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Tissue Residues in Male Chickens Fed a 50 ng/kg Dietary Concentration of 2,3,7,8-Tetrachlorodibenzo-p-dioxin

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The uptake of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) by food animals is of concern from a human health standpoint. Equally important is the effect diet may play in the depuration of TCDD residues. Olson et al (1) reported that 73.5% of a single oral dose of TCDD to the Syrian hamster remains unsecreted after 24 hours, and only 1% of the dose is found in the bile. Hamsters eliminated a single oral dose of ^{14}C-TCDD with 34.6 % in the urine and 50 % in the feces by day 35(Olson et al. 1980). Half-life values for ^{3}H-TCDD range from 10.8 (i.p. single dose) to 14.8 (p.o. single dose) days based on urine from hamsters, but half-lives of 30 to 31 days were reported for the rat and guinea pig (Olson et al. 1980; Rose et al. 1976). The implication is that withdrawal of TCDD is faster in the hamster which is the least sensitive organism based on LD_{50} to TCDD (Kociba and Schwetz 1982). The mink (Hochstein et al. 1988) appears to be the next most sensitive species after the guinea pig followed by the chicken and the rat (Schwetz et al. 1973). TCDD provided as a single oral dose on each of 21 days at 100 ng per day to young chickens produced no observable effects, but 10 times the dosage produced 80 % mortality (Schwetz et al. 1973). The toxic factor of chick edema disease that resulted in the destruction of millions of chickens in 1957-1958 was due to 1,2,3,7,8,9-hexachloro-dibenzo-p-dioxin (Cantrell et al. 1969; Firestone 1973). The most toxic dioxins were noted to be a mixture of tri- and tetrachlordibenzo-p-dioxins, while a mixture of the hexa-, hepta-, and octachlorodibenzo-p-dioxin had about 20 % of the toxic activity in a feeding experiment with chickens (Flick et al. 1972). About 80 % mortality occurred when chickens were fed a mixture of tri- and tetra-dioxins at 10 µg/kg with a total intake of 1900 ng. A reduced feed intake was observed with all dioxins when fed at 10 µg/kg (Flick et al. 1972).

Our interest in feeding TCDD was the determination of tissue residue concentrations resulting from feeding a very low concentration (50 µg/kg), one most likely to be found in the environment, and to determine if the techniques used to hasten the withdrawal of polychlorinated or brominated biphenyls (Polin et al. 1985; Polin et al. 1987) would be effective in removing TCDD.

MATERIALS AND METHODS

White Leghorn male chickens (egg type) were procured from a commercial hatchery and reared in battery brooders until 3 weeks of age using standard husbandry procedures. They were then transferred to an isolation room in Michigan State University's Animal Clinical Laboratory for the feeding studies where they were housed in a wire-floored, modified quail battery. The environmental temperature was 22 C, and the lights were on an 18:6 (1ight:dark) schedule. The chickens were weighed at the start of the experiment, at day 14, and 21 days after withdrawal of the TCDD diets. Four chickens were fed a clean commercial starter-grower diet throughout the experiment, and 14 were fed the same diet with 50 ng/kg TCDD (98 % purity). The TCDD was introduced into the diet by the same technique used to add cycanocobalamin (vitamin B_{12}) in mg quantities into experimental diets. TCDD. 0.6 mg, was dissolved in 50 ml of acetone and then pipetted drop by drop on to a flat mound of about 300 g of diet that had been sifted through a # 20 sieve. After preliminary blending with a spatula and allowing the acetone to evaporate, this mixture was thoroughly mixed by passing it through a # 20 sieve three times. The blended mixture was diluted into 3 kg of diet to which 3 % corn oil had been added to reduce dust. This mixture was then blended by tumbling end over end in a sealed container for 5 minutes. Finally, the three kg portion was divided into three 1 kg portions and each added to 3 kg of clean diet for a total of 12 kg. These were tumbled, individually, end over end in sealed containers for 5 minutes. The three 4 kg portions were then combined into one large container to give 12 kg of diet contaminated at the 50 ng/kg level.

Four control chickens were fed non-contaminated diet and 14 were fed the TCDD diet for 14 days. Two chickens fed non-contaminated diet were euthanized on day 14 and the remaining two on day 35. Of the 14 chickens fed TCDD contaminated diet, four were euthanized on day 14 and ten on day 35. Of these 10 chickens, 4 were fed a clean diet and six the same diet with 10 % mineral oil during the withdrawal period (day 14 through 35). Five of these six were also restricted in feed intake to 45% of the controls. The chickens were euthanized with excess CO_2. The following tissues were removed at the time the birds were euthanized: blood (plasma), brain, abdominal adipose, heart, kidney, breast muscle, cervical skin. and liver.

TCDD was determined by the method of Lamparski et al (12). The following criteria were used to identify and quantitate TCDD: 1) correct high resolution retention window, 2) correct chlorine isotope ratio at m/z 320/322 and 332/334, 3) correct ratio of the loss of COCl at m/z 257/320, 4) response exceeds limit of detection (3 x peak to valley noise), and 5) recovery of ^{13}C-TCDD internal stanard was between 70 to 120 %. Sample weights of tissues analyzed and detection limits are given in Table 1. Average sample weight was 9.0 g and average limit of quantitation (LOQ) was 2 ± 1 ng/kg for the eight types of tissue analyzed.

Table 1. Summary of weights and detection limits for tissues analyzed from chickens fed TCDD.

Tissue	Sample Weights (g)	LOQ (ng/kg)
Brain	5.14±2.02	3
Heart	8.69±3.27	2
Liver	10.36±0.33	1
Fat	10.07±0.44	3
Plasma	7.15±1.52	2
Muscle	10.42±O.19	2
Kidney	11.94±4.81	2
Skin	8.23±2.52	1

RESULTS AND DISCUSSION

The overall average body weight of the chickens (4 weeks of age) at the start of the experiment was 335 g. Weight gain to day 14 of feeding was 180 g and to day 35 was 464 g, with no significant difference ($p^3 0.05$) between the controls and the chickens fed TCDD. During the 1st 14 days of feeding, each chicken ate 589 g (3.1 g feed/ g gain) or 42 g feed/day. This translates into a total intake of TCDD of 29 ng/bird. Chickens that were on a restricted feed intake gained 446 g, an amount not significantly different ($p>0.05$) from those fed ad libitum (464 g).

Upon analysis only liver samples were found to contain TCDD (Table 2). Brain, heart, fat, plasma, muscle, kidney, and skin tissues all gave not detectable results for all samples and for all treatments. The residue levels in liver ranged from 12 to not detectable. At day 14 the livers of the two control chickens had measurable TCDD residues (2-8 ng/kg), while all 4 birds on the TCDD diet had measurable residue levels (4-12 ng/kg). At day 35, 21 days after withdrawal of the TCDD diet, none of the controls had contaminated livers while 2 of 4 chickens fed TCDD then clean diet had TCDD residues (2-12 ng/kg) and all six of the chickens fed TCDD then clean diet with mineral oil were contaminated (2-12 ng/kg) (Table 2).

The consequence of keeping control birds in the same room as those fed TCDD is shown in the contamination of the two controls. In an experiment (Polin and Leavitt 1984) concerning residue concentrations of PCB and PBB in chickens, some controls within the same room were also found to be contaminated despite precautions to prevent such cross contamination. This cross contamination may be due to dust being transported in air between the cages. Feeding TCDD on a

continuous basis to chickens at 50 ng/kg is very close to the borderline concentration at which TCDD can be detected in their tissues, and then only in their livers. If whole body analysis were conducted to detect body burdens, the feed level of 50 ng/kg would be too low to allow TCDD detection. Livers from hamsters (Olson et al. 1980) contained the highest concentrations of TCDD after a single oral injection. Livers of guinea pigs (Gasiewicz and Neal 1979) had concentrations less than those in adipose, skin and adrenals soon after dosing but equal to adipose three weeks later. Thus the tissue to monitor for TCDD detection and quantitation appears to favor liver tissue. Chickens appear to be more like a hamster than a guinea pig in its manner of accumulating TCDD residues. At these low dosing levels and concomitant low residue levels the half-life for the chicken is between that of the guinea pig and hamster. Liver concentrations declined from a mean of 7.0 to 6.0 ng/kg for the birds fed a restricted clean diet with mineral oil and to a mean of 3.5 ng/kg for the birds fed just clean diet. It appears that non-restricted diet without mineral oil was the best treatment to hasten the removal of TCDD residues which is opposite to the results found for the removal of PCB and PBB (Polin and Leavitt 1984; Polin et al. 1985).

Table 2. Residues in liver tissues of male, white leghorn chickens fed 50 ng/kg of TCDD. Treatment during withdrawal was clean diet (CD), clean diet with mineral oil (MO), and clean diet with mineral oil and a 45 % feed restriction (FR).

Day	Treatment	Conc. (ng/kg)	Day	Treatment	Conc. (ng/kg)
14	CD	8	35	TCDD-CD	12
14	CD	2	35	TCDD-CD	2
	Mean ± SD	5 ±	35	TCDD-CD	ND
14	TCDD	12	35	TCDD-CD	ND
14	TCDD	5		Mean ± SD	4 ±
14	TCDD	4	35	TCDD-MO	6
14	TCDD	7	35	TCDD-FR	10
	Mean ± SD	7 ±	35	TCDD-FR	12
35	CD-CD	ND	35	TCDD-FR	3
35	CD-CD	ND	35	TCDD-FR	2
	Mean ± SD	ND	35	TCDD-FR	3
				Mean ± SD	6 ± 4

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