THE PHARMACOKINETICS OF 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN TRANSFER FROM ADULT WHITE LEGHORN CHICKEN TO THEIR YOUNG

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Introduction

In the 1970s, waste oil containing TCDD was sprayed to control dust in horse arenas in Missouri, resulting in the death of at least 65 horses, several cats and dogs, hundreds of birds and numerous rodents (4). One such area that was sprayed and therefore contaminated was a town known as Times Beach, where 2,3,7,8-TCDD concentrations were found to be as high as 1200 parts per billion (5). The town was evacuated, torn down and the soil was remediated, bringing the soil concentrations down to one part per billion or less. As part of the remediation, new topsoil was added and the site now plays host to some wildlife species. It was, therefore, important to assess the risk posed to the wildlife, namely a flock of wild turkey, by the one part per billion 2,3,7,8-TCDD.

This study was part of a larger project to assess the risk posed to some wildlife living on by 2,3,7,8-TCDD residues left over from a contaminated oil spray used to combat dust on site. Other parameters studied were embryo lethality, developmental toxicity, teratogenicity, reproduction-related endocrine effects, immunotoxicity and neurotoxicity (1,2,3). It is,important to correlate between effects observed in the chick embryo study and the 2,3,7,8-TCDD content found in eggs and tissues for use in future risk assessment. This would be of particular use in cases wherein there was a tight time constraint or when the study deals with endangered species.

Materials and Methods

Calculations used throughout this study were based on the dosing regime (Table I) and the projected hen-to-egg transfer rate of 1%, as found to be the case in ring-necked pheasants (6). It was also assumed that chicken fat tissue composed 25% of the carcass (7) and would hold 10 times more 2,3,7,8-TCDD than other tissues (8).

Table I. Chicken Dosing Regime

	Given 0.25 ml of 2,3,7,8-TCDD in olive oil (μg/ml)	Each injection (μg)	Injection (μg/ day)	Injection (μg/ kg/ day)	Cumulative injection over six weeks (μg/ kg)
Control	0	0	0	0	0
Low Dose Hens	0.12	0.03	0.009	0.006	0.24
High Dose Hens	24.0	6.0	1.714	1.143	48.0

Analytical Technique

Briefly, chicken egg samples were kept frozen in glass jars by dose group. Before extractions were started, egg samples were removed from the freezer and allowed to thaw for 2-3 hours. The contents of each jar were then weighed and homogenized using a POLYTRON tissue homogenizer. Three 6-gram tissue samples per dose group were then weighed and homogenized with anhydrous sodium sulfate, pre-baked overnight at 130^{o}C, at a ratio of 1:5 egg sample to sodium sulfate. The egg-sodium sulfate homogenate was then Soxhlet extracted for 18 hours.

One important note is that volumes of extract to be used in dosing the H4IIE cell line for the enzyme induction assay were not cleaned up using gel permeation chromatography (GPC) or the porous graphitic carbon column (PGC) as these clean up procedures may possible remove compounds that could have synergistic, antagonistic or additive interactions with 2,3,7,8-TCDD. Instead, samples removed from the Soxhlet extractor were evaporated to near dryness with nitrogen and diluted in iso-octane. Iso-octane as the solvent carrier for extracts of pure compounds optimally enhances bioassay sensitivity (9).

Extracts to be analyzed on the gas chromatogram were cleaned up by both GPC and PGC. These extracts were analyzed with a Hewlett Packard gas chromatogram using an electron capture detector (ECD) with split-less injections onto a 30mm (L) X 0.25mm (ID) J&W Scientific fused silica capillary column coated with a DB-1701 stationary phase of 0.25 μ film thickness.

H4IIE Cell Culture Technique and Enzyme Induction Bioassay

The H4IIE cell culture was brought up in Dulbecco's Modified Eagles Medium (DMEM) supplemented with sodium bicarbonate and HEPES. Once cells reached 75% confluency in a 75-cm^{2}-cell culture flask, they were plated into 96-well plates at a concentration of approximately 10^{3} cells per well. Cells were dosed 24 hours after plating.

The biological assay used to measure amount of 2,3,7,8-TCDD present in the dosing solution was the ethoxyresorufin-O-deethylase (EROD) enzyme induction assay. The optimum parameters used in this assay were 17 μM ethoxyresorufin, 1 mM NADPH and a 48-hour incubation period (10). The plates were read from the bottom on a Packard Fluorocount Microplate Fluorometer with a Quartz fiber optic connection to an external Quartz-Halogen light source. The excitation wavelength was set at 530 nm and the emission wavelength was set at 590 nm.

Protein Assay

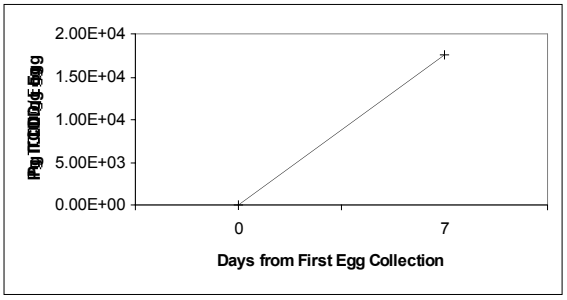
Samples were measured for protein content using the bicinchoninic protein assay.

Results and Discussion

The EROD bioassay was unable to detect any 2,3,7,8-TCDD concentrations in the control and low dose eggs and the control and low dose abdominal fat samples. Significant concentrations of 2,3,7,8-TCDD were detected in the high dose egg and abdominal fat samples. However, these concentrations were not consistent across the egg sample triplicates and the fat sample quadruplicates. No, detectable concentrations of 2,3,7,8-TCDD were found in the first of the two high-dose egg time points, H-1. In the second high-dose egg time point, H-2, detectable concentrations of 2,3,7,8-TCDD were found in only two of the three triplicate extracts and the levels found varied greatly. The same was true of the high-dose abdominal fat samples.

As a result of the limited amount of data, time point analysis could be attempted only for the high dose eggs (Figure 1). A comparison of 2,3,7,8-TCDD levels in chicken abdominal fat

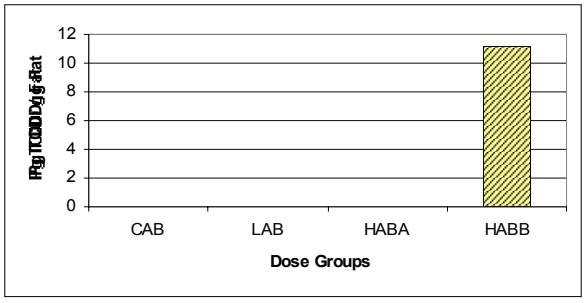
samples across dose groups is summarized in Figure 2 and a comparison of 2,3,7,8-TCDD levels in chicken egg samples across dose groups is summarized in Figure 3.



0 Days = non-detectable

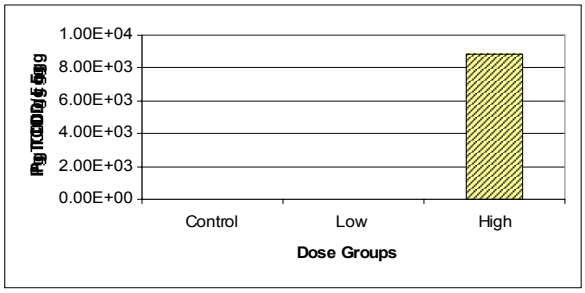
Figure 1. Time point Analysis of High Dose Eggs (EROD)

On average, gas chromatography analysis detected seven times the expected levels of 2,3,7,8-TCDD in the low dose eggs, about 42% of the expected levels of 2,3,7,8-TCDD in the high dose eggs and about 71% of the expected levels of 2,3,7,8-TCDD in the high dose abdominal fat samples. The results of



CAB = control abdominal fat extracts
LAB* = low dose abdominal fat extracts
HABA* = sacrificed immediately at end of study
HABB = sacrificed 30 days after end of study
* = non-detectable

Figure 2. Abdominal Fat 2,3,7,8-TCDD Levels Across Dose Groups (EROD)



Low = non-detectable

Figure 3. Chicken Egg 2,3,7,8-TCDD Levels Across Dose Groups (EROD)

time point analysis on the low dose and high dose egg extracts are shown in Figures 4 and 5. In both cases, 2,3,7,8-TCDD levels increased with respect to time.

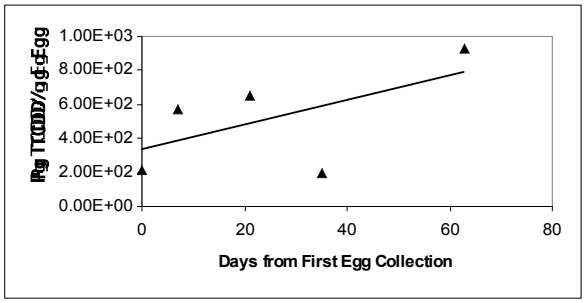


Figure 4. Time Point Analysis for Low Dose Egg Extracts (GC-ECD)

From the available data, a comparison was also made between 2,3,7,8-TCDD concentrations detected in the eggs as opposed to concentrations detected in the abdominal fat. The average concentration of 2,3,7,8-TCDD in the high dose chicken eggs was 6.1 X 10^3 picograms TCDD/ g per egg. Each egg weighs approximately 50 grams, which translates to roughly 3.05 X 10^5 picograms of 2,3,7,8-TCDD per

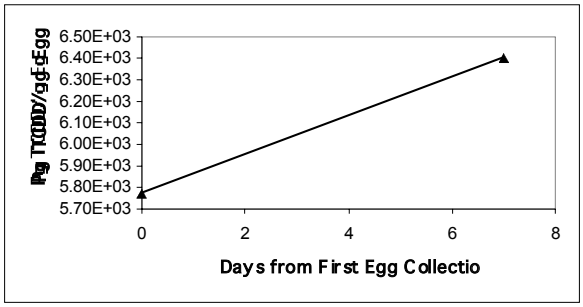


Figure 5. Time Point Analysis for High Dose Egg Extracts (GC-ECD)

egg. The average concentration of 2,3,7,8-TCDD in the high dose abdominal fat samples was 1.24 X 10^5 pg TCDD/ g fat. Each chicken weighs approximately 1500 grams, 25% of which is fat. That would translate to approximately 375 grams of fat per chicken. Therefore, each chicken would carry roughly 4.65 X 10^7 picograms of 2,3,7,8-TCDD. The egg to fat ratio would then be equivalent to 0.007 or roughly 1%, which is what Nosek et al. found to be true in their study of the metabolism and disposition of 2,3,7,8-TCDD in ring-necked pheasants.

In the high dose egg extracts, the EROD bioassay detected an average concentration of 8.80 X 10^3 picograms TCDD/ g egg which is comparable to the 6.08 X 10^3 pg TCDD/g egg detected using GC-ECD analysis but only about half the expected 2,3,7,8-TCDD concentrations. We believe this is due to the fact that egg production ceased before the expected concentrations of 2,3,7,8-TCDD could accumulate in the hens and transfer to the eggs.

Both the EROD and GC-ECD analysis indicate that 2,3,7,8-TCDD concentrations did not fall in the second group of hens (HABB) that were sacrificed 30 days after the end of the study as opposed to the first group (HABA) which were sacrificed immediately at the end of the study.

Due to the fact that the EROD bioassay was not able to detect concentrations of 2,3,7,8-TCDD in the low dose egg and abdominal fat samples, it was not possible to insert the data into a pharmacokinetic model for prediction of 2,3,7,8-TCDD transfer from adult white leghorn chicken to their eggs. With future methods development and studies to improve assay sensitivity, this should be attempted again in the future.

Acknowledgements

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