Distribution and Excretion Studies of Octachlorodibenzo-p-Dioxin in the Rat

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Early toxicity studies of chlorodibenzo-p-dioxins on a variety of animals were based primarily on the feeding of a toxic fat extract which contained a complex and incompletely characterized mixture of chlorodibenzo-p-dioxins and possibly other toxic compounds (1,2,3). Later investigations with pure samples of adequately characterized synthetic mixtures of chlorodibenzo-p-dioxins have confirmed the toxicity of these compounds (4,5,6,7). The most toxic is thought to be 2,3,7,8-tetrachlorodibenzo-p-dioxin which has been identified as a contaminant of 2,4,5-trichlorophenol and is considered to be responsible for the neurological and psychopathological disorders which have affected industrial workers involved in the manufacture of 2,4,5-trichlorophenol and 2,4,5-trichlorophenoxyacetic acid (8).

Little has been reported on the metabolism of chlorodibenzo-p-dioxins. Campbell and Friedman (9) have noted changes in the gas-liquid chromatographic pattern of chlorodibenzo-p-dioxins excreted in the faeces compared to those present in a toxic fat fed to rats. No further identification was made but it was suggested that metabolism had occurred in the liver and the metabolites had been excreted into the intestine via the bile.

Octachlorodibenzo-p-dioxin was selected for metabolic studies since it is one of the least toxic chlorodibenzo-p-dioxins and could, therefore, be fed at a sufficiently high level to facilitate detection of metabolic products. A recent report (10) indicated that chickens fed a toxic fat containing hexa-, hepta-, and octachlorodibenzo-p-dioxin did not absorb octachlorodibenzo-p-dioxin since, unlike the hexa- and heptachlorodibenzo-p-dioxins, it could be detected only in the faeces and not in any of the tissues or organs. We have, therefore, carried out a short term feeding study to determine the absorption, excretion and organ distribution of octachlorodibenzo-p-dioxin in the rat.

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Experimental

Experiment I

The absorption, excretion, distribution in various tissues and the effect on the organ weights of octachlorodibenzo-p-dioxin was studied in twenty-four male Wistar rats randomized into three groups. The three groups were fed with ground commercial rat feed (1.9 kg) mixed with corn oil (100 g) containing 0, 0.2 or 1.0 mg octachlorodibenzo-p-dioxin. The rats were housed in air conditioned rooms and were supplied with water and feed ad libitum over a two week period. Feed consumption and initial and final body weights were measured and faecal and urinary excretions were collected and stored at 0°C until analysed. The rats were killed by carbon dioxide asphyxiation and liver, kidney, spleen, testes, heart, lungs, gastro-intestinal tract and samples of skeletal muscle and adipose tissue were removed, weighed and frozen until analysed.

Experiment II

Six male Wistar rats (average weight 400 g) each were anaesthetized with sodium pentobarbital and their bile ducts were cannulated with polyethylene cannulae. Upon recovery from the anaesthesia the rats were placed in restraining cages and given by oral intubation corn oil (1 ml) containing octachlorodibenzo-p-dioxin (58 µg). Feed and water were provided ad libitum and during the next 48 to 72 hr the faeces, urine and bile were collected separately and stored at 0°C until analysed. Two control rats were cannulated in the same manner and dosed with corn oil (1 ml).

Octachlorodibenzo-p-dioxin analyses

Organs and tissues were analysed for octachlorodibenzo-p-dioxin by the AOAC method for the chick edema factor (11). The organ or tissue was homogenised with petroleum ether (4 x 20 ml) and the petroleum ether fractions were combined and shaken with concentrated sulfuric acid (15 ml). The petroleum ether solution was then concentrated to a small volume and chromatographed on an alumina column. The column was eluted with petroleum ether (100 ml), 5% ethyl ether in petroleum ether (50 ml) and 25% ethyl ether in petroleum ether (100 ml). The latter fraction was collected, concentrated to dryness under a stream of nitrogen and the residue dissolved in a known volume (100 µl or 200 µl) of distilled benzene.

This solution was analysed for octachlorodibenzo-p-dioxin by electron capture gas chromatography using a Varian 2100 gas chromatograph fitted with a tritium electron capture detector and a 3' x 1/4" o.d. glass column packed with 2.5% SE-52 on Gas-Chrom Q, 80-100 mesh. The operating conditions were: temperatures; column 210°C, detector 220°C, injection port 220°C, the carrier gas was helium at a flow rate of 60 ml/min.

The octachlorodibenzo-p-dioxin content was estimated by comparison with standard solutions and peak areas were measured by the triangulation method. The lower limit of detection for a 1 g sample of tissue was approximately 10 ppb. Recoveries from spiked samples of liver and faeces were 80 ± 5%.

Urine samples were extracted with petroleum ether (4 x 20 ml) and analysed as above. Faeces samples were homogenised with petroleum ether (600 ml) and a portion of the petroleum ether extract was analysed.

Results and Discussion

Experiment I

No significant differences in feed consumption or weight gain were detected between the control rats and those fed octachlorodibenzo-p-dioxin (Table I).

TABLE I

Relation of intake of octachlorodibenzo-p-dioxin to change in body weight

Initial wt. (g)	Final wt. (g)	Feed Intake (g)	Dioxin Ingested (µg)
106.9[a]±1.9	181.6±5.0	226.2±8.3	0
106.1 ±3.1	185.8±5.8	227 ±9.6	22.7±1.0
110.4 ±3.1	190.7±4.6	241.4±5.5	120.7±2.8

[a]Mean of eight values

[b]Standard error of the mean

Rats fed with toxic fat (1) or extracts of toxic fat (6) have shown growth depression, enlarged and fatty livers, involution of the thymus and enlarged adrenals. None of these effects nor any significant ($P<0.05$) differences in organ weights were noted with our dietary levels of octachlorodibenzo-p-dioxin (Table II). The only gross pathological change noted was congestion of the liver in the rats with the high level of ingested octachlorodibenzo-p-dioxin.

TABLE II

Organ weights expressed as a % of body weight

Dioxin Ingested (µg)	Liver	Kidney	Spleen	Testes	Heart
0	5.91[a]±0.31[b]	1.15±0.03	0.39±0.02	1.16±0.05	0.46±0.02
22.7	5.73 ±0.39	1.12±0.05	0.42±0.03	1.14±0.03	0.43±0.02
120.7	5.88 ±0.23	1.13±0.03	0.36±0.01	1.12±0.02	0.43±0.02

[a]Mean of eight values

[b]Standard error of mean

Octachlorodibenzo-p-dioxin was detected in the faeces, liver and adipose tissue (Table III). The gastrointestinal tracts, including contents, were found to contain from 1-3% of total intake of octachlorodibenzo-p-dioxin. No octachlorodibenzo-p-dioxin was detected in the heart, kidney, spleen, lungs, skeletal muscle, testes and urine of the dosed rats nor in any tissues or organs of the control rats.

TABLE III

Residues of octachlorodibenzo-p-dioxin

Dioxin Ingested (µg)	Dioxin (µg) in Faeces	Dioxin (µg) in Liver	Dioxin (µg) in Adipose tissue
0	0	0	0
22.7	13.9[a]±0.7[b]	0.46±0.09	-
120.7	45.2 ±1.7	1.35±0.19	0.04±0.02

[a]Mean of eight values

[b]Standard error of mean

Studies have shown that chlorodibenzo-p-dioxins present in toxic fats fed to rats (9) and chickens (9,10) were detected mainly in the liver. Firestone (10) reported that approximately 84% of the absorbed hexa- and heptachlorodibenzo-p-dioxins were found in the chicken liver with trace amounts in other tissues. No octachlorodibenzo-p-dioxin could be detected in the liver or tissues. Since the octachlorodibenzo-p-dioxin is only a minor component of the toxic fat the concentration retained in the chicken liver may well have been below the level of detection. There may also be a difference in the absorption or rate of metabolism of this compound between rats and chickens.

Experiment II

The presence of large quantities of octachlorodibenzo-p-dioxin in the faeces compared to that in the bile (Table IV) indicates that the dioxin present in the faeces is mainly unabsorbed dioxin. No octachlorodibenzo-p-dioxin could be detected in the faeces or bile of control rats and none could be detected in the urine of any of the rats.

TABLE IV

Residues in faeces and bile of rats orally dosed with octachlorodibenzo-p-dioxin (58 µg)

Rat		1	2	3	4	5	6
Dioxin (µg) in faeces	24 hr	10	10.3	-	9.9	6.9	7.5
	48 hr	-	6.0	-	10.1	7.0	8.3
	72 hr	-	-	-	3.3	4.9	-
Dioxin (µg) in bile	24 hr	trace	0.24	trace	0.02	trace	trace
	48 hr	-	0.50	trace	0.02	trace	trace
	72 hr	-	-		0.02	trace	trace

It may be concluded that octachlorodibenzo-p-dioxin is absorbed by the rat and that the absorbed dioxin is located mainly in the liver with small amounts also present in the adipose tissue and bile. The fact that detectable amounts of dioxin can accumulate in the liver without any appreciable toxicity, indicates that if it is metabolized, detectable amounts of metabolic products may also be present. Further studies are underway to reveal the presence and nature of these products.

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