional Animal Care and Use Committee approved the use of animals in the present study. Seventy-five first-year (virgin), natural dark, female mink and 30 first-year, natural dark, male mink from the Michigan State University Experimental Fur Farm herd were assigned to the six treatment groups on December 26, 2006. Littermates were not placed in the same treatment group, to minimize genetic predisposition to PCB toxicity. The control and targeted 5.4- and 7.2-µg ∑PCBs/g feed groups had 15 females and five males each and the targeted 0.90-, 1.8-, and 3.6-µg ∑PCBs/g feed groups had 10 females and five males each. The number of mink placed on trial balanced a reasonable expectation of detecting biologically meaningful events subject to the limitations of available time and resources to conduct the study. The control and the two highest treatment groups were assigned 15 females rather than 10 to increase sample sizes at dietary concentrations where severe effects were anticipated.

Housing

Female mink were housed individually in suspended wire cages (76 cm L × 61 cm W × 46 cm H) on the outside aisles of an open-sided mink shed. Five animals per treatment group

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were assigned to a bank of five cages. Assignment of treatments to banks of cages was done randomly. A wooden nest box (38 cm L × 28 cm W × 27 cm H) bedded with aspen shavings and excelsior (wood wool) was attached to the outside of each cage. Males were assigned to banks of five cages (61 cm L × 30 cm W × 38 cm H) on an inside aisle of the same shed. Feed and water were available ad libitum. The "Standard Guidelines for the Operation of Mink Farms in the United States" [23] were followed for housing and maintenance of animals.

Exposure period

Mink were started on their respective treatment diets on January 3, 2007, after a one-week acclimation period. Fresh feed was provided daily, and water was available ad libitum. One hour prior to feeding the treatment diets each day, animals were given 10 g of control feed to which thiamine had been added (0.25 mg thiamine hydrochloride/animal) to prevent Chastek's paralysis that could result from the incorporation of thiaminase-containing fish into the feed [22]. Feed consumption was determined for the same 2-d period each week, and body mass was recorded every two weeks. On February 14, 2007, in response to a decrease in feed consumption in the groups fed the diets that contained predominantly herring, the decision was made to incorporate a flavor enhancer (Proliant B3301 Spray-Dried Beef Flavor) and additional vitamin E into all treatment diets at rates of 66 and 4.4 g/kg feed, respectively. Animals were provided the diets containing the flavor enhancer beginning February 15, 2007, through the end of the trial. Measurement of feed consumption and determination of body mass were discontinued at the initiation of breeding, to reduce stress to the females. Measurement of feed consumption was not resumed after whelping because the females and their litters consumed the same feed until weaning. After weaning, kits were group-housed until they were approximately 10 weeks old, making accurate determination of individual feed consumption difficult. Because adult feed

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consumption cannot be measured from initiation of breeding through weaning of kits, the value for average daily feed consumption determined for the first seven weeks of the trial was applied for the total duration of the trial.

Females were mated to males within their respective treatment groups between March 1 and March 21, 2007. Each female was given an opportunity to mate every fourth day until a presumed successful mating occurred as determined by posture during mating and postcoital appearance of the female's vaginal area. A mated female was given an opportunity to breed with a different male the day following a successful mating and on the eighth and ninth days after a successful mating (a common commercial mink breeding practice).

The whelping period began on April 22 and ended on May 9, 2007. Nest boxes were checked on a daily basis for the presence of mink kits. The gender of the kits was determined at birth, and the live and stillborn young were counted. Body mass of kits was recorded at birth and at three and six weeks of age. Body mass of adult females was recorded at the time their litters were weighed.

All surviving adult mink were killed with CO_2 and necropsied between June 6 and June 19, 2007. Adult males were necropsied on June 6, 2007; adult females that did not whelp or lost their kits before weaning were necropsied on June 7, 2007; and adult females that whelped and successfully weaned their kits were necropsied on June 12 and June 19, 2007, with the exception of one female in the 7.2-µg ∑PCBs/g feed group that was still nursing a kit. The necropsy dates were chosen to allow assessment of placental scarring in females that did not whelp and in females that lost their kits before the uterine horns regressed and to allow those females that had kits to continue nursing until kits were six weeks of age. Altogether, 14 control females, nine 0.90-µg ∑PCBs/g feed females, nine 1.8-µg ∑PCBs/g feed females, two 3.6-µg ∑PCBs/g feed

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females, and three 5.4-µg ∑PCBs/g feed females were killed and necropsied during the last phase. The single surviving female in the 7.2-µg ∑PCBs/g feed group was necropsied on June 28, 2007.

On June 12 and June 19, 2007, a sample of weanling kits (approximately six weeks old) was killed and necropsied as previously described. This sample included 15 control kits (seven females, eight males), nine 0.90-µg ∑PCBs/g feed kits (four females, five males), and 13 1.8-µg ∑PCBs/g feed kits (seven females, six males). There were not sufficient numbers of kits in the 3.6-, 5.4-, and 7.2-µg ∑PCBs/g feed groups to justify necropsy of weanlings. Sampling was done to insure that as many litters as possible were represented.

The remaining kits were maintained on their respective treatment diets. On July 17, 2007, littermates were separated, with animals being assigned to individual cages (61 cm L × 30 cm W × 38 cm H) in the two interior rows of the open-sided shed. Body mass was determined on a monthly basis until termination of the trial during the first week in December. On December 4 and 5, 2007, seven-month-old animals in the two remaining treatment groups (16 females and 14 males in the control group and 11 females and 12 males in the 0.90-µg ∑PCBs/g feed treatment group) were killed and necropsied as previously described.

Statistical analyses

Toxic equivalents were calculated by summing the products of individual PCDD, PCDF, and PCB congener concentrations and their respective toxic equivalency factors (TEFs) [24]. Congeners that had concentrations less than the detection limit were assigned a concentration equal to one-half the detection limit. The choice of assigned value (0, one-half detection limit, or detection limit) had no substantive influence on ∑PCB congener or TEQ concentrations based on a quantitative assessment.

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Measurement end points of interest were classified into one of three data types and statistically analyzed according to data type. Possible data types were continuous measurements, such as ΣPCBs in livers; counts, such as kits per litter; or binary outcomes, such as whether or not an individual kit survived to three weeks. Statistical analyses of measurement end points were conducted using a generalized linear model framework [25] where the most appropriate class of linear models was selected based on classification of data type and correlation structure (e.g., repeated measures, kits clustered within litters). A summary of endpoints classified by data type and analysis method is provided in Table 1.

Continuous endpoints were analyzed by analysis of variance (ANOVA) [26] when the endpoint was measured at the experimental unit level and the experimental units within a treatment group were expected to be independent (e.g., adult mink). For example, gestation lengths for pregnant females in the same treatment group were expected to be independent. Continuous endpoints having repeated measures on an individual animal or kits clustered within litters were analyzed with linear generalized estimating equation (GEE) regression. The GEE models are a contemporary extension of generalized linear models for clustered data that adjust for within-cluster correlation [27]. Examples of clustered data in the present study were repeated measures of adult female body mass and kit liver ΣPCB concentrations within litters. The adult female body mass model for the prebreeding period was adjusted for baseline body mass and days on treatment. Likewise, the adult female feed consumption model was adjusted for days on treatment.

Count endpoints measured on adults, including number of kits whelped and kits whelped alive, were analyzed using negative binomial regression models. Treatment effects on the rate of kits whelped alive were estimated as differences (%) in rate of kits whelped alive per litter from

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control. Refitting the model with ΣPCBs in feed as a continuous variable, the relative rate of live kits per litter for a given increase in micrograms ∑PCBs/g feed (Δ) was estimated.

The minimum dietary concentrations necessary to induce 20 and 50% kit mortality (LC20 and LC50) were estimated based on the maximum likelihood estimates provided by the beta-binomial regression. Dose–response relationships were estimated for ΣPCBs as well as TEQs. The LC20 and LC50 values expressed as ΣPCBs and TEQs were estimated (with 95% CIs) for stillbirth, mortality at three weeks, and mortality at six weeks of age. Control-adjusted LC20 and LC50 values were also estimated based on the same maximum likelihood estimates of the beta-binomial regression and summarized in Supplemental Data, Table S2.

An LC50 expressed as dietary ΣPCB and TEQ concentrations for mortality by 31 weeks of age was estimated as the concentration corresponding to 1 minus the joint probability of survival from birth to the six-week necropsy period and from the six-week necropsy period to the end of the juvenile growth trial (31 weeks of age). This method accounted for the kits necropsied postweaning. The survival models were beta-binomial models previously described for modeling mortality at three and six weeks of age. The LC50 was determined by evaluating the joint probability over a range of ΣPCBs.

The product of these survival probabilities is the joint probability of surviving to 31 weeks of age, and the complement (1 minus the joint probability) is the probability of death before 31 weeks of age. This method accounted for the kits necropsied postweaning. The survival models were beta-binomial models previously described for modeling mortality at three and six weeks of age. The LC50 was determined by iteratively solving for ΣPCBs.

All statistical analyses were conducted using R statistical software (http://www.r-project.org/) including the additional R packages MASS for negative binomial models, aod for

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beta-binomial models, geepack for GEE models, and doBy for linear functions of estimated GEE regression parameters.

RESULTS

Dietary contaminant concentrations

The analyzed concentrations of ∑PCBs in feed were similar to the nominal doses, and PCDDs and PCDFs contributed less than 2% of the TEQs (Table 2). The analyzed concentrations of ∑PCBs (±standard deviation) were 0.0074 (0.0016), 0.72 (0.12), 1.5 (0.21), 2.8 (0.38), 4.5 (0.49), and 6.1 (0.51) µg/g feed. These values will be used subsequently rather than the targeted concentrations. The corresponding concentrations of $TEQs_{WHO\ 2005}$ (±standard deviation) were 0.41 (0.022), 4.8 (0.15), 10 (0.074), 18 (0.13), 28 (0.12), and 38 (0.38) pg/g feed, respectively. The percentage of contribution of PCDDs, PCDFs, non-ortho PCBs, and mono-ortho PCBs to the $TEQs_{WHO\ 2005}$ in the treatment diets averaged 1.5, 1.4, 75, and 22%, respectively. Thus, 97% of the $TEQs_{WHO\ 2005}$ in the diet was from PCBs with 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126 using International Union of Pure and Applied Chemistry nomenclature) contributing 74% of the $TEQs_{WHO\ 2005}$. The predominant organochlorine pesticide present in the feed was total toxaphene at a maximum concentration of 0.24 µg/g feed, followed by ∑dichlorodiphenyltrichloroethane (DDT) at a maximum concentration of 0.019 µg/g feed (data not shown). Mercury was present at a maximum concentration of 0.031 µg/g feed. The maximum ∑PBDE concentration was 0.032 µg/g feed, with BDE 47 accounting for 73% of the ∑PBDE concentration (Supplemental Data, Table S1).

Hepatic ∑PCB and $TEQ_{WHO\ 2005}$ concentrations

Hepatic ∑PCB and $TEQ_{WHO\ 2005}$ concentrations of PCB-exposed adult female mink were significantly greater ($p < 0.025$ and $p < 0.004$, respectively) than control concentrations and

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increased monotonically with feed ∑PCB concentrations (Table 3). Concentrations in livers of adult males (not shown) were not significantly different from those in females. The percentage of contribution of PCDDs, PCDFs, non-ortho PCBs, and mono-ortho PCBs to the $TEQs_{WHO\ 2005}$ in the maternal livers averaged 0.80, 1.4, 82, and 16%, respectively. Thus, 98% of hepatic $TEQs_{WHO\ 2005}$ was from PCBs, with 82% being contributed by PCB 126.

Hepatic ∑PCB and $TEQ_{WHO\ 2005}$ concentrations (based only on non-ortho and mono-ortho PCBs) in livers of six-week-old kits (nine animals) and 31-week-old juveniles (23 animals) were significantly greater ($p < 0.001$) in the 0.72-µg ∑PCBs/g feed group compared to controls (15 kits and 30 juveniles; Table 4). The percentage of contribution of non-ortho and mono-ortho PCBs to the $TEQs_{WHO\ 2005}$ averaged 77 and 23% in the kit livers and 81 and 19% in the juvenile livers, with PCB 126 contributing all of the non-ortho $TEQs_{WHO\ 2005}$.

Adult feed consumption

Feed consumption of adult female mink did not differ significantly ($p = 0.084$) across treatment groups during the seven-week period prior to breeding. Average daily feed consumption for adult female mink was 115 g feed/d.

Reproductive performance and offspring survivability

Although all 75 adult females on the trial were successfully mated during the three-week breeding period, the mean number of kits whelped alive per litter was less at higher dietary PCB concentrations compared to lower dietary PCB concentrations ($p < 0.004$). The percentage of females whelping at least one kit (dead or alive) decreased slightly with increasing dietary PCB concentration, although this decrease was not statistically significant ($p = 0.110$). The percentage of females whelping at least one kit was 100% for females in the control and 0.72- and 1.5-µg ∑PCBs/g feed groups and 90, 87, and 73% for females in the 2.8-, 4.5-, and 6.1-µg ∑PCBs/g

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feed groups, respectively. One female each in the 2.8- and 4.5-µg ∑PCBs/g feed groups and three females in the 6.1-µg ∑PCBs/g feed group did not whelp. Of these, one female each in the 2.8- and 6.1-µg ∑PCBs/g feed groups had uterine implantation sites, indicating conception, while the female in the 4.5-µg ∑PCBs/g feed group and two females in the 6.1-µg ∑PCBs/g feed group did not. Average gestation lengths, which ranged from 46.3 to 48.7 d, were similar for all treatment groups ($p = 0.74$). Compared to controls, the mean number of kits whelped alive per litter was 44% less (CI 17–63%, $p = 0.0039$) in the 4.5- and 49% less (CI 21–67%, p = 0.0023) in the 6.1-µg ∑PCBs/g feed groups (Table 5). The estimated relative rate of live kits per litter for a given increase in micrograms ∑PCBs/g feed (Δ) was $e^{-0.17\Delta}$ ($p < 0.001$). Increases of 1 and 2 µg ∑PCBs/g feed were associated with 15.9 and 29.3% reductions in mean live kits whelped per litter.

Kit mortality increased over time, ultimately resulting in no kits surviving to the end of the trial with the exception of those in the control and 0.72-µg ∑PCBs/g feed groups. The odds of kit mortality at six weeks of age were 22 (CI 3.7–129, $p < 0.001$) times greater in the 2.8-, 9.4 (CI 2.1–42, $p = 0.0035$) times greater in the 4.5-, and 40 (CI 4.4–369, $p = 0.0011$) times greater in the 6.1-µg ∑PCBs/g feed groups than the control group (Table 6). The numbers of kits alive at weaning, taking into consideration animals that were necropsied at six weeks of age, were 51, 30, 38, 9, 15, and 2 in the control and 0.72-, 1.5-, 2.8-, 4.5-, and 6.1-µg ∑PCBs/g feed groups, respectively. Between six and 10 weeks of age (when young mink were housed individually), 4 (7.8%), 6 (20%), 33 (87%), 8 (89%), 15 (100%), and 2 (100%) animals in the control and 0.72-, 1.5-, 2.8-, 4.5-, and 6.1-µg ∑PCBs/g feed groups died, respectively. The seven juvenile deaths that occurred from July 12, 2007, to the termination of the trial were one juvenile in the 0.72-µg ∑PCBs/g feed group, the remaining five juveniles in the 1.5-µg ∑PCBs/g feed group, and the

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remaining juvenile in the 2.8-µg ∑PCBs/g feed group. Thus, at the end of the trial, the only animals remaining were 47 juveniles in the control group and 23 juveniles in the 0.72-µg ∑PCBs/g feed group. Figure 2 depicts offspring mortality between 6 and 31 weeks of age, and Supplemental Data, Table S3 presents the number of offspring alive at key time points throughout the study.

Body mass

At the start of the experiment, adult female body mass did not differ significantly across treatment groups ($p = 0.916$) but generally decreased in all treatment groups over the first eight weeks of the trial. Body mass was significantly greater in the 1.5- (1,166 g, CI 1,119–1,213, $p = 0.014$), 4.5- (1,190 g, CI 1,156–1,224, $p < 0.001$), and 6.1- (1,206 g, CI 1,179–1,232, $p < 0.001$) µg ∑PCBs/g feed groups compared to controls (1,103 g, CI 1,072–1,134).

Body mass of individual kits was not significantly different at whelping ($p = 0.21$), but average kit masses in the 1.5-, 2.8-, and 4.5-µg ∑PCBs/g feed groups (10, 19, and 28 $TEQs_{WHO\ 2005}$/g feed, respectively) were, respectively, 27 g (CI 8.3–45.0, $p = 0.0044$) or 25%, 29 g (CI 3.7–54.6, $p = 0.025$) or 27%, and 50 g (CI 29.1–71.3, $p < 0.001$) or 46% less than controls at three weeks of age and 47 g (CI 8.5–86.0, $p = 0.017$) or 21%, 47 g (CI 2.0–92.5, $p = 0.041$) or 21%, and 73 g (CI 23.8–123.1, $p = 0.0037$) or 33% less than controls at six weeks of age. The 6.1-µg ∑PCBs/g feed group was omitted from analysis at three and six weeks of age because the regression models were not estimable with only two live kits remaining in this treatment group (Supplemental Data, Table S4).

The only significant treatment effect on juvenile growth was that males in the 0.72-µg ∑PCBs/g feed group gained 253 g (CI 82–401, $p = 0.003$) or 34% more than males in the control group. At the end of the trial, mean change in body mass in the control group was 476 g for

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females and 750 g for males, and in the 0.72-µg ∑PCBs/g feed group mean change in body mass was 488 g for females and 1,003 g for males (data not presented).

Lethal concentration estimates

Estimated dietary and maternal hepatic LC20 and LC50 values based on kit stillbirths and kit mortality at three and six weeks of age were derived in terms of ∑PCBs/g and $TEQs_{WHO\ 2005}$/g (Supplemental Data, Table S5). Control adjusted LC20 and LC50 values were also estimated (Supplemental Data, Table S2). Plots illustrating dose–response curves used to derive LC20 and LC50 values for kit mortality at six weeks of age based on dietary ∑PCB and $TEQ_{WHO\ 2005}$ concentrations and maternal hepatic ∑PCB and $TEQ_{WHO\ 2005}$ concentrations are presented in Figures 3 and 4, respectively. The estimated LC50 for mortality by 31 weeks of age was 0.78 µg ∑PCBs/g feed.

DISCUSSION

Dietary contaminant concentrations

The analyzed concentrations of ∑PCBs (0.72–6.1 ∑PCBs/g feed) in the treatment diets and the derived $TEQs_{WHO\ 2005}$ (4.8–38 pg $TEQs_{WHO\ 2005}$/g feed) were similar to those used in other mink feeding studies with a similar design [12–17]. In addition, the congener profile in most cases was similar across studies. Non-ortho PCBs (75%), and PCB 126 specifically (74%), contributed the majority of dietary $TEQs_{WHO\ 2005}$ derived from HR fish. A mink feeding study of a similar design was conducted using PCB-contaminated fish collected from the Housatonic River (Massachusetts, USA) [16,17]. Housatonic River diets contained ∑PCB concentrations ranging from 0.34 to 3.7 µg/g feed and $TEQ_{WHO\ 2005}$ concentrations ranging from 2.5 to 51 pg/g feed (recalculated using TEF values presented in Van den Berg et al. [24]), with 87% of the $TEQs_{WHO\ 2005}$ being contributed by non-ortho PCBs and 81% by PCB 126 specifically. Two

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additional mink feeding studies of a comparable design used fish containing PCBs collected from Saginaw Bay (Lake Huron, Michigan, USA) or the mouth of the Saginaw River (Michigan, USA), which empties into Saginaw Bay. In the Saginaw Bay study [12–14], dietary concentrations ranged from 0.72 to 2.6 µg ∑PCBs/g feed (17–66 pg $TEQs_{WHO\ 2005}$/g feed, recalculated using TEF values presented in Van den Berg et al. [24]), with 64% of the $TEQs_{WHO\ 2005}$ contributed by non-ortho PCBs (62% by PCB 126). In the Saginaw River study [15], which was conducted 14 years after the Saginaw Bay study, dietary ∑PCB concentrations ranged from 0.83 to 1.7 µg ∑PCBs/g feed and 22 to 57 pg $TEQs_{WHO\ 2005}$/g feed (recalculated using TEF values presented in Van den Berg et al. [24]). In this study, non-ortho PCBs and PCDDs each contributed 35% of the $TEQs_{WHO\ 2005}$ and PCDFs contributed 25%. The single largest contributor of $TEQs_{WHO\ 2005}$ was PCB 126 (33%). It should be pointed out that there are some limitations with the TEQ approach. Toxic equivalency factors are consensus values of the relative potencies of the various PCB/PCDD/PCDF congeners rather than precise values. They may vary depending upon species, measurement end points, and the relative proportions of individual congeners in complex mixtures. Some TEFs are based on in vitro studies that do not account for the potential differences between animal species in absorption, distribution, metabolism, and elimination. Other TEFs are based on quantitative structure–activity relationships because of a lack of toxicity data for some congeners, which can introduce a degree of uncertainty into the determination of TEQs. As such, TEFs may under- or overestimate the relative potencies of congeners and, thus, should be considered as protective estimates as opposed to predictors of effect thresholds [20].

The dietary concentrations of ∑PCBs used in the present study bracket dietary concentrations of ∑PCBs that are relevant for wild mink residing along the upper Hudson River.

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The National Oceanic and Atmospheric Administration (NOAA) database of tissue residue data for specific watershed projects was searched for whole-body analyses of fish sampled in the Hudson River between latitudes 42.87804 and 43.199263, which encompasses the three carp collection sites for the present study and fish sizes of 7 to 20 cm, which is the expected size of typical prey fish consumed by mink [17]. The data used were from sampling studies conducted by NOAA in 1993, 1995, and 1999 and by the General Electric Company from 2004 to 2009. Fish species sampled in this region included the largemouth bass (*Micropterus salmoides*), pumpkinseed sunfish (*Lepomis gibbosus*), redbreast sunfish (*Lepomis auritus*), yellow perch (*Perca flavescens*), golden shiner (*Notemigonus crysoleucas*), bluntnose minnow (*Pimephales notatus*), spottail shiner (*Notropis hudsonius*), common shiner (*Luxilus cornutus*), spotfin shiner (*Notropis spilopterus*), and mimic shiner (*Notropis volucellus*). The average ∑PCB concentration for all fish sampled between these two latitudes was 4.0 μg/g tissue (n = 430, range 0.32–25). Fish collected at sites closest to the carp collection sites in the present study had average ∑PCB concentrations of 4.6 μg/g tissue for the Moses Kill area (n = 55, range 0.73–16), 4.5 μg/g tissue for the Northumberland Pool area (n = 36, range 0.81–15), and 2.4 μg/g tissue for the above Lock 2 site (n = 70, range 0.48–5.5). The average ∑PCB concentration for fish sampled at these three sites on the Hudson River was 3.8 μg/g tissue (Supplemental Data, Table S6).

Daily doses (based on dietary concentrations and an average daily feed consumption of 97 g/kg body mass that was calculated by dividing average feed consumption per animal [115 g] by average body wt of adult females across the six treatment groups [1,186 g]) of the various organochlorine pesticides as well as the potentially toxic metals (arsenic, cadmium, chromium, copper, mercury, nickel, lead, selenium, and zinc) present in the treatment diets were less than the toxicity reference values for mink reported by Hink et al. [28]. Similarly, dietary

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concentrations of PBDEs were less than those reported to cause adverse effects in mink [29].

Hepatic ∑PCB and TEQ$_{WHO\ 2005}$ concentrations

Hepatic concentrations of ∑PCBs and TEQ$_{WHO\ 2005}$ concentrations increased with dietary concentration and were similar between adult male and female mink. The percentage of hepatic TEQs$_{WHO\ 2005}$ compared to dietary TEQs$_{WHO\ 2005}$ contributed by non-ortho PCBs increased slightly (from 75 to 82%), while the contribution by mono-ortho PCBs decreased by 5%. The PCB 126 contribution increased from 74 to 82% when comparing dietary to hepatic TEQs$_{WHO\ 2005}$, respectively. Bioaccumulation factors (BAFs) were derived by dividing the hepatic concentration (wet wt) of ∑PCBs and TEQs$_{WHO\ 2005}$ by dietary ∑PCB and TEQ concentrations (wet wt), respectively. The BAF for ∑PCBs was 1.2 and the BAF for TEQs$_{WHO\ 2005}$ was 5.9. These BAFs are similar to those based on dietary and hepatic ∑PCB and TEQ$_{WHO\ 2005}$ concentrations reported for the Housatonic River study (1.2 and 4.0, respectively) [16,17]. The BAFs for individual PCB congeners that contributed the majority of TEQs$_{WHO\ 2005}$ resembled the BAF for ∑TEQs$_{WHO\ 2005}$ (PCB 126 = 6.5, PCB 105 [2,3,3′,4,4′-pentachlorobiphenyl] = 4.1, PCB 118 [2,3′4,4′,5-pentachlorobiphenyl] = 4.3, and PCB 156 [2,3,3′,4,4′,5-hexachlorobiphenyl] = 7.7). Hepatic concentrations of ∑PCBs and TEQs$_{WHO\ 2005}$ in six-week-old kits and 31-week-old juveniles were similar to adult concentrations, as was reported for mink exposed to PCBs derived from fish collected from the Housatonic River [17].

Adult feed consumption

Adult feed consumption did not differ significantly over the first seven weeks of the trial, although a flavor enhancer was added to feed beginning in week 6 following the observation during week 5 that feed consumption decreased by approximately 50% in those groups fed diets containing primarily herring (control and 0.72- and 1.5-µg ∑PCBs/g feed groups). The inclusion

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of a flavor enhancer in all of the treatment diets resulted in an immediate increase in feed intake such that feed consumption was again comparable across all groups by week 6. The average daily feed consumption of 115 g reported here is identical to the value reported for adult female ranch mink [30] and similar to the 127-g/day rate reported for mink fed diets containing fish collected from the Housatonic River [16].

Reproductive performance and offspring mortality

The effects of consumption of diets containing PCB-contaminated fish collected from the Hudson River on reproductive performance were similar to those reported for mink fed diets containing PCB/PCDD/PCDF-contaminated fish collected from Saginaw Bay [12,14]. There was no significant effect on the percentage of females whelping at least one kit, but there was significant reduction in the mean number of kits whelped alive per litter in both studies. The no observed adverse effect levels (diet-based)/concentrations (tissue-based; NOAELs/NOAECs) and lowest observed adverse effect levels/concentrations (LOAELs/LOAECs) are presented in Table 7.

An increase in kit mortality is the effect most often reported in mink reproduction studies using PCB/PCDD/PCDF-contaminated fish. In addition to the present study, kit mortality was significantly increased in the Saginaw Bay study [12,14] and the Housatonic River study [16]. In the latter study, the contribution by non-ortho PCBs and PCB 126 to ∑TEQs was similar to the present study. The NOAEL/NOAEC and LOAEL/LOAEC for each study are presented in Table 7. It is interesting to note that an equivalent dietary concentration of $TEQs_{WHO\ 2005}$ provided only by PCB 126 had no effect on kit mortality through six weeks of age [18]. The differences in NOAEL(C)s ranged from 3.6-fold (hepatic TEQs) to 106-fold (dietary ∑PCBs). The differences

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in LOAEL(C)s for increased kit mortality were considerably less, ranging from 1.5-fold (hepatic ∑PCBs) to fivefold (dietary ∑PCBs).

A treatment-related increase in juvenile mortality between weaning at six weeks of age and the end of the growth period at approximately 30 weeks of age was reported only in the present study. Between 6 and 10 weeks of age, siblings were still group-housed and the larger, stronger animals were more effective at competing for feed compared to their smaller, weaker littermates. Smaller animals that continue to grow poorly may die, even after being housed singly at 10 to 12 weeks of age. Because dietary concentrations of 1.5 µg ∑PCBs/g feed and greater resulted in decreased kit body mass, it was not unexpected that animals in these treatment groups experienced excessive mortality after weaning. In the Saginaw Bay study [12,14], kits were not maintained beyond weaning; in the Housatonic River study [16], offspring maintained through 27 weeks had similar growth rates.

Body mass

The significantly greater body masses of adult females at the higher dietary concentrations of PCBs compared to controls could be a reflection of the initial decrease in feed intake of control animals fed diets containing 20% ocean herring. While feed consumption was equivalent across groups after beef flavoring was added to all treatment diets, it is apparent that control females continued to have a lower body mass.

Body masses of treated kits were comparable at whelping but significantly less compared to controls at six weeks of age in both the Saginaw Bay study [12,14] and the present study. The NOAELs/NOAECs and LOAELs/LOAECs for the respective studies are presented in Table 7. In contrast, body mass of kits exposed to an equivalent or greater concentration of $TEQs_{WHO\ 2005}$ provided only by PCB 126 was not adversely affected [18].

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It is not clear why male juveniles in the 0.72-µg ∑PCBs/g feed group gained 253 g more mass compared to controls by the end of the trial. In the Housatonic River study [16], exposure to PCBs had no effect on juvenile growth through 27 weeks of age.

Lethal concentration estimates

Because there are acknowledged limitations associated with use of NOAELs/NOAECs and LOAELs/LOAECs [30,31] in risk assessment, LC20 and LC50 values were derived for kit mortality in the present study. Regression estimates of lethal concentrations (LC20 and LC50) are less sensitive to the idiosyncrasies of experimental design, and estimates are not limited to the concentrations under study. The only mink feeding study of a similar design as the present study that reported lethal dietary concentrations was the Housatonic River study [16]. There is a 2.9-fold difference between the Hudson River and Housatonic River LC20 values based on ∑PCBs with overlapping confidence intervals. The LC20 values based on $TEQs_{WHO\ 2005}$ differ by 5.5-fold with no overlap in confidence intervals (Table 7). Assuming an average daily feed intake of 97 g/kg body mass (based on average feed consumption and body weight of adult females across the six treatment groups), the dose lethal to 20% of the population (LD20) was 33 µg ∑PCBs/kg body mass daily or 281 pg $TEQs_{WHO\ 2005}$/kg body mass daily. The corresponding LD20 values for the Housatonic River were 105 µg ∑PCBs/kg body mass daily or 1,730 pg $TEQs_{WHO\ 2005}$/kg body mass daily [16].

Comparing results among studies in which mink are fed site-specific fish is complicated by potential differences in the presence or absence of toxic contaminants, the ratios among them, and the presence of other dietary constituents that could alter the apparent overall toxicity of the measured contaminants. As mentioned previously, dietary concentrations of PBDEs, organochlorine pesticides, and potentially toxic metals were less than those reported to cause

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adverse effects in mink [28,29]. Difference in dietary, and thus hepatic, profiles of TCDD-like congeners between the sites could also influence toxicity. While the TEF system theoretically normalizes the contribution of individual TCDD-like chemicals to overall TCDD toxicity, the system may not adequately address agonistic and antagonistic interactions between components of a complex mixture [14,20]. The addition of thiamine to the feed to counteract the effects of thiaminase present in the fish could potentially ameliorate the effects induced by PCBs in that there is evidence that dietary exposure to PCBs and other organochlorine chemicals interferes with thiamine metabolism, resulting in reduced thiamine concentrations in tissues [33,34].

The results of the present study indicated that mink reproductive performance as well as offspring survivability and growth were adversely affected by consumption of feed containing PCB-contaminated fish collected from the Hudson River from two months prior to breeding through the growth period. All offspring exposed to dietary concentrations of 1.5 μg ∑PCBs/g feed and above died by 31 weeks of age. A dietary concentration of 0.34 μg ∑PCBs/g feed (2.6 pg $TEQs_{WHO\ 2005}$/g feed) was predicted to result in 20% kit mortality by six weeks of age. Assuming that mink residing along the upper Hudson River consume fish containing an average of 4.0 μg ∑PCBs/g and that fish is the only component of the mink's diet that contains PCBs or other aryl hydrocarbon receptor–active compounds, a diet comprised of less than 10% fish with these environmentally relevant PCB concentrations could be expected to result in kit mortality.

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SUPPLEMENTAL DATA

Tables S1–S6. (48 KB XLS)

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Fig. 1. Map indicating the three sites on the upper Hudson River (New York, USA) from which carp (*Cyprinus carpio*) was collected.

Fig. 2. Number of live kits in each feed group based on dietary concentration at 6, 10, and 31 weeks of age. The number of live kits at six weeks of age reflects the number alive after necropsy. The percentage of live kits at 10 and 31 weeks of age compared to six weeks of age is given in parentheses.

Fig. 3. Lethal concentration (LC) 50 and LC20 with 95% confidence interval (horizontal interval) for increased mortality of six-week-old kits based on micrograms ∑polychlorinated biphenyls (PCBs)/g feed (top) and picograms toxic equivalents (TEQs)/g feed (bottom).

Fig. 4. Lethal concentration (LC) 50 and LC20 with 95% confidence interval (horizontal interval) for increased mortality of six-week-old kits based on maternal hepatic concentrations of ∑polychlorinated biphenyls (PCBs; micrograms per gram liver, wet wt; top) and toxic equivalents (TEQs; picograms per gram liver, wet wt; bottom).

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Table 1. Summary of study end points, data types, and statistical methods

Endpoint	Data type	Statistical methods
Adult body mass	Continuous	GEEs, regression for repeated measures
Adult feed consumption	Continuous	GEEs, regression for repeated measures
Length of gestation	Continuous	Linear regression/ANOVA
Females whelping	Binary	Fisher's exact test
Number kits whelped per female	Count	Negative binomial regression
Number kits whelped live per female	Count	Negative binomial regression
Kit mass at birth, three and six weeks of age	Continuous	Linear GEE regression
Stillbirth and kit mortality at three and six weeks of age	Binary	Beta-binomial regression
Change in body mass of seven-month-old juveniles during growth trial	Continuous	Linear GEE regression
∑PCBs and ∑TEQs in adult livers	Continuous	Linear regression/ANOVA
∑PCBs and ∑TEQs in six-week-old kit livers	Continuous	Linear GEE regression
∑PCBs and ∑TEQs in seven-month-old juvenile livers	Continuous	Linear GEE regression

PCB = polychlorinated biphenyl; TEQ = toxic equivalent; ANOVA = analysis of variance; GEE = generalized estimating equation.

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Table 2. Mean concentrations of total polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), non-*ortho* PCBs, and mono-*ortho* PCBs as well as toxic equivalents (TEQs) [a] in experimental mink diets

		Dietary concentration (µg ∑PCBs/g feed)					
Compound	TEF [b]	Control	0.72	1.5	2.8	4.5	6.1
PCDDs [c]							
2,3,7,8-TCDD (ng/g)	1	0.000074	0.000073	0.000074	0.000074	0.000073	0.000079
TEQs (pg/g)		0.074	0.073	0.074	0.074	0.073	0.079
1,2,3,7,8-PeCDD (ng/g)	1	0.000074	0.000073	0.000074	0.000073	0.000073	0.00011
TEQs (pg/g)		0.074	0.073	0.074	0.073	0.073	0.11
1,2,3,4,7,8-HxCDD (ng/g)	0.1	0.000074	0.000073	0.000074	0.000073	0.000073	0.000073
TEQs (pg/g)		0.0074	0.0073	0.0074	0.0073	0.0073	0.0073
1,2,3,6,7,8-HxCDD (ng/g)	0.1	0.000074	0.000073	0.000074	0.00018	0.00022	0.00034
TEQs (pg/g)		0.0074	0.0073	0.0074	0.018	0.022	0.034
1,2,3,7,8,9-HxCDD (ng/g)	0.1	0.000074	0.000073	0.000074	0.000073	0.000073	0.000073
TEQs (pg/g)		0.0074	0.0073	0.0074	0.0073	0.0073	0.0073
1,2,3,4,6,7,8-HpCDD (ng/g)	0.01	0.00015	0.00027	0.00035	0.00060	0.00072	0.00092
TEQs (pg/g)		0.0015	0.0027	0.0035	0.0060	0.0072	0.0092

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OCDD (ng/g)	0.0003	0.0013	0.0015	0.0017	0.0020	0.0025	0.0031
TEQs (pg/g)		0.00039	0.00046	0.00050	0.00061	0.00074	0.00093
∑PCDDs (ng/g)		0.0018	0.0022	0.0024	0.0031	0.0037	0.0047
PCDD TEQs (pg/g)		0.17	0.17	0.17	0.19	0.19	0.25
% of ∑TEQs		42	3.6	1.7	1.0	0.68	0.65
PCDFs [c]							
2,3,7,8-TCDF (ng/g)	0.1	0.00022	0.00029	0.00035	0.00060	0.00054	0.00079
TEQs (pg/g)		0.022	0.029	0.035	0.060	0.054	0.079
1,2,3,7,8-PeCDF (ng/g)	0.03	0.000074	0.000073	0.000074	0.000077	0.000073	0.00016
TEQs (pg/g)		0.0022	0.0022	0.0022	0.0023	0.0022	0.0047
2,3,4,7,8-PeCDF (ng/g)	0.3	0.000074	0.00019	0.00024	0.00045	0.00059	0.00069
TEQs (pg/g)		0.022	0.058	0.073	0.14	0.18	0.21
1,2,3,4,7,8-HxCDF (ng/g)	0.1	0.000074	0.000073	0.000074	0.000073	0.000073	0.000073
TEQs (pg/g)		0.0074	0.0073	0.0074	0.0073	0.0073	0.0073
1,2,3,6,7,8-HxCDF (ng/g)	0.1	0.000074	0.000073	0.000074	0.000073	0.000073	0.000073
TEQs (pg/g)		0.0074	0.0073	0.0074	0.0073	0.0073	0.0073
1,2,3,7,8,9-HxCDF (ng/g)	0.1	0.000074	0.000073	0.000074	0.000073	0.000073	0.000073

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TEQs (pg/g)		0.0074	0.0073	0.0074	0.0073	0.0073	0.0073
2,3,4,6,7,8-HxCDF (ng/g)	0.1	0.000074	0.000073	0.000074	0.000073	0.000073	0.000073
TEQs (pg/g)		0.0074	0.0073	0.0074	0.0073	0.0073	0.0073
1,2,3,4,6,7,8-HpCDF (ng/g)	0.01	0.000074	0.000073	0.000074	0.000099	0.00014	0.00021
TEQs (pg/g)		0.00074	0.00073	0.00074	0.0010	0.0014	0.0021
1,2,3,4,7,8,9-HpCDF (ng/g)	0.01	0.000074	0.000073	0.000074	0.000073	0.000073	0.000073
TEQs (pg/g)		0.00074	0.00073	0.00074	0.00073	0.00073	0.00073
OCDF (ng/g)	0.0003	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037
TEQs (pg/g)		0.00011	0.00011	0.00011	0.00011	0.00011	0.00011
∑PCDFS (ng/g)		0.0012	0.0014	0.0015	0.0020	0.0021	0.0026
PCDF TEQs (pg/g)		0.077	0.12	0.14	0.23	0.26	0.32
% of ∑TEQs		19	2.5	1.4	1.3	0.94	0.85
Non-*ortho* PCBs [c]							
PCB 77 (ng/g)	0.0001	0.0048 [d]	0.28	0.58	1.1	1.7	2.2
TEQs (pg/g)		0.00048	0.028	0.058	0.11	0.17	0.22
PCB 81 (ng/g)	0.0003	0.00016 [d]	0.040	0.081	0.16	0.23	0.32
TEQs (pg/g)		0.000048	0.012	0.024	0.048	0.069	0.095

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PCB 126 (ng/g)	0.1	0.0013 [d]	0.034 [d]	0.075	0.13 [d]	0.21	0.28
TEQs (pg/g)		0.13	3.4	7.5	13	21	28
PCB 169 (ng/g)	0.03	0.00031 [d]	0.00064 [d]	0.0014 [d]	0.0021 [d]	0.0037 [d]	0.0049 [d]
TEQs (pg/g)		0.0094	0.019	0.042	0.062	0.11	0.15
∑Non-*ortho* PCBs (ng/g)		0.0066	0.36	0.73	1.4	2.1	2.8
Non-*ortho* PCB TEQs (pg/g)		0.14	3.4	7.6	14	21	29
% of ∑TEQs		33	72	76	76	76	76
Mono-*ortho* PCBs [c]							
PCB 105 (ng/g)	0.00003	0.16[d]	9.3	19	34	56	78
TEQs (pg/g)		0.0048	0.28	0.56	1.0	1.7	2.4
PCB 114 (ng/g)	0.00003	0.0087	0.85	1.8	3.4	5.0	7.2
TEQs (pg/g)		0.00026	0.025	0.053	0.10	0.15	0.21
PCB 118 (ng/g)	0.00003	0.61	21	42	82	127	177
TEQs (pg/g)		0.018	0.64	1.3	2.4	3.8	5.3
PCB 123 (ng/g)	0.00003	0.0073	0.63	1.3	2.4	3.7	5.2
TEQs (pg/g)		0.00022	0.019	0.039	0.071	0.11	0.15

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PCB 156 (ng/g)	0.00003	0.044	1.3	2.6	4.9	7.6	11
TEQs (pg/g)		0.0013	0.039	0.078	0.15	0.23	0.32
PCB 157 (ng/g)	0.00003	0.017	0.34	0.67	1.3	1.9	2.6
TEQs (pg/g)		0.00052	0.010	0.020	0.038	0.058	0.079
PCB 167 (ng/g)	0.00003	0.027	0.55	1.1	2.0	3.2	4.3
TEQs (pg/g)		0.00081	0.016	0.033	0.061	0.095	0.13
PCB 189 (ng/g)	0.00003	0.0037	0.071	0.14	0.26	0.40	0.55
TEQs (pg/g)		0.00011	0.0021	0.0042	0.0079	0.012	0.017
∑Mono-*ortho* PCBs (ng/g)		0.88	34	69	129	205	286
Mono-*ortho* PCB TEQs (pg/g)		0.026	1.0	2.1	3.9	6.1	8.6
% of ∑TEQs		6.4	21.4	20.6	21.6	22.0	22.6
∑PCBs (µg/g) [c]		0.0074	0.72	1.5	2.8	4.5	6.1
SD		0.0016	0.12	0.21	0.38	0.49	0.51
∑TEQs (pg/g)		0.41	4.8	10	18	28	38
SD		0.022	0.15	0.074	0.13	0.12	0.38
% Lipids		12.16	12.40	12.30	12.20	12.44	12.74

[a] TEQs based on nondetects = 0.5 × detection limit.
[b] Toxic equivalency factors (TEFs) from Van den Berg et al. [24].

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[c] Concentrations of PCDD and PCDF are means of three samples. Concentrations of PCB and ∑PCB are means of five samples.
[d] Some values used in the calculation of these means were estimated as one-half of the detection limit.
TCDD = tetrachlorodibenzo-*p*-dioxin; PeCDF = pentochlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; HpCDD = heptachlorodibenzo-*p*-dioxin; OCDD = octachlorodibenzo-p-dioxin; TCDF = tetrachlorodibenzofuran; PeCDF = pentachlorodibenzofuran; HxCDF = hexachlorodibenzofuran; HpCDF = heptachlorodibenzofuran; OCDF = octachlorodibenzofuran. Nomenclature for the PCB congeners follows that of the International Union of Pure and Applied Chemistry: PCB 77 = 3,3′,4,4′-tetrachlorobiphenyl; PCB 81 = 3,4,4′,5-tetrachlorobiphenyl; PCB 126 = 3,3′,4,4′,5-pentachlorobiphenyl; PCB 169 = 3,3′,4,4′,5,5′-hexachlorobiphenyl; PCB 105 = 2,3,3′,4,4′-pentachlorobiphenyl; PCB 114 = 2,3,4,4′,5-pentachlorobiphenyl; PCB 118 = 2,3′,4,4′,5-pentachlorobiphenyl; PCB 123 = 2,3′,4,4′,5′-penachlorobiphenyl; PCB 156 = 2,3,3′,4,4′,5-hexachlorobiphenyl; PCB 157 = 2,3,3′,4,4′,5′-hexachlorobiphenyl; PCB 167 = 2,3′,4,4′,5,5′-hexachlorobiphenyl; PCB 189 = 2,3,3′,4,4′,5,5′-heptachlorobiphenyl.

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Table 3. Mean toxic equivalents (TEQs) contributed by polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), non-*ortho* polychlorinated biphenyls (PCBs), and mono-*ortho* PCBs as well as ∑ PCBs in livers of adult female mink fed diets containing contaminated fish from the Hudson River

		Feed group based on dietary concentration (µg ∑PCBs/g feed)					
Compound	TEF [a]	Control [b]	0.72	1.5	2.8	4.5	6.1
TEQs contributed by PCDDs (pg/g wet wt)							
2,3,7,8-TCDD	1	0.15 [c]	0.21 [c]	0.24 [c]	0.24 [c]	0.36 [c]	0.52 [c]
1,2,3,7,8-PeCDD	1	0.17 [c]	0.21 [c]	0.19 [c]	0.22 [c]	0.22 [c]	0.29 [c]
1,2,3,4,7,8-HxCDD	0.1	0.017 [c]	0.020 [c]	0.019 [c]	0.022 [c]	0.032 [c]	0.038 [c]
1,2,3,6,7,8-HxCDD	0.1	0.15 [c]	0.18 [c]	0.23 [c]	0.23	0.35	0.39
1,2,3,7,8,9-HxCDD	0.1	0.017 [c]	0.020 [c]	0.019 [c]	0.022 [c]	0.023 [c]	0.026 [c]
1,2,3,4,6,7,8-HpCDD	0.01	0.033	0.040	0.063	0.060	0.099	0.11
OCDD	0.0003	0.00080	0.0018	0.0037	0.0050	0.0092	0.011
∑PCDD TEQs		0.53	0.69	0.75	0.80	1.1	1.4
TEQs contributed by PCDFs (pg/g wet wt)							
2,3,7,8-TCDF	0.1	0.017 [c]	0.020 [c]	0.019 [c]	0.022 [c]	0.020 [c]	0.022 [c]
1,2,3,7,8-PeCDF	0.03	0.0045 [c]	0.0059 [c]	0.0056 [c]	0.0066 [c]	0.0060 [c]	0.0065 [c]

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2,3,4,7,8-PeCDF	0.3	0.23	0.45	0.69	1.1	2.0	2.6
1,2,3,4,7,8-HxCDF	0.1	0.015 [c]	0.022 [c]	0.027 [c]	0.055 [c]	0.12	0.14
1,2,3,6,7,8-HxCDF	0.1	0.015 [c]	0.023 [c]	0.027 [c]	0.047	0.09	0.11
1,2,3,7,8,9-HxCDF	0.1	0.015 [c]	0.020 [c]	0.019 [c]	0.022 [c]	0.020 [c]	0.022 [c]
2,3,4,6,7,8-HxCDF	0.1	0.032	0.036 [c]	0.055 [c]	0.090	0.075 [c]	0.099 [c]
1,2,3,4,6,7,8-HpCDF	0.01	0.0015 [c]	0.0032 [c]	0.0034 [c]	0.0037 [c]	0.0074	0.0085
1,2,3,4,7,8,9-HpCDF	0.01	0.0015 [c]	0.0020 [c]	0.0019 [c]	0.0022 [c]	0.0020 [c]	0.0022 [c]
OCDF	0.0003	0.00023 [c]	0.00029 [c]	0.00028 [c]	0.00033 [c]	0.00030 [c]	0.00032 [c]
∑PCDF TEQs		0.34	0.58	0.85	1.4	2.3	3.0
TEQs contributed by non-*ortho* PCBs (pg/g wet wt)							
77	0.0001	0.00028 [c]	0.0033	0.0049	0.0076	0.0097	0.0093
81	0.0003	0.00084 [c]	0.010	0.012	0.017	0.022	0.028
126	0.1	1.2	23	45	82	152	185
169	0.03	0.092 [c]	0.13	0.21	0.24	0.40	0.24 [c]
∑Non-*ortho* PCB TEQs		1.3	23	45	82	153	186
TEQs contributed by mono-*ortho* PCBs (pg/g wet wt)							

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105	0.00003	0.038	2.0	3.5	4.3	6.2	8.1
114	0.00003	0.0019	0.18	0.31	0.33	0.47	0.58
118	0.00003	0.14	5.1	9.3	10	15	19
123	0.00003	0.00076	0.044	0.058	0.042	0.039	0.055
156	0.00003	0.013	0.49	0.88	1.0	1.8	2.1
157	0.00003	0.0046	0.11	0.19	0.24	0.39	0.47
167	0.00003	0.0073	0.16	0.27	0.29	0.44	0.52
189	0.00003	0.0014	0.029	0.049	0.055	0.088	0.10
∑Mono-*ortho* PCB TEQs		0.20	8.1	15	17	25	30
∑TEQs (pg/g wet wt) [d]		2.4 (0.97)	33 (16)	61 (25)	101 (33)	181 (55)	220 (63)
∑PCBs (μg/g wet wt) [d]		0.064 (0.032)	1.5 (0.86)	2.9 (1.8)	3.4 (2.2)	5.0 (3.7)	6.2 (4.8)
% Lipid (95% CI)		12.5 (8.8–16.3)	11.0 (6.2–15.8)	12.2 (7.3–17.2)	9.1 (5.9–12.2)	8.5 (5.9–11.1)	9.5 (7.0–12.1)

[a] Toxic equivalency factors (TEFs) from Van den Berg et al. [24].

[b] Values are TEQs based on nondetects = 0.5 * detection limit.

[c] Some values used in the calculation of these means were estimated as one-half of the detection limit.

[d] Number in parentheses is the standard deviation.

TCDD = tetrachlorodibenzo-*p*-dioxin; PeCDF = pentochlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; HpCDD = heptachlorodibenzo-*p*-dioxin; OCDD = octachlorodibenzo-p-dioxin; TCDF = tetrachlorodibenzofuran; PeCDF = pentachlorodibenzofuran; HxCDF = hexachlorodibenzofuran; HpCDF = heptachlorodibenzofuran; OCDF = octachlorodibenzofuran. Nomenclature for the PCB congeners follows that of the International Union of Pure and Applied Chemistry: PCB 77 = 3,3′,4,4′-tetrachlorobiphenyl; PCB 81 = 3,4,4′,5-tetrachlorobiphenyl; PCB

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126 = 3,3′,4,4′,5-pentachlorobiphenyl; PCB 169 = 3,3′,4,4′,5,5′-hexachlorobiphenyl; PCB 105 = 2,3,3′,4,4′-pentachlorobiphenyl; PCB 114 = 2,3,4,4′,5-pentachlorobiphenyl; PCB 118 = 2,3′,4,4′,5-pentachlorobiphenyl; PCB 123 = 2,3′,4,4′,5′-penachlorobiphenyl; PCB 156 = 2,3,3′,4,4′,5-hexachlorobiphenyl; PCB 157 = 2,3,3′,4,4′,5′-hexachlorobiphenyl; PCB 167 = 2,3′,4,4′,5,5′-hexachlorobiphenyl; PCB 189 = 2,3,3′,4,4′,5,5′-heptachlorobiphenyl.

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Table 4. Mean concentrations of total polychlorinated biphenyls (PCBs), non-ortho PCBs, and mono-ortho PCBs as well as toxic equivalents (TEQs) in livers of six-week-old kits and 31-week-old juvenile mink fed diets containing contaminated fish from the Hudson River

		Feed group based on dietary concentration (µg ∑PCBs/g feed)							
		Control[a]		0.72		Control[a]		0.72	
		6-week-old kits				31-week-old juveniles			
Compound	TEF[b]	Concentration (ng/g wet wt)	TEQ (pg/g wet wt)	Concentration (ng/g wet wt)	TEQ (pg/g wet wt)	Concentration (ng/g wet wt)	TEQ (pg/g wet wt)	Concentration (ng/g wet wt)	TEQ (pg/g wet wt)
		Non-ortho PCBs							
77	0.0001	0.0031[c]	0.00031	0.068	0.0068	0.0020[c]	0.00020	0.028	0.0028
81	0.0003	0.00063[c]	0.00019	0.022	0.0067	0.00033[c]	0.00010	0.041	0.012
126	0.1	0.014	1.4	0.30	30	0.010	0.99	0.19	19
169	0.03	0.0013[c]	0.040	0.0059[d]	0.18	0.0013[c]	0.038	0.0027	0.080
∑Non-*ortho* PCBs,		0.019	1.4	0.40	30	0.014	1.0	0.26	19

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∑TEQs									
		Mono-ortho PCBs							
105	0.00003	1.9	0.056	89	2.7	1.4	0.042	44	1.3
114	0.00003	0.095	0.0029	7.6	0.23	0.059	0.0018	3.4	0.10
118	0.00003	4.7	0.14	166	5.0	3.9	0.12	93	2.8
123	0.00003	0.039	0.0012	1.0	0.031	0.022	0.00066	1.1	0.032
156	0.00003	0.45	0.014	20	0.61	0.36	0.011	8.0	0.24
157	0.00003	0.16	0.0048	4.4	0.13	0.13	0.0039	1.9	0.056
167	0.00003	0.24	0.0071	5.9	0.18	0.20	0.0059	2.9	0.087
189	0.00003	0.033	0.0010	0.89	0.027	0.030	0.00091	0.46	0.014
∑Mono-ortho PCBs, ∑TEQs		7.6	0.23	296	8.9	6.11	0.18	155	4.6

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PCBs (µg/g wet wt) ∑TEQs (pg/g wet wt)[e]	0.038 (0.017)	1.7 (0.85)	1.2 (0.99)	39 (39)	0.044 (0.021)	1.2 (0.63)	0.89 (0.28)	23 (5.7)
% Lipid (95% CI)	Males: 11.5 (9.2–13.9) Females: 12.3 (9.8–14.8)		Males: 7.8 (5.4–10.3) Females: 11.4 (6.3–16.5)		Males: 14.4 (11.6–17.2) Females : 14.5 (11.7–17.3)		Males: 14.0 (11.7–16.4) Females: 14.2 (11.5–16.9)	

[a] Values are based on nondetects = 0.5 * detection limit.

[b] Toxic equivalency factors (TEFs) from Van den Berg et al. [24].

[c] Some values used in the calculation of these means were estimated as one-half of the detection limit.

TCDD = tetrachlorodibenzo-*p*-dioxin; PeCDF = pentochlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; HpCDD = heptachlorodibenzo-*p*-dioxin; OCDD = octachlorodibenzo-p-dioxin; TCDF = tetrachlorodibenzofuran; PeCDF = pentachlorodibenzofuran; HxCDF = hexachlorodibenzofuran; HpCDF = heptachlorodibenzofuran; OCDF = octachlorodibenzofuran. Nomenclature for the PCB congeners follows that of the International Union of Pure and Applied Chemistry: PCB 77 = 3,3′,4,4′-tetrachlorobiphenyl; PCB 81 = 3,4,4′,5-tetrachlorobiphenyl; PCB 126 = 3,3′,4,4′,5-pentachlorobiphenyl; PCB 169 = 3,3′,4,4′,5,5′-hexachlorobiphenyl; PCB 105 = 2,3,3′,4,4′-pentachlorobiphenyl; PCB 114 = 2,3,4,4′,5-pentachlorobiphenyl; PCB 118 = 2,3′,4,4′,5-pentachlorobiphenyl; PCB 123 = 2,3′,4,4′,5′-penachlorobiphenyl; PCB 156 = 2,3,3′,4,4′,5-hexachlorobiphenyl; PCB 157 = 2,3,3′,4,4′,5′-hexachlorobiphenyl; PCB 167 = 2,3′,4,4′,5,5′-hexachlorobiphenyl; PCB 189 = 2,3,3′,4,4′,5,5′-heptachlorobiphenyl.

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Table 5. Mean number of kits whelped alive per litter from mink fed diets containing polychlorinated biphenyl (PCB)–contaminated fish collected from the Hudson River [a]

		95% CI			95% CI		
Feed group based on dietary concentration	Mean live kits whelped per litter (68) [b]	LCL	UCL	Difference (%) in rate from control	LCL	UCL	p [c]
Control	5.5	4.4	7.0	NA	NA	NA	NA
0.72 µg ∑PCBs/g feed	4.9	3.6	6.6	–11	–39	29	0.53
1.5 µg ∑PCBs/g feed	7.2	5.6	9.3	30	–7.9	84	0.13
2.8 µg ∑PCBs/g feed	5.1	3.7	7.0	–7.6	–37	36	0.69
4.5 µg ∑PCBs/g feed	3.1	2.2	4.3	–44	–63	–17	0.0039
6.1 µg ∑PCBs/g feed	2.8	2.0	4.1	–49	–67	–21	0.0023

[a] Treatment effects were estimated with negative binomial regression models.
[b] Results based on 68 litters with at least one kit born dead or alive.
[c] p value for comparison to control group.
CI = confidence interval; LCL = lower confidence limit; UCL = upper confidence limit.

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Table 6. Mortality of kits whelped and nursed by mink fed diets containing polychlorinated biphenyl (PCB)–contaminated fish collected from the Hudson River [a]

			Litter-adjusted			
				95% CI		
End point	Feed group based on dietary concentration	Kit mortality (%)[b]	Kit mortality (%)	LCL	UCL	p [c]
Kit mortality at three weeks ($p < 0.001$) [d]	Control	19	18	7.6	38	NA
	0.72 µg ∑PCBs/g feed	14	13	3.2	41	0.66
	1.5 µg ∑PCBs/g feed	27	24	9.2	50	0.65
	2.8 µg ∑PCBs/g feed	83	86	57	96	<0.001
	4.5 µg ∑PCBs/g feed	72	71	44	89	0.0021
	6.1 µg ∑PCBs/g feed	96	92	59	99	<0.001
Kit mortality at six weeks ($p < 0.001$) [d]	Control	21	20	8.5	40	NA
	0.72 µg ∑PCBs/g feed	14	12	2.8	39	0.51
	1.5 µg ∑PCBs/g feed	29	29	13	54	0.49
	2.8 µg ∑PCBs/g feed	83	84	56	96	<0.001
	4.5 µg ∑PCBs/g feed	72	70	43	88	0.0035
	6.1 µg ∑PCBs/g feed	96	91	58	99	0.0011

[a] Treatment effects were estimated with beta-binomial regression models.
[b] Results based on 321 kits born live from 61 litters with a least one live birth.

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[c] *p* value for comparison to control group.
[d] *p* value for overall test of treatment effect.
CI = confidence interval; LCL = lower confidence limit; UCL = upper confidence limit; NA = not available.

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Table 7. Comparison of threshold concentrations of ∑polychlorinated biphenyls (PCBs) and toxic equivalents (TEQs) relating to offspring mortality and growth

Threshold type	Location	Critical effect	∑PCBs (µg/g feed)	TEQs (pg/g feed)	∑PCBs (µg/g liver)	TEQs (pg/g liver)	Source
No observed adverse effect level/concentration (NOAEL/NOAEC)	PCB 126 study	Females whelping	0.00024 [a]	24			[18]
	Hudson River	Kit stillbirth	2.8	19	3.4	101	Present study
	Saginaw Bay		1.5	33	3.1	403	[12,14]
	Hudson River	Kit mortality	1.5	10	2.9	61	Present study
	Housatonic River		1.6	12	3.1	50	[16,17]
	Saginaw Bay		0.015	0.70	0.09	17	[12,14]
	Hudson River	Juvenile mortality	0.72	4.8	1.5	33	Present study
	Hudson River	Kit body mass	0.72	4.8	1.5	33	Present study
	Saginaw Bay		0.015	0.70	0.09	17	[12,14]
Lowest observed adverse effect level/concentration (LOAEL/LOAEC)	PCB 126 study	Females whelping	0.0024 [a]	240			[18]

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	Hudson River	Kit stillbirth	4.5	28	5.0	181	Present study
	Saginaw Bay		2.6	66	6.3	627	[12,14]
	Hudson River	Kit mortality	2.8	18	3.4	101	Present study
	Housatonic River		3.7	51	3.1	189	[16,17]
	Saginaw Bay		0.72	17	2.2	226	[12,14]
	Hudson River	Juvenile mortality	1.5	10	2.9	61	Present study
	Hudson River	Kit body mass	1.5	10	2.9	61	Present study
	Saginaw Bay		0.72	17	2.2	226	[12,14]
Concentration lethal to 50% of the population (LC50)	Hudson River	Kit stillbirth	6.8	42	7.9	251	Present study
	Hudson River	Kit mortality	2.4	15	3.0	92	Present study
Concentration lethal to 20% of the population (LC20)	Hudson River	Kit stillbirth	1.4	9.0	1.7	56	Present study
	Hudson River	Kit mortality	0.34	2.6	0.80	13	Present study

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Housatonic River	0.98	16	[16,17]

[a] Concentration given is for 3,3',4,4',5-pentachlorobiphenyl (PCB 126).

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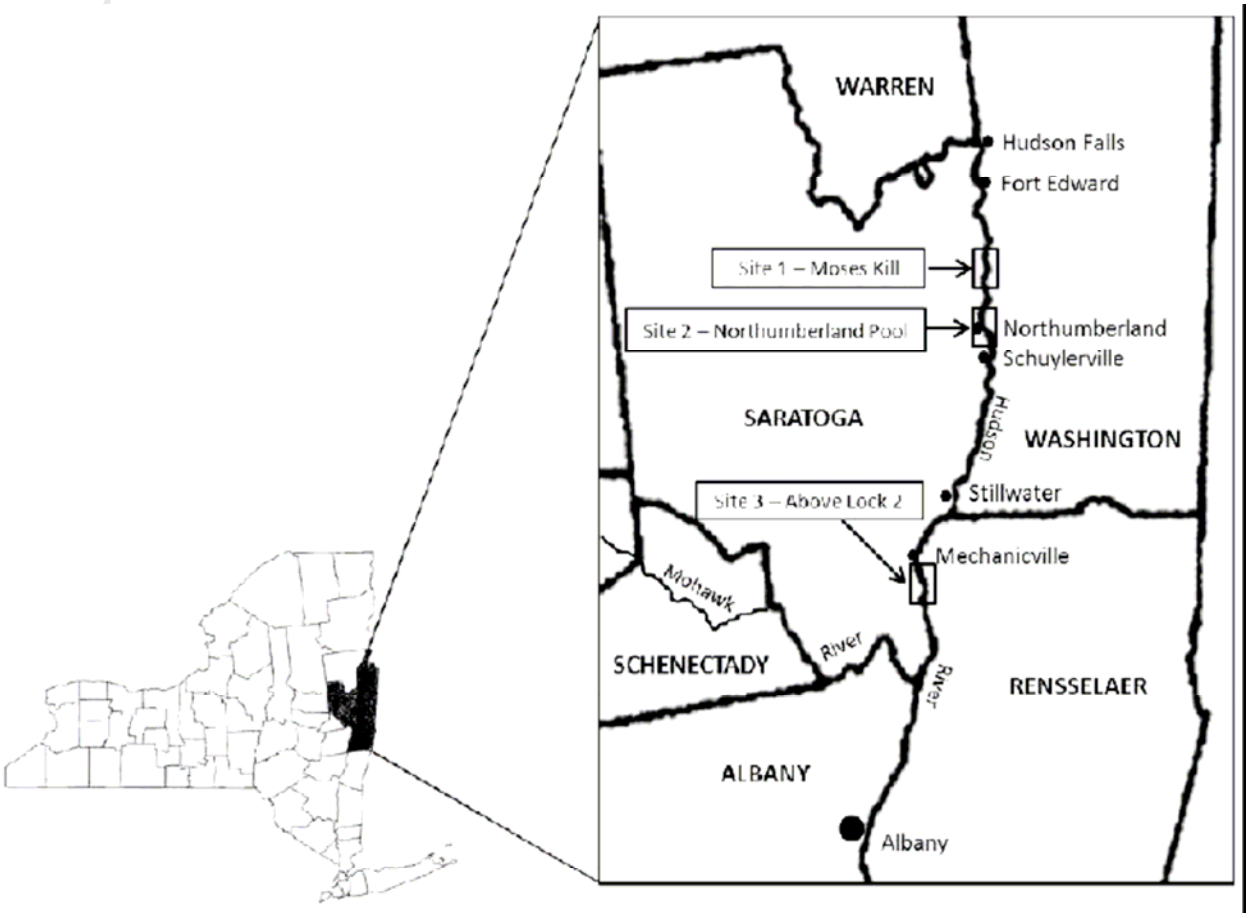

Figure 1

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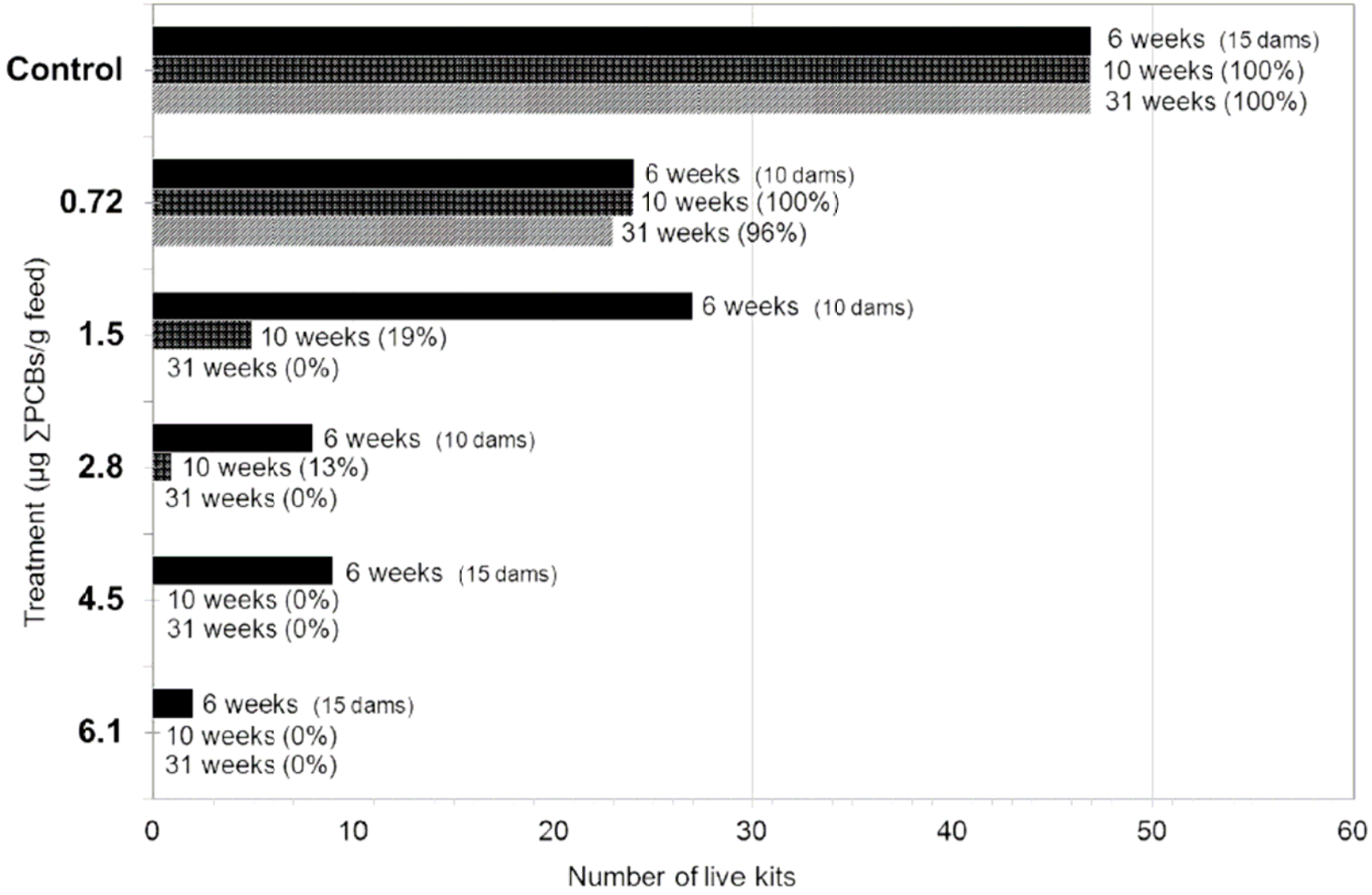

Figure 2

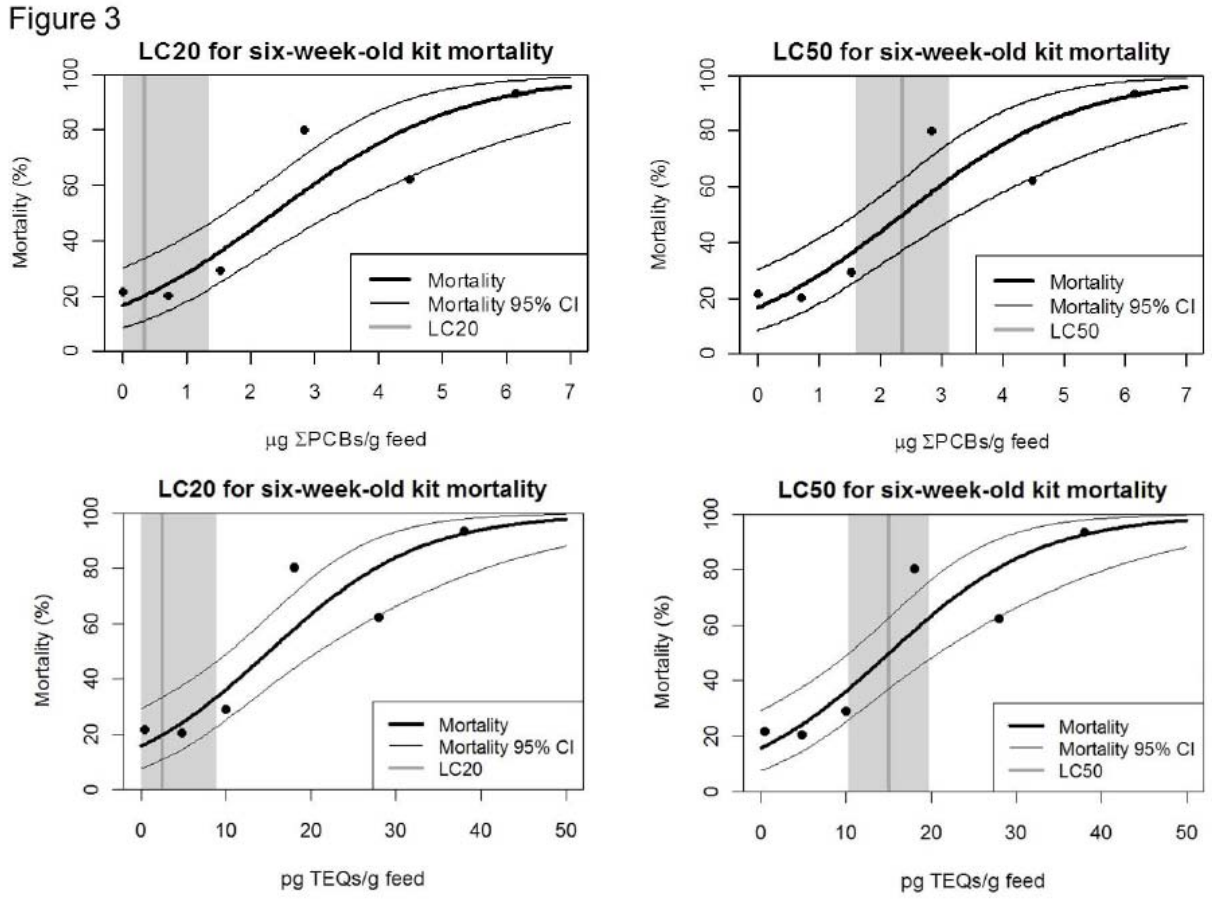

Figure 3

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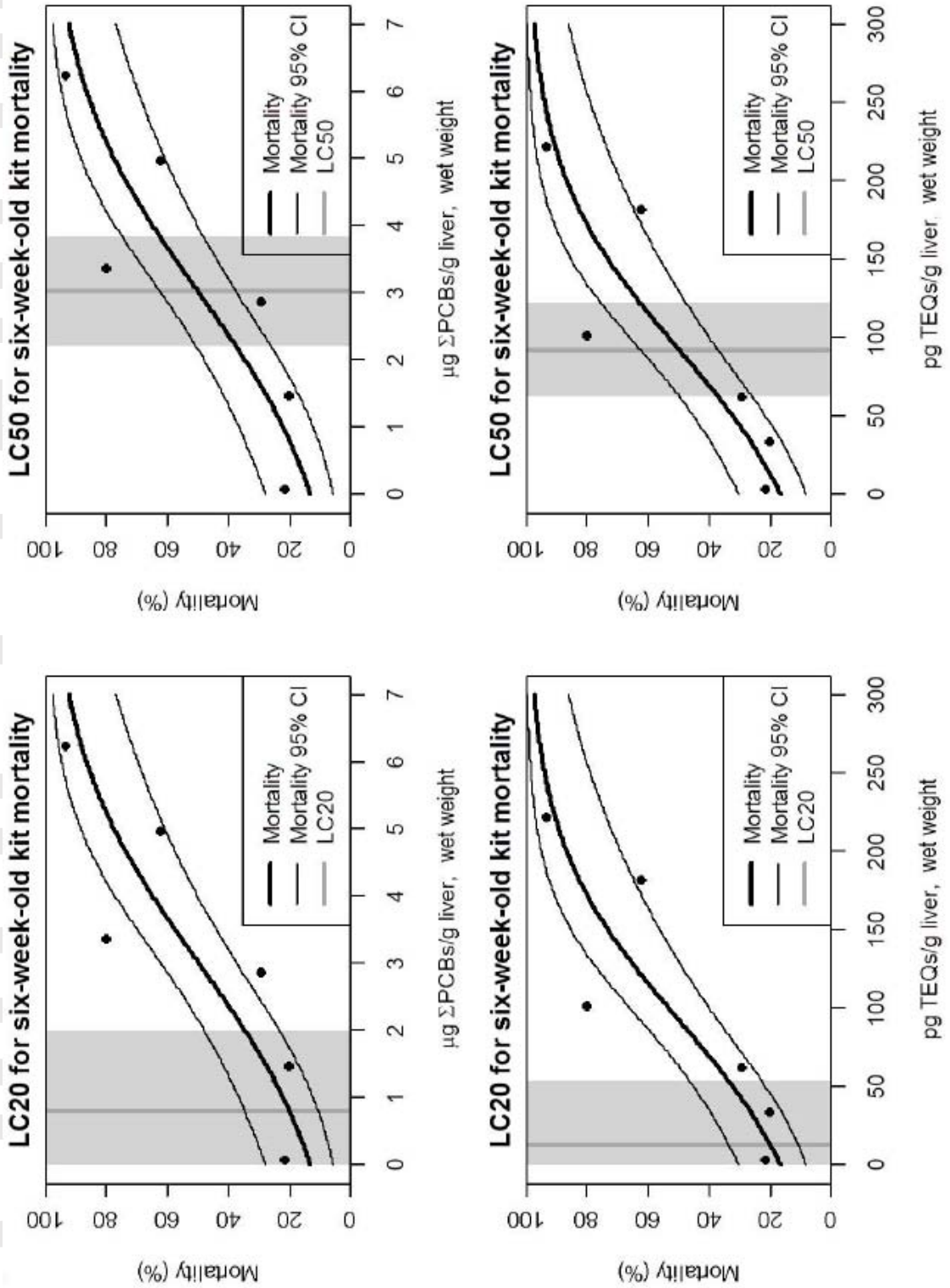

Figure 4

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