BIOACCUMULATION OF PCDD/PCDFs IN CHICKENS: CONTROLLED EXPOSURE STUDIES

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ABSTRACT

Controlled exposure studies in the laboratory and the field have been initiated to investigate the transfer of low soil concentrations of PCDD/Fs to chickens. The accumulation over time of specific isomers is being tracked in eggs, blood, feces, liver, adipose, and edible flesh. Measurable increases of PCDD/F concentration in eggs of exposed chickens, as compared to controls, were seen after 30 days of exposure. The exposure characterization, as well as initial data on isomer specific accumulation rates in eggs are presented.

INTRODUCTION

Previous reports from this laboratory[1,2] have presented data on the bioaccumulation of PCDD/Fs in foraging farm animals in areas with soil PCDD/Fs with a CA-TEF[3] in the 20-50 ppt range. In one of these sites, near a wood treatment plant in Oroville, California, the PCDD/Fs showed a pentachlorophenol profile[2]. In our previous investigations, neither the level nor the duration of exposure could be controlled. One of the major objectives of the current studies is to estimate the rates and levels of bioaccumulation of PCDD/Fs in chickens. The studies are carried out under controlled conditions and the results are anticipated to provide an improved understanding of the relationship between agricultural soil contamination by PCDD/Fs and the accumulation of these compounds in foraging animals.

EXPERIMENTAL

1. Study Design. The study consists of two parts: a laboratory phase, conducted at the University of California, at Davis, where the chickens are fed a formulated diet containing soil from an Oroville, California, backyard found contaminated with PCDD/Fs, and a field phase where the chickens are raised on PCDD/F contaminated soil at that Oroville backyard. The purpose of the two phases is to study the potential for PCDD/F buildup in eggs and bioaccumulation in tissues through ingestion of contaminated soil. Rates and levels of bioaccumulation will be determined during the laboratory phase and will be compared to rates observed in the field phase in an effort to isolate the contribution of the soil vector, as compared to other exposure pathways.

A. Laboratory phase

Exposure groups. This phase involves three exposure groups with 20 chickens in each group. The groups are housed in separate animal rooms, with the individually caged chickens having free access to food and water. The three groups are: 1. Control group,

where chickens are fed a formulated laying-bird diet containing 10% uncontaminated soil. 2. Low exposure group, where chickens are fed the same diet as above containing 10% "low level" contaminated soil. The soil came from the same backyard where the field phase is conducted and is macroscopically similar to the control soil (sandy loam). 3. High exposure group, where chickens are fed the same diet as above containing 10% spiked contaminated soil. The soil is the same low level soil used in the low exposure group with some isomers fortified through spiking.

Spiking procedure. One ^{13}C-2,3,7,8 isomer per congener group was fortified to ~20 times the original level in the low level soil. The other 2,3,7,8-isomers will serve as internal controls for the spiking effect. The required amounts of each isomer were put in a toluene solution that was added to four batches of 400 g each of low level soil. After allowing the toluene to evaporate, the dried soil was left for 2 months undisturbed in the dark to increase binding of the spiked isomers onto the soil particles. Each batch of spiked soil was then mixed with 8 kg of low level soil in a ballmill to obtain a homogeneous mixture of the desired PCDD/F profile. Random samples from each soil level were analysed for PCDD/Fs and found satisfactorily homogeneous.

The PCDD/F content of the soil and feed mix for each exposure group is given in Table 1. The soil/feed mixture is prepared over time in 20 kg batches. Every time a new batch of feed is mixed with the soil, 4 samples are drawn and submitted for analysis to determine homogeneity by ICP-MS screening of soil elements and a composite of the 4 samples is analysed for PCDD/F by GC-MS. The first batches of mixed feed were homogeneous and at the right concentrations. In fact, given the long half-life of PCDD/F isomers in animals, small variations in the composition of the feed, and therefore in the PCDD/F intake, are not important.

Table 1. PCDD/F isomer concentrations (ppt) in soil and feed.

ISOMER	LEVELS IN SOIL			LEVELS IN FEED MIX			
	CONTROL	LOW	HIGH	CONTROL	LOW	HIGH	HIGH/LOW
* 2,3,7,8 TCDD	NA	NA	NA	NA	NA	NA	NA
* 1,2,3,7,8 PeCDD	<0.3	11.8	242.5	<0.1	0.7	13.9	19.9
* 1,2,3,4,7,8 HxCDD	<0.5	16.4	415.0	<0.07	1.7	22.5	13.2
1,2,3,6,7,8 HxCDD	3.2	54.3	89.0	<0.1	6.1	5.0	0.8
1,2,3,7,8,9 HxCDD	<1.7	40.0	56.0	<0.2	5.0	2.9	0.6
1,2,3,4,6,7,8 HpCDD	20.0	707.0	1088.5	2.0	52.0	96.5	1.8
* OCDD	105.0	4250.0	108736.0	9.8	359.0	8000.0	22.3
* 2,3,7,8 TCDF	<1.0	6.8	82.0	<1.0	<1.4	8.6	>6.1
1,2,3,7,8 PeCDF	<0.1	1.1	<1.0	<0.04	<0.1	<0.2	-
* 2,3,4,7,8 PeCDF	<0.1	4.4	133.5	0.07	0.5	8.9	29.7
1,2,3,4,7,8 HxCDF	<0.1	45.0	97.5	0.1	3.2	9.0	2.8
* 1,2,3,6,7,8 HxCDF	<0.4	10.1	370.0	0.05	0.8	27.0	33.8
2,3,4,6,7,8 HxCDF	<0.1	20.3	21.5	<0.03	0.8	2.3	2.9
1,2,3,7,8,9 HxCDF	<0.2	<0.1	<0.1	<0.04	<0.1	<0.1	-
1,2,3,4,6,7,8 HpCDF	2.7	177.5	547.0	0.30	14.0	30.5	2.2
* 1,2,3,4,7,8,9 HpCDF	<0.4	40.5	1128.0	<0.20	2.2	101.0	45.9
* OCDF	6.2	249.5	9660.0	0.42	19.0	787.0	41.4
CA-TEF	0.6	57.3	566.4	0.1	3.3	40.4	
EPA-TEF	0.0	4.4	171.1	0.0	0.8	10.5	
I-TEF	0.5	43.2	408.7	0.1	3.5	30.4	

NA = Not analysed
* = Isomer spiked in the High exposure dose group

B. Field phase

This phase mimics the historic exposure of chickens raised at the field site that led to the elevated PCDD/F levels found in eggs and tissues. Two exposure groups are employed in this phase. 1. Exposed group, consisting of 15 chickens maintained in range-type conditions within a fenced enclosure where surface soil contains 20-50 ppt CA-TEF PCDD/Fs. The chickens are given the same commercial feed as the birds in the control group, but at reduced amounts in order to encourage foraging. 2. Control group, consisting of 9 chickens housed in elevated wire cages with no access to the soil and fed the same commercial diet.

2. Analysis

For both study phases, eggs are collected throughout the study every 5 days during the first month and every 10 days thereafter. Chickens are culled according to a schedule and samples of blood, feces, edible meat, liver and adipose tissue are collected and stored for subsequent analysis. For both eggs and tissues, three samples are composited prior to extraction and analysis. PCDD/Fs in soil and feed samples are extracted with toluene and purified using AX21 carbon, followed by basic and acidic silica gel mini-

column. PCDD/Fs in egg samples are purified using C-18 solid phase extraction cartridges, followed by Carbopack C and alumina column cleanup[5]. All samples are analysed by HRGC-LRMS in methane NCI mode. The cleanup of split samples sent to the University of Umeå is based on the Smith et al.[6] method with the analysis performed by HRGC-HRMS. Tissue specimens are available for future toxicity studies such as hepatic enzyme assays and immunologic testing.

RESULTS AND DISCUSSION

Thus far, in both the field and laboratory studies, egg production and animal health appear to be normal. Table 2 shows the PCDD/PCDF results for pre-exposure eggs, as well as eggs laid following 30 and 50 days of exposure in the field study for both the exposed and the control groups. Table 3 shows the pre-exposure and 30-day PCDD/F egg results from the laboratory study for the control, the low and the high exposure dose groups. The PCDD/F profile in feed corresponding to that exposure period is shown on Table 1.

Field phase. The initial results from this phase indicate a gradual increase over the pre-exposure levels for the exposed chickens with lower and rather stable levels for the control chickens. However, these levels are much lower than what was previously observed in eggs from chickens raised on that property which had CA-TEFs in the 100 to 200 pg/g fat range[1,2]. This difference may be due to changes in food consumption, i.e. more reliance on commercial feed as opposed to foraging, or to depletion of soil PCDD/Fs and/or soil organisms in the enclosure area to which the chickens have access. The data could, however, reflect a slow rise to the expected levels. This issue should be resolved when more egg samples are analysed later in time.

Table 2. PCDD/F isomer concentrations (pg/g fat) in eggs from the field phase of the study.

ISOMER	BASELINE	DAY-30		DAY-50	
		CONTROL	EXPOSED	CONTROL	EXPOSED
2,3,7,8 TCDD	NA	< 0.42	< 0.39	NA	NA
1,2,3,7,8 PeCDD	< 0.15	< 0.32	1.31	< 0.15	1.82
1,2,3,4,7,8 HxCDD	< 0.20	< 0.26	2.20	0.41	2.41
1,2,3,6,7,8 HxCDD	0.72	0.75	5.64	1.20	4.67
1,2,3,7,8,9 HxCDD	< 0.53	< 0.83	0.39	< 0.29	2.93
1,2,3,4,6,7,8 HpCDD	4.78	5.79	27.38	9.69	31.23
OCDD	40.29	59.25	55.30	40.77	46.68
2,3,7,8 TCDF	0.89 *	0.89 *	0.94 *	1.04 *	1.21 *
1,2,3,7,8 PeCDF	0.07	< 0.06	0.25	0.29	0.33
2,3,4,7,8 PeCDF	0.32	0.31	2.82	0.43	0.60
1,2,3,4,7,8 HxCDF	0.36	0.33	1.42	0.57	1.29
1,2,3,6,7,8 HxCDF	0.33	< 0.09	0.95	0.45	0.88
2,3,4,6,7,8 HxCDF	0.18	< 0.10	0.83	0.41	0.98
1,2,3,7,8,9 HxCDF	< 0.13	< 0.19	< 0.22	< 0.14	< 0.09
1,2,3,4,6,7,8 HpCDF	0.55	0.97	3.58	2.33	4.16
1,2,3,4,7,8,9 HpCDF	< 0.31	< 0.23	< 0.27	< 0.32	< 0.37
OCDF	2.06	< 1.05	< 1.40	2.70	< 0.71
CA-TEF	1.7	1.9	6.7	2.4	5.6
EPA-TEF	0.3	0.4	1.5	0.4	1.6
I-TEF	0.7	0.8	3.7	0.9	3.1

* = Interference
NA = Not analysed

Laboratory phase. The PCDD/F levels in eggs following 30 days of exposure in the laboratory phase are clearly higher than in the pre-exposure eggs. Also, the levels in the eggs from the low exposure group are higher than the levels in eggs following 30 and 50 days of exposure in the field. Since the low exposure dose group is fed the same soil that the field chickens are exposed to, the differences in accumulation are probably due to differences in the intake. In the laboratory, the chickens are given 10% soil in their feed, whereas it appears that, at least at this time, the field chickens ingest much less.

The levels of PCDD/Fs in the eggs are proportional to the levels in the feed for both the low and the high exposure groups, as shown on Tables 1 and 3. Additionally, the congener profile and the isomer pattern of the eggs and the respective feed are very similar. As expected, lower chlorinated isomers appear to accumulate at a faster rate than higher chlorinated isomers.

The data from the Day-30 laboratory eggs suggest no preferential bioavailability of the spiked isomers as opposed to the unspiked 2,3,7,8-isomers in the same congener group. This is indicated by the relatively constant ratios of the concentration of each isomer in the high and low exposure eggs versus the concentration in the high and low feed. For example, when the 1,2,3,4,7,8 HxCDD (spiked isomer) is compared to the 1,2,3,6,7,8 HxCDD (control isomer), the ratio of H/L in the feed is 13.2 : 0.8 = 16.1 *(Table 1) and in the eggs it is 30.5 : 1.6 = 18.8 (Table 3). Similarly, for the 1,2,3,4,7,8,9 HpCDF (spiked) and the* 1,2,3,4,6,7,8 HpCDF (control isomer), the ratio of H/L in the feed is 45.9 : 2.2 = 21, very close to the ratio in the eggs 65.7 : 2.8 = 23.5.

Table 3. PCDD/F isomer concentrations (pg/g fat) in eggs from the laboratory phase of the study.

ISOMER	BASELINE	DAY 30			
		CONTROL	LOW	HIGH	HIGH/LOW
2,3,7,8 TCDD	< 0.60	NA	NA	NA	NA *
1,2,3,7,8 PeCDD	< 0.39	0.40	4.50	146.39	32.5 *
1,2,3,4,7,8 HxCDD	< 0.25	< 0.14	8.50	259.49	30.5 *
1,2,3,6,7,8 HxCDD	1.02	< 0.17	31.35	50.86	1.6
1,2,3,7,8,9 HxCDD	< 0.77	2.25	15.56	21.56	1.4
1,2,3,4,6,7,8 HpCDD	6.35	9.49	223.17	411.18	1.8
OCDD	42.77	29.60	554.22	17352.11	31.3 *
2,3,7,8 TCDF	0.50 *	1.80 *	1.78 *	44.89	25.2 *
1,2,3,7,8 PeCDF	< 0.05	0.18	1.39	2.44	1.8
2,3,4,7,8 PeCDF	0.29	1.08	2.64	99.83	37.8 *
1,2,3,4,7,8 HxCDF	0.41	0.39	28.12	74.28	2.6
1,2,3,6,7,8 HxCDF	0.28	0.26	6.24	255.35	40.9 *
2,3,4,6,7,8 HxCDF	0.18	0.22	6.32	12.54	2.0
1,2,3,7,8,9 HxCDF	< 0.21	< 0.10	< 0.16	< 0.11	-
1,2,3,4,6,7,8 HpCDF	1.27	1.24	43.16	121.03	2.8
1,2,3,4,7,8,9 HpCDF	< 0.24	< 0.31	6.30	413.92	65.7 *
OCDF	3.88	1.51	24.76	1355.29	54.7 *
CA-TEF	1.6	3.9	21.4	342.2	
EPA-TEF	0.4	0.6	5.8	105.6	
I-TEF	0.8	1.4	16.7	223.3	

* = Interference
NA = Not analysed
\+ = Isomer spiked in the high exposure dose feed

In conclusion, these preliminary data indicate that chickens can take up PCDD/Fs from soil at very low ppt levels and transfer them into their eggs, at levels directly proportional to the levels in the feed. As both phases of the study proceed, the distribution of individual isomers into liver, adipose tissue and edible flesh will be determined. These data, in conjunction with the analysis of feces, will allow for an approximation of a mass balance for PCDD/Fs. Even at this early stage of the study it appears that consumption of soil with PCDD/Fs concentrations as low as 50 ppt CA-TEF can lead to measurable bioaccumulation. It is hoped that the final results will provide information relevant to regulations and health guidelines on raising range food animals on soil with low levels of PCDD/F contamination

REFERENCES

1. Chang, R., Hayward, D., Goldman, L., Harnly, M., Flattery, J., Stephens, R.D.: Foraging farm animals as biomonitors for dioxin contamination. Chemosphere, 19 481, 1989.

2. Stephens, R.D., Harnly M., Hayward, D.G., Chang, R., Flattery, J., Petreas, M.X., Goldman, L.; Bioaccumulation of dioxins in food animals II: Controlled exposure studies. Paper presented at Dioxin 89, Toronto, Canada. 1989.

3. California Department of Health Services: Health effects of 2,3,7,8-Tetrachlorodibenzo-p-dioxin and related compounds. California Department of Health Services: Report to the California Air Resources Board, Berkeley, CA, 1985.

4. Umbreit, T.H. Personal communication, 1989.

5 Chang, R., Jarman, W.M., King, C.C., Esperanza, C.C., Stephens, R.D.; Bioaccumulation of PCDDs and PCDFs in food animals III: *A rapid cleanup of biological materials using reverse phase adsorbent columns. Paper presented at Dioxin 89,* Toronto, Canada. 1989

6. Smith, L.M., Stalling, D.L. and Johnson, J.L; Determination of part-per-trillion levels of polychlorinated dibenzofurans and dioxins in environmental samples. Analytical Chemistry, 56, 1830, 1984.