DIOXIN EXPOSURE AND EFFECTS ASSESSMENT OF RED-TAILED TROPICBIRDS NESTING ON JOHNSTON ISLAND, CENTRAL PACIFIC OCEAN

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INTRODUCTION

This study evaluated dioxin exposure hazard and adverse effects to Red-tailed Tropicbirds nesting in the dioxin contaminated former Herbicide Orange storage area (HO site) on Johnston Island, Central Pacific Ocean, the storage location of 5.4 million kg. of herbicide orange following the Vietnam war. Tropicbirds do not build a nest, but lay a single egg directly on the soil surface. Tropicbird chicks sit in place on the soil for 12-13 weeks, until they fledge, and are at risk from exposure as a consequence of dust inhalation or soil ingestion.

The study included four independent measures of exposure, response, and adverse effect, which would not kill any chicks: 1) blood sampling for direct analytical chemistry of TCDD and other dioxins and furans in serum; 2) cell culture assay (CALUX) to detect dioxin-like activity in serum by induction of luciferase activity coupled to the Ah receptor for induction of cytochrome P450 activity; 3) surgical liver biopsies to determine induction of EROD (ethoxyresorufin-o-deethylase); and 4) high resolution radiography of the chick heads to evaluate developmental asymmetry of the cerebral hemispheres and cranium. We assumed that the dioxin sensitivity of tropicbirds is similar to that of cormorants and herons, and that adverse effects would be similar.

METHODS

Seventeen chicks between 7 and 12 weeks of age were sampled from the HO site, and 13 reference chicks were samples from nests approximately 2 miles upwind from the HO site. Each bird was intubated with a human pediatric tracheal catheter, and ventilated with a combination of isoflurane and oxygen for anesthesia. Surgical biopsies of 0.1gm liver were obtained by sterile surgery, and six radiographic views were taken for analysis of potential skeletal malformations. 5 ml. blood was taken from the brachial or metatarsal vein for measurement of dioxin in serum.

Chemical Activated Luciferase Gene Expression Assay (CALUX):

The CALUX (Chemical activated luciferase gene expression) assay (Garrison et al., 1996) is based upon the induction potential of dioxin like compounds in a mouse hepatoma cell line transfected with a reporter gene for luciferase. In this assay, 25ul samples were analyzed in triplicate giving a sensitivity of about 128 pg/ml serum.

Analytical Chemistry of Dioxins in Serum Samples

Five serum samples from tropicbird chicks were submitted to the USGS Columbia Environmental Research Center (CERC) for analysis of 2,3,7,8 chlorinated dibenzodioxins, dibenzofurans, and dibenzothiophenes. Extracts were cleaned-up using acid- and base-treated silica gels and adsorbent chromatography on activated silica gel, followed by high performance gel permeation chromatography, and fractionated on high performance Porous Graphitic Carbon, PCDD/PCDF fractions were eluted through basic alumina for removal of potential co-contaminants. All samples were spiked with surrogate standards of ^{13}C-labeled 2,3,7,8 substituted dioxins/furans before extraction. PCDDs, PCDFs, and PCDTs were determined by gas chromatography/high resolution mass spectroscopy (GC/HRMS)

Liver Enzyme Analysis (EROD):

Microsomes were prepared from liver biopsy samples and analyzed for EROD with a kinetic assay modified from the method of Burke and Mayer, (1974) miniaturized for a 96 well microtiter plate fluorescent plate reader. Microsomal protein was quantified by the method of Bradford (1976) using a Coomasie Blue protein kit from Pierce Chemical Company. EROD activity of tropicbird liver microsomes was compared to archived samples of double-crested cormorant liver samples from San Francisco Bay, (Davis, Fry and Wilson, 1996, 1997). Data was reported as pico moles resorufin produced per min per mg microsomal protein (pmol/min/mg).

Radiographic analysis of Tropicbird brain asymmetry.

Radiography was conducted at 50KVA, with high-resolution Trimax-2™ film and intensifying cassettes designed for avian veterinary practice. Chicks were radiographed an oblique rostrocaudal view at 45° to optimize resolution of the cranial cavity surrounding the cerebral hemispheres. Radiographs were scanned at 0.08 mm resolution and analyzed by Kodak Imaging for Windows™. Each image was evaluated for dorsoventral alignment of the mid-sagital bones. The radiographic images were enlarged 6 fold, and measured to the nearest 0.5mm., equivalent to detection of differences as small as 0.08mm.

RESULTS:

CALUX Estimation of TCDD Exposure:

Serum samples from three birds had detectable activity of dioxin-like compounds (Table 1). These three samples, plus the serum sample from JH877, the only chick with measurable brain asymetry, and one additional HO site chick (JH611) were submitted to CERC for PCDD/PCDF analysis.

Quantification of Dibenzodioxins/furans by Gas Chromatography/Mass Spectrometry (GC/MS).

Method detection limits for PCDD/PCDF were about 0.2 pg/g. Concentrations of 2378-TCDD in the five selected sera are presented in Table 1. Concentrations of 2,3,7,8-TCDF were below the detection limit of 1 pg/g. OCDD and OCDF were present at concentrations within three times the background. All other 2378-substituted PCDD/PCDFs were < 2 pg/g. No polychlorinated dibenzothiophenes were detected in any sera samples.

EROD determination of exposure and response.

The EROD activity of tropicbird samples is given in Table 1. The maximum activity of any sample was 28.6 pmol/min/mg protein, with the average activity of controls and HO site birds *not statistically different* from each other. Activities of both groups were very low compared to studies of mainland US and Canadian colonies of cormorants or herons.

Radiography of Tropicbird Chicks

One bird (JH877) had an asymmetry of 0.5mm. All other birds were symmetrical to within 0.16mm, below the detection limit of asymmetry described by Henshel (1998) and Henshel et al (1995, 1997a,b). The asymmetry of JH877 (Figure 11) was measurable in 3 of the 4 head radiographs, indicating that the asymmetry was not due to dorsoventral alignment projection differences. The nest location of chick JH877 was in the highest contaminated part of the HO site, with a 50 ppb soil TCDD level reported by Crockett et al (1986) for the quadrant nest site. JH877 had no detectable TCDD in serum from the CALUX assay, but had detectable levels of TCDD when analyzed by CERC (10 pg 2,3,7,8-TCDD/g serum. Chick JH877 also had one of the highest EROD values, at 27.6 pmol/min/mg protein, but this level would not be considered elevated in any other study in the literature.

DISCUSSION:

There was not complete agreement between the two TCDD estimation methods. Two samples were positive in the CALUX assay and negative in the GC/HRMS assay. The false positives could have been due to PCBs or PAHs, which would have been removed in the cleanup procedures for the GC/HRMS. The methods substantially agreed for three of the other four serum samples, although the TCDD estimates differed. The CALUX assay appeared to slightly overestimate the TCDD.

The EROD levels low compared to other studies of wildlife, and below the threshold level for detection of adverse effects in Great Blue Herons or cormorants, the most closely related species to Tropicbirds that have been studied (Bellward et al. 1990, Blus et al 1997, Sanderson and Bellward 1995, Janz and Bellward 1996, Sanderson et al. 1997a,b).

Henshel et al. (1995) discovered asymmetry in the brains of great blue herons and width differences of 0.5 and 1mm consistently correlated with exposures greater than 100pg/g (ppt). The probit-determined ED50 concentrations for width differences were 32 pg/g for TCDD and 64.5pg/g for TCDD-TEQ. Cormorants were the least sensitive species with an ED50 of 34pg/g TCDD and 97pg/g TCDD-TEQ.

The detection of TCDD in the serum of chicks indicates a small, and possibly significant, exposure to dioxins in the HO site.

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Table 1

Biochemical and Morphological Data on Red-tailed Tropicbird Chicks

Sample	EROD activity	Group Mean	CALUX TCDD Activity	Serum TCDD pg/g	Cranial width diff. (mm)
JC036	17.61	13.00 ±1.35	ND		-0.17
JC038	8.42		ND		0.00
JC041	5.19		ND		-0.08
JC043	15.24		No sample		0.08
JC584	22.14		ND		-0.08
JC901	9.59		ND		
JC902	15.00		ND		-0.08
JC903	11.51		176 pg/g	**<1pg/g**	0.00
JC980	10.01		ND		0.00
JC982	13.13		ND		0.00
JC983	19.82		ND		-0.17
JC989	8.86		ND		0.00
JC991	12.50		ND		0.17
JH111	5.37	13.85 ±2.05	ND		0.17
JH444	15.38		ND		0.00
JH610	16.17		ND		0.08
JH611	4.90		ND	**<1pg/g**	-0.08
JH612	7.19		ND		0.00
JH615	17.09		ND		0.00
JH866	12.52		ND		0.00
JH867	10.65		ND		-0.08
JH868	11.81		ND		-0.08
JH870	22.92		ND		-0.08
JH871	28.63		ND		-0.08
JH872	28.10		ND		-0.17
JH874	7.81		ND		-0.17
JH875	6.29		228pg/g	**<1pg/g**	0.00
JH876	3.68		128pg/g	**34pg/g**	-0.17
JH877	27.57		ND	**10pg/g**	**-0.50**
JH880	9.37		ND		0.17