Toxic Effects of 2,3,7,8-Tetrachlorodibenzo-p-dioxin

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Abstract—The toxic compound 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin) was shown to cause pericardial oedema and death in chickens after a single oral dose of 25-50 μg/kg. The extreme toxicity of dioxin to the guinea-pig was confirmed. Female rats given a single oral dose of 200 μg dioxin/kg had a depressed food intake and lost weight. The mean time to their death was 40-4 days. The animals showed no consistent post-mortem appearance, although gastric haemorrhage and jaundice were common. In the first 3 days after dosing there were significant changes in liver constitution, although there were no alterations in plasma components indicative of liver malfunction. The changes in blood cell counts, haematocrit, haemoglobin and plasma-protein levels 1 wk after dosing were most probably associated with the reduction in food intake. The liver showed pathological changes at later periods, in particular the formation of multinucleate parenchymal cells.

INTRODUCTION

Since the 1930s, chloracne has been recognized as an occupational hazard associated with the production or use of chlorinated aromatic compounds (Key & Birmingham, 1966). In particular, it has been linked with the manufacture of 2,4,5-trichlorophenol, a compound used in the preparation of the herbicide 2,4,5-trichlorophenoxyacetic acid and of the bactericide hexachlorophene (Poland, Smith, Metter & Possick, 1971). In groups of cases studied in Germany in the 1950s (Bauer, Schulz & Spiegelberg, 1961; Hofmann, 1957; Kimmig & Schulz, 1957; Schulz, 1968), the chloracne was associated with other disorders and the causative agent was identified as 2,3,7,8-tetrachlorodibenzo-p-dioxin (referred to henceforth as dioxin). Besides inducing the hyperkeratosis of chloracne, dioxin was found to be highly toxic to various animal species. In rabbits the LD₅₀, administered as a single oral dose, was 10 μg/kg (Schulz, 1968).

The formation of dioxin has been shown to occur when an alkali metal salt of 2,4,5-trichlorophenol is heated at elevated temperatures (Milnes, 1971). It is readily produced in 2,4,5-trichlorophenol manufacture, but if the process is carefully controlled dioxin contamination of the trichlorophenol is less than 1 ppm.

In an incident in the United States in 1957, some millions of chickens died, the most characteristic symptom being pericardial oedema. This condition was traced to the inclusion in the diet of a fat from which the toxic factors could be isolated in yields of approximately 100 μg/kg of fat (Harman, Davis, Ott, Brink & Kuehl, 1960; Wootton, Artman & Alexander, 1962; Yartzoff, Firestone, Banes, Horwitz, Friedman & Nesheim, 1961). One of these compounds was eventually identified by crystallography as 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin (Cantrell, Webb & Mabis, 1969). Dioxin itself has been found to be very toxic in the chick oedema bioassay (Higginbotham, Huang, Firestone, Verrett, Ress & Campbell,
Structurally similar compounds, tetra- and pentachlorodibenzofurans, are contaminants of polychlorinated biphenyls that cause hydropericardium in chicks and hyperkeratosis of the rabbit's ear (Vos & Beems, 1971; Vos & Koeman, 1970; Vos, Koeman, van der Maas, ten Noever de Brauw & de Vos, 1970). Other studies have shown that dioxin is a potent teratogen and foeticidal agent for rats and mice (Courtney & Moore, 1971; Neubert & Dillmann, 1972; Sparschu, Dunn & Rowe, 1971).

In the work now described, some effects of dioxin on various species were studied in an attempt to elucidate its mode of action.

**EXPERIMENTAL**

*Preparation of dioxin.* Dibenzo-p-dioxin was prepared by the method of Gilman & Dietrich (1957) save that the reaction was carried out in refluxing diethylene glycol dimethyl ether for 5-8 hr. Prior to crystallization, the dibenzodioxin was purified by steam-distillation, since this procedure removed a minor contaminant which otherwise conferred a pale-blue fluorescence and a yellow phosphorescence on the product. Dibenzodioxin was dissolved in glacial acetic acid and chlorine gas was bubbled through the solution under reflux for 4-8 hr (cf. Tomita, Ueda & Narisada, 1959). The solvent was evaporated and the residue was washed with ether. The resultant solid was crystallized several times from benzene to give material which was shown by gas-liquid chromatography (GLC) to contain 8-2% trichlorodibenzodioxin. (GLC was carried out on a Pye Series 104 chromatograph fitted with an electron-capture flame ionization detector head, using a 7 ft column of 3% E 30 on 100–120 mesh Diatomite CQ at 200°C and a carrier gas (nitrogen) flow rate of 67 ml/min.)

It was assumed that the flame ionization detector response to the dioxins was proportional to their molecular weight; any decreased sensitivity to dioxin itself due to the extra chlorine atom would imply a lower degree of contamination than that indicated above. Dioxin was administered to animals either as a 100 μg/ml solution in dimethylsulphoxide (DMSO) or arachis oil or, for higher doses, as a suspension in arachis oil.

*Animals and treatment.* Male and female albino rats of the Porton strain, bred in this laboratory, were given Diet 41B and water ad lib. White Leghorn chickens (4-6 wk old) from Southern Biological Ltd., Carshalton, were given Layers Pellets and water ad lib. Adult albino guinea-pigs of the Porton strain had free access to Diet IGP and water supplemented with ascorbic acid. All the test animals were given a single orally intubated dose of dioxin in solution or suspension while controls received an equivalent volume of the appropriate solvent. For all three species, body weights were recorded at intervals throughout the observation period and autopsies were performed on all animals. In addition some of the treated rats given single doses up to 500 μg dioxin/kg were used for more extensive studies, covering food-consumption, haematology and liver weight and histology, in addition to growth and mortality. Details of the doses given and the periods of observation are reported in the appropriate parts of the ‘Results’ section. Six weanling SPF and two adult germ-free rats were also treated with dioxin in a small comparative mortality study.

*Blood assays.* For the assay of plasma components, blood was taken on days 1, 3, 9 and 21 after treatment in heparinized syringes from the heart of female rats, following the opening of the thorax under ether anaesthesia. The blood was centrifuged at 5°C as soon as possible after collection and the plasma was separated and stored frozen. Blood samples for cell counts and haemoglobin assays were taken weekly from the tail. Portions of these samples were diluted in Isoton containing 30% bovine serum albumin solution (100:1, Trade name of Coulter Electronics Ltd., Dunstable, Beds.)
Albunin stabilized the erythrocytes against haemolysis. Blood cell counts were performed on a Coulter counter. Total plasma bilirubin was assayed by the method of Ferro & Ham (1967) save that all operations were performed in a dimly-lit room, since it was found that bilirubin solutions, the colour reagent and mixtures of the two were all photo-labile. Plasma protein was estimated with the biuret reagent. Kits for the assay of plasma alkaline phosphatase, glutamic-oxalacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) were obtained from Sigma Chemical Co., Ltd., London, and a kit for the assay of plasma cholesterol by the Liebermann-Burchard reaction was obtained from Roche Products Ltd., London.

Liver studies. The weight and water content of liver were determined 24 and 72 hr after dioxin treatment on samples removed as rapidly as possible following decapitation and dried to constant weight at 110 ± 5°C. The livers were from male and female animals which had been used for the determination of hexobarbitone sleeping times (Greig, 1972).

Histology. Female rats were killed 2-60 days after oral doses of dioxin (50-400 µg/kg body weight). Samples were taken from all the major organs except the brain and fixed in formal alcohol. Paraffin sections were prepared in the usual way and stained with Harris' haematoxylin and eosin.

Statistical methods. The significance of the results was assessed by Student's t test, unless otherwise indicated.

RESULTS

Toxicity of dioxin to chickens

The toxicity of dioxin towards chickens was confirmed, a dose of 25-50 µg/kg given orally killing them 12-21 days later. The only immediate effect of the dioxin on the birds was that they gained weight less rapidly than controls. Prior to death some birds lost weight and were in poor condition, with laboured breathing, the beak agape and feathers ruffled. The most common post-mortem finding was some accumulation of serous fluid in the pericardial sac, as has been described for the chick oedema syndrome (Allen & Carstens, 1966; Schmittle, Edwards & Morris, 1958). It thus appears that a single dose of dioxin is as effective as prolonged feeding in causing pericardial oedema (Allen & Carstens, 1966).

Toxicity of dioxin to guinea-pigs

The effect of dioxin (2, 4 or 10 µg/kg) on groups of three male and three female adult guinea-pigs was a 15-30% loss of body weight within 8-24 days. Two animals (a female given 10 µg/kg and a male given 4 µg/kg) died within this period and the others were killed when in poor condition. Autopsy revealed no obvious abnormalities of the major viscera, except that there was little or no food in the gastro-intestinal tract and the stomachs were distended with gas. This confirmed the extreme susceptibility of the guinea-pig to dioxin (Sparschu et al. 1971).

Effects of dioxin in rats

Growth, mortality and post-mortem studies. The oesophageal intubation of female rats (8-11 wk old, body weight 180-200 g) with solutions of 200 µg dioxin/kg in DMSO led to an immediate and prolonged reduction in food intake, with a loss of body weight (Fig. 1). There was a wide variation in the response of the animals to dioxin, as is shown by the greater standard errors of body-weight measurements on dosed animals compared to their...
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controls. A few animals maintained a nearly constant weight for 3–7 wk after dosing and then underwent a rapid loss of weight. The effect on weanling female rats (4–5 wk old, 60–70 g) of single, oral doses of 25–200 μg/kg was a lower or zero growth rate compared with controls.

Attempts to estimate an LD₅₀ value in female rats given a single oral dose of dioxin failed because of the irregular distribution of deaths in the treated groups. The mortality figures from two experiments are set out in Table 1. The mean time to death in these experiments was 40·4 ± 4·5 (SEM) days.

Table 1. 90-Day mortality of female rats given a single oral dose of 0–300 μg dioxin/kg

<table>
<thead>
<tr>
<th>Dosage (μg/kg)</th>
<th>Deaths/no. in group</th>
<th>Dosage (μg/kg)</th>
<th>Deaths/no. in group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0/12</td>
<td>0</td>
<td>0/6</td>
</tr>
<tr>
<td>30</td>
<td>1/6</td>
<td>126</td>
<td>3/6</td>
</tr>
<tr>
<td>48</td>
<td>1/6</td>
<td>199</td>
<td>1/5</td>
</tr>
<tr>
<td>75</td>
<td>1/6</td>
<td>315</td>
<td>1/6</td>
</tr>
<tr>
<td>120</td>
<td>1/6</td>
<td>500</td>
<td>4/6</td>
</tr>
<tr>
<td>190</td>
<td>3/6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>3/5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Rats (170–200 g, 8–9 wk old) received dioxin in dimethylsulphoxide, of which controls received 3 ml/kg.
†Rats (170–200 g, 9–10 wk old) received dioxin in arachis oil, of which controls received 5 ml/kg.
‡Excluding one animal killed at 42 days with severe swelling of the limbs and urine staining in the genital area.
In rats dying after dioxin treatment, no consistent changes were observed at autopsy. A common finding was gross haemorrhage, usually originating from the mucosa of the glandular stomach but on occasions involving the ileum or the retroperitoneal tissue. Some rats were jaundiced and this was sometimes associated with distension of the bile duct as far as the duodenum. No physical obstruction other than abnormal amounts of mucus in the duodenum could be found in these rats. Changes in the lungs were common and ranged from petechial haemorrhage or perivascular oedema to massive infection. Six weanling SPF Grade IV and two adult germ-free rats dosed with dioxin and maintained in a sterile isolator showed no signs of infection at autopsy, but deaths still occurred (two SPF rats by 24 days and one of the germ-free adults by 21 days). One of these rats had bile-duct enlargement and another had duodenal haemorrhage associated with jaundice.

A tenfold increase in the oral dose of dioxin (1·25-5·0 mg/kg suspended in arachis oil) administered to six female rats (170-190 g) did not accelerate the appearance of toxic effects. The weight loss of these animals after 7 and 14 days (17·1 ± 2·8 and 19·7 ± 3·4 %, respectively) did not differ significantly from that in rats given 200 μg/kg (18·8 ± 2·8 and 18·9 ± 3·1 %). Two of the animals on the high doses died at 16 and 21 days, but the post-mortem appearance of the four survivors, which were killed at 23 days, was similar to that of the animals given a lower dose and killed after 3-4 wk, save that excessive peritoneal fluid was present in three of the animals.

**Haematology.** A single oral dose of dioxin (200 μg/kg) administered to female rats (180-200 g) raised their red cell count by the end of 1 wk (Table 2). This increase persisted for 2 and 3 wk after dosing and was associated with a rise in the haematocrit (Table 3) and haemoglobin content of the blood. Leucocyte counts in both dosed and control groups fluctuated considerably but were significantly elevated in the dosed group at 2 and 3 wk.

**Liver function and weight.** The jaundice seen in some rats at autopsy suggested that the liver might be a target organ. Therefore the levels of various plasma components indicative of liver function were measured (Table 3). None of these were altered to any large extent either 1 or 3 days after the administration of an oral dose of dioxin; later the level of plasma bilirubin was raised and that of plasma protein was lowered.

Although measures of liver function were not affected by dioxin at 1 and 3 days, there were significant alterations in liver components (Table 4). The wet and dry weights of the liver, expressed as a percentage of body weight, were increased in male and female rats on days 1 and 3 after treatment, respectively. In both sexes the water content was significantly increased on days 1 and 3. This effect was not due to the altered food intake of dioxin-treated rats, since when food was withheld immediately after treatment from both dosed and control male rats, similar increases were observed in the dosed group 24 hr later.

**Histology.** Scattered areas of necrosis were present in the centrilobular zone of the rat liver 3 wk after dioxin treatment. This loss of parenchymal cells resulted in apparent dilatation of surrounding sinusoids. The scattered necrosis adjacent to the central veins continued throughout the period studied and was associated with a prominent infiltration of mononuclear cells. By day 60 the walls of the central veins were grossly thickened by connective tissue and mononuclear cells and the sinusoidal dilatation persisted (Fig. 2). The arrangement of liver trabeculae was distorted. The parenchymal cells varied in size and shape, with many multinucleate forms containing 4-30 nuclei (Fig. 3). These were found in the centrilobular area. Large hepatocytes with between four and six nuclei appeared as early as day 21. Within the multinucleate cells an occasional pyknotic nucleus was observed. No mitotic figures were seen in the multinucleate cells. The continuing necrotic lesion was associated
Table 2. Effect of a single oral dose of 200 µg dioxin/kg on the haematology of female rats

<table>
<thead>
<tr>
<th>Time after treatment (days)</th>
<th>Treatment†</th>
<th>Body weight (g)</th>
<th>Erythrocytes ((10^6/mm^3))</th>
<th>Leucocytes ((10^3/mm^3))</th>
<th>Haemoglobin (g/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Control</td>
<td>209·6 ± 2·1</td>
<td>7·75 ± 0·15</td>
<td>8·58 ± 0·89</td>
<td>16·0 ± 0·3</td>
</tr>
<tr>
<td></td>
<td>Dioxin</td>
<td>202·8 ± 3·6</td>
<td>8·00 ± 0·22</td>
<td>10·22 ± 1·60</td>
<td>16·5 ± 0·5</td>
</tr>
<tr>
<td>7</td>
<td>Control</td>
<td>217·2 ± 3·9</td>
<td>7·36 ± 0·17</td>
<td>4·20 ± 0·60</td>
<td>15·1 ± 0·4</td>
</tr>
<tr>
<td></td>
<td>Dioxin</td>
<td>176·7 ± 6·2***</td>
<td>9·40 ± 0·31***</td>
<td>6·72 ± 1·25</td>
<td>19·0 ± 0·6***</td>
</tr>
<tr>
<td>14</td>
<td>Control</td>
<td>224·4 ± 4·7</td>
<td>7·56 ± 0·15</td>
<td>3·16 ± 0·38</td>
<td>16·1 ± 0·2</td>
</tr>
<tr>
<td></td>
<td>Dioxin</td>
<td>160·3 ± 6·9***</td>
<td>10·43 ± 0·43***</td>
<td>7·15 ± 1·72*</td>
<td>20·2 ± 0·5***</td>
</tr>
<tr>
<td>21</td>
<td>Control</td>
<td>231·8 ± 5·7</td>
<td>8·24 ± 0·30</td>
<td>5·90 ± 0·73</td>
<td>16·5 ± 0·5</td>
</tr>
<tr>
<td></td>
<td>Dioxin</td>
<td>156·8 ± 9·7***</td>
<td>10·65 ± 0·85*</td>
<td>14·27 ± 1·70**</td>
<td>19·7 ± 1·7</td>
</tr>
</tbody>
</table>

†Dioxin (200 µg/kg) was dissolved in dimethylsulphoxide, while controls received an equivalent volume of the vehicle alone. Values are means ± SEM for five control or six treated rats and those marked with asterisks differ significantly from the controls: *P < 0·05; **P < 0·01; ***P < 0·001.
Table 3. Effect of a single oral dose of 200 \( \mu \)g dioxin/kg on blood constituents of female rats

<table>
<thead>
<tr>
<th>Time after treatment (days)</th>
<th>Treatment</th>
<th>Haematocrit (%)</th>
<th>Protein (mg/ml)</th>
<th>Cholesterol (mg/100 ml)</th>
<th>Bilirubin (mg/100 ml)</th>
<th>Alkaline phosphatase (Karmen units/ml)</th>
<th>GOT (Karmen units/ml)</th>
<th>GPT (Karmen units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>45·6 ± 1·1</td>
<td>76·9 ± 0·9</td>
<td>73·3 ± 6·8</td>
<td>0·50 ± 0·1</td>
<td>3·02 ± 0·63</td>
<td>181·0 ± 63·0</td>
<td>30·2 ± 2·3</td>
</tr>
<tr>
<td></td>
<td>Dioxin</td>
<td>47·0 ± 0·8</td>
<td>76·5 ± 0·2</td>
<td>74·2 ± 4·4</td>
<td>0·55 ± 0·1</td>
<td>1·73 ± 0·07</td>
<td>75·3 ± 5·3</td>
<td>26·1 ± 1·3§</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>45·9 ± 0·7</td>
<td>68·7 ± 1·2</td>
<td>63·3 ± 5·3</td>
<td>0·25 ± 0·04</td>
<td>2·3 ± 0·2</td>
<td>81·2 ± 3·3</td>
<td>26·5 ± 1·6</td>
</tr>
<tr>
<td></td>
<td>Dioxin</td>
<td>48·0 ± 0·8</td>
<td>69·6 ± 2·5</td>
<td>73·9 ± 5·0</td>
<td>0·49 ± 0·11</td>
<td>2·1 ± 0·2</td>
<td>98·9 ± 4·5*</td>
<td>24·2 ± 3·4</td>
</tr>
<tr>
<td>9</td>
<td>Control</td>
<td>43·3 ± 0·7</td>
<td>68·7 ± 1·2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dioxin</td>
<td>48·4 ± 1·1**</td>
<td>62·3 ± 2·5*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Control</td>
<td>42·1 ± 0·7</td>
<td></td>
<td></td>
<td>0·33 ± 0·13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dioxin</td>
<td>47·7 ± 1·0*</td>
<td></td>
<td></td>
<td>10·97 ± 4·63§</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GOT = Glutamic-oxalacetic transaminase  GPT = Glutamic-pyruvic transaminase

†Dioxin (200 \( \mu \)g/kg) was dissolved in dimethylsulphoxide, while controls received an equivalent volume of the vehicle alone.

‡Haemolysis affected results.

§One sample lost.

||Value differs significantly (ranking test) from control: \( P = 0.008 \).

Values are means ± SEM for groups of six rats (or five in the case of controls at 1 and 21 days). Those marked with asterisks differ significantly from the controls: \( *P < 0.05; **P < 0.01 \).
Table 4. Weight and water content of the liver in rats given an oral dose of 200 μg dioxin/kg

<table>
<thead>
<tr>
<th>Time after treatment (hr)</th>
<th>Sex</th>
<th>Treatment</th>
<th>Liver weight (g/100 g body weight)</th>
<th>Water content of liver (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Wet weight</td>
<td>Dry weight</td>
</tr>
<tr>
<td>24</td>
<td>M</td>
<td>Control</td>
<td>4.74 ± 0.10</td>
<td>1.42 ± 0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dioxin</td>
<td>5.28 ± 0.10*</td>
<td>1.54 ± 0.03*</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>Control</td>
<td>4.30 ± 0.06</td>
<td>1.24 ± 0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dioxin</td>
<td>4.64 ± 0.17*</td>
<td>1.33 ± 0.04</td>
</tr>
<tr>
<td>72</td>
<td>M</td>
<td>Control</td>
<td>4.71 ± 0.17</td>
<td>1.39 ± 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dioxin</td>
<td>4.95 ± 0.22</td>
<td>1.40 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>Control</td>
<td>3.81 ± 0.06</td>
<td>1.13 ± 0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dioxin</td>
<td>4.61 ± 0.09***</td>
<td>1.32 ± 0.02***</td>
</tr>
<tr>
<td>24‡</td>
<td>M</td>
<td>Control</td>
<td>3.61 ± 0.09</td>
<td>1.05 ± 0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dioxin</td>
<td>4.02 ± 0.08*</td>
<td>1.15 ± 0.02**</td>
</tr>
</tbody>
</table>

†Dioxin (200 μg/kg) was dissolved in dimethylsulphoxide, while controls received an equivalent volume of the vehicle alone.
‡Food was withheld from both groups immediately after dosing. The vehicle was arachis oil.
Values are means ± SEM for groups of six rats (or 12 in the case of treated and control females at 72 hr).
Those marked with asterisks differ significantly from controls: *P < 0.05; **P < 0.01; ***P < 0.001.

with an increased incidence of parenchymal cell mitoses throughout the liver lobule. Identical histological changes were produced at each dose level studied and there was no evidence that higher doses increased the parenchymal cell loss.

No lesions attributable to dioxin were observed in the rat lungs, but many animals that died following dioxin dosage had severe chronic and acute inflammatory lung lesions. Several animals had pronounced congestion of the mucosa and submucosa of the stomach and duodenum. No definite ulcers were seen in the sections examined. No significant abnormality was seen in the spleen and lymph nodes.

**DISCUSSION**

The findings of this study were generally in accord with others already reported. Thus a depression in growth rate or a loss of weight has been reported to follow a dose of 1 μg-10 mg dioxin/kg given ip or orally (Buu-Hoï, Pham-Huu Chanh, Sesqué, Azum-Gelade & Saint-Ruf, 1972a; Cunningham & Williams, 1972). An increase in plasma bilirubin and reduction in plasma protein was also described by Buu-Hoï, Pham-Huu Chanh, Sesqué, Azum-Gelade & Saint-Ruf (1972b) following massive (10 mg/kg) doses of dioxin given ip. It is possible that the prolonged reduction in food intake may have contributed to the depression in plasma-protein levels, and also the rises seen in the red and white blood cell counts, haematocrit and haemoglobin levels. Similar changes associated with inanition have been reported by Weimer (1961). Norback & Allen (1972) observed moderate hypertrophy of the liver in male rats receiving a diet containing 0.002% "chlorinated diphenyl-p-dioxin". In the present study, the increase in dry liver weight did not correlate with any changes in the protein or DNA content of the organ but may have been due to an increase in liver lipids (Cunningham & Williams, 1972).

The delayed deaths of rats and guinea-pigs after a single small dose of dioxin cannot be explained by any consistent pathological change in the dead or dying animals. Although
Fig. 2. Liver taken 60 days after treatment from a rat given an oral dose of 100 μg dioxin/kg, showing thickening of the wall of the central vein and two multinucleate cells. Haematoxylin and eosin ×200.

Fig. 3. Multinucleate cells in rat liver 60 days after oral administration of 100 μg dioxin/kg. Other features illustrated are variation in cell and nuclear size and the presence of parenchymal-cell mitoses in these livers Haematoxylin and eosin × 380.
all rats had liver damage, the extent of this was such that it was difficult to attribute the death of the animals to a gross hepatic failure. The histological appearance of the liver was unusual in that there was a progressive lesion with the formation of multinucleate cells and some fibrosis. These changes developed in the absence of extensive focal or zonal necrosis when the livers were examined by conventional histological methods. The multinucleate cells may have been formed by fusion of parenchymal cells and the mechanism of this is under investigation. Liver lesions similar to those seen in this study have been reported in Macaca mulatta monkeys fed toxic fat (Allen & Carstens, 1967) and in male Wistar rats given 10 mg dioxin/kg body weight by ip injection (Buu-Hoi et al. 1972a). Both of these groups described multinucleate cells and a centrilobular lesion, although the abnormal cells were less bizarre than those described in this study.

The deaths and similar morbidity in germ-free and SPF rats given dioxin indicated that the terminal pulmonary infection in conventional animals was not the primary cause of death.

Our interpretation of the findings is that dioxin interferes with the capacity of the liver cells to maintain their correct organization; in some cells this leads to death and in others to disorganization of structure. This is not inconsistent with a mode of action of a cascade-type involving a series of biochemical lesions (Buu-Hoi et al. 1972b). We have confirmed that the liver microsomal mixed-function oxidase system of rats given dioxin is seriously disturbed (Greig, 1972; J. B. Greig, unpublished results 1972). The possibility that the mode of action of dioxin is linked with this prolonged disturbance is being studied.

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REFERENCES


TOXIC EFFECTS OF DIOXIN

Troxic Effects de la 2,3,7,8-tétrachlorodibenzo-p-dioxine

Résumé—On a démontré qu'une seule dose orale de 25 à 50 µg/kg du composé toxique 2,3,7,8-tétrachlorodibenzo-p-dioxine (dioxine) provoque de l’œdème du pericarde et la mort chez le poulet. L’extrême toxicité de la dioxine chez le cobaye est confirmée. Une dose orale unique de 200 µg de dioxine/kg a fait diminuer la consommation de nourriture et le poids chez des femelles, qui n’ont plus survécu, en moyenne, que de 40,4 jours. Quoique des hémorragies gastriques et des jaunissements fussent courantes, le tableau d’autopsie des animaux n’était pas uniforme. La constitution du foie s’était modifiée significativement dans les 3 jours suivant l’administration, sans toutefois que les composants du plasma présentassent des altérations révélatrices d’une mauvaise fonction du foie. Les modifications de la concentration globulaire, de l’hémoglobine, et du taux de protéines du plasma observées une semaine après l’administration étaient très probablement associées à la diminution de la consommation de nourriture. Des modifications pathologiques du foie se sont manifestées plus tard; il s’agissait plus particulièrement de formations de cellules parenchymatrices multinucléées.

Toxische Wirkungen von 2,3,7,8-Tetrachlordibenzo-p-dioxin