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ON-REFUGE INVESTIGATIONS SUB-ACTIVITY

Title: PI- Black-Footed Albatross Contamination due to Global and Local Contamination

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by

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II. INTRODUCTION

Recent work done on the albatross in the north Pacific have found some startling results. First is that albatrosses forage wide areas of the Pacific year round. Second is that despite this wide geographical range of food sources, that due to its position at the top of the food chain albatrosses are highly contaminated with persistent organochlorine compounds. This apex position also makes the albatross a sentinel for effects of global pollution. The third finding is that despite the global range of the birds, local contamination can have dramatic effects. An example of this is the occurrence of 'droop-wing' in albatross chicks on Midway due to ingestion of lead-paint chips scavenged from the ground around peeling WWII edifices.

This study was initiated due to the high levels of PCBs (2300ppm in soil, 660ppb in sediment, 40 ppb groundwater) and lead (2800 ppm soil) found on Tern Island in French Frigate Shoals (FFS). Pearl and Hermes Atoll was not thought to have been used for human exploitation or habitation. Recently our office has learned that the Atoll was used to refuel float planes during WWII. At Midway, DDT contamination is found with every excavation. Recent monitoring results found PCBs in the Midway Atoll fish as high as 25ppm (dry weight) and 2300 ppb in the sediments. These sediment results are even more alarming in that they were taken from a high energy area where all debris was removed more than 2 years ago, suggesting an unknown source. One third of Sand Island of Midway Atoll is manmade landfill. No records exist as to what was used for fill besides coral dredge waste. This year two previously unknown USTs were found on Eastern Island, Midway Atoll. Each year, over 1 million albatross nest on Midway. Several hundred thousand more nest on the other atolls of the Northwest Hawaiian chain. This study is to determine what effect local contamination has on albatross reproduction and survival.

II. A. Background and Justification

In 1909 President Theodore Roosevelt established a reserve in the Northwestern Hawaiian islands to protect seabirds from unregulated commercial exploitation, which became the Hawaiian Islands National Refuge in 1940. The present-day Hawaiian Islands National Wildlife Refuge consists of the leeward islands and atolls extending over a 1000 miles northwest of the main Hawaiian islands terminating with Midway and Kure Atolls. These small bodies of land serve as the primary nesting areas in the north Pacific for many species of seabirds including; noddies, terns, boobies, frigates, and albatrosses. Over 500,000 pairs of albatross nest each year on Midway, on much smaller Tern Island in French Frigate Shoals (about 500 miles from the main Hawaiian Islands), about 3200 pairs of albatross nest each year. Albatross are wide ranging foragers that only come to land in November each year where they conduct their elaborate breeding rituals, lay one egg which both parents share responsibility for incubating and raising the chick until it fledges in June/July. Recently, researchers (Sievert and Anderson, pers. comm.) have placed transmitters on the foraging parents and found that they are ranging as far as the coast of Alaska in search of food.

Albatrosses are one of the longest-lived birds. A few individuals banded just after World War II are still being seen on Midway. There are several immature age classes, and age of first nesting occurs from 8-13 years. They lay one egg every one to three years and are incapable of laying a

replacement if lost. They form life-long pair bonds and mate loss will cause the loss of one to five breeding seasons by the widowed bird (Fisher 1976). Natural annual adult mortality has been estimated to be very low, about 4 to 9% or less (Fisher 1976, Weimerskirch and Jouventin 1987). All these attributes are indicative of species that are slow or not able to react and/or adapt to changes in their habitat. The late age of maturity can conceal population changes in the subadult classes for a decade. Then even if the changes are corrected, it can take more than a decade to repair.

Greatest threats to albatross survival include fishing by-catch (formerly driftnets, currently long-line), habitat destruction (anthropogenic and volcanos) (Croxall and Gales 1998), and environmental pollution (Ludwig *et al.* 1998, Auman *et al.* 1997, Jones *et al.* 1996). A recent study (Ludwig *et al.* 1998) done on possible impacts of global dispersion of organochlorine chemicals on oceanic organisms found that the albatrosses of the Pacific were contaminated with PCBs and DDT. It had been thought that since they forage the pelagic oceans, far from coastal point sources of pollutants, that they would be minimally contaminated. The opposite was found to be true. As the albatross is near the top of the food chain (Harrison 1990), they were found to contain significant amounts of biomagnifying organochlorine contaminants. Analyses of eggs of both the Laysan (*Diomedea immutabilis*) and black-footed (*Diomedea nigripes*) albatrosses found that average DDT levels, while high, were not to levels known to cause egg-shell thinning in other fish-eating birds. However, PCBs in the black-footed albatross eggs had TCDD-EQs and TEQs that can produce elevated embryo mortality and a potentially a wide range of other effects documented in sensitive avian species in the Great Lakes (*i.e.*, GLEMEDS syndrome). While these species may not be as sensitive as some species tested, all bird species tested to date have been found to have very steep dose-response curves for dioxin-like chemicals in the embryo stage and once a concentration present is associated with an effects threshold (Tillet *et al.* 1992, Giesy *et al.* 1994a,b, Devito and Birnbaum 1994). Additionally, before embryo death is seen, other, much more subtle effects may be present due to *in ovo* exposure. For example, asymmetries in brain development of TCDD-exposed Great Blue Herons (*Ardea jerodias*) occurred in the ranges of 30-60 TCDD-EQs with no increase in egg death or other overt signs of GLEMEDS (Henshel *et al.* 1995). Other delayed reproductive effects associated with endocrine disruption of developmental processes may occur when the birds mature. The authors concluded that the concentrations found in black-footed albatross eggs are high enough, on average, that any increases in the TCDD-EQs or TEQs will cause increases in egg death rates and other wide-ranging effects characteristic of GLEMEDS. Hazard quotients (HQ) based on TCDD-EQs and TEQs were calculated for three years of data and compared to an average of all the lowest reported LOAELs for avian species. The HQs for Laysan Albatross eggs ranged from 5 to 33 and Black-footed HQs ranged from 12 to 83. The average HQ for Laysan Albatross at 7.4 is greater than one, and indicates a potential risk, that might be within extrapolation error (*i.e.*, LOAELs used were from other species). However, the Black-footed, with an average HQ of 17.7, and as high as 83, is well above parity and therefore this population is most likely at risk to dioxin-like affects in the egg (Auman *et al.* 1997, Ludwig *et al.* 1997).

Endocrine disrupting compounds (EDCs) can mimic estrogens, antiestrogens, and other hormones that can disrupt the endocrine system of developing embryos. This developmental disruption can produce permanent modifications in the reproductive, immunological, and neurological capabilities of the organism, and in turn, the population. Demasculinization and

feminization of males resulting from exposure to exongenous estrogens or other endocrine-disrupting compounds has been shown in a wide variety of organisms such as mice (Newbold *et al.* 1989), alligators (Guillette *et al.* 1994), and turtles (Guillette *et al.* 1994, Bergeron *et al.* 1994). Guillette et al (1994) found elevated plasma estradiol (E_2) levels in female alligators when compared to references. Juvenile males from the same system showed elevated E_2 but normal testosterone (T) levels suggesting that abnormal ratios of E_2/T are an indicator of EDC exposure.

Suspected EDCs such as PCBs and DDT can express adverse affects as parent compounds, especially persistent compounds, and as metabolized congeners, such as DDE's role in eggshell thinning. Similarly, several PCB metabolites are structurally similar to thyroxine so they can compete for thyroxine binding sites on the transport protein, transthyretin (TTR) (Lans *et al.* 1993). This competitive binding can cause reduction in plasma tetraiodothyroxine (T_4) levels and serum transport of vitamin A in rodents (Lans *et al.* 1993, Brouwer *et al.* 1998). Hydroxylated metabolites of PCBs have been shown *in vitro* to have binding affinities 10 times greater for TTR than for T_4 (Lans *et al.* 1993). Bergman *et al.* (1994) found that this preferential binding resulted in the persistence of these metabolites in blood of both seals and humans exposed to PCBs in the environment. Competitive binding by PCB metabolites with T_4 for TTR or other endogenous proteins could cause toxic effects such as thyroid dysfunction resulting from the imbalance in the synthesis and regulation of thyroid hormones (Klasson-Wehler *et al.* 1998). Brouwer *et al.* (1989) suggested that the significantly lower plasma levels of retinol found in harbor seals contaminated with PCBs were caused by a competitive interference by a hydroxylated metabolite of PCB-77. They further suggested that this lowered plasma retinol concentrations cause vitamin A deficiencies.

MIDWAY ATOLL NWR

Midway Atoll National Wildlife Refuge (NWR) is a coral atoll, with three islands located in a semi-circular lagoon. Midway Atoll was discovered in 1859 when Captain N.C. Brooks of the *Gambia* ran aground and named the islands "Middlebrook Islands". Midway hosts the world's largest colony of nesting Laysan albatross (*P. immutabilis*), and the second-largest colony of black-footed albatross (*P. nigripes)*.
Midway has seen much of human and military disturbances. At the turn of the 20th century, Japanese feather and egg poachers decimated both albatross species. In 1903, President Roosevelt assigned jurisdiction and control of Midway, including the surrounding reefs and territorial waters, to the US Navy (Presidential Executive Order 199-A, January 20, 1903) (Rauzon, 2001). Additionally, in 1903, US Marines were sent to Midway to enforce the protection of the Cable Company and birds (Cousins and Cooper, 1998). In the 1930's Sand Island, Midway became a destination stopover for the Pan American Clipper seaplane service flights. During World War II, Midway had a population of approximately 10,000 military personnel.

In 1993, US Navy Midway Airfield began closure under the Base Realignment and Closure Act of 1990 (BRAC). During the BRAC operation, over 120 underground and aboveground storage fuel tanks were removed, buildings were demolished, and active landfills were investigated. During this remediation, soil contaminated with DDT{1,1,1-trichloro-2,2-bis(p-

chlorophenyl)ethane}, petroleum, and polychlorinated biphenyls (PCB) was removed from all over the island. Despite the large remediation effort, one site called the old bulky waste landfill (OBWLF) could still be of concern since evidence of contamination of that site has surfaced since cleanup of the atoll. OBWLF is an artificial peninsula that extends out into the lagoon on the south shore of Sand Island.

The investigation found elevated levels of pesticides, PCBs, and metals detected in all environmental media at the OBWLF site, possibly due to the disposal of wastes that contained PCBs (Ogden Environmental and Energy Services Co., 1997). OBWLF was formed by depositing bulky metal wastes, construction debris, scrap metal, old machinery, and salvaged vehicles into the peninsula and offshore (Ogden Environmental and Energy Services Co., 1997). During closure of the landfill and BRAC a 2.5-4 foot thick cap of clean soil was placed on OBWLF. However, in 1996, 2.4-80 mg/kg (ppm) of Arochlor-1260 (PCB) were found in subsurface soil samples (Ogden Environmental and Energy Services Co., 1997), and during preliminary analyses in 2000, PCB concentrations as high as 8.61 mg/kg were found in OBWLF soil samples. From previous research suspected contaminants in the soil at the dump are PCBs and pesticides such as DDT. Possible exposure pathways exist to terrestrial receptors such as seabirds that nest directly on the soil such as the black-footed albatross. Direct exposure to PCB-contaminated soil through dermal contact, incidental ingestion, inhalation of vapors, or ingestion of PCB-contaminated prey could be the primary exposure pathways to PCB contaminants in the soil currently at OBWLF. Preliminary soil analyses in October 2000 at OBWLF for this research concluded that there was a need for further investigation for contaminants such as PCBs of the soil at OBWLF on Midway Atoll NWR.

TERN ISLAND, HAWAIIAN ISLANDS NWR

Like Midway Atoll, Tern Island has seen its share of anthropogenic alterations. There have been a number of investigations dealing with environmental issues as a result of past US military usage (Woodward-Clyde 1999). In 1991 as part of the Defense Environmental Restoration Program, 21 underground fuel storage tanks were closed (Woodward-Clyde 1999). Over the years, large quantities of unclassified debris have been disposed of in a landfill on Tern Island and pushed into the sea around the island (Miao et al. 2000). On Tern the major contaminants of concern in the soil where albatross are nesting are from an eroding US Coast Guard landfill known to contain scrap metal, capacitors, batteries, and transformers. A geophysical survey revealed an area thought to be composed of landfill debris approximately 2,690 feet in the east-west direction, and from 30-140 feet in the north-south direction (Woodward-Clyde 1999). This old landfill has been the center of concern for years, and past evidence has shown that there is still contamination in the soil at Tern. During an environmental investigation conducted by the US Coast Guard, 346 soil samples were collected from Tern Island. Of these samples, 278 samples were collected from the surface (0-6") of the soil and 68 samples were collected from the subsurface (Woodward-Clyde 1999). The majority of these samples were collected directly from two sites known to be "hotspots" for PCB contamination (Woodward-Clyde 1999), both of these were found in the old landfill area mentioned above. In this investigation PCBs were detected in 200 surface soil samples with concentrations of 0.1 to 2300 mg/kg, and PCBs were detected in 44 of the 65 subsurface samples with detection limits ranging from 0.0099 to 24

mg/kg (Woodward-Clyde 1999). This study at Tern investigates if the hormone levels of black-footed albatross (*Phoebastria nigripes*) could be affected by nesting in PCB-contaminated soil.

II.B. Scientific Objectives

(1) Determine if black-footed albatross eggs on Tern have equivalent or higher concentrations of PCBs than those recorded by Auman et al. (1997) and Ludwig et al. (1997).

(2) Determine if estradiol and testosterone concentrations in adult Black-footed Albatross are correlated with adult contaminant burdens, contamination of soil in nesting areas, or diet contamination.

(3) Determine if there is a correlation between plasma concentrations and uropigial oil concentrations for use as a surrogate estimate for blood.

II.C. Management Actions

To date we have used the data and small reports to bring reality to the five-year review of the Old Bulky Waste Dump at Midway. Navy contractors ignored tours and photos of conditions on the dump and attempted to get a final 'No Further Action' for this BRAC site. With our data and reports we have kept this site open for further monitoring and potential action.

III. METHODS

Field Methods: Midway

Preliminary Soil Analyses; Fall 2000

Eleven soil samples were collected from Old Bulky Waste Landfill on Sand Island, Midway Atoll NWR in October, 2000. For this study, Old Bulky Waste Landfill was broken up into eleven sections for sampling and a reference plot was located on the shoreline approximately 150 meters west of OBWLF. Twelve composite surface soil samples were collected using disposable sampling spoons. Sampling involved collecting a portion of soil at even intervals along a paced-off grid within a section. Preferentially soil was collected from a Bonin petrel (*Pterodroma hypoleuca*) burrow entrance if it was at or near a sample location. All samples were collected in the same manner and approximate size by using local vegetation as boundaries.

Soil samples were sieved through mesh sieves to sort out the fine soil for analysis. Samples were extracted with methanol using the SDI Sample Extraction Kit (Strategic Diagnostics Inc., Newark, Delaware). Extracted soil samples were analyzed using a PCB RaPID Assay Kit; (Strategic Diagnostics Inc., Newark, Delaware).

2001 Study Sites

Two study sites were designated for the breeding year of 2001: Bulky Dump (contaminated site) and Black-foot 8 (reference site). The reference site was a 20 x 20 m plot that had been monitored by the US Fish and Wildlife Service since 1996. In preliminary analysis, this site showed no detectable PCB contamination in the soil. The contaminated site was an old landfill that the Navy used to dispose of unwanted debris. This plot at Bulky Dump was approximately

130 x 300 m. The plots were located near each other on the southern shore facing the east on Sand Island, Midway Atoll. The main difference was the age groups that occupied the plots. Bulky Dump was constructed recently, and seemed to have only a younger age-class nesting. Black-foot 8 was a plot that the US Fish and Wildlife Service has been monitoring since 1996, and Bulky Dump was a plot set up for the first time in 2001.

Soil Collection

In January 2001 nest soil samples were collected from active nests at Black-foot 8 and Bulky Dump. Surface soil was collected using nitrile gloves and scooping 3-4 teaspoons of soil into individual plastic bags. The nest soil was collected from the middle of the nest cup while the adult albatross was incubating an egg or newly hatched chick. A total of 64 soil samples from Black-foot 8 and 100 soil samples from Bulky Dump were collected.

Blood Sampling

Blood samples were collected from adult albatross on Sand Island, Midway Atoll NWR located in the north-central Pacific Ocean (28º 13'N, 177º 22'W) during the 2001 nesting season. Adult data collection consisted of hand-capturing incubating or chick-brooding adults and drawing 5 ml of blood from the ular wing vein using 21-gauge needles and Vacutainer tubes (BD Vacutainer ™ containing freeze-dried lithium heparin, Becton, Dickinson and Company, Franklin Lakes, NJ). For blood sampling, two assistants were required, one holding the bird's beak and body across their lap and the other holding the feet and the wing to be sampled flat against a padded mat. This position allowed the wing to be draped outward and the blood to rapidly flow to the vein to be sampled. At times, blood from both wings was sampled. In addition, birds were banded (if not previously banded) and weighed. Blood was kept in a cooler with ice packs and then centrifuged for 10 minutes at 3,000 x g within 4 hours of sampling. Approximately 2 ml of plasma were obtained from 5 ml blood collected. Plasma was divided into two microcentrifuge tubes and kept frozen at -40º C for later analysis of hormones and contaminants.

Chick blood was collected by hand-capturing temporary color-banded chicks (monitored from day of egg-lay) in late May 2001. Five ml of blood were drawn from the ular wing vein using 21-gauge needles and vacutainers containing lithium heparin anticoagulant, as described for adult birds.

Sampling of parent birds took place February 12 to February 23, 2001. During this time blood was collected from 174 adults and we were able to obtain samples from approximately 25 pairs of albatross at both plots. When the same plot was examined the following day, the brooding parents were usually switched. During the breeding season, the parents were sharing brooding duties and exchanged places daily or every few days.

Eggs and Chicks

The samples were collected in January to June of 2001. Three chick carcasses from nests B8 32, Bulky Dump (BD) 77 and BD 88 were approximately 1 month old. Three older chick (OC) carcasses were collected from the BD sites and were approximately 4-5 months old, just about ready to fledge. The carcasses were placed in a plastic bag and stored until transfer to the laboratory. Unhatched and/or abandoned eggs were collected from sites B8 and BD. The

contents of the egg were placed into a clean jar and stored until transport to the laboratory. All samples were stored at –20 ºC until preparation for analysis.

Field Methods Tern Island, French Frigate Shoals

Study Sites

The Old Landfill (contaminated site) was selected based on findings described in Chapter 1 from investigations that pinpointed "hotspots" of PCB soil contamination (Ogden Environmental and Energy Services Co., 1997; Woodward-Clyde, 1999) on the landfill used for years by the US Coast Guard to dispose of unwanted debris. The plot at Old Landfill was approximately 123 x 45 m. East End (reference site) was a 25 x 33 m plot that was located the farthest from the buildings and the landfill site and shown not to be contaminated in an investigation in 2000 (Chase Environmental Group, 2002).

Birds

Chicks of black-footed albatross (*Phoebastria nigripes*), 12-15 weeks of age, were selected based on the probable location of their nest cup. I was not able to identify the sex of chicks. From April 16 to May 12, 2001, blood samples were taken from a total of 90 chicks. They were selected from two sites on Tern Island, Old Landfill and East End. In addition to blood samples, 86 soil samples were collected from directly under each chick.

Blood Sampling

Blood samples were collected from chick albatross on Tern Island, HINWR during the 2001 nesting season. Chick data collection consisted of hand-capturing chicks located within the designated plots and drawing 5 ml of blood from the ulnar wing vein using 21-gauge needles and Vacutainer tubes (BD Vacutainer ^{TM} containing freeze-dried lithium heparin, Becton, Dickinson and Company, Franklin Lakes, NJ). For blood sampling, two assistants were required, one holding the bird's beak and body across their lap and the other holding the feet and the wing to be sampled flat against a padded mat. This position allowed the wing to be draped outward and the blood to rapidly flow to the vein to be sampled. At times, blood from both wings was sampled. In addition, chicks were banded and weighed. Blood was kept in a cooler with ice packs and then centrifuged for 10 minutes at 3,000 x g within 4 hours of sampling. Approximately 2 ml of plasma were obtained from 5 ml blood collected. Plasma was divided into two microcentrifuge tubes and kept frozen at -40º C for later analysis of hormones and contaminants. During this sampling period 45 chicks were sampled from both sites on Tern for a total of 90 blood samples collected.

Soil Collection

In May of 2001, soil samples were collected from active nest cups located near chicks within designated plots. Immediately after blood sampling, nest soil samples were collected using nitrile gloves and scooping 3-4 teaspoons of soil into individual plastic bags.

PCB Analyses

Plasma

All of the samples were collected in January to June of 2001. USFWS personnel collected all of the samples. The plasma was already separated from the red blood cells before shipment to our

laboratory, and was stored at -20 °C until extraction. The soil collected from the individual nests was also stored at -20 °C until extraction. Prior to extraction, the soil samples were air-dried to remove excess moisture.

The plasma was extracted with pressurized fluid extraction (PFE). The instrument used was a Dionex (Sunnyvale, CA) ASE 200 extractor. Plasma samples were treated with methanol prior to extraction, and were allowed to sit overnight for solvent evaporation. The samples were then combined with sodium sulfate for PFE extraction. Approximately 3 g of acidified alumina was placed at the bottom of the extraction cell to retain some lipids from the extract. The plasma samples were extracted with hexane acetone (1:1, v/v) at a pressure of 1500 psi and temperature of 100 °C. Two static cycles of 20 mins each were performed.

The PFE plasma extracts were cleaned up with concentrated sulfuric acid, followed by an aqueous sodium chloride. The extracts were passed through sodium sulfate and concentrated to 1 mL. The 1 mL of extract was further cleaned up with column chromatography. The column was composed of 3% deactivated silica, 6% deactivated alumina, and 1 cm of anhydrous sodium sulfate on the top. The extract was loaded onto the column, and eluted with 20 mL hexane. The eluent was concentrated and ready for GC-ECD/MS analysis.

Uropygial oil

The uropygial oil samples (most samples less than 1 mg total) were manually extracted with hexane. The hexane extracts were then cleaned up with concentrated sulfuric acid followed by column chromatography as discussed above for the plasma. The samples were then analyzed by GC-ECD/MS.

The air-dried soil samples were extracted with PFE. Samples of ~10 g soil were loaded into the extraction cell, and were extracted with hexane:acetone (1:1, v/v) at a pressure of 2000 psi and a temperature of 100 □C, with two static cycles of 5 mins. The extracts were filtered through sodium sulfate and concentrated prior to GC-ECD/MS analysis.

PCB Determination

The samples were analyzed on a Varian Saturn 2000 gas chromatograph (GC) with simultaneous mass spectrometric and electron capture detection (GC-MS and GC-ECD, respectively). The column flow was split between the ECD and the MS in a 1:10 ratio. The column used was a capillary column ZB 1 (60 m x 0.25 mm i.d. x 0.25 □m film; Phenomenex, Torrance, CA). Helium grade 5.5 was used as the carrier gas, and nitrogen was used as the make-up gas for the ECD. The initial oven temperature was 120 ºC and it was linearly ramped at 1 ºC/min to 260 ºC. The injector and ECD detector were set to 280 ºC and 330 ºC, respectively. Identification of the individual PCB congeners was based on retention time matching to PCB standards. Concentrations of the individual PCB congeners were calculated from external PCB standards (Accustandard Inc., New Haven, CT) with the ECD data, and confirmation of the congener identification was performed with the MS.

Recoveries of PCBs

Table 1 shows the PFE and cleanup recoveries of PCBs containing 3 to 8 chlorine atoms in albatross plasma and soil. Plasma samples were spiked with a 12 component PCB standard

mixture (Accustandard, C-CS-08 standard (PCBs 30, 43, 55, 58, 76, 109, 112, 120, 159, 186, 192, 198)) at a concentration of 20 ng/g. Soil samples were spiked with an 18 component PCB standard mixture (Accustandard, C-CS-07 (PCBs 36, 72,… plus PCBs 77, 126, 169, and 209) at a concentration of 24 ng/g.

PCB Congener Identification and Quantitation

Toxic equivalents (TEQs) were calculated for the various parts using toxic equivalency factors (TEFs) for birds from M. Van den Berg et al.{M. Van den Berg, 1998 #22}. It is believed that these compounds have a common toxicological mechanism in the organism, with the initial step being the binding of the aryl hydrocarbon receptor (Van den Berg reference and more). This concept of the TEF involves toxicity comparison of the given compound to that of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the most toxic congener in this group of compounds (Van den Berg reference and more). The combination of TEFs with chemical concentration data results in TEQ concentrations, and it is assumed that the combinations of congeners are concentration additive. TEF and TEQs are used for risk management purposes, and using these numbers for biological and toxicological assessment is still uncertain due to the complexity of biological systems.

Eggs and Chicks

In the laboratory, after the feathers were removed and the total mass was weighed, the bird was then dissected into bone, head and neck, liver, muscle, organs (other than liver) and skin. The various parts were homogenized separately with dry ice and lyophilized. The dried material was placed in an airtight container until supercritical fluid extraction (SFE).

Egg Extraction and Cleanup.

The lyophilized tissues and eggs were extracted with an Isco SFX 220 extractor (Isco, Inc.; Lincoln, Nebraska) following the procedure of Miao et al. (2000, 2001). Samples (~ 1.0 g) were placed into the 10 mL extraction cells, and acidic alumina (~ 3 g) was placed at the bottom of the cell to remove some of the fats from the extract. A static extraction at 6000 psi pressure and temperature of 150 □C for 10 min followed by a dynamic extraction at the same pressure and temperature with 80 mL of supercritical CO2 at a flow rate of 2 mL/min. The extracts were collected in hexane. The SFE extracts were cleaned up with concentrated sulfuric acid, followed by column chromatography (column consisted of 3% deactivated silica, 6% deactivated alumina, and 1 cm of anhydrous sodium sulfate) that was eluted with 20 mL hexane. The eluent was concentrated for GC-ECD/MS analysis.

PCB Determination.

The samples were analyzed on a Varian Saturn 2000 (Palo Alto, CA) gas chromatograph with simultaneous mass spectrometric (ion trap) and electron capture detection (GC-MS and GC-ECD, respectively). The column flow is split between the ECD and the MS in a 1:10 ratio, respectively. The column is a capillary column ZB 1 (60 m □ 0.25 mm i.d. □ 0.25 □m film; Phenomenex, Torrance, CA) with helium as the carrier gas and nitrogen as the makeup gas for the ECD. The oven temperature is linearly ramped from 120 ºC at 2 ºC/min to 275 ºC (hold 10 min).. The injector and ECD detector are set at 280 ºC and 330 ºC, respectively. The individual PCB congeners are identified with retention time correlation to PCB standards (Accustandard Inc., New Haven, CT) and mass spectral matches. Concentrations of each PCB congener were

calculated from external PCB standards with ECD data, and confirmed with the MS. All PCB concentrations were calculated on a lipid weight basis (lipid weight determined by gravimetric analysis). PCB recovery.

The different tissue samples were fortified with standard C-CS-08 (Accustandard, New Haven, CT) which contained PCBs 30, 43, 55, 58, 76, 109, 112, 120, 159, 186, 192 and 198). The spike level of each PCB was approximately 50 ng/g. Table 1 shows the average recoveries of PCBs containing 4 to 8 chlorine atoms from the various parts. The average recoveries ranged from 47% to 258% .

Hormone Analyses

Radioimmunoassays of Steroid Hormones

Plasma concentrations of estradiol-17β, testosterone, progesterone, total and free thyroxine (T4), and tri-iodothyronine (T3) were determined by radioimmunoassay using commercially available kits with modifications (see below). Since albatross feed primarily on flying-fish eggs and squid with high lipid content, it was necessary to separate interfering substances in the plasma by using Sep-Pak C-18 light cartridges (Waters, Milford, MA) to estimate steroid hormones. Albatross chick plasma was used in validation for both adult and chick radioimmunoassays.

Estradiol, testosterone, and progesterone were extracted from 250 µl of plasma with Sep-Pak C - 18 cartridges. Briefly, the cartridge, preconditioned with 2 ml of 2-propanol and 2 ml of 0.1% aqueous trifluoroacetic acid (TFA), was loaded with the plasma sample. The cartridges were washed with 2 ml of 0.1% TFA, followed by 2 ml of 25% acetonitrile in 0.1% TFA. Estradiol and testosterone were eluted with 1.5 ml 40% acetonitrile in 0.1% TFA, and progesterone with 1.5 ml of 60% acetonitrile in 0.1% TFA. The eluates were evaporated to dryness using a SpeedVac (Savant, Atens, GA), and the dried residues were reconstituted with 250 µl of assay buffer consisting of 10 mM phosphate buffer (pH 7.3), containing 0.1% Triton X-100 (Sigma), 1% bovine serum albumin (Sigma), and 0.01% sodium azide (Sigma).

Estradiol 17-β

Plasma concentrations of estradiol-17β were determined by radioimmunoassay using a commercially available kit with modification (Immuchem Double Antibody Direct Estradiol-17β ^{125}I RIA Kit; ICN Biochemicals, Costa Mesa, CA). Estradiol-17β standards were prepared by dissolving estradiol-17β (Sigma) in 100% ethanol (1 mg/ml) and then diluting to the desired concentrations with an assay buffer consisting of 10 mM phosphate buffer (pH 7.3), containing 0.1% Triton X-100 (Sigma), 1% bovine serum albumin (Sigma), and 0.01% sodium azide (Sigma). Glass tubes (12 x 75 mm) were used. To each tube were added 50 µl of standard or plasma sample, and 250 µl of ^{125}I estradiol-17β and 250 µl of anti-estradiol-17β (provided with the kit) were added. After incubation at 37°C for 90 minutes, 250 µl of precipitant solution (provided with the kit) was added. After centrifugation at 2000 x g for 20 min at 4°C, the tubes were aspirated and ^{125}I was counted in a gamma counter (Cobra II, Packard, Meriden, CT).

The validity of the assays was assessed by demonstrating that parallel displacement curves are generated with serial dilutions of plasma samples and also by the absence of cross-reaction with stripped plasma. In the preliminary experiment, dilution of neat plasma of the chick was not parallel with estradiol standards (Figure 2.1). Plasma was then extracted with ether, following the procedure established in this laboratory for angelfish (genus, species) plasma. Briefly, 2 ml of ethyl ether (Anhydrous, Fisher Scientific, Pittsburgh, PA) was added to the plasma sample. The tubes were vortexed and frozen at -80°C for 10 min, and the aqueous organic layer was decanted into a new glass tube. The ether extract was evaporated to dryness in a water bath at 40°C for 1 hour, and then placed under nitrogen for 5 min to ensure complete evaporation. Extracts were then reconstituted with assay buffer. For stripping, an aliquot of the pooled plasma was incubated at room temperature for 15 min with 2% activated carbon (Norit A, Aldrich, Milwaukee, WI). As shown in Figure 2.1, however, the ether-extracted and stripped plasma still cross-reacted with the estradiol antibody, indicating the presence of interfering substances after ether extraction. Thus, estradiol 17-β was extracted from 250 µl of albatross plasma with a Sep-Pak C-18 cartridge, and a fraction was eluted with 25-40% acetonitrile. In a preliminary experiment, 10 µCi of ^{3}H-estradiol ([2, 4, 6, 7, 16, 17-^{3}H] oestradiol, Amersham, Piscataway, NJ) in 10 µl ethanol was diluted with 250 µl assay buffer, and added to the cartridge. As shown in Figure 2.2, estradiol was eluted in fractions between 25% and 35% acetonitrile. Serial dilution of the chick plasma after the Sep-Pak purification was parallel with the estradiol standard, and no cross-reaction was seen with the stripped plasma (Figure 2.1). The interassay and intra-assay coefficients of variation were 14.3% (n = 5) and 23% (n=10), respectively.

Figure 2.1. Displacement curves for estradiol 17-β and serial dilution of albatross chick plasma. Closed circles represent estradiol 17-β standards. Other symbols represent diluted albatross plasma after various treatments. Each E_2 point represents the mean of duplicate determinations.

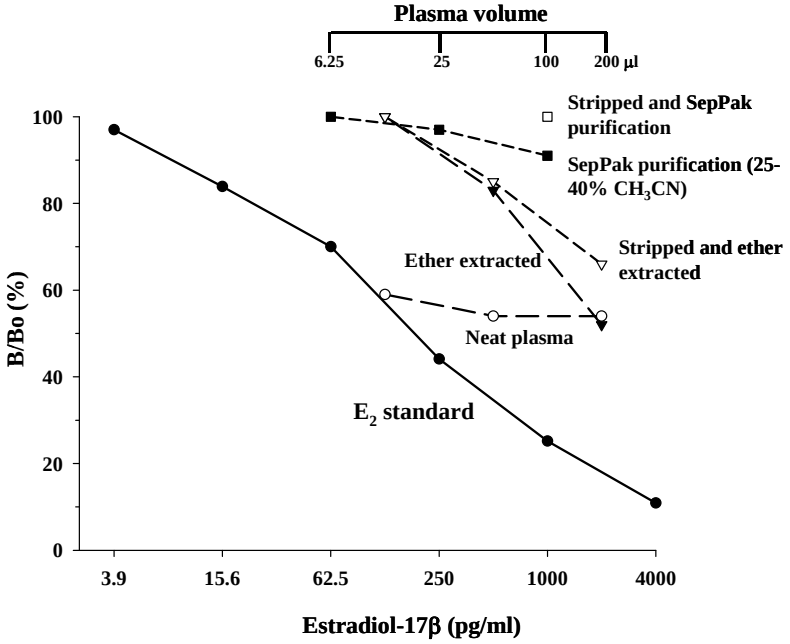
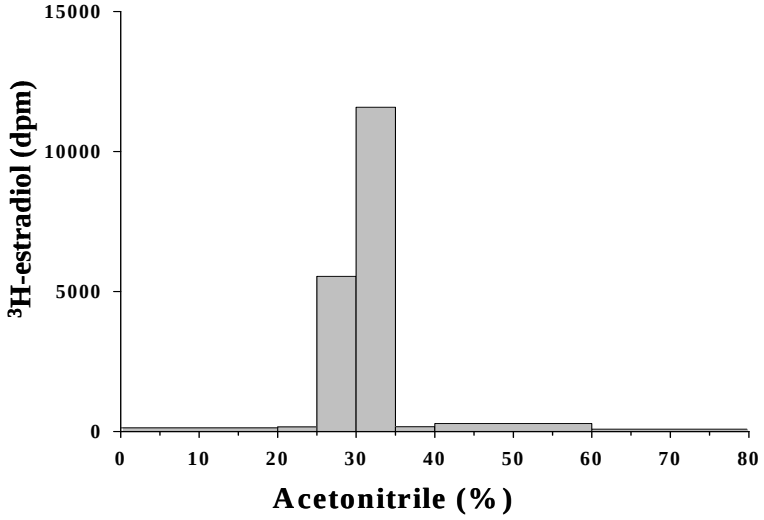


Figure 2.2. Elution profile of ^{3}H-estradiol 17-β through Sep-Pack C18 cartridge. 10 µCi ^{3}H-estradiol 17-β in 10 µl ethanol was diluted with 250 µl assay buffer and added to the cartridge.



Testosterone

Plasma concentrations of testosterone were determined by radioimmunoassay using a commercially available kit with modification (Immuchem Double Antibody Testosterone ^{125}I RIA Kit; ICN Biochemicals). Testosterone standards were prepared by dissolving testosterone (Sigma) in 100% ethanol (1 mg/ml) and then diluting to desired concentrations with assay buffer as above. To each tube were added 50 µl of standard or plasma sample, and 250 µl of ^{125}I testosterone and 250 µl of anti-testosterone (provided with the kit) were added. After incubation at 37°C for 120 min, 50 µl of the second

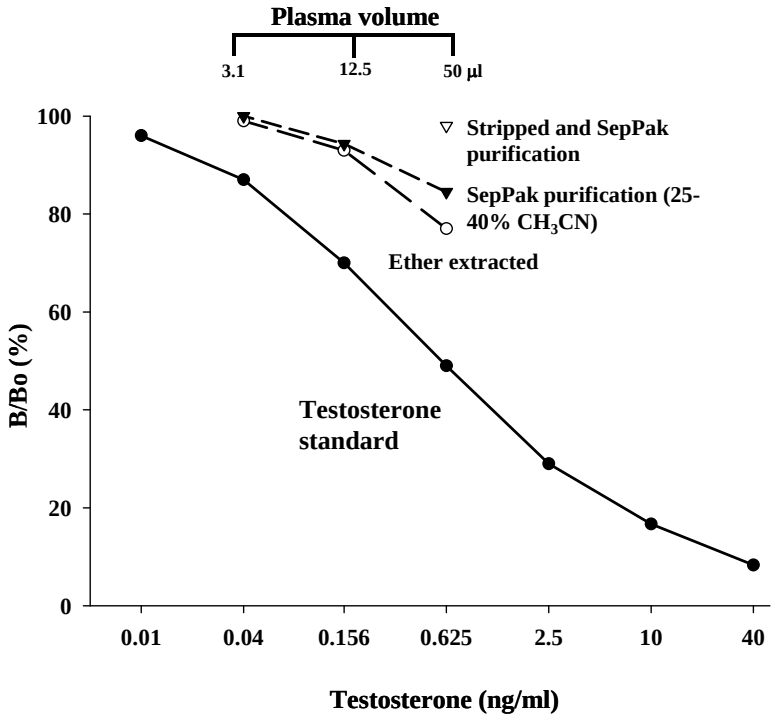


Figure 2.3. Displacement curves for testosterone and serial dilution of albatross chick plasma. Closed circles represent testosterone standards. Other symbols represent diluted albatross plasma after various treatments. Each point represents the mean of duplicate determinations.

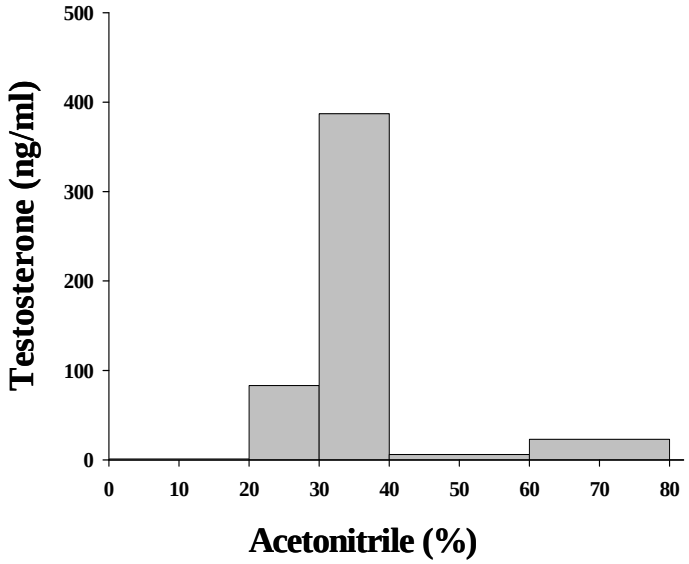


Figure 2.4. Elution profile of testosterone through Sep-Pack C-18 cartridge. Testosterone (200 ng in 400 µl assay buffer) was applied to the cartridge.

antibody (provided with the kit) was added, and incubated again at 37°C for 60 min. After centrifugation at 2000 x g for 20 min at 4°C, the tubes were aspirated and counted ^{125}I in a gamma counter.

At first, ether extract of the chick plasma was prepared as described above for estradiol radioimmunoassay. The dilution curve of the ether-extracted plasma seemed parallel with testosterone standard (Figure 2.3). Thus, the same plasma fraction as used for estradiol (25-40% acetonitrile) was used for the assay. The serial dilution of this fraction was parallel with the testosterone standard, and no cross reaction was seen after stripping (Figure 2.3). The interassay and intra-assay coefficients of variation were 47% (n=6) and 0.8% (n=10) respectively.

Progesterone

Plasma concentrations of progesterone were determined by radioimmunoassay using a commercially available kit with modification (Immuchem Double Antibody Progesterone ^{125}I RIA Kit; ICN Biochemicals). Progesterone standards were prepared by dissolving progesterone (Sigma) in 100% ethanol (1 mg/ml) and then diluting to desired concentrations with assay buffer as above. To each tube were added 50 µl of standard or plasma sample, and 250 µl of anti-progesterone, and 100 µl of ^{125}I progesterone (provided with the kit) were added. After incubation at 37°C for 60 min, 250 µl of precipitant solution (provided with the kit) was added. After centrifugation at 2000 x g for 20 min at 4°C, the tubes were aspirated and counted for ^{125}I in a gamma counter.

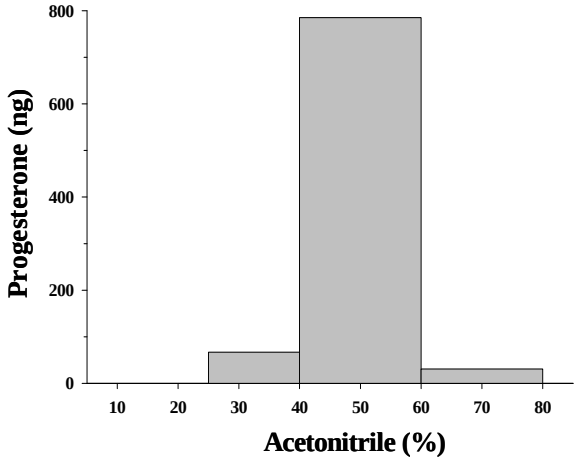


Figure 2.5. Elution profile of progesterone through Sep-Pack C-18 cartridge. Progesterone (200 ng in 400 µl assay buffer) was applied to the cartridge.

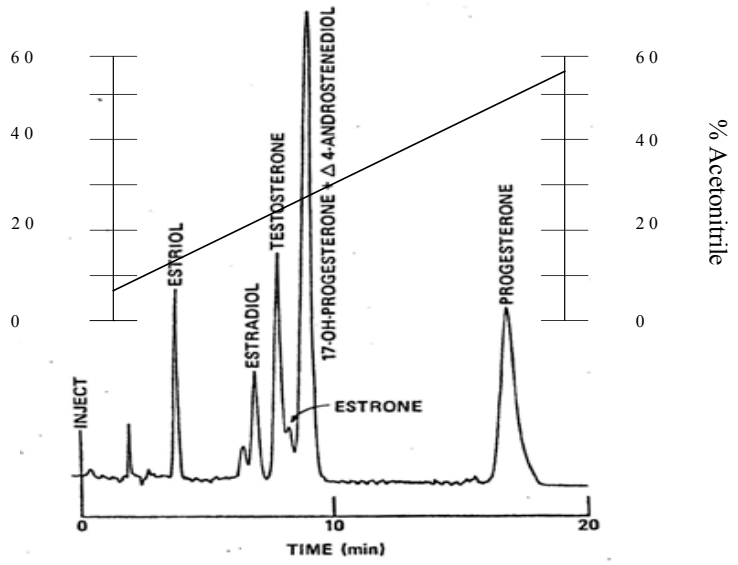


Figure 2.6. Separation of sex steroids by reverse-phase HPLC. Packing: µBONDAPAK C18, Solvent: 55% acetonitrile, Flow rate: 2 ml/min, Detector: UV at 254 nm.

In order to examine the elution profile of progesterone, 1000 ng progesterone in 100 µl assay buffer was applied to Sep-Pak C-18 cartridge, and eluted by stepwise increase in acetonitrile. Progesterone concentrations in each fraction were determined by radioimmunoassay as described above. As shown in Figure 2.5, progesterone was eluted exclusively in fractions between 40% and 60% acetonitrile. This is in accord with the elution profile of progesterone in reverse-phase high performance liquid chromatograph (HPLC), the progesterone peak appearing toward the end of the elution using 55% acetonitrile, and estradiol and testosterone appearing between 25-40% acetonitrile (Figure 2.6). The interassay and intra-assay coefficients of variation were 13% (n=5) and 11% (n=10) respectively.

In summary, estradiol 17-β, testosterone and progesterone were extracted from albatross plasma with the Sep-Pak C-18 cartridge as follows. The cartridge, preconditioned with 2-propanol and 0.1% TFA, was loaded with 250 µl of plasma sample. The cartridge was washed with 2 ml of 0.1% TFA, followed by 2 ml of 25% acetonitrile in 0.1%TFA. Estradiol and testosterone were eluted with 1.5 ml 40% acetonitrile in 0.1% TFA, and then progesterone was eluted with 1.5 ml 60% acetonitrile in 0.1% TFA. The eluates were evaporated to dryness, and the dried residues were reconstituted with 250 µl of assay buffer.

Total thyroxine (T4) and tri-iodothyronine (T3)

Plasma concentrations of total T4 were determined by radioimmunoassay using a commercially available kit with modifications (T4 Monoclonal solid Phase Radioimmunoassay; ICN Biochemicals). To each tube were added 12.5 µl of T4 standard or plasma sample, and then 500 µl of ^{125}I T4 (provided with the kit) were added. After incubation at room temperature for 60 min, the tubes were aspirated and counted for ^{125}I in a gamma counter.

Similarly, plasma concentrations of total T3 were determined by radioimmunoassay using a commercially available kit with modification (T3 Solid Phase Radioimmunoassay; ICN Biochemicals). To each tube were added 50 µl of T3 standard or plasma sample, and then 500 µl of ^{125}I T3 (provided with the kit) were added. After incubation at 37°C for 60 min, the tubes were aspirated and counted for ^{125}I in a gamma counter. These assays were validated using parallel dilution curves of albatross plasma with T4 or T3 standards (Figure 2.7). Interassay and intra-assay coefficients of variation for total T4 were 13% (n=3) and 13% (n=10), respectively. The interassay and intra-assay coefficients of variation for total T3 were 8% (n=4) and 14% (n=7), respectively.

Free T4 and T3

Plasma concentrations of free T4 were determined by radioimmunoassay using a commercially available kit with modification (Free T4 Solid Phase Radioimmunoassay; ICN Biochemicals). To each tube were added 25 µl of T4 standard or plasma sample, and then 500 µl of ^{125}I T4 (provided with the kit) were added. After incubation at 37°C for 90 min, the tubes were aspirated and rinsed with 1 ml of distilled water. After aspiration, the tubes were counted for ^{125}I in a gamma counter. Similarly, plasma concentrations of total T3 were determined by radioimmunoassay using a commercially available kit with modification (Free T3 Solid Phase Radioimmunoassay; ICN

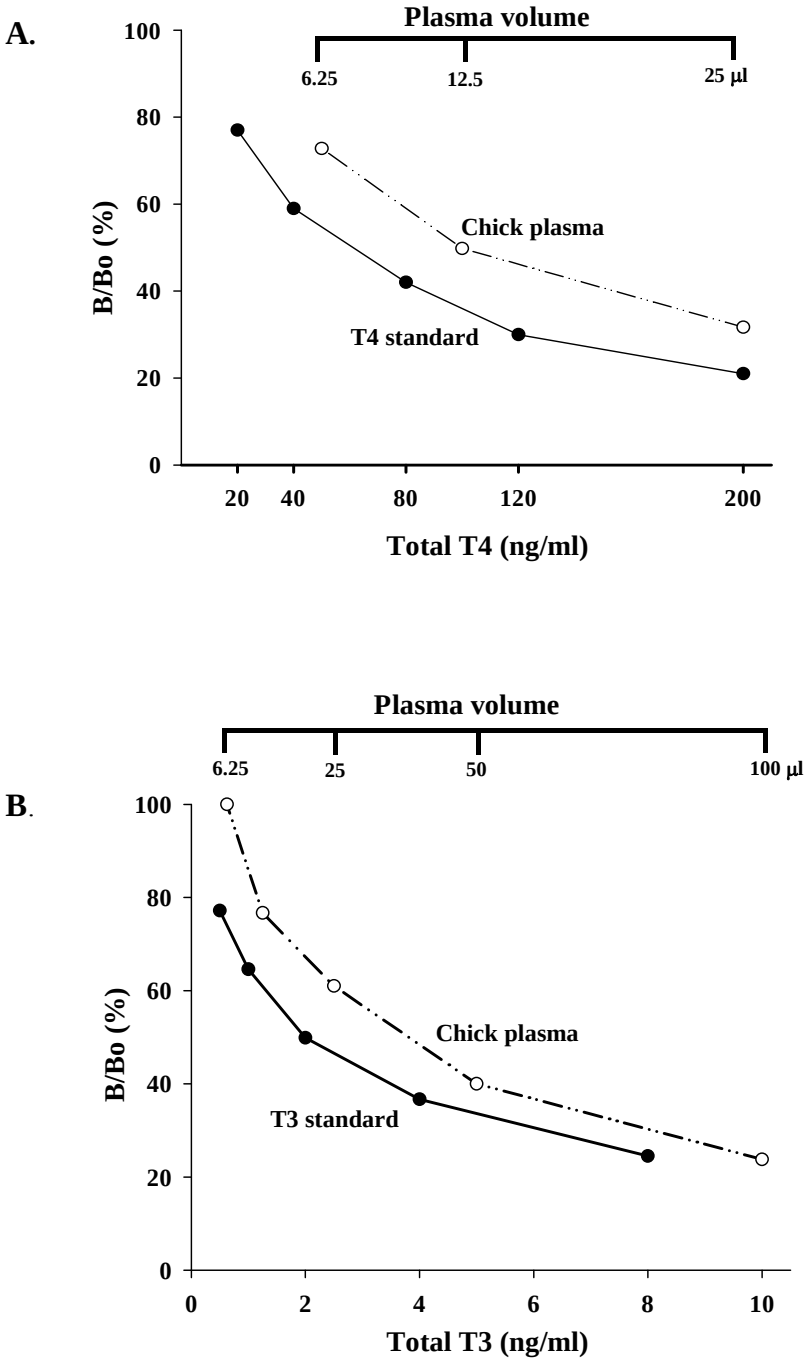


Figure 2.7. Displacement curves for total T4 (A) and T3 (B) and serial dilution of albatross chick plasma. Closed circles represent T4 and T3 standards. Open circles represent diluted albatross plasma. Each point represents the mean of duplicate determinations.

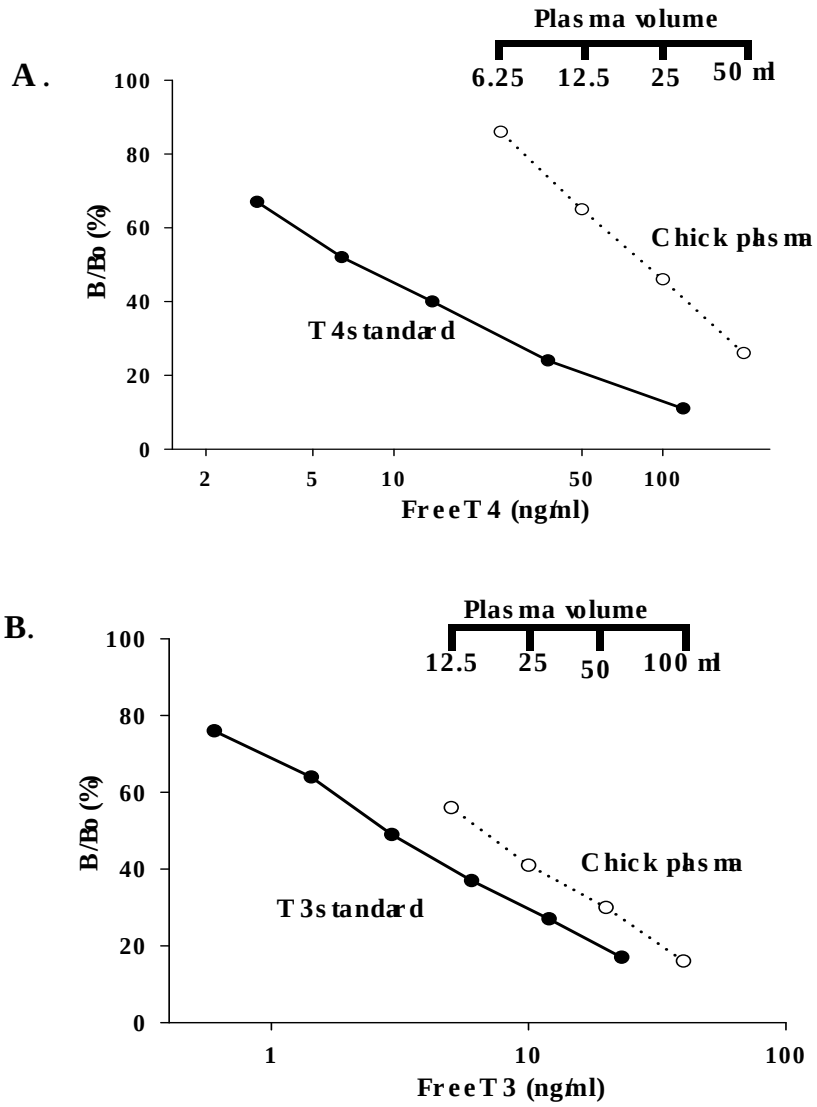


Figure 2.8. Displacement curves for free T4 (A) and T3 (B) and serial dilution of albatross chick plasma. Closed circles represent T4 and T3 standards. Open circles represent diluted albatross plasma. Each point represents the mean of duplicate determinations**.**

Biochemicals). To each tube were added 50 µl of T3 standard or plasma sample, and then 500 µl of ^{125}I T4 (provided with the kit) were added. After incubation at 37°C for 2.5 h, the tubes were aspirated and rinsed with 1 ml of distilled water. After aspiration, the tubes were counted for ^{125}I in a gamma counter. These assays were validated by parallel dilution curve of albatross plasma with the T4 or T3 standards. As shown in Figure 2.8, serial dilution of chick plasma was parallel with the T3 standard, whereas no parallelism was seen with the T4 standard. This seems to be unavoidable, as plasma samples could not be diluted with either serum or buffer, because of the nature of the T4/protein equilibrium. Interassay coefficients of variation for free T4 were 5% (n=5) and free T3 10% (n=3).

IV. RESULTS

PCB in Plasma

In most cases, the chicks had a higher weight than the adults, especially the Bulky Dump BFAL sampled, but did not necessarily contain the highest total PCB concentrations. The total PCB concentrations of the chicks from the B8 sampling site ranged from 26.9 ng/g to 223.8 ng/g. The adults from B8 Site contained total PCB concentrations ranging from 27.3 to 298.6 ng/g. Nest 44 showed high total PCB concentrations for the adults (264.5 ng/g, 257.2 ng/g) and chick (223.8 ng/g) as well as Nest 25, with the adults containing 264.8 ng/g and 203.4 ng/g and the chick 152.0 ng/g.

The total PCB concentrations in the chicks from the Bulky Dump sampling site ranged from 4.7 to 140.4 ng/g. The adults from the Bulky Dump sampling site contained PCBs ranging from 22.8 to 504.5 ng/g. One of the adults from Nests 84 and 185 contained very high concentrations of PCBs (504.5 ng/g and 498.8 ng/g, respectively).

B8 Plasma Samples

Chicks
Mean= 91.9 ng/g (wet wt.)
Range= 26.9-223.8 ng/g (wet wt.)

Adults
Mean= 145.3 ng/g (wet wt.)
Range= 27.3-298.6 ng/g (wet wt.)

Soil/Sand Taken from Nests

Mean=73.5 ng/g
Range= 8.4-262.9 ng/g

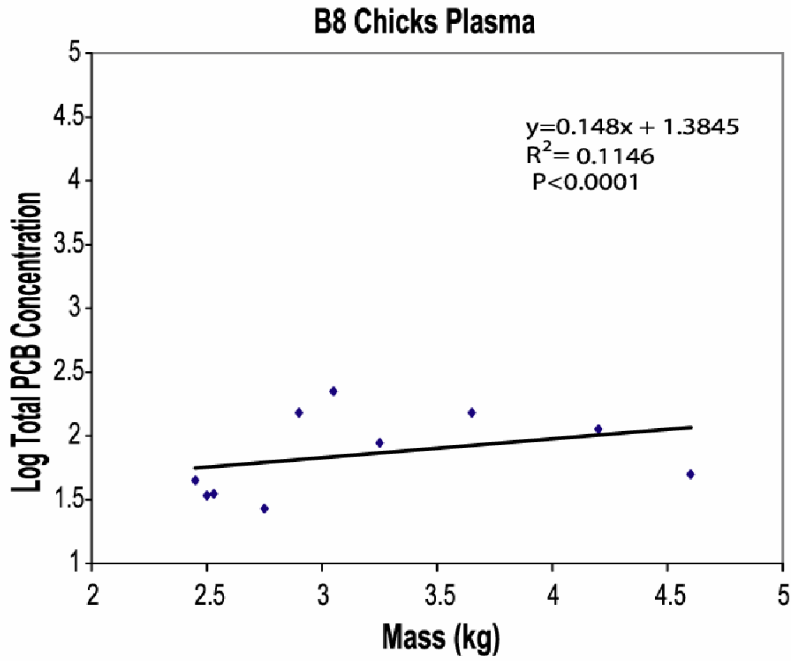
BD Plasma Samples

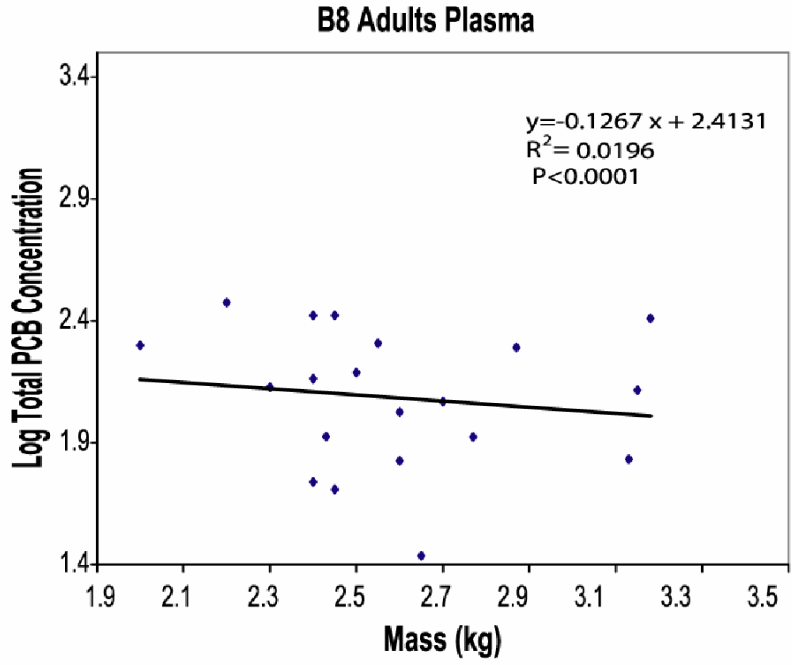
Chicks
Mean= 68.3 ng/g (wet wt.)
Range= 2.3-140.4 ng/g (wet wt.)

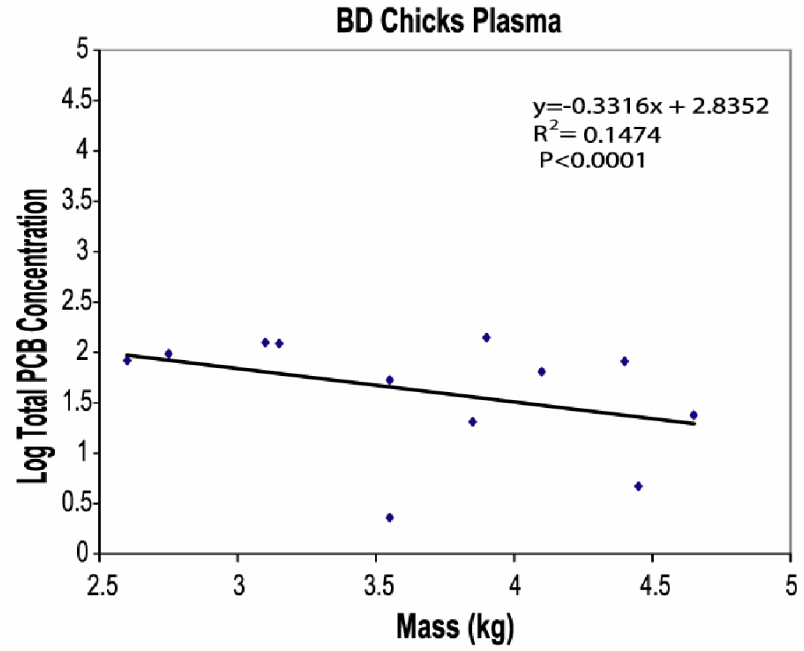
Adults
Mean= 171.9 ng/g (wet wt.)
Range= 22.8-504.5 ng/g (wet wt.)

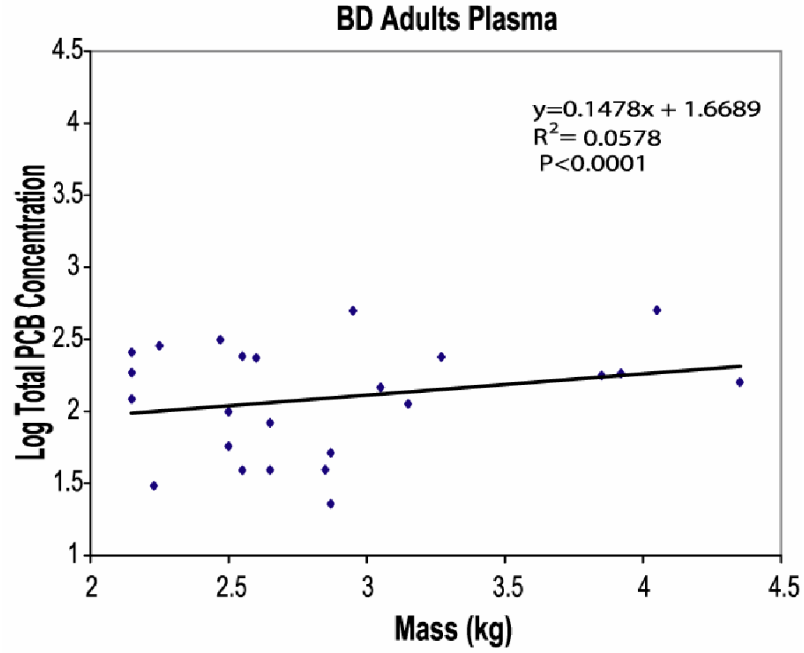
Soil/Sand Taken from Nests

Mean=32.8 ng/g
Range= 5.1-74.2 ng/g

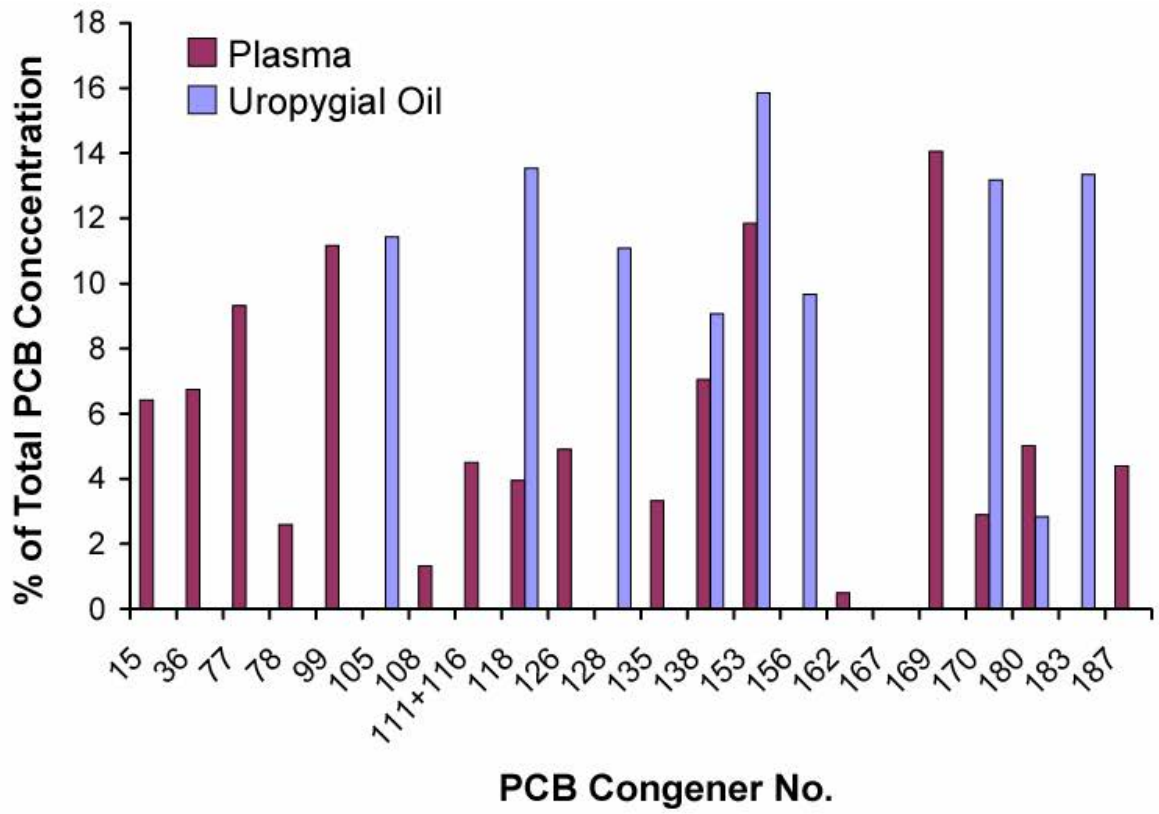

B8 Chicks Plasma
y=0.148x + 1.3845
R2= 0.1146
P<0.0001
Log Total PCB Concentration
Mass (kg)
5
4.5
4
3.5
3
2.5
2
1.5
1
2
2.5
3
3.5
4
4.5
5

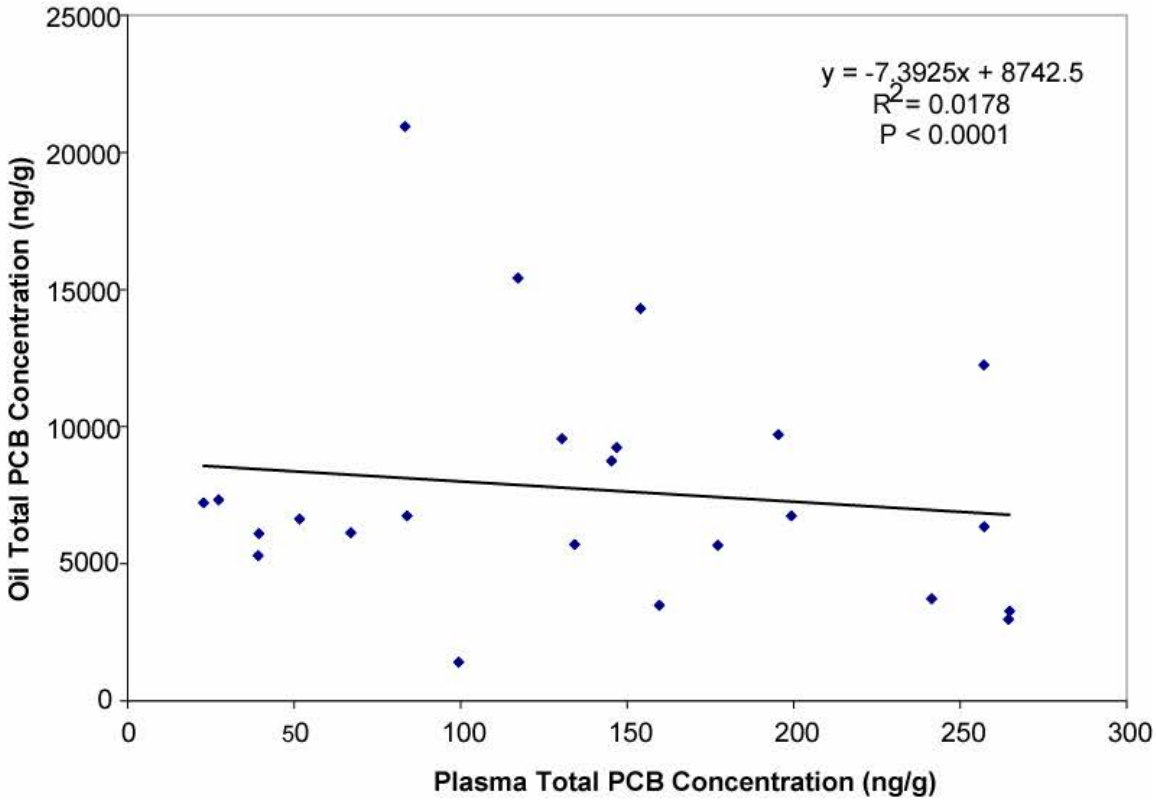

B8 Adults Plasma
y=-0.1267 x + 2.4131
R2= 0.0196
P<0.0001
Log Total PCB Concentration
Mass (kg)
3.4
2.9
2.4
1.9
1.4
1.9
2.1
2.3
2.5
2.7
2.9
3.1
3.3
3.5

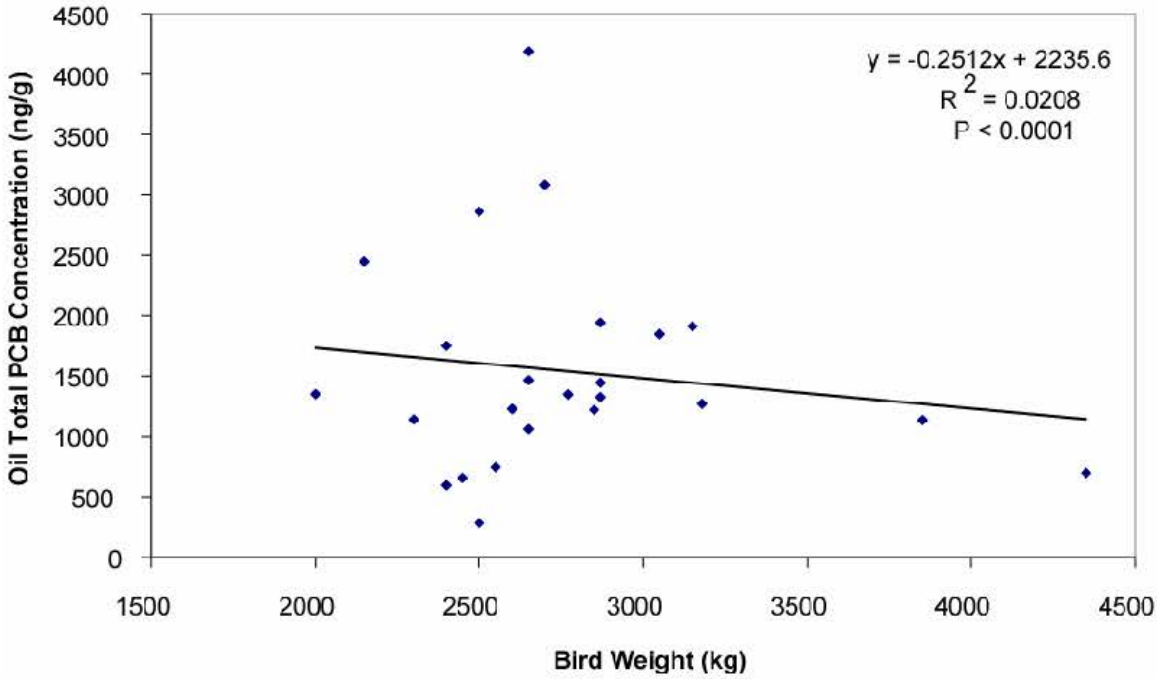

BD Chicks Plasma
y=-0.3316x + 2.8352
R2= 0.1474
P<0.0001
Log Total PCB Concentration
Mass (kg)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
2.5
3
3.5
4
4.5
5


BD Adults Plasma
y=0.1478x + 1.6689
R2= 0.0578
P<0.0001
Log Total PCB Concentration
Mass (kg)
4.5
4
3.5
3
2.5
2
1.5
1
2
2.5
3
3.5
4
4.5

PCB in Uropygial Oil




y = -7.3925x + 8742.5
R2 = 0.0178
P < 0.0001
Oil Total PCB Concentration (ng/g)
Plasma Total PCB Concentration (ng/g)
25000
20000
15000
10000
5000
0
0
50
100
150
200
250
300


y = -0.2512x + 2235.6
R 2 = 0.0208
P < 0.0001
Oil Total PCB Concentration (ng/g)
Bird Weight (kg)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
1500
2000
2500
3000
3500
4000
4500

PCB Concentrations in Eggs and Toxicity Assessment.
Total PCBs in four black-footed albatross (BFAL) eggs and three Laysan albatross eggs were analyzed and compared. The BFAL eggs of B8-49, BD-4, BD-113, and BD-184 contained 10.5, 8.0, 9.6, and 2.8 µg/g lw of total PCBs, respectively. The egg B8-49 contained the highest concentration of total PCBs, 10.5 µg/g, and the oldest BFAL on record was incubating this egg before abandoning it. This high concentration could be accounted for by the age and PCB accumulation of the BFAL producing the egg. The three Laysan albatross eggs contained 4.0-5.4 µg/g lw of total PCBs with similar TEQs (53-87 pg/g). The TEQs of BFAL egg BD-4 and BD-184 (37 and 17 pg/g lw, respectively) were smaller those of BFAL egg B8-49 and BD-113 (137 and 170 pg/g lw, respectively).

Auman and coworkers (1997) found 3.8 µg/g ww of total PCBs in a number of BFAL eggs. PCB concentrations in the BFAL eggs from Midway Atoll were similar to the eggs of several fish-eating birds from the Great Lakes, indicating that the albatross nesting on Midway Atoll are at a similar toxicological risk to the fish-eating birds found near the Great Lakes. The PCB levels detected in the BFAL eggs from Midway Atoll are close to the levels where population-level adverse effects are expected to occur.

Jones *et al*. (1996) found the total PCB concentrations in BFAL eggs to be 688 ng/g ww and TEQ concentrations of 124 pg/g ww. Auman *et al*. (1997) found total PCB levels to be 3800 ng/g (ww) and calculated the PCB-TEQ for the BFAL eggs to be 87 pg/g ww. At this level Auman *et al.* (1997) expected that the BFAL would be experiencing slight population-level effects. The total PCB concentrations in the eggs of this study were higher than those found by Jones *et al*. (1996) and Auman *et al*.(1997), although our concentrations are calculated on a lw basis. The total TEQ levels in the BFAL eggs ranged from 16.5-170.2 pg/g (lw) and the Laysan 53.1-87.4 pg/g (lw). This suggests that the BFAL would be experiencing population-level effects. Black-footed albatross eggs contained considerably higher concentrations of PCBs than Laysan albatross eggs, and this may be due to differences in diet and migration patterns (Jones et al., 1996).

Egg 4 contained the highest percentage of PCB 78 (25%), while the commonly accumulated congeners made up a lower percentage of the total PCB concentration (PCBs 99 (3%), 118 (3%), 138 (4%), 153 (4%), 170 (2%), 180 (3%) and 183 (2%)). PCB 78 was also the most abundant congener in Egg 163 (33 % of the total PCB concentration). The other abundant congeners in egg 163 were PCBs 99 (3%), 118 (4%), 138 (5%), 153 (5%), 170 (2%), 180 (3%) and 183 (3%). Egg 184 contained the highest concentration of PCB 4 (23% of the total PCB concentration), PCBs 8 (4%), 99 (4%), 118 (2%), 138 (5%), 153 (5%), 170 (3%), 180 (3%) and 183 (2%) were abundant congeners in this egg. The percentages of PCBs 99, 118, 138, 153, 170, 180 and 183 most closely resemble those found in the older chicks (4-5-month old chicks). This is indicative of the fact that the PCBs passed from the mother BFAL to the egg would also show metabolism. During the time of egg formation, lipids and PCBs are transferred to the eggs, and therefore provide information of the female at the time of laying (Braune and Norstrom, 1989). Jones *et al*. (1996) points out the contaminants found in the eggs tend to be of local origin and differ from the accumulated contaminants found in the tissues of the adult BFAL. During the breeding season, energy reserves are mobilized in preparation for egg laying, and the concentrations of contaminants in eggs reflect the pollutant uptake by the female a few days prior to egg laying (Munoz Cifuentes et al., 2003).

The egg from Nest 4 contained the highest TEQ value, 320.6 pg/g, and PCB 126 made up 99% of the total TEQ concentration. Egg 163 and 184 contained very similar total TEQ values, 155.3 and 156.3 pg/g, respectively. PCB 81 contributed 98% to the total TEQ value for Egg 163, and PCB 126 was 36% of the total TEQ for Nest 184.

Embryo metabolism is also a factor influencing the PCB congener pattern, and the stage of embryo development will effect the congener distribution of the egg (Mora, 1996). The PCB congener distribution of the contaminant exposure influences the congener distribution of the egg, and the absence of lower chlorinated PCBs can be due to the metabolism of the less persistent congeners by the liver of the female bird prior to egg laying (Munoz Cifuentes et al., 2003).

By looking at the ratio of the egg weight to the weight of the female, one can assess if the PCBs released by egg laying is able to reduce the levels of the female (Lemmetyinen et al., 1982). In the Arctic Terns and Herring Gulls, the percentages of egg weight to female weight were 32.4 and 29.2, respectively (Lemmetyinen et al., 1982). Tanabe *et al*. (1986) found that this ratio for Adelie Penguins was only 7.4%, and that the excretion of contaminants through egg laying is dependent on the species. This low percentage also applies to the BFAL, who lay one egg per nesting season. The average weight of the eggs analyzed is 277 g, and an approximate average weight of the female BFAL is 2500 g, making the percentage about 11 %. Therefore, the excretion of PCBs by the female BFAL during egg laying is not a major factor when comparing male and female BFAL total PCB levels. PCB egg concentrations are approximately the same or less than the maternal PCB concentrations (lipid wt), and the PCB congener distribution will be dependent on the mother's PCB congener content (due to metabolic capacity) (Barron et al., 1995). Contaminants in the egg would be diluted by the body fat reserves in the chicks (Guruge et al., 2000b).

The relative potencies of eggs from Green Bay ranged from 6 to 56 TEQ/□g PCB, and total PCB content is a poor indicator of biological potency of toxicity (even though the TEQ values are calculated from the PCB concentrations) (Giesy et al., 1994). The concentrations of TEQ in eggs of colonial fish eating water birds measured by bioassays were found to be 100-1000 pg/g, and this is within the range of concentrations that are able to cause toxicological effects (Giesy et al., 1994). The chemicals transferred to the egg comes from the maternal blood, and because the liver is in high contact with the blood, the egg levels may reflect the maternal body burdens at the time of egg formation quite well (Hario et al., 2004). After mating, the female BFAL leaves to build up fat and food reserves before returning to lay the egg (Warham, 1990). During this time, the lipid reserves are being mobilized for the formation of the egg and accumulated contaminants are transferred to the egg (Warham, 1990; Hario et al., 2004).

PCB Concentrations in 1-Month Old Chicks and Toxicity Assessment.

Table 2 shows the concentrations of total PCBs in the individual parts of the 1-month old BFAL chicks. The total PCB concentrations in the three birds are inversely related to the total body weights. BFAL B8-32 is the smallest 1-month old chick, weighing only 146 g, and contained the highest total PCB concentration (191 □g/g lw). BFAL BD-88 (309 g) and BD77 (413 g) contained 118 and 37 □g/g lw of total PCBs, respectively. The livers B8-32 and BD-88 contained the highest portion of total PCBs. It is interesting that the bone and head and neck contained the

highest concentrations of total PCBs. The major congeners in most of the albatross parts included PCBs 99, 118, 138, 153, 170, 180 and 183. These seven congeners accounted for 78, 75, 68, 80, 36, and 71% of the total PCBs in bone, head and neck, liver, muscle, internal organs, and skin, respectively. The seven major congeners in BD-77distributed, as relative to the total PCBs, 75% in bone, 81% in head and neck, 85% in liver, 82% in muscle, 82% in organs and 86% in skin. The seven PCB congeners in BD-88 made up the percentages of the total PCB concentration of 88% in the bone, 87% in head and neck, in liver (10%), 79% in muscle, 88% organs and 88% skin.

Twenty of the 209 PCB congeners have mono and non-ortho chlorine substituents, and these congeners can form a planar structure similar to the structure of the highly toxic 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDF) and as the number of ortho chlorines increases, the planarity of the congener decreases (Hope et al., 1997; Parham et al., 1997). PCBs 77, 126, and 169 contain 4, 5, and 6 Cl atoms in the non-ortho positions, respectively, and are very toxic and with toxicity similar to TCDD (Hope et al., 1997). Less toxic are PCBs 105, 114, 118, 123, 156, 157, 167, and 189, which are mono-ortho substituted, and the di-ortho substituted PCBs (PCBs 138, 153, 170, 180 etc.) are not very toxic, but these congeners can all still cause adverse effects in wildlife (Falandysz et al., 1994; Hope et al, 1997). The concept of toxic equivalent factors (TEF) involves toxicity comparison of the given compound to that of TCDD, and the combination of TEFs with chemical concentration data results in toxicity equivalent (TEQ) concentrations (Hope et al., 1997; Van den Berg et al., 1998). This assumes that the combinations of congeners are concentration additive, but this is not always accurate (Giesy et al., 1994). TEF and TEQs are used for risk management purposes, and using these numbers for biological and toxicological assessment is still uncertain due to the complexity of biological systems. Toxic effects on wildlife appear to be better correlated with total TCDD equivalent toxicities than to total PCB concentrations (Hope et al., 1997). In the Great Lakes fish-eating birds, the planar or co-planar PCBs have a greater contribution to the TCDD-TEQ than the PCDDs or PCDFs, and it is suspected to be a trophic level transfer from their food source (Giesy et al., 1994). This creates a greater potential for bioaccumulation, and selective accumulation of the more toxic non-ortho PCB congeners in tissues of the organism can result in a congener mixture more toxic than the original Aroclor, up to 4-6 times greater (Giesy et al., 1994).

TEQs were calculated for the various parts using TEFs for birds from Van den Berg et al. (1998). The PCB congeners 81 and 126 have a TEF value of 0.1 in birds, while PCB 77 has a TEF of 0.05 (Van den Berg et al., 1998). PCB 114 and PCB 169 both a TEF value of 0.001 for birds, and these 5 congeners have the highest toxic effect on birds (Van den Berg et al., 1998).

Table 3 shows the TEQ values for the 1-month-old chicks. BFAL B8 32 has the highest total TEQ of the 1-month-old chicks (11165.4 pg/g). The liver of B8 BFAL 32 Chick showed the highest TEQ (4014.7 pg/g) and PCB 118 accounted for 37% of the total TEQ concentration, followed by PCB 114 (27%), PCB 156 (19%), PCB 155 (16%) and PCB 167 (1 %). The bone contains the second highest total TEQ concentration (3934.1 pg/g) and PCBs 118 (36%), 114 (31%), 105 (14%), 156 (11%), 169 (5%), 157 (3%), 167 (0.5%) and 189 (0.5%) contribute to the total TEQ. The muscle has a total TEQ concentration of 1117.2 pg/g and PCB 118 contributed 55% to the total TEQ followed by PCB 114 (27%), PCB 105 (18%) and PCB 167 (0.6%). The head and neck contains PCBs 105 (24%), 118 (75%), 167 (1 %) and 189 (0.2%), and the total

TEQ concentration is 993.6 pg/g. The skin has a total TEQ concentration of 894.9 pg/g, and PCB congeners 105 (25%), 118 (49%), 156 (19%), 157 (5%) and 167 (1%) contributed to the total TEQ concentration. The organs have the lowest total TEQ concentration (210.9 pg/g) and PCBs 105 (11%), 114 (60%), 118 (17%), 123 (0.2%), 157 (6%), 167 (0.9%), 169 (5%) and 189 (0.1%) were all present in the organs. All of the parts contained PCBs 105, 118 and 167 and PCB 118 was the congener in the highest abundance in all parts except the organs (PCB 114 is the highest). PCBs 77 and 126 were not found in any of the parts of BFAL B8 32 chick, but PCB 169 was found in the bone and organs.

BFAL BD 77 Chick has lower total TEQs than the other 1-month old chicks (124.3 pg/g). The bone had the highest total TEQ (35.6 pg/g) with PCB 118 making up 74% of the total TEQ. The liver had a comparable total TEQ value of 32.2 pg/g, and PCB 118 made up 93 % of the total TEQ. The head and neck has a total TEQ concentration of 23.64 pg/g and the concentration PCB 118 is 93% of the total TEQ. PCB 118 makes up 88% of the total TEQ of the organs (22.5 pg/g), and PCB 118 makes up 96% of the muscle total TEQ (8.5 pg/g). The lowest TEQ was found in the skin (1.9 pg/g) and PCB 118 contributed 92% to the total TEQ. PCB 118 was found in all of the samples and was the highest concentration congener in all parts. PCB 157 was found in all of the parts except for the skin, and PCB 105 was found only in the bone and skin.

BFAL BD 88 Chick contained moderate TEQ values in comparison to the other 1-month old chicks (total TEQ 2621.8 pg/g). The muscle had the highest total TEQ at 855.9 pg/g, and PCBs 118, 114, 156, 105 and 157 contributed to the total TEQ. The bone and the head and neck contained very similar total TEQ concentrations (784.5 pg/g and 770.2 pg/g, respectively) and PCB 118 was the found in the greatest abundance. The organs contained 102.9 pg/g total TEQ, and PCB 118 made up 52% of the total TEQ. The skin has a total TEQ of 55.2 pg/g and PCB 118 is 76% of the total TEQ. Surprisingly the liver contained the lowest TEQ concentration (52.99 pg/g) and PCBs 167 (51% of total TEQ), 105 (39%) and 118 (10%) were present. All of the samples contained PCB congeners 105, 118. PCB 167 is found in all of the samples except for the muscle, and PCB 114 occurs in most of the parts (not present in the liver and skin).

PCB Concentrations in 4-5 Month Old Chicks and Toxicity Assessment.

Table 4 shows the concentrations of total PCBs in the individual parts of the 4-5-month old chicks. BFAL BD OC-2 (723 g), OC-1 (882 g) and OC-3 (1352 g) contained 387, 233 and 311 µg/g lw of sum total PCBs in all the dissected tissues, respectively. The concentrations of total PCBs in the livers of all the three chicks on a dry weight (dw) basis were apparently higher than those in the other tissues. The total PCB body burdens were in OC-1, OC-2 and OC-3, 10.4 mg, 6.0 mg and 12.0 mg (dw), respectively. It should be noted that PCBs 99, 118, 138, 153, 170, 180 and 183 in OC-1 are present, but at lower concentrations (7-18%) in various tissues than those seen in the 1-month-old chicks. These seven PCBs make up 24% in bone, head and neck, liver and organs, 8% in muscle and 21% in skin of OC-2. The muscle shows a higher abundance of the lower chlorinated congeners than the more highly chlorinated PCBs. The concentrations of the seven PCBs accounted for 19% in bone, 22% in head and neck, 6% in liver, 15% in organs, 18% in skin and 26% in muscle of OC-3.

Table 4. Total PCB distribution among various body parts of three 4-5-month old BFAL chicks collected from BD nests

Body part	**Total PCB concentration, µg/g**					
	BD OC-2		BD OC-1		BD OC-3	
	dw[a]	lw[b]	dw	lw	dw	lw
Bone	0.9	44	0.3	49	0.8	47
Head and neck	2.4	87	0.7	39	1.4	43
Liver	5.7	97	2.7	39	2.5	49
Muscle	2.8	109	0.9	58	0.8	116
Internal organs	1.1	24	1.2	22	2.6	35
Skin	1.5	26	1.1	26	1.0	21
Sum concentration	14.4	387	6.9	233	9.0	311
Total body wet weight, g	723		882		1352	
Total PCB body burden, mg	10.4	280	6	206	12	420

[a] dw = PCB concentrations based on dry weight.
[b] lw = PCB concentrations based on lipid weight.

Table 4 shows the total PCB concentrations in the individual parts of the 4-5- month old chicks. BD BFAL OC 1 is the medium sized 4-5-month old chick analyzed, and is 882 g total mass. The muscle contained the highest total PCB concentrations (58 µg/g). The bone contained 49 µg/g total PCB concentrations, followed by the head and neck (39 µg/g total PCB concentration). The liver contained 39 µg/g total PCB concentrations, and the skin contained 26 µg/g total PCBs. The organs contained the lowest total PCB concentration (22 µg/g). The major congeners detected in all of the older chick samples showed a greater diversity than those in the younger chick samples. PCBs 99, 118, 138, 153, 170, 180 and 183 are present, but at lower concentrations. The percentage of these 7 congeners in bone (16%), head and neck (7%), liver (10%), organs (9%), muscle (7%) and skin (18%) is considerably lower than the percentages seen in the 1-month-old chicks.

BD BFAL OC 2 is the smallest 4-5-month old chick, with a total mass of only 723 g. This chick contained the highest total PCB concentrations (387 µg/g, total PCB concentration for all parts). The liver contained the highest total PCB concentration (97 µg/g), followed by muscle (109 µg/g), head and neck (87 µg/g), bone (44 µg/g), skin (26 µg/g), and organs (24 µg/g). PCBs 99, 118, 138, 153, 170, 180 and 183 make up the following percentages in the parts of BD BFAL OC 2: bone (24%), head and neck (24%), liver (24%), muscle (8%), organs (24%) and skin (21%). The muscle shows a higher abundance of the lower chlorinated congeners than the more highly chlorinated PCBs.

BD BFAL OC 3 is the largest 4-5-month old chick analyzed, total mass of 1352 g. The muscle contained the highest concentrations of total PCBs (116 µg/g), and the liver contained 49 µg/g total PCB concentration. The bone contained 47 µg/g total PCB concentration. The head and neck contained 43 µg/g of total PCB concentration, followed by the organs (35 µg/g), and the skin (21 µg/g). The parts of BD BFAL OC 3 contain the following percentages of PCBs 99, 118, 138, 153, 170, 180 and 183 concentration: bone (19%), head and neck (22%), liver (6%), organs (15%), skin (18%) and muscle (26%).

The congener distribution was quite uniform with the 1-month old chicks, and PCB congeners 99, 118, 138, 153, 170, 180 and 183 were the most abundant congeners in all of the parts and in all of the birds in the younger age group. This is illustrated in Figure 1A where the PCB congener

distribution is shown for the parts of BFAL chick 32. A majority of the congeners are very similar among the parts, but the organs show a more diverse distribution, possibly due to food and/or decomposition.

Figure 1B shows the PCB congener distribution in BFAL OC-2, and there is much more variety in the congener distribution among the parts. There is a higher abundance of lower chlorinated congeners and the PCBs 99, 118, 138, 153, 170, 180 and 183 are no longer the highest congeners in abundance. The congeners are relatively similar among the different parts of BFAL OC-2, but a greater variation exists in the lower chlorinated PCBs.

The hexa-chlorinated PCBs made up 40-50% of the total PCBs in all 6 of the parts analyzed, and the Cl6PCBs had the highest percentage for all parts of B8-32. The hexa-chlorinated PCBs made up 50-60% of the total PCBs in all of the parts from BFAL-77 chick, and the Cl7PCBs were the second highest in abundance. The hexa-chlorinated PCB congeners were the most abundant congeners present in BFAL-88 chick.

Figure 2 shows the distribution of PCBs by the number of chlorine atoms in the different parts of a 1-month old chick, a 4-5-month old chick and the distribution in the Aroclor mixtures. Figure 2A shows that the Cl6PCBs are the most abundant congeners in all of the parts of BFAL chick 77. There is the closest resemblance to Aroclor 1260, but the match is not exact. A small percentage of lower chlorinated congeners (Cl2-4 PCBs) are found in the parts of BFAL chick 77, and this may be due to metabolism or breakdown. The same patterns are seen in the other 2 1-month old chicks. Figure 2B shows the chlorine distribution of the parts of BFAL OC-2. From this plot, it can be seen that the degree of substitution is much more varied in the OC and that the pattern does not match very closely with any of the Aroclor mixtures. This same variation is also seen with the other 4-5 month old chicks. The change in congener substitution is representative of breakdown of congeners in the older chicks, and the metabolism of PCBs in the 4-5 month old chicks is much greater than that of the 1-month old chicks. The 1-month old chicks are in a state of building up fat reserves, so the PCBs ingested are being stored and the 1-month old chicks are not able to metabolize PCBs as easily as the older chicks. An immature white-tailed sea eagle was found to contain different PCB congener pattern than the adults, suggesting that the immature sea eagles are not able to metabolize PCBs like the adults (Falandysz et al., 1994). This makes quantification by Aroclor standards difficult since the congener distribution would be different due to metabolism, and therefore individual congener analysis is needed. By analyzing for individual PCBs, a better understanding of which congeners are metabolized vs. accumulated is achieved and a more accurate concentration level is determined.

Comparison and Discussion of Chick Data

The position in the food chain exposes the seabird to different PCBs, and this determines the overall congener profile (Focardi et al., 1988). The differences in congener distributions from one trophic level to another is dependent on environmental "weathering" and the sorting of compounds by solubilities, volatilities, or degradation rates (Giesy et al., 1994). The congener profiles in biota are dependent on a variety of factors: foraging range, food preferences, behavior, metabolic capabilities, species lipid contents, depuration rates, uptake of certain congeners through diet, biotransformation (Hope et al., 1997) In migratory species, the concentrations of individual components of the mixtures are a function of time and space, and exposure of an

organism at one location may differ from exposure at a different location (Braune and Norstrom, 1989; Parham et al., 1997). Fish-eating birds tend to have a predominance of higher chlorinated congeners, and generally the proportions of some higher chlorinated congeners (≥ 5 Cl) increase through the food chain, while the less chlorinated PCB congeners make up a small percentage of the congeners (Mora, 1996; Hope et al., 1997). The amount of lower chlorinated congeners decreases as the trophic level increases, but atmospheric transport of PCBs is still applicable to migratory open ocean seabirds (Jones et al., 1996). Atmospherically deposited PCB congeners tend to be lower chlorinated ones (Jones et al., 1996). Jones et al. (1996) found the congener profiles in the BFAL of Midway unique because of the low abundance of the lower chlorinated PCB congeners (< 5 Cl atoms).

The stereochemistry of PCBs can influence the metabolism of the PCB congener. The position of the Cl atoms on the phenyl ring(s) creates a diminished ability of the congener to be metabolized due to the incorrect orientation of the biphenyl rings in relation to the active site of cytochrome P-450 (Borlakoglu et al., 1990a). The less planar, highly asymmetric congeners will more readily be sequestered in fat than the symmetric, planar congeners (Parham et al., 1997).

Congeners that are metabolized contain at least one pair of adjacent unsubstituted meta-para carbons in the biphenyl rings because oxidative metabolism is favored at the meta-para carbon atoms in the least chlorinated phenyl ring (Borlakoglu et al., 1990a,b). When the site is sterically hindered by chlorine atoms, the probability of metabolism decreases as the degree of chlorination increases (Borlakoglu et al., 1990a,b). The absence of chlorine substituents at adjacent positions in at least one of the phenyl rings increases the capacity for metabolism (Braune and Norstrom, 1989). Orientation of Cl positions relative to the bond joining the phenyl rings is also related to metabolism. The PCBs are metabolized by the hepatic microsomal enzyme that can be separated into the phenobarbital (PB-type) and methylcholanthrene (MC-type) induced microsomal enzymes (Guruge et al., 2001b). The phenobarbital-type enzymes are responsible for the metabolism of PCBs in birds, and congeners with meta-para chlorine unsubstituted carbon atoms in the biphenyl ring are metabolized by the PB-type enzymes (Guruge et al., 2001b) This makes the highly toxic ortho-meta chlorine unsubstituted congeners more likely to be accumulated (Braune and Norstrom, 1989; Guruge et al., 2001b).

The absence of hydrogen atoms at adjacent carbon atoms is an essential structural feature for the accumulation of a particular PCB congener (Borlakoglu et al., 1990b). PCBs 138, 153, 170, 180, and 187 are the most common congeners found in the highest abundance in biota samples, and structurally, these congeners contain chlorine atoms in positions 2,4,5-, 2,3,4- or 2,3,5- positions in one or both of the phenyl rings, para positions on both rings, and the absence of adjacent unsubstituted meta and para positions (Focardi et al., 1988; Gonzalez et al., 1991; Hope et al., 1997). PCBs 138, 153, 170, 180, and 183 are the major components of Aroclors 1254 and 1260 (Borlakoglu et al., 1990a). PCBs with no adjacent chlorines in the meta-para locations are metabolized to the greatest degree (PCBs 101, 110, 141, 149, 174), followed by meta-ortho locations (PCBs 60+56, 66, 74, 99, 105, 118, 128, 137, 138, 170+190, 171, 177) (Braune and Norstrom, 1989). PCB congeners with no adjacent unsubstituted positions are metabolized very slowly or not at all (PCBs 146, 153+132, 172+197, 178, 180, 182+187, 183, 194, 200+157, 201, 203+196) (Braune and Norstrom, 1989).

Munoz Cifuentes et al. (2003) state that PCB degradation in birds constantly exposed to high levels of PCBs is faster because the liver enzymes involved in metabolism are induced to a greater extent. Generally, birds do not have the ability to metabolize the highly chlorinated PCBs, but the albatross can induce some xenobiotic metabolizing enzyme activities that other bird species cannot (Jones et al., 1996).

Jones et al. (1996) found that the ratio of PCB 101: PCB 99 was approximately 1:10 in albatross samples from Midway Atoll. PCBs 99 and 101 are similar physically and chemically, but the predominance of PCB 99 over PCB 101 may be due to differential metabolism by the BFAL (Jones et al, 1996). PCB 101 contains adjacent unsubstituted meta and para positions, which are required for oxidative metabolism and elimination, and it has been shown that PCB 101 is metabolized by fish-eating sea birds.(Borlakoglu, 1990a) PCB 99 has chlorine substituents at both para positions, and therefore does not have unsubstituted adjacent meta and para positions, making this congener more likely to be accumulated. The average PCB 101: PCB 99 in the 1-month-old chicks is 1:40, while the ratio is 1:10 in the 4-5-month old chicks. These results suggest that the 1-month old chicks are accumulating PCB 99 to a greater extent than the older chicks, and the older chicks may be metabolizing PCB 99 to a greater extent compared to the 1-month old chicks.

Phillips et al. (2003) compared the chick metabolic rate and growth of three species of albatross (not including the black-footed albatross). Seabirds grow slowly, but accumulate large lipid reserves during the nestling stage, and albatross chicks peak in their body mass during the late rearing period, but lose mass prior to fledging (Phillips et al., 2003). The chick feeding varied because of an unpredictable food supply from the parents. The accumulation of lipids is high in the early rearing period (when energy requirements are lower and the reserves can be used in later development when energy requirements are greater). The food supply is energy rich, but low in nutrition, and there is an accumulation of reserves for use in the post-fledgling period (when foraging skills have not been fully developed) (Phillips et al, 2003). The difference in lipid percentages between the BFAL age groups was quite substantial. The 1-month old chicks contained a much higher percentage of lipids in all of the parts, with averages ranging from 5.6% (bone) to 26.0% (skin). The 4-5-month old chicks showed lower lipid percentages, and the average bone lipid percentage was 1.1%, for the skin was 4.7%, and for the liver was 5.9%. In their early development stage, the younger chicks are building up their fat reserves, and the 4-5-month old chicks are larger in size so the lipids are not as concentrated as in the small chicks. These older chicks may already be utilizing some of their fat reserves if the parents have stopped coming back to feed them. The 4-5-month old BFAL chicks are getting ready to fledge, so these chicks are losing some weight prior to flying.

The total PCB concentrations in the 4-5-month old chicks are higher than the levels in the 1-month old chicks, but not proportional to the increase in mass of the chicks. Generally, the total PCB concentrations increase with the growth stage, but Guruge et al. (2000a) observed a dilution of total PCBs during the rapid growth stage in nestlings. Lemmetynin et al. (1982) looked at the PCB levels at different life stages of artic terns and herring gulls, and found the highest levels in the adults and the newly hatched chicks, and in both species, the chicks showed a sharp decrease in total PCB concentrations at 2-4 weeks, with the herring gulls decreasing even more prior to fledging. Nestling juveniles provide the best indicators for local environment contamination

because they are stationary during this time and would be most affected by their surrounding environment (Guruge et al., 2000b). There was a general increase in PCB concentration and age observed with the common cormorants from Japan (Guruge et al., 2000b).

The smaller chicks in both age groups, BD BFAL Nest 32 and BD OC-2, contained the highest levels of total PCBs, although only 3 chicks were sampled in each group. There could be some correlation between chick size and PCB contamination, and that either the highly loaded PCBs could slow the development of the chicks or the smaller body size concentrated the PCB levels. Falandysz et al. (1994) observed that white-tailed eagles that contained high PCB concentrations were emaciated and contained a low fat content in the breast muscle. Due to dilution, the PCB levels in a lighter weight chick tend to be higher than in heavier-weight chicks (Lemmetyinen et al., 1982; Hario et al., 2004). PCB tissue concentrations increase in times of starvation when lipids are mobilized, and PCBs are redistributed from adipose tissue to muscle and then from muscle to adipose tissue after feeding (Barron et al., 1995). Also, these smaller chicks may have been abandoned in a state of starvation and therefore their PCB levels would be higher due to lipid utilization and slow PCB metabolism and secretion (Barron et al., 1995). Food supply is one of the main factors in the growth rate of chicks (Phillips et al., 2003). The Procellariiformes chicks show patterns of infrequent weight gains from feedings and weight loss due to metabolism between feedings (Ricketts and Prince, 1984; Phillips et al., 2003).

The albatross close to Midway Atoll are exposed to several non-point contamination sources and a local source of contamination occurs (Jones et al., 1996; Guruge et al., 2001a). Air samples collected south of Midway Atoll contained a mean total PCB concentration of 130 pg/m3 and samples of seawater taken along this route in the North Pacific Ocean showed a mean total concentration of 24 pg/L (1993).

During the incubation and guarding periods, adult BFALs on Midway are in a state of fasting and lose approximately 25% of their body weight (Warham, 1990; Jones et al., 1996). Therefore, feedings when the adult guarding is in a starvation state, would be from the adult's lipid reserves and the PCB levels would tend to be high. During the nesting period, the adult albatross feed 300 to 500 miles north of the Hawaiian Islands chain at the major oceanic currents, not near Midway (Jones et al., 1996). The adult BFALs are not accumulating organochlorines from a local source of contamination (from Midway Atoll itself), and the contamination seen in the adult BFAL is representative of the more general contamination of the North Pacific (Jones et al., 1996; Guruge et al., 2001a). The food supplied to the chicks by their parents would be representative of this general contamination of the North Pacific. However, prior to fledging, the young albatross chicks spend their entire time on the ground at Midway Atoll. During this period of time, the chcicks are susceptible to local sources of PCB contamination on the atoll.

The greatest volume of work in avian toxicity effects of PCBs has been performed in the Great Lakes region, and these studies are the usual standard when comparing PCB levels.(Tilbury et al., 1997) The organochlorine levels detected in the BFAL on Midway Atoll are comparable to the concentrations found in birds from heavily contaminated areas, such as the Great Lakes (Jones et al., 1996).

The hepatic monoxygenase system is an active metabolic pathway, may become saturated, and PCB congeners transported from different tissues to the liver may be redistributed in the body (Rozemeijer et al., 1995). This redistribution of PCBs is limited by the rate of transport of PCB congeners from one area to the liver, and would regulate particular congener concentrations in the liver and the rate of metabolism of this congener (Rozemeijer et al., 1995). The accumulation of PCBs is determined by the neutral lipid (triglycerides) concentration in a tissue, and expressing the concentration of PCBs on a lipid weight basis makes comparing the PCB concentration of different tissues more reliable (Tilbury et al., 1997; Hario et al., 2004). The neutral lipid levels determine the degree of PCB partition between the adipose tissue and the blood (Haddad et al., 2000). The blood/brain barrier prevents PCB penetration, but it has been seen in sheep that this barrier is not completely functional at birth (Jan et al., 1999). The low levels of PCBs in the brain may be due to the blood/brain barrier, but also to the lower neutral lipid contents in the brain (Tilbury et al., 1997; Karlson et al., 2000). In the Caspian seal, the PCB concentrations in the various tissues follow an order of brain < lung < kidney < intestines < muscle < heart < blubber, and dependent on the lipid content of the tissue (Tanabe et al., 1986). Parham et al. (1997) correlated PCB structures with congener distribution, metabolism and accumulation. After entering the blood, a number of the PCB congeners may preferentially distribute into the liver, muscle, skin and adipose tissue (Parham et al., 1997). Those congeners with unsubstituted meta-para carbons tend to be more quickly eliminated than congeners with meta-para substitution (Parham et al., 1997) (check this sentence). There is a large inter-individual variability of lipid content in tissues, and is dependent on sex, diet, etc. (Parham et al, 1997).

The TEQs of the 4-5-month old chicks were considerably larger than those of the 1-month old chicks. The toxic PCB congeners are being accumulated to a greater extent as the chick becomes older, and that as the BFAL becomes older the more toxic PCB congeners increase in concentration. The BFAL is at risk for PCB toxicological effects, and the concentrations of these toxic congeners would be expected to increase over the lifetime of the BFAL. PCBs 77, 81 and 126 (have the highest TEFs for birds) all contain no ortho chlorine atoms and Cl substituents at the meta-para carbon atoms, making these congeners more likely to be accumulated rather than metabolized.

Hormone analyses of black-footed albatross at Midway and Tern Island

Estradiol

Levels of estradiol 17-β for adult and chick albatross at the two sites on Midway Atoll NWR ranged from 15 to 40 pg/ml (Figure 2.9). There was a significant difference between the nesting sites in adult albatross in Midway Atoll NWR. The mean levels of estradiol 17-β in the adults at Bulky Dump (BD) (contaminated) were significantly higher than the levels in those nesting at Black-foot 8 (B8) (reference site) ($P < 0.05$). There was no significant difference in estradiol 17-β in chicks from the two sites.

Testosterone

The testosterone levels for adults and chicks at the two sites ranged from 10 to 50 pg/ml (Figure 2.10). In contrast to estradiol 17-β results, plasma concentrations of testosterone in adults at Bulky Dump were significantly lower than the levels found at Black-foot 8 ($P < 0.001$). There was no significant difference ($P = 0.624$) in testosterone levels in chicks from the two sites. However, testosterone levels in the chicks at both sites were significantly lower than those of adults in Black-foot 8 and Bulky Dump ($P < 0.001$).

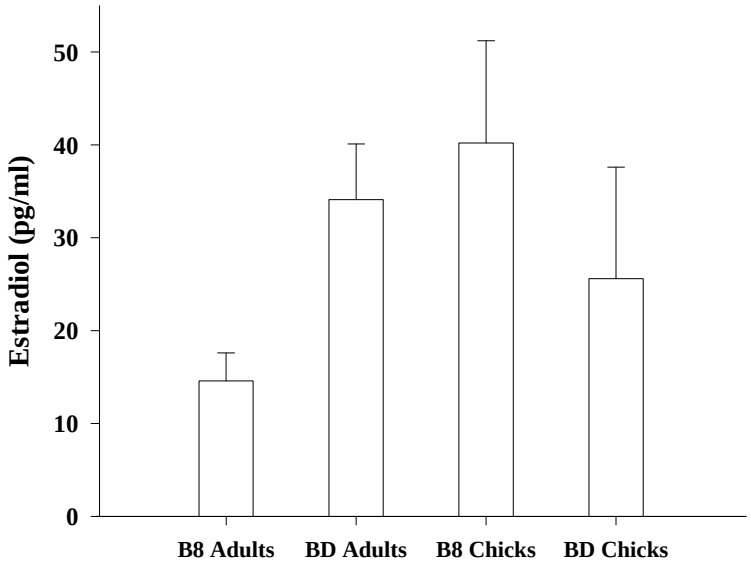


Figure 2.9. Estradiol levels (pg/ml) of adult and chick albatross at two sites on Midway Atoll, Black-foot 8 (B8) and Bulky Dump (BD). Vertical bars represent means ± SE (B8 adult, n=75; BD adult, n=72; B8 chick, n=48; BD chick, n=43). Values not sharing common letters are significantly different ($P < 0.05$).

Figure 2.10. Testosterone levels (pg/ml) of adult and chick albatross at two sites on Midway Atoll, Black-foot 8 (B8) and Bulky Dump (BD). Vertical bars represent means ± SEM (B8 adult,

n=75; BD adult, n=72; B8 chick, n=48; BD chick, n=43). Values not sharing common letters are significantly different ($P < 0.05$).

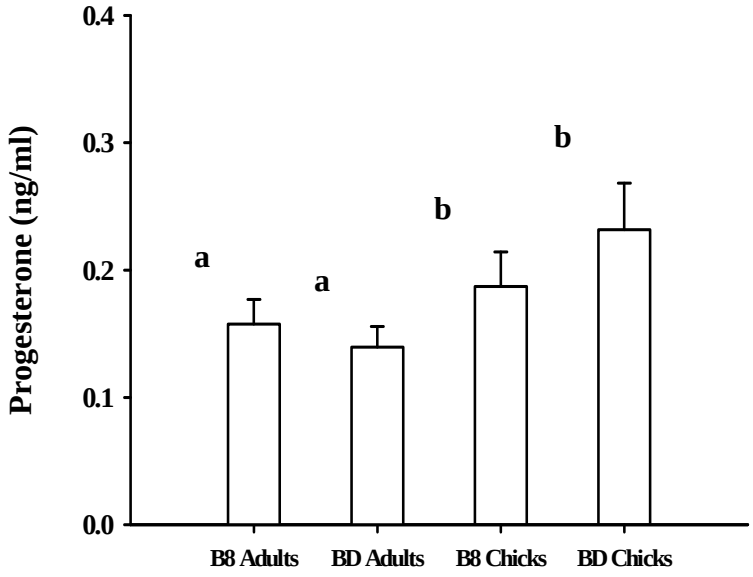


Figure 2.11. Progesterone levels (ng/ml) of adult and chick albatross at two sites on Midway Atoll, Black-foot 8 (B8) and Bulky Dump (BD) Vertical bars represent means ± SEM (B8 adult, n=75; BD adult, n=72; B8 chick, n=48; BD chick, n=43). Values not sharing common letters are significantly different ($P < 0.05$).

Progesterone

Plasma levels of progesterone for adults and chicks at the two sites were 0.1 to 0.3 ng/ml. No significant difference was observed among levels in adults at the two sites. Similarly, there was no significant difference in progesterone levels in chicks between the two sites (Figure 2.11)

Total T3 and T4

Total T3 levels in adult and chicks ranged from 1.3 to 2.2 ng/ml. Total T3 in the adults nesting at Bulky Dump was significantly lower than in those at Black-foot 8 ($P < 0.05$). There was no significant difference ($P = 0.772$) in T3 levels in chicks between the two sites, although the levels of the chicks were significantly higher ($P < 0.05$) than in adults (Figure 2.12).

Plasma concentrations of total T4 for adults and chicks were between 45 and 75 ng/ml. The total T4 levels were significantly higher in adults nesting at Bulky Dump compared with adults nesting at Black-foot 8 ($P < 0.001$). There was no significant difference ($P = 0.366$) in total T4 concentrations in chicks. However, the levels in the chicks were significantly ($P < 0.001$) higher than in the adults at Black-foot 8 (Figure 2.13).

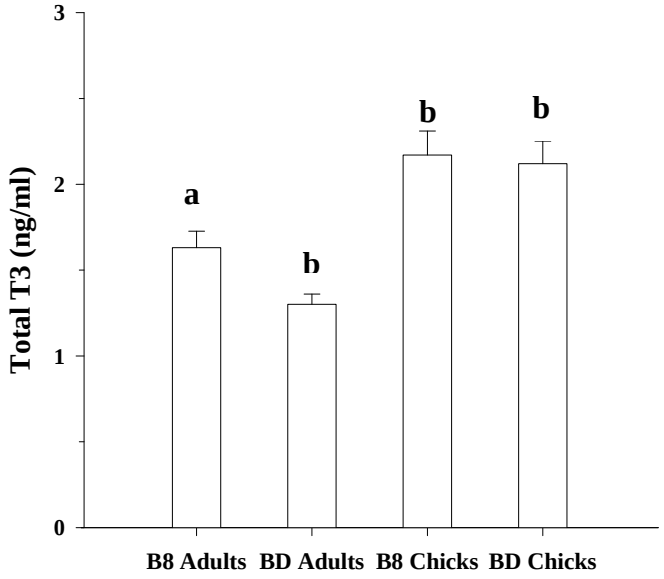


Figure 2.12. Total T3 levels (ng/ml) of adult and chick albatross at two sites on Midway Atoll, Black-foot 8 (B8) and Bulky Dump (BD). Vertical bars represent means ± SE (B8 adult, n=75; BD adult, n=72; B8 chick, n=48; BD chick, n=43). Values not sharing common letters are significantly different ($P < 0.05$).

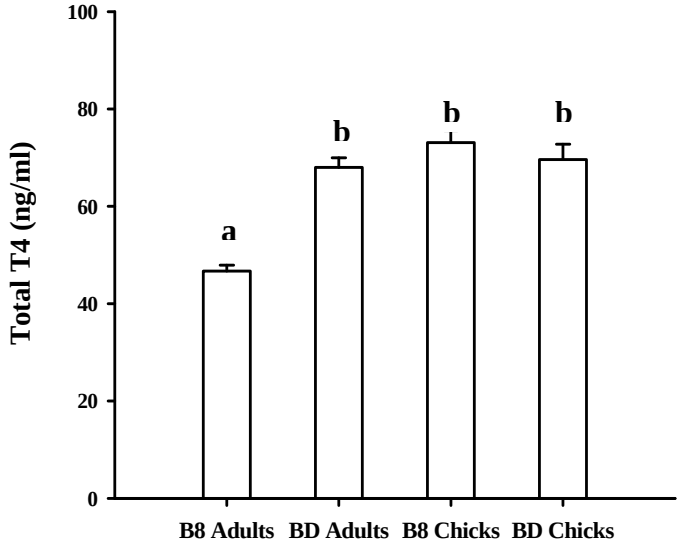


Figure 2.13. Total T4 levels (ng/ml) of adult and chick albatross at two sites on Midway Atoll, Black-foot 8 (B8) and Bulky Dump (BD). Vertical bars represent means ± SE(B8 adult, n=75; BD adult, n=72; B8 chick, n=48; BD chick, n=43). Values not sharing common letters are significantly different ($P < 0.05$).

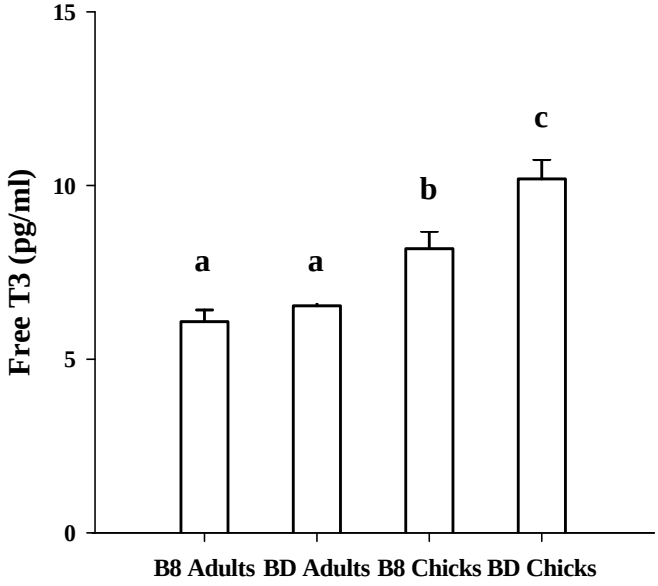


Figure 2.14. Free T3 levels (pg/ml) of adult and chick albatross at two sites on Midway Atoll, Black-foot 8 (B8) and Bulky Dump (BD). Vertical bars represent means ± SEM (B8 adult, n=75; BD adult, n=72; B8 chick, n=48; BD chick, n=43). Values not sharing common letters are significantly different ($P < 0.05$).

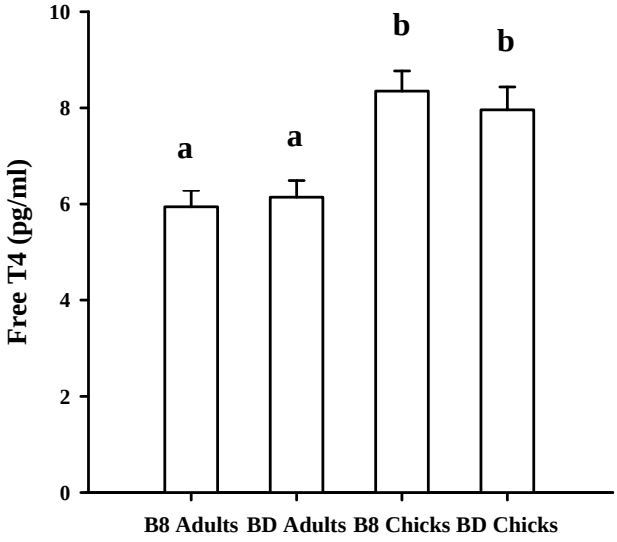


Figure 2.15. Free T4 levels (pg/ml) of adult and chick albatross at two sites on Midway Atoll, Black-foot 8 (B8) and Bulky Dump (BD). Vertical bars represent means ± SEM (B8 adult, n=75; BD adult, n=72; B8 chick, n=48; BD chick, n=43). Values not sharing common letters are significantly different ($P < 0.05$).

Free T3 and T4

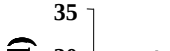
Free T3 levels for adult and chick albatross at both sites were 6-10 pg/ml. There was no significance difference ($P = 0.302$) between adults at Black-foot 8 and those at Bulky Dump. Free T3 levels in chicks at Bulky Dump were significantly higher ($P < 0.001$) than those at Black-foot 8. The levels in chicks were significantly ($P < 0.001$) higher than those in adults at both sites (Figure 2.14).

Free T4 levels for adults and chicks ranged from 6-8 pg/ml. There was no significant difference ($P = 0.795$) among adults at Black-foot 8 and those at Bulky Dump. There was also no significant difference ($P = 0.537$) observed among chicks nesting a Black-foot 8 and Bulky Dump. Chicks nesting at both Black-foot 8 and Bulky Dump had significantly ($P < 0.001$) higher Free T4 levels than adults nesting at both sites (Figure 2.15).

Correlation between PCB Concentrations in the Nest Cup and Plasma Hormone Levels

In January 2001, soil samples were collected from each nest cup for the analysis PCB concentrations. PCB concentrations are available for only 12 of 100 nests from Bulky Dump and 10 of 64 nests from Black-foot 8. Available data show no significant difference in PCB concentration between Bulky Dump (32.8 ± 6.9 □g/kg, Mean ± SEM, n = 12) and Black-foot 8 (73.5 ± 27.0 □g/kg, Mean ± SEM, n = 10) . Correlations between PCB concentrations in the nest cup and plasma hormone levels in the chicks

Figure 2.16. Correlation between PCB levels in the nest cup and plasma levels of estradiol, testosterone, and progesterone in albatross chicks from Bulky Dump (BD, filled circles) and Black-foot 8 (B8, open circles).



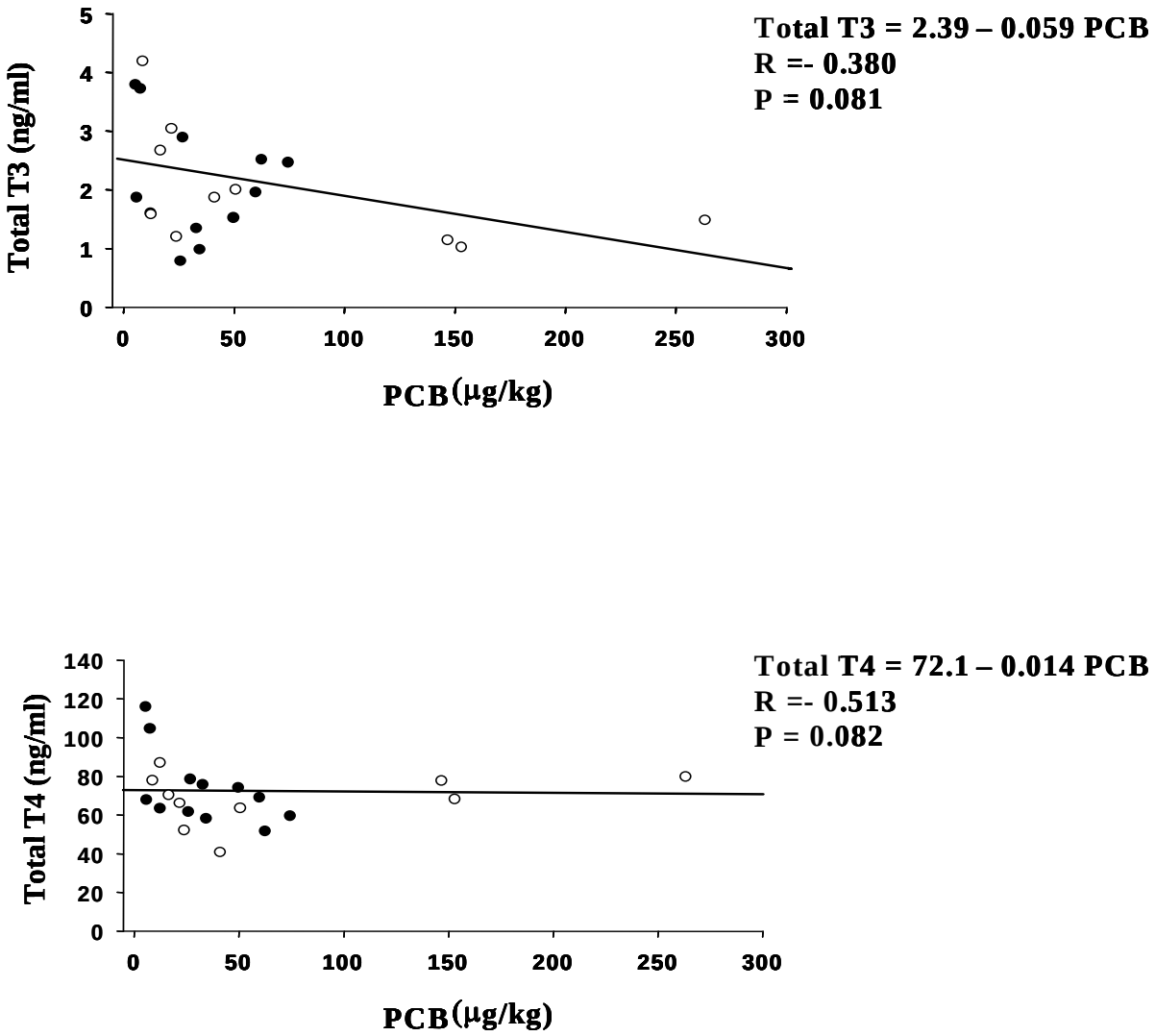


Figure 2.17. Correlation between PCB levels in the nest cup and plasma levels of total T3 and total T4 in the albatross chicks from Bulky Dump (BD, filled circles) and Black-foot 8 (B8, open circles).

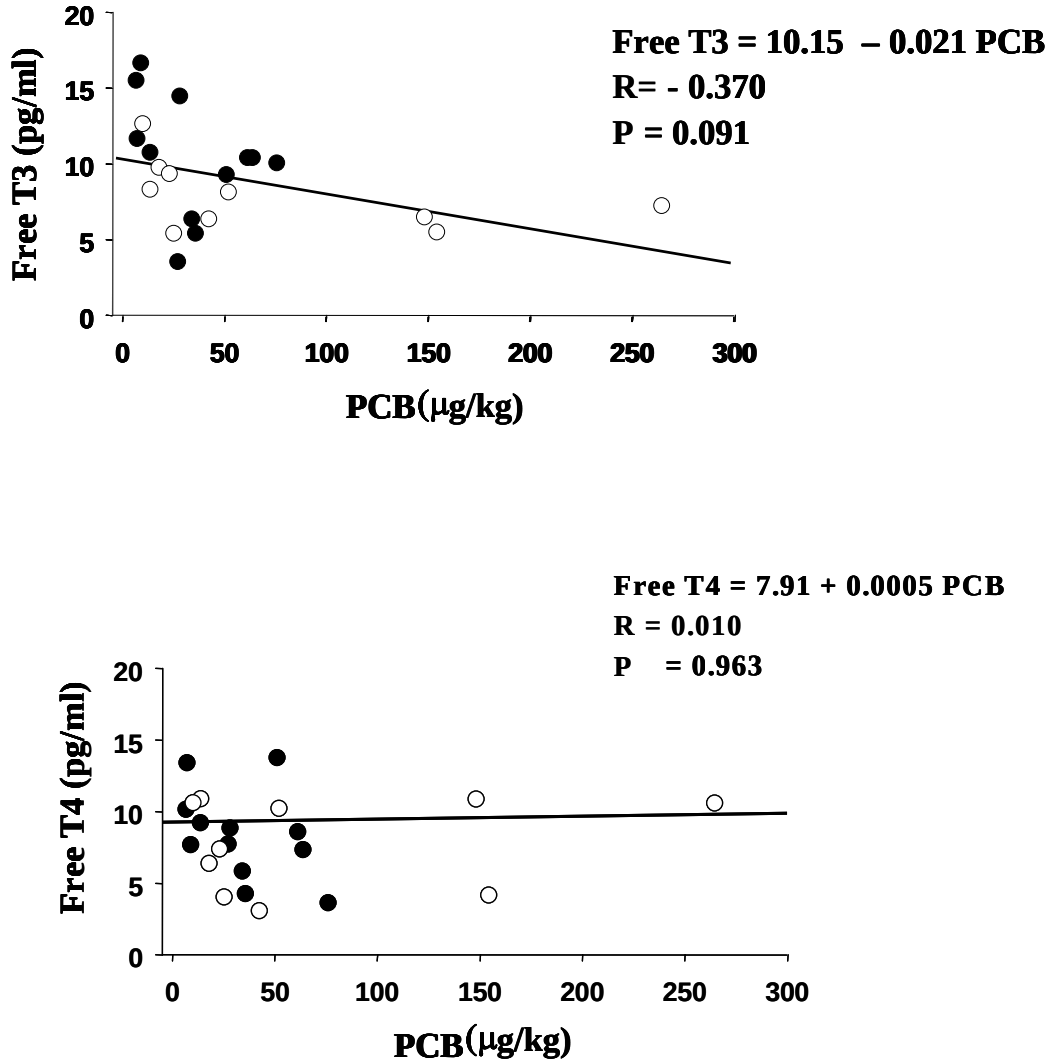


Figure 2.18. Correlation between PCB levels in the nest cup and plasma levels of free T3 and free T4 in the albatross chicks from Bulky Dump (BD, filled circles) and Black-foot 8 (B8, open circles).

were analyzed by combining the data obtained from Bulky Dump and Black-foot 8, since there was no difference in PCB levels between the two sites. As shown in Figures 2.16-2.18, no significant correlation was observed between nest cup PCB levels and plasma levels of estradiol, testosterone, progesterone, or total and free T3 and T4 in the chick. When the correlation was analyzed for Bulky Dump and Black-foot 8 separately, there was no significant correlation either, except for total T4 levels at Bulky Dump (Total T4 = 88.8 – 0.463 PCB, $r = -0.578$, $P < 0.05$).

Nesting activities of black-footed albatross (Phoebastria nigripes) were monitored on Midway Atoll National Wildlife Refuge during the 2001 breeding season. Blood samples were taken from adult and chick albatross, and analyzed for hormones indicative of endocrine disruption. Adults nesting at Bulky Dump (contaminated site) had significantly higher estradiol levels ($P < 0.05$) than those nesting at Black-foot 8 (reference site), whereas those nesting at Bulky Dump had significantly lower ($P < 0.001$) testosterone levels. Adults nesting at Bulky Dump had significantly higher ($P < 0.001$) total thyroxine (T4) levels than those nesting at Black-foot 8. In contrast, the adult albatross nesting at Bulky Dump had significantly lower ($P < 0.05$) total tri-iodothyronine (T3) levels than those nesting at Black-foot 8. There was no significant difference in progesterone, free T3 or T4 concentrations in adult albatross between the two sites. Chicks sampled from Bulky Dump had significantly higher ($P < 0.001$) levels of free T3 than chicks nesting at Black-foot 8. When PCB soil concentrations and plasma hormone levels were analyzed no relationship was found. These results indicate that although there are significant differences in the plasma hormone profiles in both adult and chick black-footed albatross nesting at two sites at Midway Atoll, no conclusive biological evidence of endocrine disruption from the nesting soil at the sites was obtained.

Tern Island

Estradiol, Testosterone and Progesterone

The estradiol levels for chicks at the two sites on Tern Island ranged from 30 to 48 pg/ml. There was a significant difference in estradiol and testosterone levels in chick albatross from nests at two locations on Tern Island. As shown in Figure 3.1, the mean levels of estradiol in the chicks at Old Landfill were significantly higher then the levels of estradiol in those at East End ($P < 0.05$).

The testosterone levels for chicks at the two sites ranged from 6 to 22 pg/ml. In contrast to estradiol, plasma concentration of testosterone in chicks at the Old Landfill was significantly ($P < 0.05$) lower than the levels found at East End (Figure 3.2).

On the other hand, plasma levels of progesterone for chicks at the two sites were .25 to .27 ng/ml. There was no significant difference ($P = 0.691$) observed in their progesterone levels in chicks between the two sites (Figure 3.3).

Total T3 and T4

Total T3 levels in chicks ranged from 1.9 to 2.2 ng/ml, and there was no significant difference ($P = 0.064$) observed in total T3 levels in chicks between the two sites on Tern Island (Figure 3.4).

Plasma concentrations of total T4 in chicks were between 54 to 95 ng/ml. The total T4 levels were significantly ($P < 0.001$) higher in chicks nesting at Old Landfill than in chicks nesting at East End (Figure 3.5).

Free T3 and T4

Free T3 levels for chick albatross at both sites were about 10 pg/ml. There was no significant difference ($P = 0.201$) between chicks at East End and those at Old Landfill (Figure 3.6).

Free T4 levels for chicks at Tern ranged from 4 to 6 pg/ml. There was a significant difference between the nesting sites in free T4 levels in chick albatross in Tern Island. Chicks from Old Landfill had significantly ($P < 0.001$) higher free T4 levels than chicks from East End (Figure 3.7).

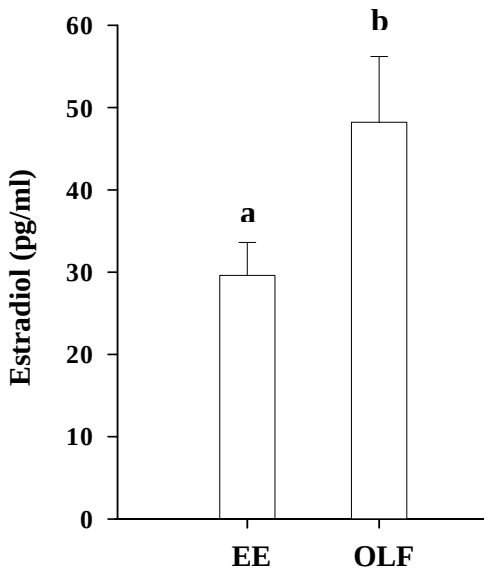


Figure 3.1. Estradiol levels (pg/ml) of albatross chicks at East End (EE) and Old Landfill (OLF) on Tern Island. Vertical bars represent means ± SEM (EE chick, n=45; OLF chick, n=45). Values not sharing common letters are significantly different ($P < 0.05$).

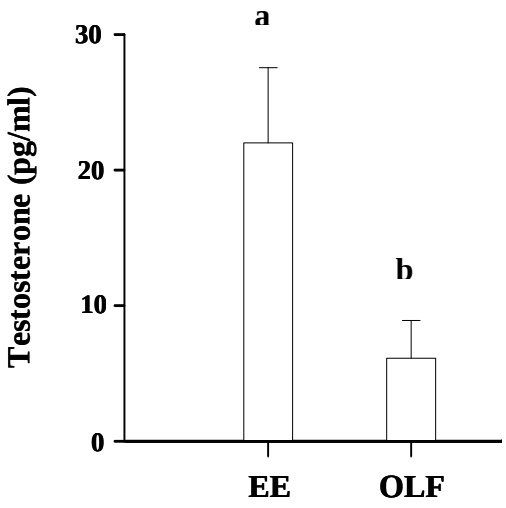


Figure 3.2. Testosterone levels (pg/ml) of albatross chicks at East End (EE) and Old Landfill (OLF) on Tern Island. Vertical bars represent means ± SEM (EE chick, n=45; OLF chick, n=45). Values not sharing common letters are significantly different ($P < 0.05$).

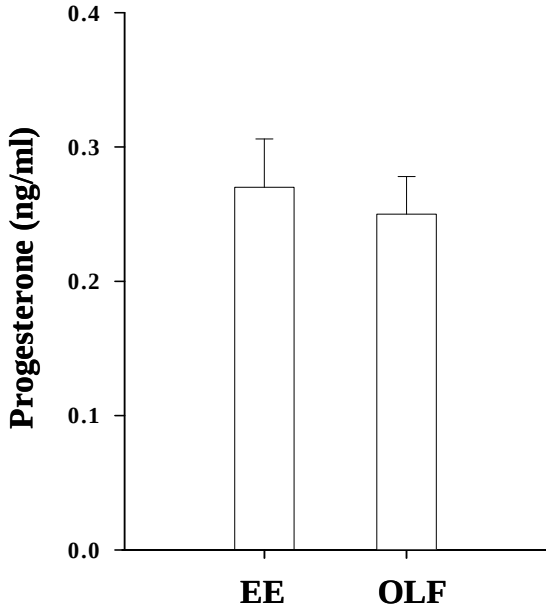


Figure 3.3. Progesterone levels (ng/ml) of albatross chicks at East End (EE) and Old Landfill (OLF) on Tern Island. Vertical bars represent means ± SEM (EE chick, n=45; OLF chick, n=45).

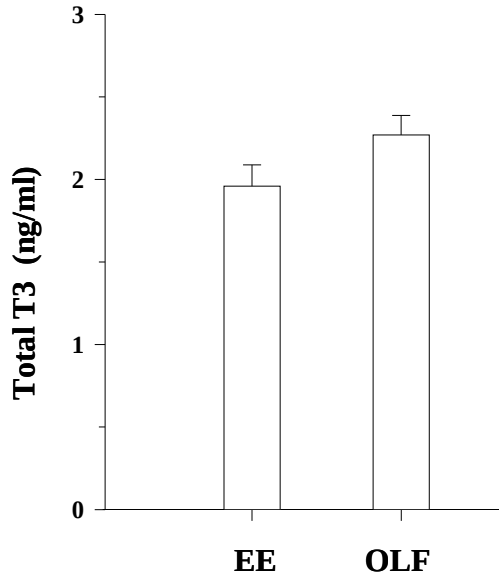


Figure 3.4. Total T3 (ng/ml) of albatross chicks at East End (EE) and Old Landfill (OLF) on Tern Island. Vertical bars represent means ± SEM (EE chick, n=45; OLF chick, n=45).

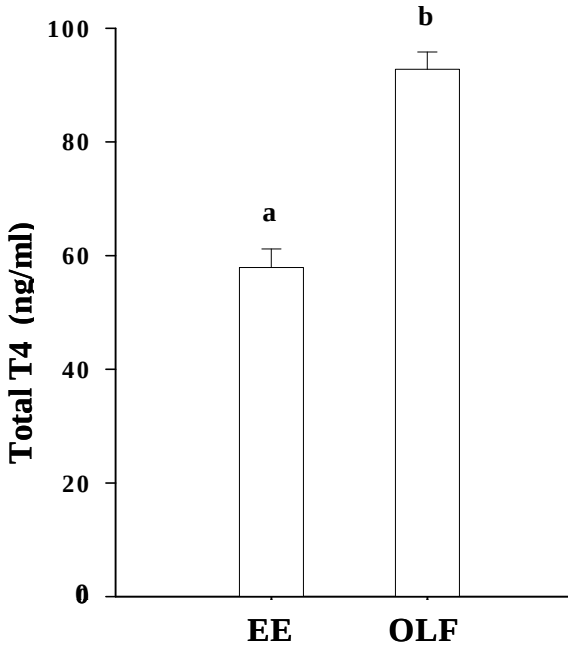


Figure 3.5. Total T4 (ng/ml) of albatross chicks at East End (EE) and Old Landfill (OLF) on Tern Island. Vertical bars represent means ± SEM (EE chick, n=45; OLF chick, n=45). Values not sharing common letters are significantly different ($P < 0.05$).

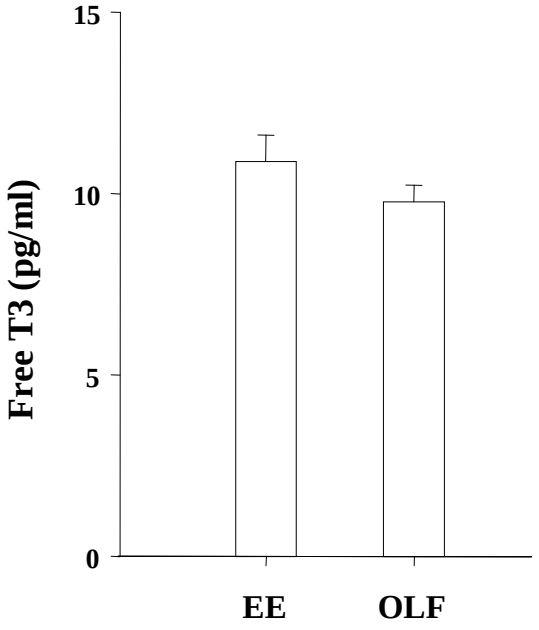


Figure 3.6. Free T3 (pg/ml) of albatross chicks at East End (EE) and Old Landfill (OLF) on Tern Island. Vertical bars represent means ± SEM (EE chick, n=45; OLF chick, n=45).

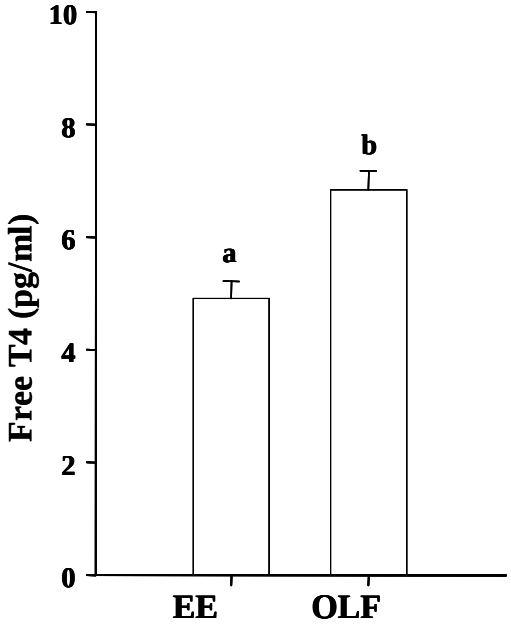


Figure 3.7. Free T4 (pg/ml) of albatross chicks at East End (EE) and Old Landfill (OLF) on Tern Island. Vertical bars represent means ± SEM (EE chick, n=45; OLF chick, n=45). Values not sharing common letters are significantly different ($P < 0.05$).

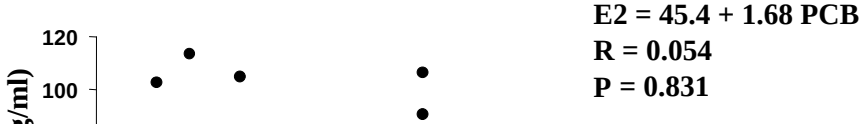


Figure 3.8. Correlation between PCB levels in the nest cup and plasma levels of estradiol, testosterone, and progesterone in the albatross chicks from Old Landfill.

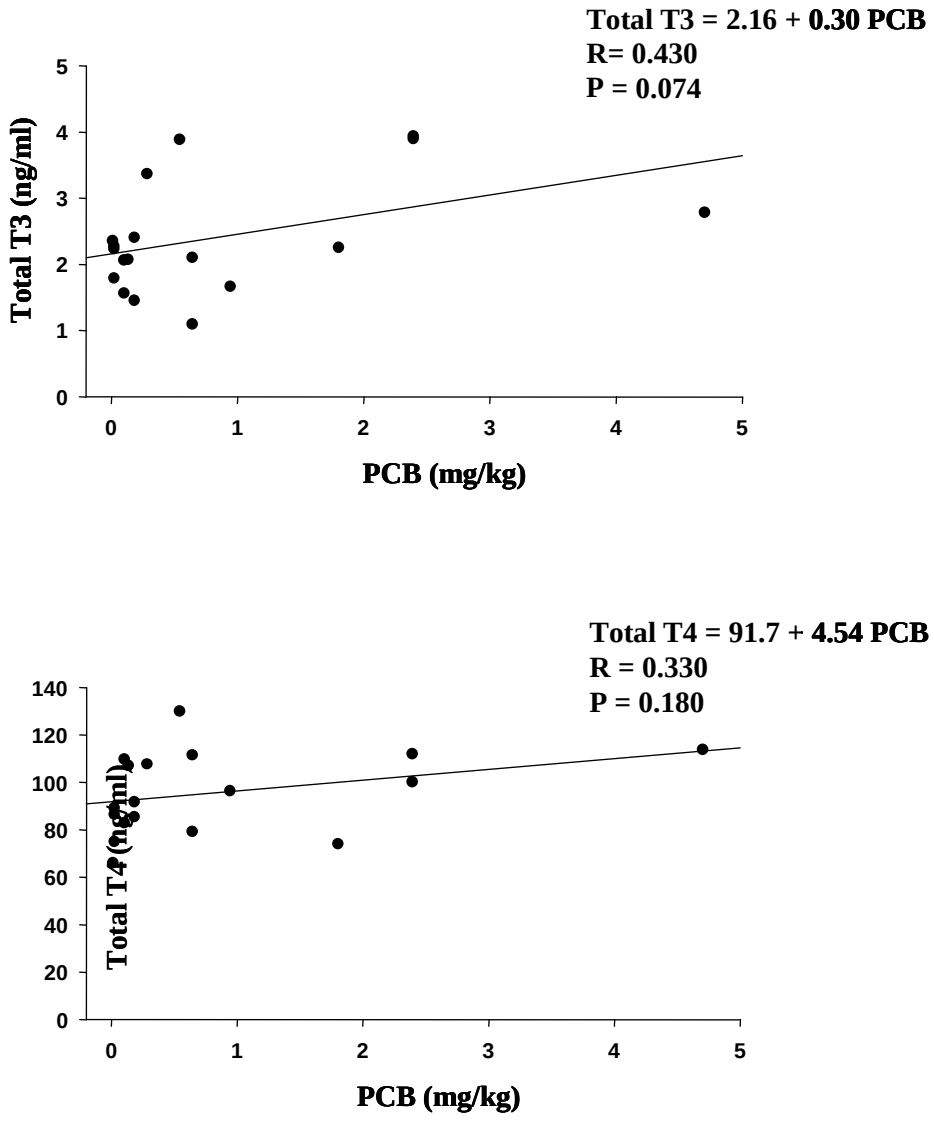


Figure 3.9. Correlation between PCB levels in the nest cup and plasma levels of total T3 and total T4 in the albatross chicks from Old Landfill.

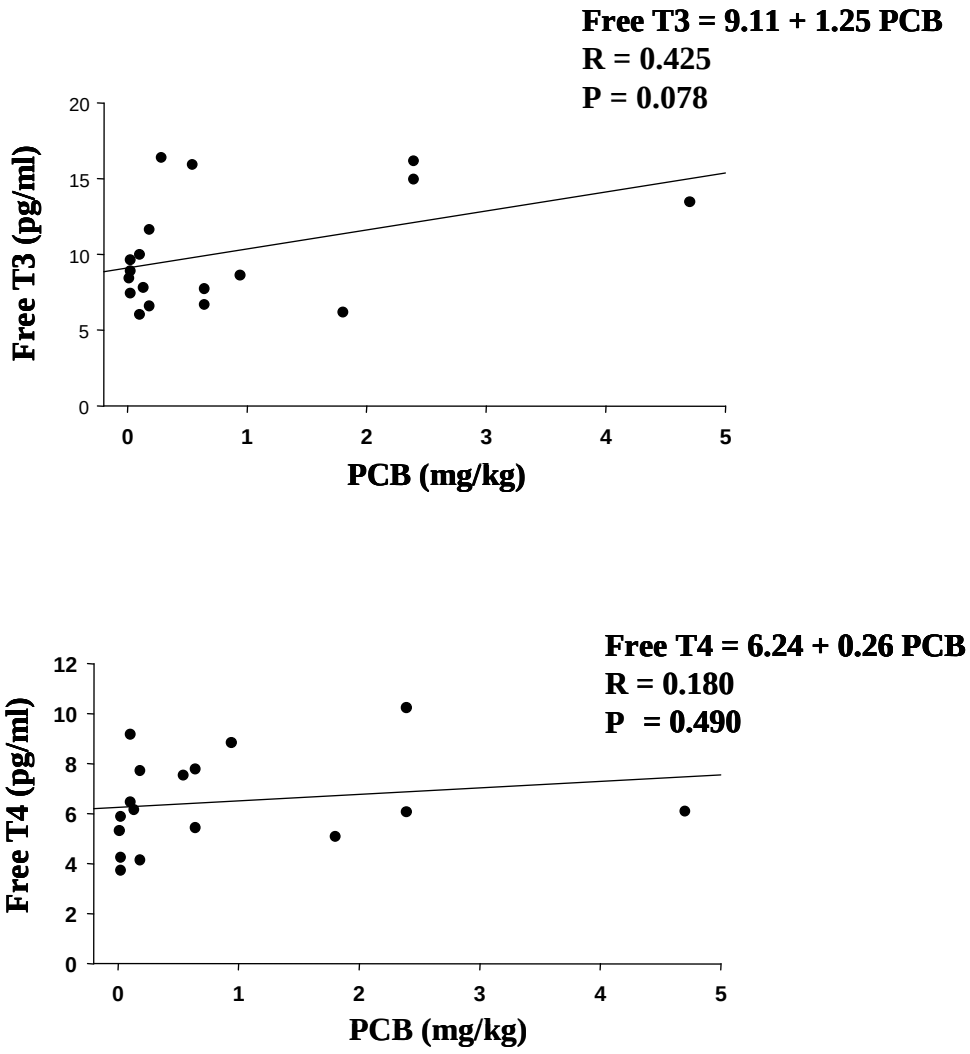


Figure 3.10. Correlation between PCB levels in the nest cup and plasma levels of free T3 and free T4 in the albatross chicks from Old Landfill.

Correlation between PCB Concentrations in the Nest Cup and Plasma Hormone Levels

In May of 2001, soil samples were collected from active nest cups located near chicks within designated plots. PCB concentrations were available for areas around 18 nests in Old Landfill from a previous investigation (Woodward-Clyde, 1999), ranging from 0.01 to 0.54 mg/kg (0.84 ± 0.29 mg/kg, Mean ± SEM, n = 18). Results of correlation analyses between PCB concentrations in the nest cup and plasma hormone levels in the chick were analyzed are shown in Figures 3.8 – 3.10. No significant correlation was observed between soil of a nest area PCB levels and plasma levels of estradiol, testosterone, progesterone, or total and free T3 and T4 in the chick.

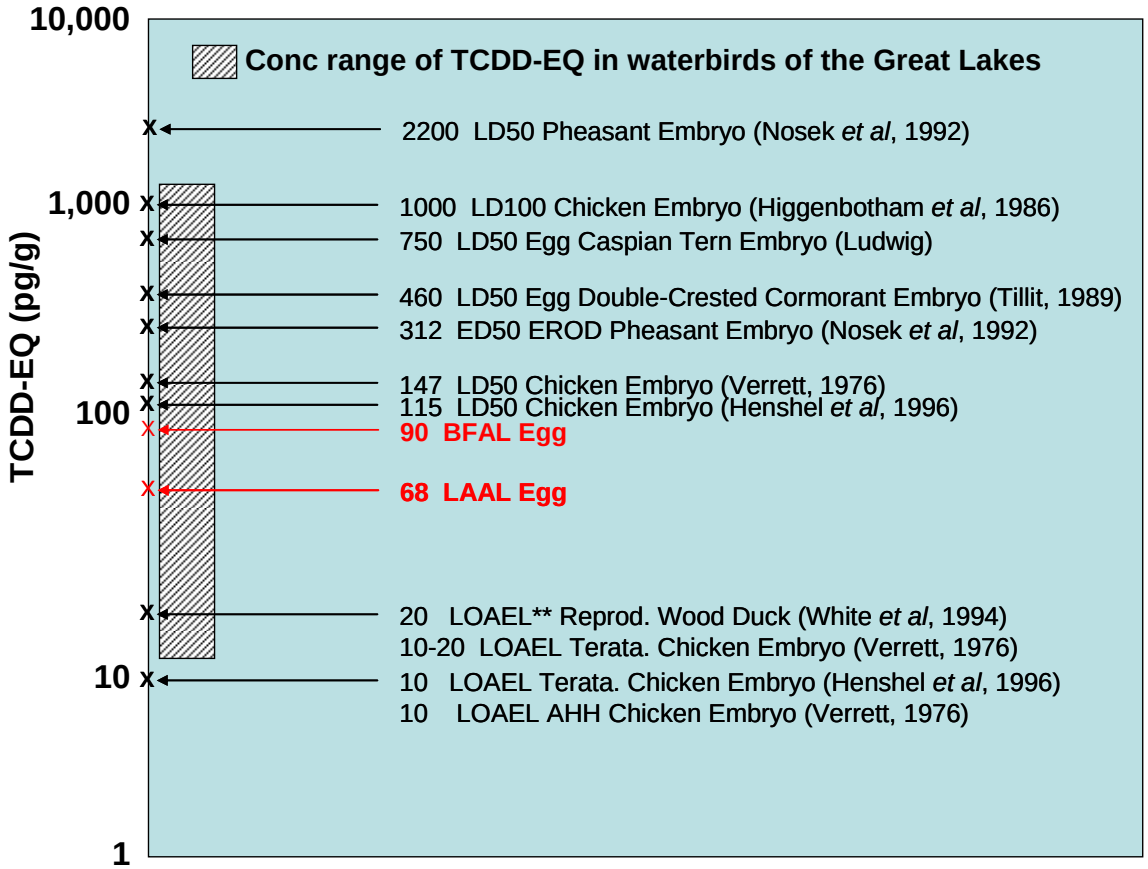
V CONCLUSIONS

The importance of individual PCB congener analysis is exemplified here. The degradation of PCB mixtures in the environment and in biota makes the comparison to Aroclor standards difficult, making quantitation of individual PCB congeners absolutely necessary. This study shows that GC is able to separate nearly all of the 209 PCB congeners and the sensitivity of the ECD allows pg/g levels of PCBs to be detected, and the MS provides mass confirmation. The analysis of individual congeners makes the calculation of concentrations of potential toxic congeners possible as well as individual congener distribution within individual tissue samples (Gonzalez et al., 1991).

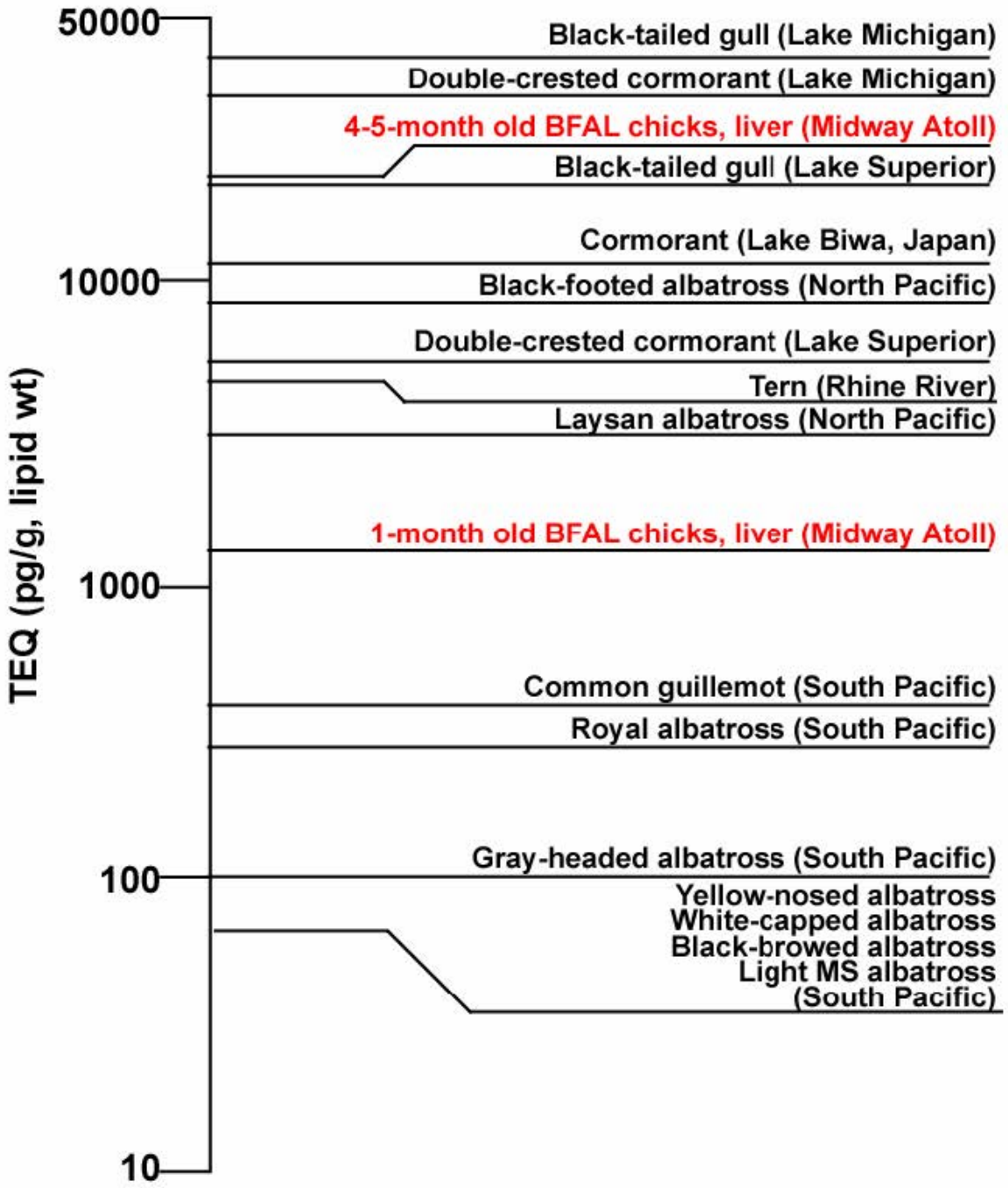
Total PCB concentrations in tissues of these young BFAL chicks are in the □g/g concentration levels, lipid weight. Because the BFAL migrate and forage over a great area, it is hard to determine the exact source of the PCBs. Every year, the BFAL spend the nesting season on Midway Atoll, and when they are feeding it is far from the atoll; so it is unlikely that the sole source of these PCBs is at Midway Atoll. The concentrations of PCBs in the BFAL chicks from feeding are more an average concentration of PCBs in the North Pacific Ocean, but exposure of the young chicks prior to fledging to PCBs on Midway Atoll is a potential toxicological risk.

Hormonal profiles of chicks of black-footed albatross (Phoebastria nigripes) were examined at two sites on Tern Island, Hawaiian Islands National Wildlife Refuge (HINWR) during the 2001 breeding season. Plasma samples were analyzed for estradiol, testosterone, progesterone, total and free thyroxine (T4) and tri-iodothyronine (T3) as indicators of endocrine disruption. Chicks nesting at the Old Landfill (contaminated site) had significantly higher ($P < 0.05$) estradiol levels than those nesting at the East End (reference site), whereas chicks nesting at Old Landfill had significantly lower ($P < 0.05$) testosterone levels than those nesting at East End. On the other hand, chicks nesting at Old Landfill had significantly higher ($P < 0.001$) total T4 and ($P < 0.001$) free T4 than those nesting at East End. There was no difference in the albatross chicks between the two sites in progesterone, total T3, and free T3 concentrations. When correlations between soil PCB and plasma hormone levels were determined, no relationship was found in any of the hormones. These data indicate that although there were significant differences in the plasma hormone profiles in the chicks of black-footed albatross nesting at two sites at Tern Island, no conclusive biological evidence of endocrine disruption from the nesting soil at the sites was obtained based on this limited data set.

2,3,7,8-TCDD Avian Toxicological Thresholds*



* Taken from J.P Giesy *et al*., Dioxins and Health, A. Schecter (Ed.), 1994, p 249-307.


50000
10000
1000
100
10
TEQ (pg/g, lipid wt)
Black-tailed gull (Lake Michigan)
Double-crested cormorant (Lake Michigan)
4-5-month old BFAL chicks, liver (Midway Atoll)
Black-tailed gull (Lake Superior)
Cormorant (Lake Biwa, Japan)
Black-footed albatross (North Pacific)
Double-crested cormorant (Lake Superior)
Tern (Rhine River)
Laysan albatross (North Pacific)
1-month old BFAL chicks, liver (Midway Atoll)
Common guillemot (South Pacific)
Royal albatross (South Pacific)
Gray-headed albatross (South Pacific)
Yellow-nosed albatross
White-capped albatross
Black-browed albatross
Light MS albatross
(South Pacific)

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VI. BUDGET

VII. BUDGET					
EXPENDITURES	**Year 1 FY 2000**	**Year 2 FY 2001**	**Year 3 FY 2002**	**Year 4 FY 2003**	**All Years**
Field Operations: University of Hawaii					
Field Work	$ 5,000.00	$ 20,000.00	$ 20,000.00	$ 0.00	**$ 45,000.00**
Chemical Analysis (UH post-doc)	$ 0.00	$ 40,000.00	$ 20,000.00	$10,000.00	**$ 70,000.00**
Supplies/equipment	$ 5,000.00	$ 7,000.00	$ 10,000.00	$ 5,000.00	**$ 27,000.00**
UH Subtotal	$ 10,000.00	$ 67,000.00	$ 50,000.00	$ 15,000.00	**$ 142,000.00**
UH Overhead (8%)	$ 800.00	$ 5,360.00	$ 4,000.00	$ 1,200.00	**$ 11,360.00**
U-Hawaii Total	**$ 10,800.00**	**$ 72,360.00**	**$ 54,000.00**	**$ 16,200.00**	**$ 153,360.00**
Travel	$ 5,000.00	$ 10,000.00	$ 10,000.00	$ 1,000.00	**$ 26,000.00**
oordination/Logistics	$ 8,000.00	$ 8,000.00	$ 2,000.00	$ 2,000.00	**$ 20,000.00**
Report Writing	$ 2,000.00	$ 2,000.00	$ 2,000.00	$ 8,000.00	**$ 14,000.00**
Operational Subtotal	$ 25,800.00	$ 92,360.00	$ 68,000.00	$ 27,200.00	**$ 213,360.00**
Refuge Overhead (8.7%)	$ 2,244.60	$ 8,035.32	$ 5,916.00	$ 2,366.40	**$ 18,562.32**

VII. FY 2008 REVIEW AND APPROVAL

Title: PI- Black-Footed Albatross Contamination due to Global and Local Contamination

Project ID#: 1N54

Submitted by: _____ Date: 1 May 2007
Contaminant Specialist, Field Office

Reviewed by: _____ Date: _____
FO Supervisor

Reviewed by: _____ Date: 05/01/07
Refuge Manager, (required for On-Refuge Investigations)

Reviewed by: _____ Date: _____
Regional Environmental Contaminants Coordinator

Approved by: _____ Date: _____
Assistant Regional Director

NOTE: All On-Refuge interim reports must be signed by the Refuge Manager. In addition to the required signatures, authors are free to add to this signature page to include other Project Leaders and Offices (especially when other Service programs are involved) and/or to accommodate the required surname process of their respective Regional and Field Offices.

Table 1. Average recoveries of PCBs from various albatross body parts and eggs

Body parts	Recoveries (%) ± standard deviations				
	Cl_4PCBs	Cl_5PCBs	Cl_6PCBs	Cl_7PCBs	Cl_8PCBs
Bone	47 ± 20	78 ± 9	100 ± 12	91 ± 8	71 ± 11
Head and neck	117 ± 37	143 ± 31	81 ± 16	84 ± 21	55 ± 7
Liver	104 ± 3	246 ± 2	258 ± 2	119 ± 2	62 ± 4
Muscle	27 ± 17	55 ± 4	70 ± 9	72 ± 7	64 ± 8
Other organs	56 ± 13	81 ± 10	107 ± 10	88 ± 2	72 ± 5
Skin	160 ± 132	176 ± 116	201 ± 107	105 ± 3	85 ± 4
Egg	84 ± 8	81 ± 3	81 ± 16	75 ± 4	63 ± 10

Table 2. Total PCB distribution among various body parts of three 1-month old BFAL chicks collected from B8 and BD nests

Body part	**Total PCB concentration, µg/g**					
	B8-32		BD-88		BD-77	
	dw[a]	lw[b]	dw	lw	dw	lw
Bone	2.2	40	0.7	13	0.6	10
Head and neck	2.0	15	1.4	11	1.2	9
Liver	10.8	79	3.7	75	1.1	8
Muscle	2.1	15	1.5	11	0.3	2
Internal organs	3.6	26	0.9	6	0.9	7
Skin	4.1	16	0.4	2	0.2	0.6
Sum concentration	25	191	5.0	118	4.3	37
Total body wet weight, g	146		309		413	
Total PCB body burden, mg	3.7	27.9	1.5	36.5	1.8	15.3

[a] dw = PCB concentrations based on dry weight.
[b] lw = PCB concentrations based on lipid weight.

Table 4. Total PCB distribution among various body parts of three 4-5-month old BFAL chicks collected from BD nests

Body part	**Total PCB concentration, µg/g**					
	BD OC-2		BD OC-1		BD OC-3	
	dw[a]	lw[b]	dw	lw	dw	lw
Bone	0.9	44	0.3	49	0.8	47
Head and neck	2.4	87	0.7	39	1.4	43
Liver	5.7	97	2.7	39	2.5	49
Muscle	2.8	109	0.9	58	0.8	116
Internal organs	1.1	24	1.2	22	2.6	35
Skin	1.5	26	1.1	26	1.0	21
Sum concentration	14.4	387	6.9	233	9.0	311
	723		882		1352	
Total body wet weight, g						
Total PCB body burden, mg	10.4	280	6	206	12	420

[a] dw = PCB concentrations based on dry weight.
[b] lw = PCB concentrations based on lipid weight.

Table 6. Comparison of total PCB concentrations and TEQs[a] in BFAL eggs and Laysan albatross eggs collected from Midway Atoll nests.

Sample	Mass of egg (ww, g)	Total PCB conc. (lw, μg/g)	Total TEQ[a] (lw, pg/g)	TEQ/total PCB ratio (pg TEQ/μg PCB)
B8-49 (BFAL)	284	10.5	136.5	13.0
BD-4 (BFAL)	270	8.0	37.3	4.6
BD-113 (BFAL)	302	9.6	170.2	17.7
BD-184 (BFAL)	276	2.8	16.5	5.9
BD-163 (Laysan)	279	5.4	64.6	12.0
BD-164 (Laysan)	254	4.0	53.1	13.3
BD-169 (Laysan)	NA[b]	4.5	87.4	19.6

[a] Toxic equivalents (TEQs) to 2,3,7,8-TCDD, based on TEF values from Van Den Berg *et al*. for birds (Van den Berg et al., 1998).
[b] Not Available.

Table 3. Toxic Equivalents (TEQs) for the 1 Month-Old BFAL Chicks from Midway Atoll (pg/g, lipid weight).

PCB Congener	77	81	105	114	118	123	126	156	157	167	169	189	Total TEQ (pg/g)
TEFs*	0.05	0.1	0.0001	0.001	0.0001	0.00001	0.1	0.0001	0.0001	0.00001	0.001	0.00001	--
BFAL B8 32 Chick TEQs (pg/g, lipid weight)												**Total**	**11165.4**
Bone	--	--	540.6	1224.5	1411.3	--	--	436.2	103.0	21.1	178.8	18.7	3934.1
Head & Neck	--	--	238.5	--	743.4	--	--	--	--	9.6	--	2.1	993.6
Liver	--	--	648.5	1086.1	1489.3	--	--	751.1	--	39.7	--	--	4014.7
Muscle	--	--	197.8	299.4	613.0	--	--	--	--	7.1	--	--	1117.2
Organs	--	--	22.6	126.8	35.6	0.4	--	--	13.3	1.8	10.2	0.2	210.9
Skin	--	--	227.5	--	441.7	--	--	172.2	42.4	11.1	--	--	894.9
BFAL BD 77 Chick TEQs (pg/g, lipid weight)												**Total**	**124.3**
Bone	--	--	5.9	--	26.3	--	--	--	3.4	--	--	--	35.6
Head & Neck	--	--	--	--	22.0	--	--	--	1.6	--	--	--	23.6
Liver	--	--	--	--	30.7	--	--	--	1.5	--	--	--	32.2
Muscle	--	--	--	--	8.1	--	--	--	0.4	--	--	--	8.5
Organs	--	--	--	--	19.8	--	--	--	2.7	--	--	--	22.5
Skin	--	--	0.2	--	1.8	--	--	--		--	--	--	1.9
BFAL BD 88 Chick TEQs (pg/g, lipid weight)												**Total**	**2621.8**
Bone	--	--	119.3	153.1	503.8	--	--	--	--	8.3	--	--	784.5
Head & Neck	--	--	101.9	190.9	329.7	--	--	115.8	25.6	6.4	--	--	770.2
Liver	--	--	20.9	--	5.1	--	--	--	--	26.9	--	--	53.0
Muscle	--	--	111.2	142.9	448.5	--	--	129.9	23.5	--	--	--	855.9
Organs	--	--	13.0	33.1	53.2	--	--	--	3.1	0.6	--	--	102.9
Skin	--	--	12.1	--	42.1	--	--	--	--	1.1	--	--	55.3

* TEFs for birds from Van den Berg *et al*. (1998).

Table 5. Toxic Equivalents (TEQs) for the 4-5-Month Old BFAL chicks from Bulky Dump on Midway Atoll (pg/g, lipid weight).

PCB Congener	77	81	105	114	118	123	126	156	157	167	169	189	Total TEQ (pg/g)
TEFs*	0.05	0.1	0.0001	0.001	0.0001	0.00001	0.1	0.0001	0.0001	0.00001	0.001	0.00001	--
BFAL OC 1 TEQs (pg/g, lipid weight)												**Total**	**17542.9**
Bone	--	--	10.6	208.9	185.7	--	8980.7	20.6	2.4	191.2	79.2	43.9	9723.1
Head & Neck	--	--	--	1325.4	39.2	--	--	--	28.3	--	42.2	--	1435.0
Liver	--	--	--	503.1	--	--	--	--	--	--	--	--	503.1
Muscle	--	--	149.5	192.5	65.3	--	4567.6	--	4.8	290.1	81.8	--	5351.5
Organs	--	--	--	487.7	--	0.2	--	--	--	--	38.7	--	526.6
Skin	--	--	--	--	--	1.5	--	--	--	--	--	2.1	3.7
BFAL OC 2 TEQs (pg/g, lipid weight)												**Total**	**69254.1**
Bone	3051.1	--	158.5	183.4	128.0	0.5	4613.3	--	6.6	1.7	234.5	--	8377.4
Head & Neck	--	--	34.6	211.8	--	--	11371.9	20.1	--	0.8	111.6	0.2	11751.0
Liver	3126.4	--	146.3	121.2	117.0	1.4	17596.2	117.3	--	7.0	134.5	0.7	18241.5
Muscle	--	--	--	--	103.5	--	15539.7	19.6	--	1.0	141.5	0.1	15701.8
Organs	--	--	36.6	--	110.0	0.2	8703.9	10.6	3.6	1.0	--	0.2	8829.5
Skin	--	--	--	--	92.0	--	6272.1	17.5	--	0.9	62.2	0.1	6352.9
BFAL OC 3 TEQs (pg/g, lipid weight)												**Total**	**94577.6**
Bone	--	--	--	--	105.7	--	2199.3	--	--	--	136.1	--	2335.3
Head & Neck	--	28564.2	--	--	127.6	--	6452.4	--	--	--	114.3	--	35258.4
Liver	--	--	--	--	--	--	45399.1	9.7	136.8	23.1	--	11.2	45579.9
Muscle	--	--	--	--	372.0	--	2744.1	3.3	--	--	1792.9	--	4912.2
Organs	--	5964.4	17.0	--	82.7	0.01	4040.9	2.6	--	--	49.8	--	4192.9
Skin	2182.8	--	--	--	30.1	--	--	2.0	--	--	84.0	--	2298.9

* TEFs for birds from Van den Berg *et al*. (1998).

Figure 1

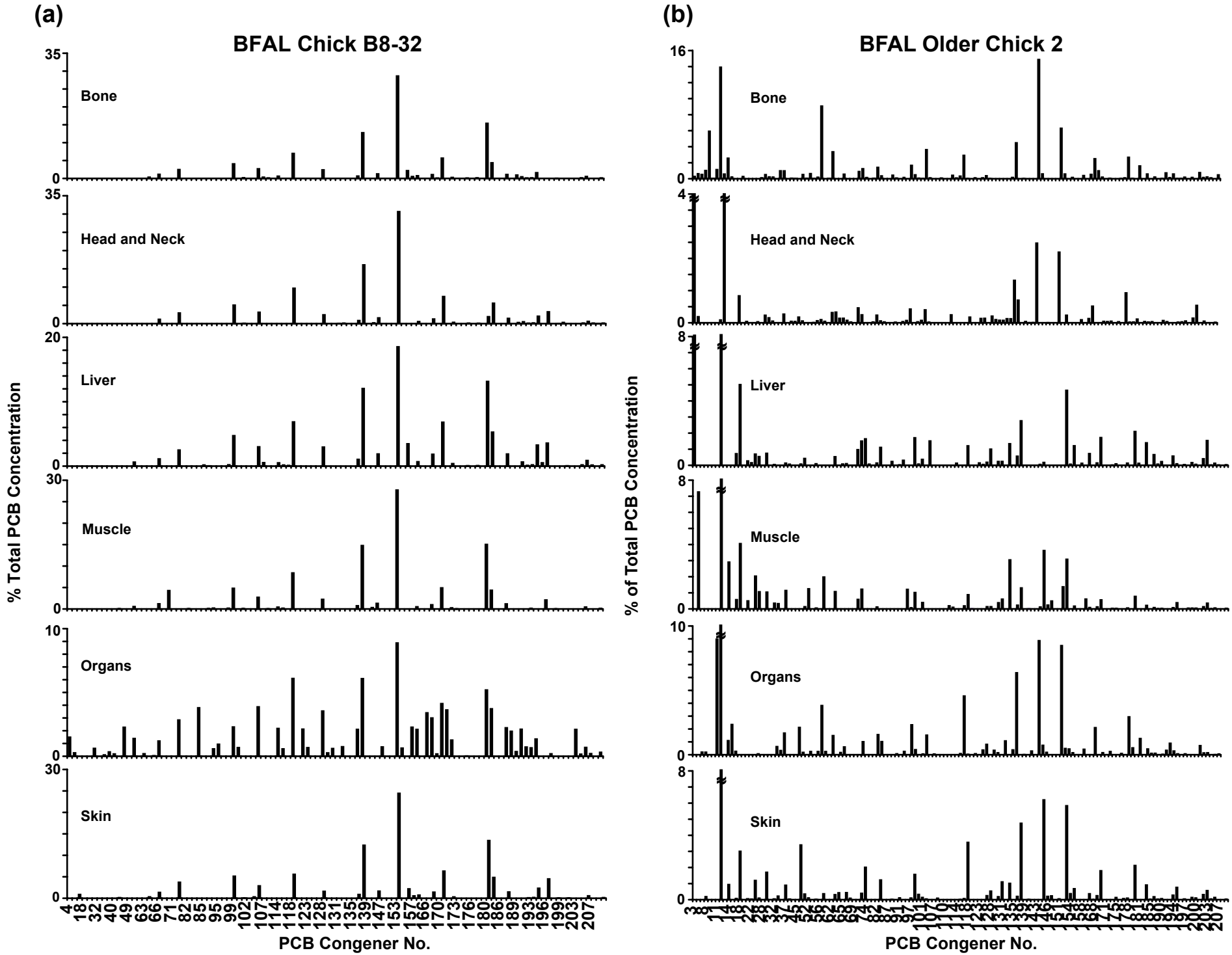

(a)
BFAL Chick B8-32
Bone
Head and Neck
Liver
Muscle
Organs
Skin
% Total PCB Concentration
PCB Congener No.
(b)
BFAL Older Chick 2
Bone
Head and Neck
Liver
Muscle
Organs
Skin
% of Total PCB Concentration
PCB Congener No.

Figure 2

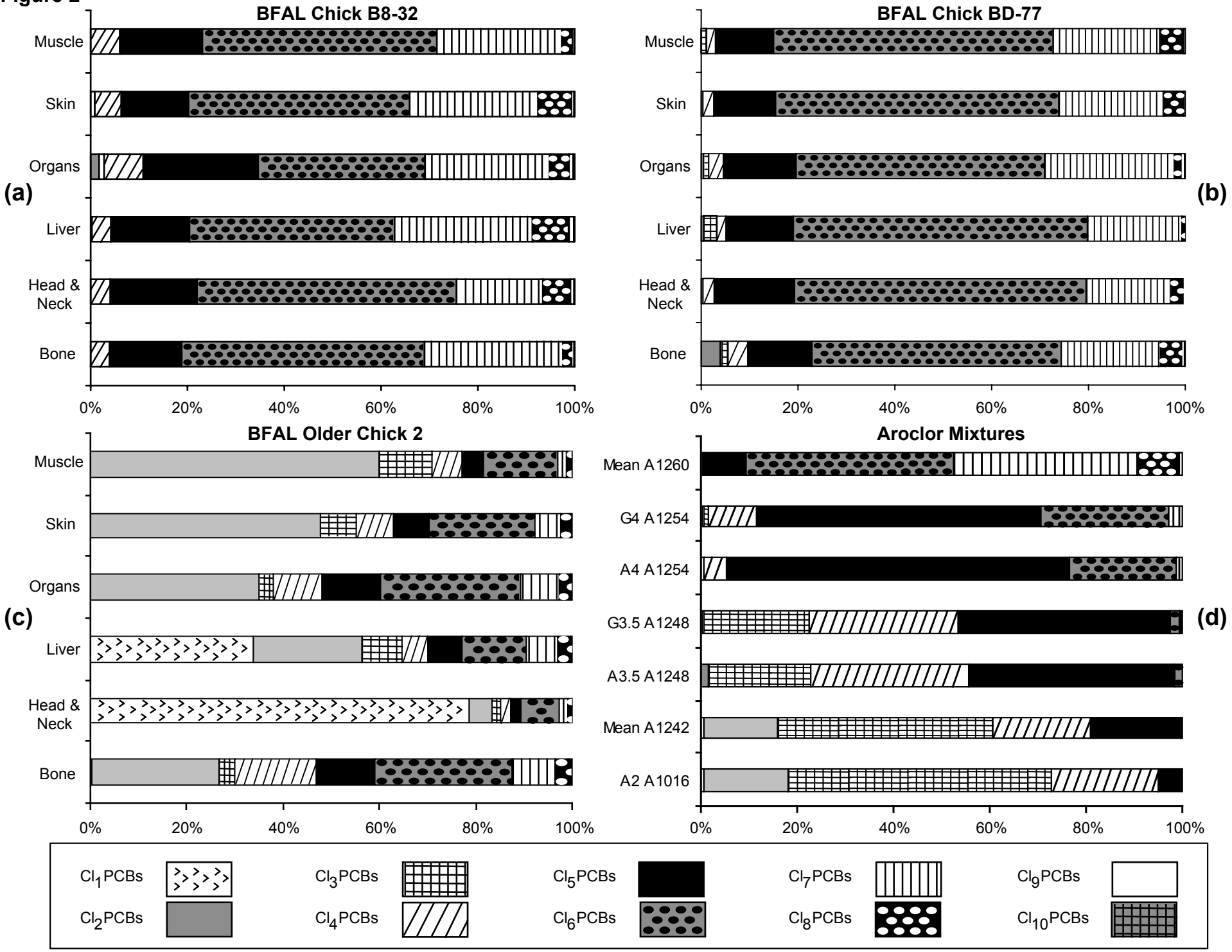

BFAL Chick B8-32
Muscle
Skin
Organs
Liver
Head & Neck
Bone
0%
20%
40%
60%
80%
100%
(a)
BFAL Chick BD-77
(b)
BFAL Older Chick 2
(c)
Aroclor Mixtures
Mean A1260
G4 A1254
A4 A1254
G3.5 A1248
A3.5 A1248
Mean A1242
A2 A1016
(d)
Cl1PCBs
Cl2PCBs
Cl3PCBs
Cl4PCBs
Cl5PCBs
Cl6PCBs
Cl7PCBs
Cl8PCBs
Cl9PCBs
Cl10PCBs

Figure 3

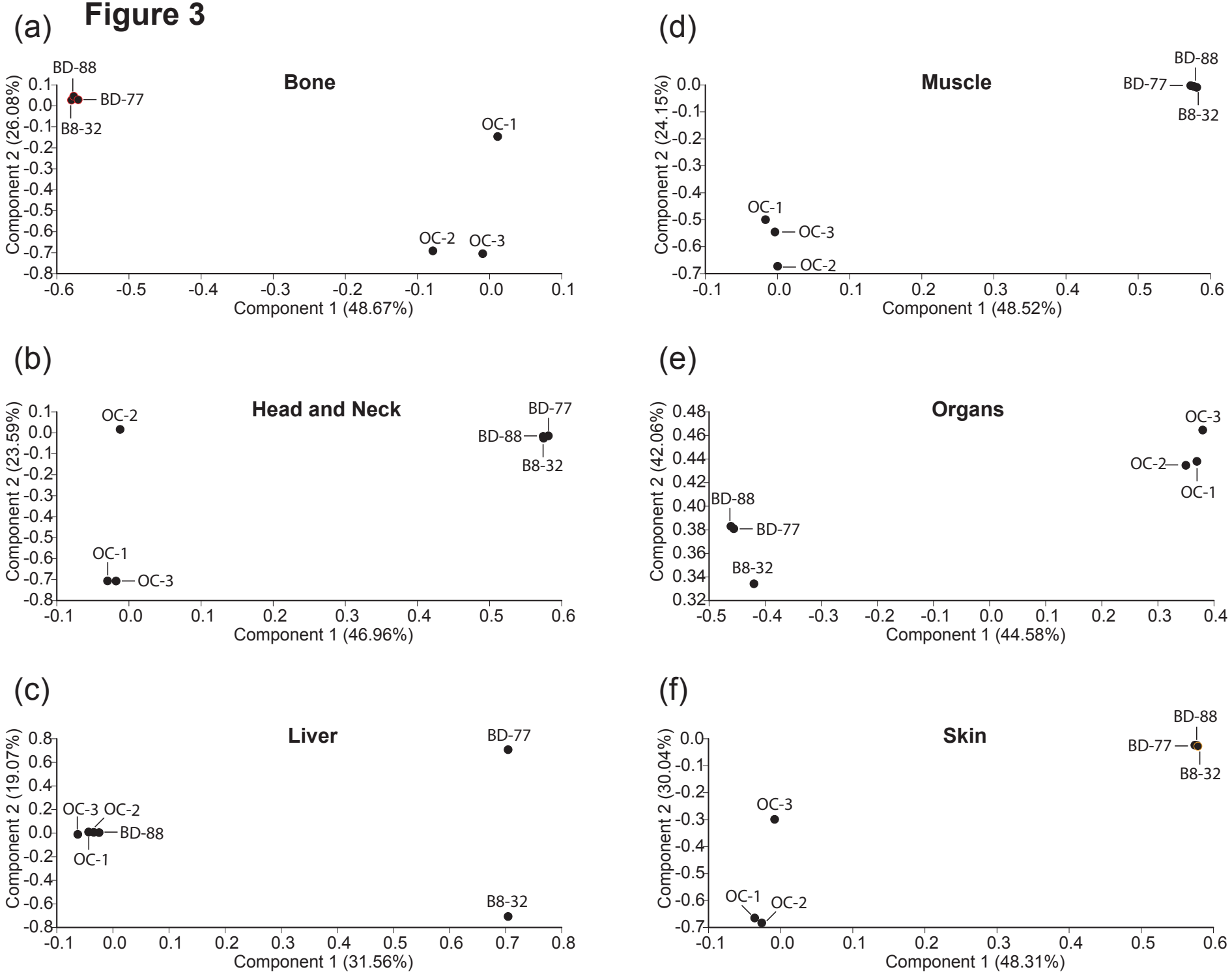

(a)
Bone
BD-88
BD-77
B8-32
OC-1
OC-2
OC-3
Component 2 (26.08%)
Component 1 (48.67%)
(b)
Head and Neck
OC-2
BD-77
BD-88
B8-32
OC-1
OC-3
Component 2 (23.59%)
Component 1 (46.96%)
(c)
Liver
BD-77
OC-3
OC-2
BD-88
OC-1
B8-32
Component 2 (19.07%)
Component 1 (31.56%)
(d)
Muscle
BD-88
BD-77
B8-32
OC-1
OC-3
OC-2
Component 2 (24.15%)
Component 1 (48.52%)
(e)
Organs
OC-3
OC-2
OC-1
BD-88
BD-77
B8-32
Component 2 (42.06%)
Component 1 (44.58%)
(f)
Skin
BD-88
BD-77
B8-32
OC-3
OC-1
OC-2
Component 2 (30.04%)
Component 1 (48.31%)

Figure 4

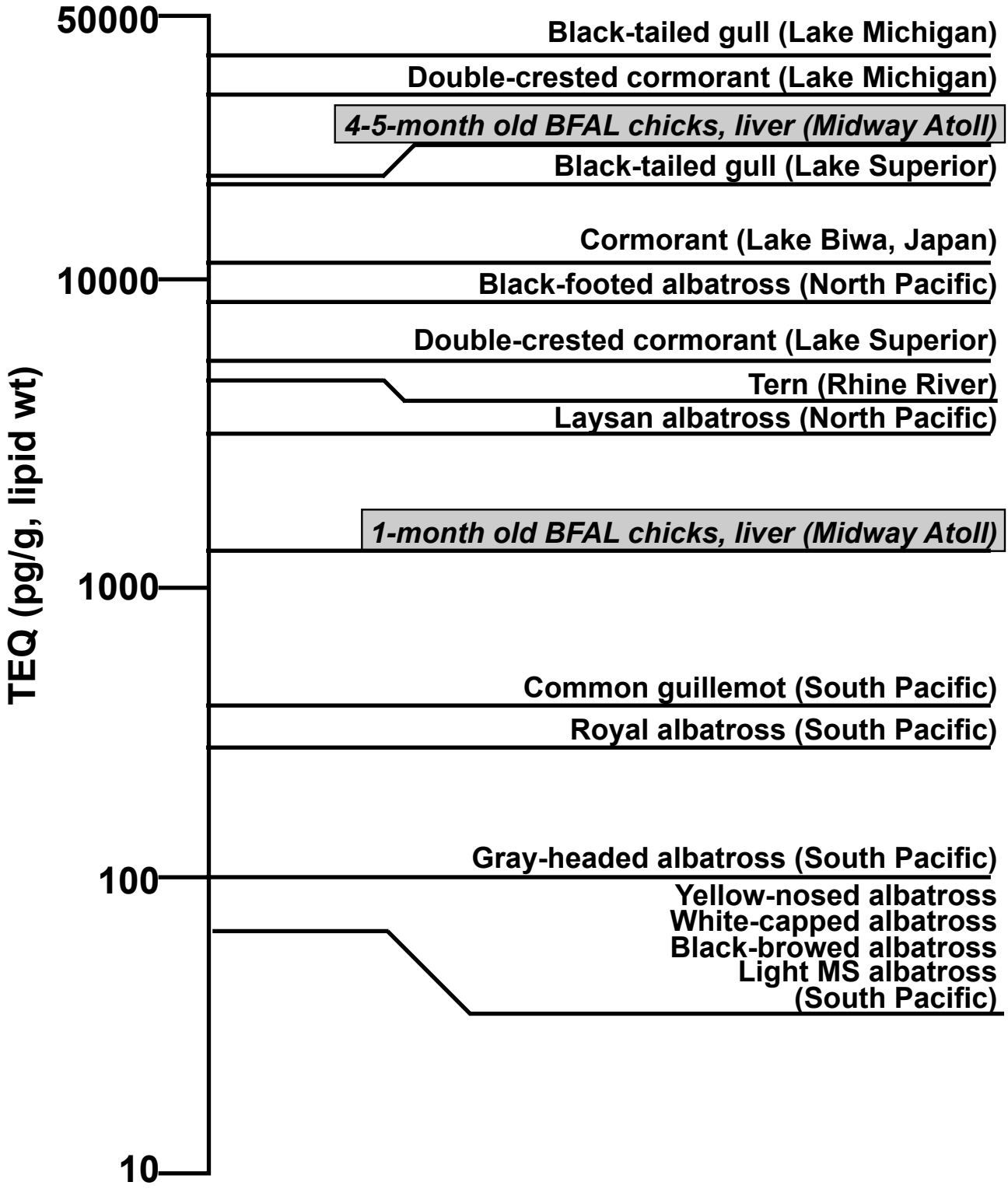
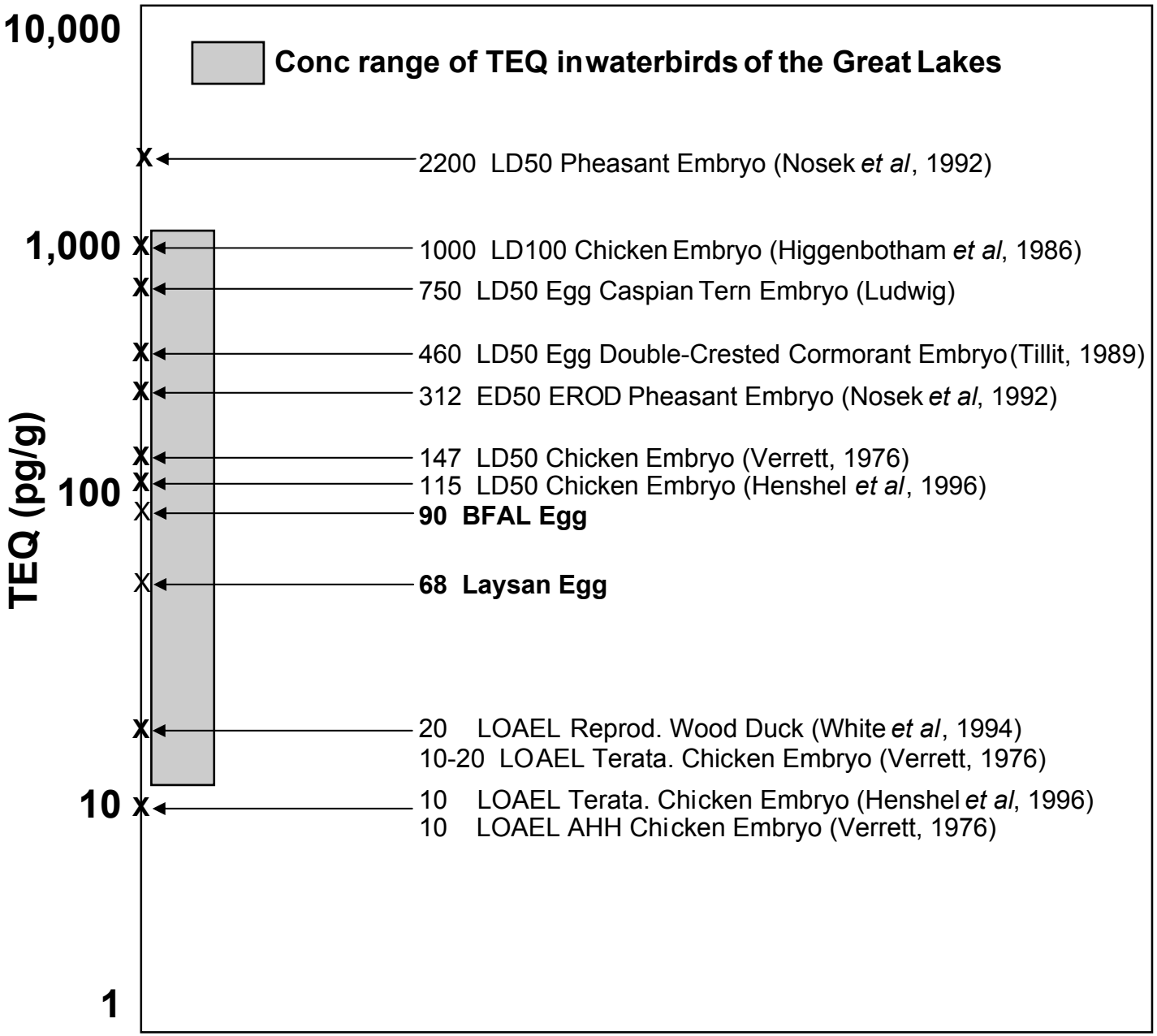

50000
10000
1000
100
10
TEQ (pg/g, lipid wt)
Black-tailed gull (Lake Michigan)
Double-crested cormorant (Lake Michigan)
4-5-month old BFAL chicks, liver (Midway Atoll)
Black-tailed gull (Lake Superior)
Cormorant (Lake Biwa, Japan)
Black-footed albatross (North Pacific)
Double-crested cormorant (Lake Superior)
Tern (Rhine River)
Laysan albatross (North Pacific)
1-month old BFAL chicks, liver (Midway Atoll)
Common guillemot (South Pacific)
Royal albatross (South Pacific)
Gray-headed albatross (South Pacific)
Yellow-nosed albatross
White-capped albatross
Black-browed albatross
Light MS albatross
(South Pacific)

Figure 5


10,000
1,000
100
10
1
TEQ (pg/g)
Conc range of TEQ in waterbirds of the Great Lakes
2200 LD50 Pheasant Embryo (Nosek et al, 1992)
1000 LD100 Chicken Embryo (Higgenbotham et al, 1986)
750 LD50 Egg Caspian Tern Embryo (Ludwig)
460 LD50 Egg Double-Crested Cormorant Embryo (Tillit, 1989)
312 ED50 EROD Pheasant Embryo (Nosek et al, 1992)
147 LD50 Chicken Embryo (Verrett, 1976)
115 LD50 Chicken Embryo (Henshel et al, 1996)
90 BFAL Egg
68 Laysan Egg
20 LOAEL Reprod. Wood Duck (White et al, 1994)
10-20 LOAEL Terata. Chicken Embryo (Verrett, 1976)
10 LOAEL Terata. Chicken Embryo (Henshel et al, 1996)
10 LOAEL AHH Chicken Embryo (Verrett, 1976)