Toxicity of 1, 2-Dibromo-3-chloropropane (DBCP)

I. Histopathological Examination of Male Rats Exposed to DBCP Vapour

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Abstract: A histopathological examination was made of adult male rats which inhaled 1,2-dibromo-3-chloropropane (DBCP). The rats were continuously exposed to 10 ppm DBCP for 14 days and were sacrificed 1, 16 and 36 days, and 12 months after exposure. Severe injury was produced in the testicle, kidney and lung. The testicle became completely atrophied with irreversible aspermatogenesis. The renal injury was characterized by proximal tubular necrosis in the outer medulla. In the lung, necrosis and cytomegaly were observed in the bronchial and bronchiolar epithelial cells as well as the alveolar emphysema. In another experiment, adult male rats were exposed to 0, 0.3, 1, 3 and 8 ppm DBCP continuously for 14 days. The testicle was examined 1 and 15 days after exposure, revealing pathological changes in the 3 and 8 ppm exposure groups, with recovery and a progressive course, respectively.

Keywords: DBCP—Inhalation—Histopathology—Testicle—Kidney—Lung

INTRODUCTION

The nematocide 1,2-dibromo-3-chloropropane (DBCP), a widely used soil fumigant, has been shown to cause marked sperm reduction in pesticide workers engaged in DBCP production since 1977. The Occupational Safety and Health Administration established an 8 hr time weight average permissible exposure of 1 part per billion. Experimentally, DBCP is known to induce liver and kidney necrosis, testicular atrophy, stomach cancer and dominant lethality in some species of animals. Microbial assays have indicated that this compound is mutagenic. This paper deals with the histopathological findings of rats which inhaled DBCP. In a next paper, we shall report clinico-biochemical studies on DBCP inhalation.
MATERIALS AND METHODS

Two experiments were performed using male Sprague-Dawley rats aged 14 weeks (Japan CLEA, Tokyo) according to the designs which shall be reported in detail in a next paper. Six concentration levels of DBCP were prepared in mixing chambers by mixing clean air with DBCP vapour from a saturation apparatus. The DBCP was introduced into inhalation chambers respectively. A DBCP sample was supplied from Toyo Soda Co. The sample contained 10% toluene. For inhalation experiments, toluene was removed off by distillation and DBCP collected at about 196°C was used. Concentration in the inhalation chambers was determined by a gaschromatograph with a FID (Hewlett Packard, 5840A). In experiments I, 11 rats were continuously exposed to 10 ppm of DBCP in air for 14 days, and 5, 2 and 2 rats were killed 1, 16 and 36 days after exposure, respectively. Of the remaining 2 rats, one died 12 months after exposure, when the other was sacrificed. In experiment II, 19 rats were exposed to 0, 0.3, 1, 3 or 8 ppm of DBCP in air for 14 days. One and 15 days after exposure, 1 or 2 rats of each dose group were killed.

Histopathological investigations were made of the heart, lung, liver, kidney, spleen and testicle, while only the testicle was examined from the rats killed 36 days after exposure in experiment I and from all cases in experiment II. Tissue specimens were fixed in neutral buffered 10% formalin and embedded in paraffin. Sections of 5 µm in thickness were stained with hematoxylin and eosin, and selected ones were also stained with periodic acid Schiff or trichrome stain.

RESULTS

Experiment I:

Marked lesions were induced by DBCP inhalation in the testicle, kidney and lung. At all sacrifice times, the testicular lesions were characterized by diffuse atrophy of the seminiferous tubules which contained only Sertoli cells being occasionally degenerative (Figs. 1–4). Necrotic germ cells that had coalesced into aggregated spherical giant cells could be detected in the tubules at day 1 (Fig. 2). With increasing time after exposure, the degree of tubular atrophy became more prominent, resulting in irreversible aspermogenesis (Figs. 2–4). Fibrous thickening of both the tubular basement membrane and tunica albuginea was obvious at 12 months. In the interstitium without fibrous proliferation, there was moderate increase of Leydig cells as well as arterioles with edematous tunica media in cases examined at 36 days and 12 months. Hemangioma occurred in one testis from a case which spontaneously died at 12 months.

At day 1, the proximal tubules in the outer medulla of the kidney revealed a few flattened epithelial cells with hyperchromatic or bizarre nuclei (Fig. 5). Some necrotic cells were contained in the dilated lumen. The proximal tubular
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Fig. 1. Testicle of a control rat. H & E Bar = 100 μm

Fig. 2. Testicle of a rat at 1 day post exposure showing extensive loss of germ cells and aggregated giant cells in the atrophic tubules. H & E Bar = 100 μm. DBCP concentration: 10 ppm

Fig. 3. Testicle of a rat at 36 days post exposure showing diffuse atrophy of seminiferous tubules with degenerative Sertoli cells. H & E Bar = 100 μm. DBCP concentration: 10 ppm

Fig. 4. Testicle of a rat at 12 months post exposure showing atrophic tubules with a fibrous thickened basement membrane and moderate increase of interstitial arterioles. H & E Bar = 100 μm. DBCP concentration: 10 ppm
Fig. 5. Kidney of a rat at 1 day post exposure showing dilated proximal tubules having a few flattened epithelial cells with bizarre nuclei (arrows) in the outer medulla. H & E Bar = 40 μm. DBCP concentration: 10 ppm

Fig. 6. Kidney of a rat at 16 days post exposure showing considerable regeneration of proximal tubules in the outer medulla. Note the occasional appearance of giant epithelial cells (arrows). H & E Bar = 40 μm. DBCP concentration: 10 ppm

Fig. 7. Kidney of a rat at 12 months post exposure showing giant epithelial cells with brush border (arrow) of the proximal tubules in the outer medulla. PAS Bar = 40 μm. DBCP concentration: 10 ppm

Fig. 8. Kidney of a dead rat at 12 months post exposure showing an adenocarcinoma with a tubular growth pattern. H & E Bar = 40 μm. DBCP concentration: 10 ppm
epithelial cells in the cortex had a large pale nucleus and hydropic swollen cytoplasm, while similar lesions which were detected in the outer medulla were observed focally. Eosinophilic hyaline casts were present in the distal tubules and Henle's loop. At day 16, the degree of tubular regeneration was considerable (Fig. 6); however, some parts of the proximal tubules in the outer medulla were

Fig. 9. Lung of a control rat. H & E Bar = 300 μm.

Fig. 10. Lung of a rat at 1 day post exposure showing bronchial dilation and emphysematous alveolar distention. H & E Bar = 300 μm.

DBCP concentration: 10 ppm
still dilated and were devoid of epithelial cells. Occasionally, giant epithelial cells with a distinct brush border appeared in the proximal tubules of the outer medulla in cases examined at 16 days and 12 months post exposure (Figs. 6, 7). One case examined at 12 months had a single white nodule of 7 mm in diameter in the cortex of the left kidney and this was a lipoma. In some tubules in the marginal area of the nodule, epithelial hyperplasia was observed. In another case spontaneously dying at 12 months, the right kidney was grossly enlarged with an irregular shape and weighing 11.94 g. Histologically, the tumor was found to be an adenocarcinoma having a tubular or massive growth pattern with frequent

![Fig. 11. Bronchus of a rat at 1 day post exposure showing cytomegalosis in the epithelial lining. Note the giant cells with hyperchromatic nuclei and basophilic cytoplasm without cilia (arrows). H & E Bar=40 μm. DBCP concentration: 10 ppm](image1)

![Fig. 12. Bronchus of a rat at 1 day post exposure showing desquamation of necrotized epithelial cells. H & E Bar=40 μm. DBCP concentration: 10 ppm](image2)
mitotic figures (Fig. 8). Metastatic lesions were disseminated to the left kidney, lung, lymph nodes and throughout the abdomen.

Dilation of the bronchial and bronchiolar spaces as well as emphysematous alveolar distention were observed in the lung from autopsy cases at 1 and 16 days post exposure (Fig. 10). From the proximal bronchi to terminal bronchioles, some cytomegalosis in the epithelial lining had occurred and giant cells with hyperchromatic nuclei and basophilic cytoplasm without cilia were occasionally apparent (Fig. 11). There were focal desquamation of necrotized cells in day 1 cases (Fig. 12). However, the alveolar epithelium remained intact. The severity of the bronchial lesions was similar between day 1 and day 16 cases except for the epithelial necrosis, although less severe alveolar distention was present in day 16 cases. In the lung of cases examined at 12 months, metastasis of the renal adenocarcinoma was the only lesion.

The spleen of day 1 cases revealed atrophy of the white pulp and a decrease of lymphocytes in the red pulp. No remarkable changes were observed at day 16.

**Experiment II:**

As shown in Table 1, in rats exposed to less than 1 ppm, no remarkable changes were detected in the testicle. In one of 2 cases exposed to 3 ppm, a slight decrease in germ cells or some atrophy of a few seminiferous tubules was recognized at day 1 and day 15. After exposure to 8 ppm, necrosis of the germ cells as well as severe atrophy of the tubules appeared in all cases examined.

**Table 1. Production of testicular lesions in rats exposed to DBCP.**

<table>
<thead>
<tr>
<th>Days post exposure</th>
<th>DBCP (ppm)*</th>
<th>0.3</th>
<th>1</th>
<th>3</th>
<th>8</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>−**</td>
<td>−</td>
<td>−</td>
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<tr>
<td>15</td>
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* Exposed continuously for 14 days.

** Severity of individual lesions: −; no changes, +; slight, #; moderate, ##; severe.

**DISCUSSION**

Inhalation of 10 ppm DBCP in rats caused severe atrophy of the testicle with the seminiferous tubules lacking differentiating germ cells, and the condition was progressive. The changes were similar to those observed in human cases and experimental cases in some animal species. Exposure to a high concentration of DBCP might cause necrosis of all differentiating germ cells as well as depletion of stem cells, resulting in irreversible azoospermia.

In experiment II, dose-related changes were found to occur with doses of more than 3 ppm. Hasegawa et al. found that 0.3 ppm or more caused a dose-

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related oligospermia or azoospermia, while recovery was possible with less than 3 ppm, in agreement with our data. These findings indicate that more differentiated germ cells, either spermatid or spermatocytes, might be more susceptible and a lower concentration of DBCP might not always affect the stem cells.

In the kidney, the proximal tubules of the outer medulla, pars recta, were shown to be the primary target as has been reported by others, although the reasons remained unclear. By autoradiography, a high concentration of radiocarbon in the outer medulla has been shown to be selectively distributed after oral intoxication; however, other possibilities such as a capacity for xenobiotic metabolism or susceptibility to injury of this portion, should be considered.

Necrosis and cytomegaly of the bronchial epithelium as reported by Renznik et al. were observed in the lung of the present cases, while hyperplasia and squamous metaplasia of the bronchial epithelium could not be detected in this study. The duration of exposure was only 2 weeks and this might not be sufficient to cause proliferative or metaplastic lesions. On the other hand, distention of the bronchial or alveolar spaces of considerable duration was also noticed, and occurrence of emphysema after prolonged exposure might be possible, although the precise pathological mechanism remains uncertain.

Formation of giant epithelial cells was observed in the renal proximal tubules and bronchi at day 1 and day 16. Such giant cells were also detected in the kidney at 12 months. These cells might be polyploid, suggesting an irreversible chromosomal aberration due to DBCP, since the compound has been reported to be a potential alkylating agent.

DBCP has been found to induce squamous carcinoma in the stomach of rats and mice after long-term oral exposure. In addition, induction of renal adenocarcinoma and increased occurrence of hemangioma have also been noticed. Since the spontaneous occurrence of such neoplasms is very rare in Sprague-Dawley rats, the renal adenocarcinoma and testicular hemangioma observed in the case dead at 12 months might have been induced by this compound.

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REFERENCES