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# PERSISTENT SYNTHETIC CHLORINATED HYDROCARBONS IN ALBATROSS TISSUE SAMPLES FROM MIDWAY ATOLL

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**Abstract**—Anthropogenic organic contaminants have been found in even the most remote locations. To assess the global distribution and possible effects of such contaminants, we examined the tissues of two species of albatross collected from Midway Atoll in the central North Pacific Ocean. These birds have an extensive feeding range covering much of the subtropical and northern Pacific Ocean. Anthropogenic contaminants were found at relatively great concentrations in these birds. The sum of 19 polychlorinated biphenyl (PCB) congeners ranged from 177 ng/g wet weight in eggs to 2,750 ng/g wet weight in adult fat. Total toxic equivalents (TEQs) derived from polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) ranged from 17.2 to 297 pg/g wet weight in the same tissues, while the inclusion of TEQs from PCBs increased these values to 48.4 and 769 pg/g wet weight, respectively. While contaminant concentrations varied between species and tissues, the contaminant profile was relatively uniform. The profile of contaminants detected was unusual in that much of the TEQs was contributed by two pentachlorinated congeners (2,3,4,7,8-pentachlorinated dibenzofuran and 1,2,3,7,8-pentachlorinated dibenzo-*p*-dioxin), and the profiles of PCB congeners did not match known sources. When compared to other studies the concentrations detected in the Midway Atoll samples were near or above the thresholds known to cause adverse effects in other fish-eating bird species.

**Keywords**—Organochlorines Dioxins PCBs Birds Pacific Ocean

## INTRODUCTION

Planar chlorinated hydrocarbons (PCHs), such as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and some polychlorinated biphenyl (PCB) congeners, are ubiquitous contaminants in aquatic ecosystems. Planarchlorinated hydrocarbons have been detected in freshwater and marine organisms throughout the northern hemisphere [1–3]. Concentrations in the marine environment in the southern hemisphere are generally lower [4,5], and it is believed that atmospheric transport accounts for most of these materials transported to the southern oceans [5]. While considerable data describe contaminants in marine mammals [4], similar data are limited for oceanic birds [6], and data are particularly scarce for oceanic birds from remote locations [7,8].

In view of the potential toxicity of PCHs, a variety of chemicals, such as polychlorinated naphthalenes (PCNs), polychlorinated diphenylethers (PCDEs), and polychlorinated terphenyls (PCTs), have been introduced as replacements. The environmental behavior of these chemicals is not yet fully understood. However, some appear to have similar properties to the PCHs which they are replacing [9]. In view of the possible harmful effects of these replacements, monitoring of the global distribution of these chemicals seems appropriate.

Studies of freshwater ecosystems have demonstrated that fish-eating waterbirds may be particularly vulnerable to adverse effects from organochlorine contaminants [10,11]. However, as with other organisms, there are great differences in sensitivity between individual species. For example, Harris and Osborn [12] reported no effect on breeding puffins (*Fratercula arctica*) contaminated with up to 612 μg/g of technical Aroclor® in fat, while the estimated median lethal dose (LD50) for adult chickens (species not specified) is 25 to 50 ng PCBs/g body weight [13]. On the other hand, it is clear from many of the above studies that the most sensitive end points for the effects of PCHs involve reproduction and embryonic development.

Populations of various albatross species in the tropical North Pacific were decimated by hunting in the early 1900s. Since the end of this exploitation, there has been a steady increase in both Laysan albatross (*Diomedea immutabilis*) and black-footed albatross (*Diomedea nigripes*) populations [14]. The short-tailed albatross (*Diomedea albatrus*), which was thought to be extinct, is also recovering [15]. These marine bird populations may be threatened by other human impacts, such as inappropriate refuse disposal [16]. Accumulation of plastic refuse in albatross chicks has been shown to be significant [16,17] and may be causing a decrease in the reproductive capacity of these populations [15].

To evaluate the possible risks posed by anthropogenic contaminants to albatross species in the tropical North Pacific, we determined the concentrations of some anthropogenic contaminants in these birds. This report describes the chemical determination of some persistent organochlorine contaminants in samples of albatross tissue collected from Midway Atoll during the spring and summer of 1993 and 1994.

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## METHODS

### *Sample collection and handling*

Dead or injured Laysan albatross adults were collected from the islands of Midway Atoll in the central North Pacific Ocean (28°11′N, 177°22′W). After collection birds were examined externally and necropsied, and tissue samples were frozen in chemically clean I-Chem jars for subsequent analysis. Samples were collected between December 1 and 12, 1993, and again between February 1 and 14, 1994. Tissue composites (five individuals per composite) were prepared for each time period using liver and fat from the same individuals from each sampling period. Samples were composited by placing a measured portion of each sample in a blender jar and homogenizing the sample. Composites were kept frozen until analysis.

Egg pools (10 eggs per pool) were collected for both Laysan and black-footed albatross in November 1993. Egg pools were prepared by opening the eggs into chemically clean jars. Pools were homogenized before subsampling and analysis.

### *Analysis of dioxin, furan, and PCB congeners*

The PCDD, PCDF, and PCB congeners were analyzed as described previously [4,18]. Samples were fortified with $^{13}C_{12}$-PCDD, -PCDF, and -PCB congeners (Cambridge Isotope Laboratories, Andover, MA, USA) and were extracted four times by blending with 30 ml 2:1 acetone : hexane. Extracts were dried by passage through anhydrous $Na_2SO_4$, concentrated to near dryness, and redissolved in 50 ml of hexane. A 2.5-ml portion of the extract was removed for gravimetric lipid determination. The remaining extract, after any subsampling, was transferred to a separating funnel and washed repeatedly with concentrated $H_2SO_4$ (eight times), followed by repeated washing with $H_2O$ (three times). The extract was again dried by passage through $Na_2SO_4$ before being chromatographed sequentially on columns of $H_2SO_4$/silica : NaOH/silica, $Al_2O_3$, and Carbopac C (Supelco, Bellefonte, PA, USA) dispersed on Celite. The PCB congeners pass through the Carbopac column, while PCDDs and PCDFs are retained and eluted into a separate fraction. The PCB fraction was chromatographed on Florisil (Sigma Chemical Company, St. Louis, MO, USA) to isolate the three non-*ortho*-substituted congeners. All analytes were determined by high-resolution gas chromatography with high-resolution mass spectrometry for identification and quantitation using a VG70S mass spectrometer.

### *Quality assurance*

The dioxin laboratory maintains World Health Organization and New Zealand Testing Laboratory Accreditation Council accreditation for the analysis of PCDD, PCDF, and PCB congeners in a variety of environmental matrices. Subsamples of the pooled Laysan eggs were analyzed in duplicate for PCDD, PCDF, and PCB congeners. The laboratory blank performed with the samples is reported; data have not been manipulated by blank subtraction.

### *Dioxin equivalents*

Concentrations of dioxin equivalents were measured in two ways. The first method used the quotient of toxic potency (equivalency factors, TEFs) and the concentrations of the individual PCB, PCDD, and PCDF congeners in an additive model to determine toxic equivalent (TEQ) concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin TCDD. The second method determined the relative potency to induce ethoxyresorufin-*o*-deethylase (EROD) activity in H4IIE rat hepatoma cells as compared to that of TCDD.

### *Semiquantitative analysis of other persistent organic contaminants*

The occurrence of other persistent organic contaminants in these samples was investigated in one Laysan albatross fat sample. The sample was the 1993 adult fat sample as this sample contained the greatest concentrations of PCDD, PCDF, and PCB congeners. Compounds targeted were PCNs, PCDEs, PCTs, and polychlorinated diphenyltoluenes (PCDTs) (also called polychlorinated mono-methyl-substituted diphenyl methanes (Me-PCDMs)).

To maximize mass spectrometer sensitivity, persistent organics were determined in single-ion recording (SIR) mode with an operating mass resolution of approx. 8,000. As "genuine" reference materials were not available for most of the targeted compounds, these analyses must be considered as semiquantitative. The sample for analysis was removed from the extract before the Florisil chromatography step. This fraction is the combined effluents of all chromatography steps up to this stage of the analysis; therefore, it is assumed that at this stage the only analytes that have been removed are PCDD and PCDF congeners. Analyte concentrations were estimated assuming 100% recovery and a response factor of 1 relative to PCB 153 (or PCB 180 for PCTs). Compounds were quantified using $^{13}C_{12}$-PCB 153, added before extraction.

Analysis of other persistent organochlorines was focused on the presence of penta-, hexa-, and heptachlorinated congeners for several reasons. First, hexachloro-PCNs have been shown to be the most toxic members of this chemical family [19,20]. Second, what little information is available on PCTs suggests that hexa- and heptachlorinated PCT congeners are the most abundant congeners in the environment [21]. Similarly hexachlorinated PCDEs are the predominant congeners detected in biota [22]. It was also assumed that these congeners would show "average" properties in the analytical procedures used, which would result in the best recoveries.

### *TCDD TEQs (H4IIE bioassay)*

H4IIE bioassay procedures were a modification of the standard methods reported by Tillitt et al. [23] as modified by Sanderson et al. [24]. Modifications were to miniaturize and automate the procedures into a 96-well microtiter plate configuration. H4IIE cells were seeded at 5,000 cells/well in 250 μl of Dulbecco's modified Eagle's medium (DMEM) culture medium [23]. After a 24-h incubation the cells were dosed with sample extracts or standards in 5 μl of isooctane. Cells were exposed to four different concentrations of the samples in a threefold dilution series (i.e., 1.0, 0.3, 0.1, 0.03) at five replications per dose. For calibration of samples, TCDD standards were dosed at five concentrations (0.3, 1, 3, 10, and 30 pg/well) at seven replications per dose. Determination of TCDD TEQs was by slope ratio assay [25] as previously described [26]. Variance estimates were based on an additive model of variance [25] and were calculated as previously described [23,25].

## RESULTS

### *PCDD, PCDF, and PCB congeners*

Results for the congener-specific analysis of PCDD, PCDF, and PCB congeners are given in Tables 1 and 2. 2,3,7,8-Substituted PCDD and PCDF congeners were detected in all samples analyzed ($n$ = 7); wet weight concentrations were greatest

Table 1. Concentrations (pg/g wet weight) of 2,3,7,8-substituted dioxin and furan congeners in albatross tissue composites. All fat and liver samples were from Laysan albatross. Toxic equivalents (TEQs) were calculated using the toxic equivalency factors of Ahlborg et al. [27]

| Congener[a] | Laboratory blank | Fall 1993 (Fat) | Fall 1993 (Liver) | February 1994 (Fat) | February 1994 (Liver) | Laysan egg (Pool 1) | Laysan egg (Pool 2) | Black-footed egg (Pool) |
|---|---|---|---|---|---|---|---|---|
| 2,3,7,8-TeF | <0.1 | 77.1 | 3.1 | 156 | 6.1 | 8.4 | 9.6 | 12.9 |
| 2,3,7,8-TeD | <0.1 | 12.9 | <0.6 | 17.5 | <0.8 | 1.3 | 1.6 | 3.0 |
| 1,2,3,7,8-PeF | <0.2 | 91.6 | 3.1 | 153 | 5.6 | 8.9 | 10.2 | 13.4 |
| 2,3,4,7,8-PeF | <0.1 | 194 | 10.1 | 284 | 14.0 | 15.5 | 21.4 | 34.4 |
| 1,2,3,7,8-PeD | <0.3 | 82.5 | 4.0 | 124 | 6.2 | 7.5 | 8.3 | 18.6 |
| 1,2,3,4,7,8-HxF | <0.3 | 50.3 | 3.3 | 82.8 | 4.1 | 5.2 | 5.7 | 8.2 |
| 1,2,3,6,7,8-HxF | <0.2 | 58.0 | 3.0 | 87.7 | 3.6 | 5.3 | 5.9 | 10.7 |
| 2,3,4,6,7,8-HxF | <0.3 | 62.8 | 3.9 | 109 | 5.0 | 7.1 | 7.5 | 12.3 |
| 1,2,3,7,8,9-HxF | <0.5 | <4 | <0.5 | <3 | <0.9 | <0.6 | <0.6 | <0.8 |
| 1,2,3,4,7,8-HxD | <0.2 | 26.7 | 1.9 | 40.3 | 2.0 | 2.5 | 2.9 | 3.6 |
| 1,2,3,6,7,8-HxD | <0.2 | 124 | 5.1 | 167 | 6.8 | 8.9 | 10.6 | 19.5 |
| 1,2,3,7,8,9-HxD | <0.2 | 22.6 | 1.4 | 32.2 | 2.0 | 2.3 | 2.7 | 3.9 |
| 1,2,3,4,6,7,8-HpF | <0.2 | 2.7 | <0.4 | 3.2 | <0.6 | <0.4 | <0.1 | 0.8 |
| 1,2,3,4,7,8,9-HpF | <0.4 | <2 | <0.3 | <2 | <0.4 | <0.3 | <0.4 | <0.5 |
| 1,2,3,4,6,7,8-HpD | <0.6 | 11.2 | 1.4 | 17.5 | 1.8 | 1.4 | 1.9 | 2.9 |
| OCDF | <0.9 | <2 | <1 | <2 | <1 | <0.9 | <0.8 | <0.5 |
| OCDD | <3.0 | 15.3 | <3 | 21.8 | <3 | 4.3 | 4.5 | 3.9 |
| TEQs (NATO) | 0.3 | 198 | 9.7 | 297 | 13.8 | 17.2 | 21.5 | 37.4 |
| PCB TEQs | 0.3 | 333 | 8.9 | 472 | 14.4 | 31.2 | 30.2 | 86.6 |
| Total TEQs | 0.6 | 531 | 18.6 | 769 | 28.2 | 48.4 | 51.7 | 124 |
| TEQs/PCB, ppm | | 252 | 295 | 298 | 317 | 220 | 292 | 180 |

[a] HpD = heptachlorodibenzo-p-dioxin, HpF = heptachlorodibenzofuran, HxD = hexachlorodibenzo-p-dioxin, HxF = hexachlorodibenzofuran, OCDD = octachlorodibenzo-p-dioxin, OCDF = octachlorodibenzofuran, PCB = polychlorinated biphenyl, PeD = pentachlorodibenzo-p-dioxin, PeF = pentachlorodibenzofuran, TeD = tetrachlorodibenzo-p-dioxin, TeF = tetrachlorodibenzofuran.

Table 2. Concentrations (ng/g wet weight) of polychlorinated biphenyl (PCB) congeners in albatross tissue composites. All fat and liver samples were from Laysan albatross. Toxic equivalents (TEQs) were calculated using the toxic equivalency factors (TEFs) of Ahlborg et al. [30]

| PCB (IUPAC no.[a]) | Laboratory blank | Fall 1993 (Fat) | Fall 1993 (Liver) | February 1994 (Fat) | February 1994 (Liver) | Laysan egg (Pool 1) | Laysan egg (Pool 2) | Black-footed egg (Pool) |
|---|---|---|---|---|---|---|---|---|
| 77 | <0.005 | 2.1 | 0.07 | 4.06 | 0.15 | 0.26 | 0.26 | 0.39 |
| 126 | <0.005 | 2.8 | 0.07 | 4.01 | 0.12 | 0.25 | 0.25 | 0.69 |
| 169 | <0.005 | 1.0 | 0.03 | 1.48 | 0.04 | 0.09 | 0.09 | 0.27 |
| 8 | 0.2 | <3 | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 |
| 15 | <0.02 | <3 | 0.04 | <0.1 | 0.04 | 0.05 | 0.04 | 0.04 |
| 28 | 0.5 | 16.1 | 1.7 | 28.5 | 2.0 | 3.2 | 2.6 | 4.3 |
| 52 | 0.07 | 1.6 | 0.3 | 3.2 | 0.3 | 0.6 | 0.4 | 0.6 |
| 99 | 0.02 | 61.0 | 1.4 | 97.6 | 3.0 | 7.2 | 5.2 | 22.1 |
| 101 | 0.02 | 2.2 | 0.2 | 7.1 | 0.3 | 0.6 | 0.3 | 0.9 |
| 105 | 0.07 | 87.3 | 2.3 | 157 | 4.2 | 10.3 | 7.6 | 32.7 |
| 118 | 0.1 | 301 | 8.5 | 341 | 14.2 | 38.3 | 31.9 | 103 |
| 153 | 0.2 | 882 | 26.7 | 1,030 | 36.5 | 86.0 | 73.0 | 249 |
| 138 | 0.1 | 308 | 9.7 | 477 | 15.1 | 35.9 | 23.4 | 122 |
| 170 | 0.02 | 76.8 | 1.3 | 105 | 1.3 | 3.3 | 3.0 | 16.2 |
| 180 | 0.06 | 264 | 7.4 | 344 | 8.0 | 24.5 | 20.9 | 95.9 |
| 183 | 0.01 | 60.0 | 2.1 | 75.5 | 2.5 | 7.2 | 6.2 | 31.7 |
| 187 | 0.01 | 12.2 | 1.0 | 24.3 | 1.0 | 2.2 | 1.9 | 7.9 |
| 194 | 0.01 | 28.2 | 0.01 | 51.9 | <0.01 | <0.01 | 0.02 | 0.1 |
| 202 | <0.01 | 0.7 | 0.07 | 1.9 | 0.06 | 0.2 | 0.1 | 0.5 |
| Sum | 1.41 | 2,110 | 63.0 | 2,750 | 88.9 | 220 | 177 | 688 |
| TEQs | 0.3 | 333 | 8.9 | 472 | 14.4 | 31.2 | 30.2 | 86.6 |

[a] IUPAC = International Union of Pure and Applied Chemistry.

Table 3. Calculation of polychlorinated biphenyl (PCB) toxic equivalent (TEQ) values with differing toxic equivalency factor (TEF) systems.

| TEF Set | Laboratory blank | Fall 1993 (Fat) | Fall 1993 (Liver) | February 1994 (Fat) | February 1994 (Liver) | Laysan egg (Pool 1) | Laysan egg (Pool 2) | Black-footed egg (Pool) |
|---|---|---|---|---|---|---|---|---|
| NATO PCDD/F[a] | 0.3 | 198 | 9.7 | 297 | 13.8 | 17.2 | 21.5 | 37.4 |
| Ahlborg PCB[b] | 0.3 | 333 | 8.9 | 472 | 14.4 | 31.2 | 30.2 | 86.6 |
| Total TEQs | 0.6 | 531.0 | 18.6 | 769.0 | 28.2 | 48.4 | 51.7 | 123.4 |
| Safe PCB[c] | 0.4 | 779 | 13.5 | 1,020 | 29.7 | 80.9 | 67.8 | 261 |
| Total TEQs | 0.7 | 977.0 | 23.2 | 1,317.0 | 43.5 | 98.1 | 89.3 | 298.4 |
| Tillitt TEQs[d] | 2.7 | 291 | 10.9 | 439 | 16.7 | 26.6 | 29.5 | 65.3 |
| H4IIE assay | | | | | | 12.5 | | 52.2 |

[a] NATO PCDD/F = TEQ derived from PCDD and PCDF congeners using NATO TEF values [27].
[b] Ahlborg et al. [30].
[c] Safe [43].
[d] Tillitt et al. [31] TEFs for all PCDD, PCDF, and PCB congeners.

in adult fat samples, approximately 10-fold lower in egg samples, and lower still in adult liver samples. The five most abundant congeners were 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) > 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD) > 1,2,3,7,8-PeCDF > 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PeCDD) > 2,3,4,6,7,8-hexachlorodibenzofuran (HxCDF). Toxic equivalents, calculated using NATO TEFs [27], ranged from 297 pg/g wet weight in adult fat to between 17 and 37 pg/g wet weight in egg composites.

Polychlorinated biphenyl congeners were detected in all samples analyzed (Table 2). The sum of the 19 congeners analyzed varied in concentration from 2,750 ng/g wet weight in adult fat to between 220 and 688 ng/g wet weight in egg composites. Congener sums were less than 100 ng/g wet weight in adult livers. When compared to PCB congener mixtures found in technical materials and other environmental matrices, the Midway albatross PCB congener profiles were unusual in showing a paucity of lower chlorinated congeners (Cl < 5). In addition, the ratios of some diagnostic congener pairs were different from those normally observed. In particular the ratio between PCBs 101 and 99 is unusual. In the Midway albatross samples the ratio is approx. 1:10 (PCB 101:PCB 99), while in samples of southern ocean marine mammal blubber it is approx. 3:2 [4]. Due to the similar chemical and physical properties of these two congeners, it seems likely that differential metabolism of the congeners is the cause of this difference rather than differential environmental chemistry. This hypothesis is supported by the fact that of the two congeners only PCB 101 possesses adjacent unsubstituted *meta* and *para* positions, which are required for the oxidative metabolism and subsequent elimination of these compounds [28]. It has also previously been observed that congener 101 is readily eliminated by fish-eating seabirds [29].

Concentrations of PCHs were higher in samples collected in February than in samples collected in the previous fall. Due to nesting and incubation duties, the adult birds spend almost 60% of their first 2 to 3 months on the island (during the winter) in a fasting state, which results in the loss of more than 25% of body weight. This weight loss is sufficient to explain the observed increase in fat PCH concentrations between the two sampling periods.

The similarity of the congener profiles was evaluated by linear regression. The concentrations of most PCDD, PCDF, and PCB congeners were highly correlated ($r \geq 0.9$, $p < 0.01$, $n = 7$). The congeners which were less well correlated were those for which a significant number of "nondetect" values were recorded. This situation arose as a value of half the detection limit was used in the regression calculations where nondetect values were recorded.

Total TCDD TEQs were calculated for PCB mixtures using the TEFs of Ahlborg et al. [30]. Although these TEF values are designed to protect human health, they were chosen to provide an indication of the relative risk of PCDDs, PCDFs, and PCBs. Toxic equivalents derived from PCBs varied from 472 pg/g wet weight in adult fat, to 8.9 to 14.4 pg/g wet weight in adult liver, to 51.7 to 124 pg/g wet weight in egg composites (Table 3). Not all the toxic congeners listed by Ahlborg et al. were analyzed in these samples; therefore, the estimated TEQs in this report may represent a low estimate of the actual TEQs. Of the 13 congeners given TEFs [30], five mono-*ortho*-substituted congeners (114, 123, 156, 157, and 167) were not analyzed. As non-*ortho*-substituted congeners commonly account for over 50% of TEQ, the omission of these mono-*ortho*-congeners should not effect the overall TEQ to any great extent.

As an additional measure of TEQ egg pools were analyzed using the H4IIE bioassay procedure to determine total TEQs (including dioxin and PCB TEQs) based on a measured biological effect. Bioassay TEQs were higher in black-footed albatross eggs (52.5 pg/g wet weight) than in Laysan albatross eggs (12.5 pg/g). These values were also compared to the calculated concentrations of TEQs determined using TEF values measured in the same assay system [31] (Table 3). The H411E biassay TEQ concentrations accounted for 47 and 80% of the calculated TEQs in the Laysan and blackfoot egg pools, respectively. These results demonstrate that the measured PCHs account for a large part of the observed biological activity.

*Analysis of other persistent organochlorine contaminants*

The most abundant of the other persistent organochlorine compounds detected were the pentachlorinated naphthalenes, with an estimated concentration of 28 ng/g. Pentachlorinated-PCDE, -PCDT, and -PCT were detected at concentrations less than 10 ng/g wet weight (Table 4). The PCNs were the only compounds for which reference material was available. Polychlorinated naphthalene congeners detected in the fat sample were also detected in the technical material (Halowax 2141-N, Koppers Company, Pittsburgh, PA, USA) analyzed, although the congener profiles were quite different (data not shown).

Due to the low levels of PCNs, PCDEs, PCDTs, and PCTs detected and the analytical uncertainties involved in these de-

Table 4. Persistent organochlorines in the Laysan albatross fat composite from February 1994

| Compound[a] | Chlorination | m/z[b] | Concn. (ng/g) |
|---|---|---|---|
| $^{13}C_{12}$-PCB 153 | Hexa- | 371.8810, 373.8780 | 8.49[c] |
| PCN | Penta- | 299.8648, 301.8618 | 28 |
| | Hexa- | 333.8258, 335.8229 | 2.8 |
| PCDE | Penta- | 341.8750, 343.8720 | 6.6 |
| | Hexa- | 387.8730, 389.8700 | <0.1 |
| PCDT | Penta- | 353.9120, 355.9090 | 1.3 |
| | Hexa- | 387.8730, 389.8700 | <0.1 |
| $^{13}C_{12}$-PCB 180 | Hepta- | 405.8430, 407.8390 | 5.5[c] |
| PCT | Hexa- | 435.8730, 437.8700 | 1.9 |
| | Hepta- | 469.8340, 471.8310 | <0.1 |

[a] PCB = polychlorinated biphenyl, PCDE = polychlorinated diphenylether, PCDT = polychlorinated diphenyltoluene, PCN = polychlorinated naphthalene, PCT = polychlorinated terphenyl.
[b] m/z = mass to charge ratio.
[c] Concentration of $^{13}C_{12}$ PCB internal standard congeners added before sample extraction.

terminations, these compounds were not analyzed further. However, the detection of these chemicals, albeit at very low concentrations, in this remote location highlights a tendency for these contaminants to be dispersed globally. Future monitoring programs could also focus on the transport and fate of these chemicals.

## DISCUSSION

Two major questions arise from this study: (1) What is the source of the contaminants found in these birds, and (2) what, if any, are the effects of these contaminants on the birds?

### *Sources*

The consistent profile of contaminants in the fat liver and egg tissues from the two species at two time points would suggest a common contaminant source for all samples analyzed. Although these data are from a relatively small number of samples, two points should be remembered: (1) The body fat samples were collected at different times and when the birds had been feeding in very different regions of the North Pacific Ocean, and (2) in general, contaminants accumulated in egg tissue tend to be of local origin and thus distinct from contaminants accumulated in the body fat of migrating adult birds [32]. Nonbreeding Laysan albatross mainly frequent the western Pacific and Asian coasts, while black-footed albatross frequent the northeastern Pacific and North American coasts. Despite this geographical separation of the two species, the egg contaminant profiles of the two species are very similar. Together these observations suggest that the contaminants found represent the "general contamination" in the tropical and North Pacific Ocean.

The possibility of an immediate local source of the contaminants can be discounted for several reasons. First, due to nesting and incubation duties, the adult albatross are in a fasting state for almost 60% of the first 2 to 2.5 months on the atoll. Second, during local as opposed to migratory feeding, the adults travel 300 to 500 miles north of the atoll to feed at the intersection of the major oceanic currents north of the Hawaiian chain [33]; therefore, the birds do not, to any great extent, feed in the vicinity of the atoll and thus do not accumulate contaminants from an immediate local source.

Given that the contaminants detected represent the general contamination of the North Pacific Ocean, the sources of these contaminants and the means by which the contaminants are accumulated by the birds are of considerable interest. It is of particular concern that the concentrations of PCHs detected in this supposedly pristine remote location are similar to PCH concentrations detected in birds from relatively contaminated locations such as the Great Lakes of North America.

The profiles of PCBs in the Midway albatross samples are unusual for birds in terms of the paucity of lower chlorinated PCBs. Similar PCB profiles have been detected in human [34] and polar bear fat samples [35]. These mammalian species have a considerably higher capacity to metabolize PCBs than many bird species. However, comparative studies indicate that procellariform birds, which includes albatrosses, have a greater ability to induce some xenobiotic metabolizing enzyme activities than do other bird species [36]. This hypothesis is strengthened by the unusual ratio of PCB congeners 101 and 99 as discussed previously. A trend towards a similar congener ratio has been indicated in herring gulls from the Great Lakes [37]. Thus, the congener profile may be a result of metabolic depuration of the lesser chlorinated congeners, particularly since atmospherically deposited PCBs have an abundance of lower chlorinated congeners [4,5]. Although the abundance of the lower chlorinated PCBs decreases with increasing trophic level this "atmospheric transport" pattern is still apparent in open ocean carnivores [38]. The high ratio of PCDFs to PCDDs found in the samples may indicate an atmospheric source as PCDFs are known to have a longer atmospheric half-life than PCDDs [39].

The high concentrations of PCDFs, compared to PCDDs, present in the Midway albatross samples would at first suggest that technical PCB mixtures could be a major source of the PCHs since PCDFs are more abundant than PCDDs in technical PCB mixtures [40]. However, the presence of significant concentrations of PCDDs suggests that multiple sources of contamination are involved. In addition, the high ratio of PCDFs to PCBs would indicate considerable enrichment of the PCDFs compared to technical PCB mixtures [41]. The albatross in this study have very high consumption rates of plastic fragments from the ocean surface, some of which are partially burned or melted [16]. It seems possible that plastics serve to transfer PCHs to albatross.

### *Effects*

High concentrations of PCHs have been shown to adversely affect the reproductive capacity of birds [10,11]. The adverse effects observed range from direct toxic and teratogenic effects in developing embryos [32,42] to subtle changes in parental behavior [2]. A comprehensive example of these effects has been the study of reproductive effects in fish-eating waterbirds in the Great Lakes of North America [10]. In this ecosystem, elevated levels of PCHs have led to the definition of a characteristic suite of contaminant-induced reproductive impairments [42]. These studies have demonstrated a close association between contaminant accumulation, measured as TEQs, and lowered reproductive success and increased rates of chick deformity. In view of these findings, and the comparatively high levels of PCHs found in the Midway albatross samples, it is pertinent to evaluate what the possible effects of the PCHs might be on the Midway albatross populations.

When comparing calculated TEQ values for environmental samples it is essential to remember that various TEF systems may be used to calculate TEQ concentrations. The PCB TEF values chosen here [30] are the most recent values based on

Table 5. Hazard indices (HI) for polychlorinated biphenyls (PCBs) and 2,3,7,8-tetrachlorinated dibenzo-*p*-dioxin (TCDD) toxic equivalents (TEQs) in Midway Atoll albatross eggs

| Sample | Contaminant | Concn. | Reference dose[a] | HI |
|---|---|---|---|---|
| Laysan | PCB[b] | 199 ng/g[c] | 400 ng/g | 0.5 |
| Black-footed | PCB[b] | 688 ng/g[c] | 400 ng/g | 1.7 |
| Laysan | TCDD TEQs | 50 pg/g | 1.5–10 pg/g[d] | 5–33 |
| Black-footed | TCDD TEQs | 124 pg/g | 1.5–10 pg/g[d] | 12–83 |

[a] No-observable-adverse-effect level, bald eagle, egg lethality (Giesy et al. [10]).
[b] Sum of PCB congeners (Table 2).
[c] Average for the two Laysan Pools analyzed.
[d] Range of values for most sensitive species (bald eagle, 1.5 pg/g) and least sensitive species (herring gull, 10 pg/g) based on egg lethality (Giesy et al. [10]).

human intake. One major advantage of these TEF values is that they provide a comparable estimate of PCB and PCDD/PCDF toxicity when combined with the commonly used TEFs for PCDDs and PCDFs [27]. The values used are considerably lower than the values of Safe [43], which previously were the most commonly used values in environmental assessments. To determine the effect of using different TEF values, TEQs were calculated using three different sets of TEFs (Table 3). Toxic equivalents calculated using all three systems were highly correlated ($r^2 > 0.99$, $p < 0.0001$), as has been previously demonstrated [44]. Although these methods are closely correlated, the absolute concentration of TEQ predicted is different, particularly for the Safe TEFs, where TEQ concentrations are approximately twofold higher than the other estimates. It is the absolute TEQ concentration which is of most concern in evaluating possible biological effects.

Calculated TEQ concentrations in the egg samples were within the range of concentrations where sensitive avian species show adverse reproductive effects [10,11]. This is particularly so for the black-footed albatross, where egg TEQ concentrations were 124 pg/g wet weight. Of note is the fact that black-footed albatross eggs showed considerably higher concentrations of PCDF and PCB congeners than Laysan albatross eggs. This may reflect the different food habits and/or migration patterns of the species.

To further assess the possible adverse effects of contaminants on the reproduction of the albatrosses, hazard indices (HIs) were calculated for each species (Table 5). The HI is calculated as the concentration of the contaminant divided by a "reference dose," a no-observable-adverse-effect level (NOAEL) based on the species, contaminant, and end point under investigation [10]. The HI therefore reflects the likelihood of the occurrence of adverse effects. As lowest-observable-adverse-effects levels (LOAELs) are generally 10-fold higher than NOAELs, it is unlikely that adverse effects will be seen until HIs exceed 10. In general, when HIs exceed 20, adverse effects are likely to be detectable at the population level. In practice, species-specific data are not available for the species studied, so NOAELs were assumed to be similar to other fish-eating birds [10]. For PCBs the NOAEL chosen was the average of those for other birds. Due to the greater range in species sensitivity to TEQs two values ranging from least sensitive to most sensitive were used.

Hazard indices determined for total PCBs in both albatross species were well below the level at which adverse effects would be expected. However, these estimates were based on the sum of the 19 congeners analyzed, while the NOAEL was based on total PCBs. Given that the congeners determined constitute approx. 40% of the total PCB concentration (P.D. Jones, unpublished results), it is still unlikely that adverse effects due to total PCBs would be detectable. In contrast, HIs based on TEQs and the NOAEL for the most sensitive species known (bald eagle) exceeded levels where adverse effects would be expected for both species. When HIs were calculated based on TEQs and NOAEL for the least sensitive species (herring gull), the HI for the black-footed albatross still exceeded levels where adverse effects would be expected. It therefore seems probable that the PCHs present in these albatross may be having an adverse effect on reproduction. Preliminary analysis of field results indicates that the black-footed albatross are indeed less successful reproductively than Laysan albatross at Midway Atoll. Black-footed albatross had higher embryo death rates and lower egg hatchability than Laysans in 1994 [16].

## CONCLUSIONS

From a comparative perspective it appears that the greatest "risk" to the albatross is from PCDD, PCDF, and PCB contamination. The concentrations of contaminants and of dioxin toxic equivalents in these birds from a "pristine" environment are of considerable concern. Although other contaminants were detected in these samples, the concentrations present were much lower, on a mass basis, compared to the PCB concentrations present. Similarly, from a toxicological point of view, even if the TEF values for these minor contaminants were as high as PCBs, or even PCDDs, the contribution to TEQs would be small compared to PCDDs, PCDFs, and PCBs. Also, since the closeness of results from the bioassay procedure and the calculation of TEQs would indicate that the PCDDs, PCDFs, and PCBs analyzed account for most of the dioxin-like biological activity observed.

The presence of relatively high concentrations of contaminants in the two species studied suggests that similar levels of contamination may also be found in the short-tailed albatross (*Diomedea albatrus*), which also inhabits the tropical North Pacific. In view of the very small population of this species currently breeding in the Pacific, it may be pertinent to determine if the contaminants detected in this study are having effects on the recovery of this endangered species.

The origins of the synthetic chlorinated contaminants found in birds on Midway Atoll remain unknown. Further sampling of the birds and especially their food sources will be required to answer this question. The concentrations of contaminants detected are close to levels known to cause adverse effects in other bird species. The sensitivity of procellariform birds to these contaminants remains to be determined, as does the impact of these contaminants on the bird populations of the Pacific Ocean.

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