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**Liver Morphology in Guinea Pigs Fed Soot from the Binghamton
State Office Building Continuously for Ninety Days**

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**DIRECTOR
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Introduction

Soot collected from the Binghamton State Office Building (BSOB) was mixed with certified guinea pig chow and administered to Hartley guinea pigs of both sexes continuously for ninety days. Animals were divided into five groups of 10 males and 10 females, and fed either chow with no soot (controls) or chow with 0.2, 1.9, 9.3 or 46.3 ppm soot. This test was designed, performed and analyzed toxicologically by DeCaprio and co-workers from this center (see DeCaprio et al., 1983). The liver morphology of these animals was studied by routine light microscopy as well as conventional electron microscopy, and the results are reported here.

Methods

Liver samples were removed from the guinea pigs as soon after death as possible and immediately immersed in either buffered formalin or buffered 2% glutaraldehyde at 4°C. Formalin fixed samples from each animal were embedded in paraffin, and 2 randomly selected males and 2 randomly selected females from each group were embedded in JB-4 plastic. Paraffin sections were stained with hematoxylin and eosin (H&E), Perl's iron, periodic acid-Shiff (PAS) and PAS after diastase. JB-4 plastic sections were stained with toluidine blue.

The samples fixed in glutaraldehyde were immediately minced, fixed overnight, postfixed in 1% buffered O_3O_4 , and embedded in epon. Five blocks from each animal were randomly selected, semithin (0.25 μ m) sectioned, and stained with toluidine blue. Preliminary light microscopic quantitative analysis was performed using these sections, which were centered on the microscope axis at a magnification (x50) too low to distinguish the pertinent cytoplasmic details. The magnification was then increased (x800) so that a 133 x 205 μ m image plane aperture defined the observed specimen area. The number of hepatocytes with one or more vacuoles (the prominent alteration in the lowest dose group), or with one or more concentric membrane assays (CMAs) (the

prominent alteration in the highest 2 dose groups) were counted. The data set composed of the number of hepatocytes per field which had one or more vacuoles was subjected to an analysis of variance using a self-critical procedure requiring adherence to the assumptions of normality and homoscedasticity. Bartlett's test was used as a further check of homoscedasticity. In addition a regression analysis was performed and a model fit to the data. The data set consisting of the number of hepatocytes per field with one or more CMAs more closely followed a Poisson rather than a Gaussian distribution and was analyzed by maximum likelihood estimation.

Two males and 2 females were randomly selected from each group for evaluation by electron microscopy. Five additional randomly selected blocks from these animals were semithin sectioned and stained with toluidine blue. The resultant 10 blocks from each animal were evaluated qualitatively by light microscopy. Blocks were ultrathin sectioned, stained with uranyl acetate and lead citrate, and examined by electron microscopy.

Results

Gross Observations and Light Microscopy

Pale areas, single or multiple, were observed on the liver surfaces, with equal incidence in all experimental and control groups. The minute (1-2 mm) foci consisted of coagulative necrosis of hepatocytes, polymorphonuclear cells, and hemosiderin-laden macrophages at the periphery. These lesions were reported by us in the acute BSOB soot feeding test (see attached off print), and were described in otherwise normal guinea pig populations by Cuba-Caparo et al., (1977).

Although liver architecture was well preserved at the light microscopic level, hepatocyte cytoplasmic alterations were observed with increased incidence relative to the controls in all dose groups. Alterations were predominantly in the centrilobular hepatocytes and included cytoplasmic vacuolation,

acidophilic hyalin-like bodies (CMAs), hypertrophy, iron accumulation, increased glycogen, steatosis, adenofibrosis, and focal cell necrosis as evidenced by one to a few necrotic, densely acidophilic hepatocytes.

Cytoplasmic vacuoles were readily observed by light microscopy in both plastic preparations, but were extremely difficult to distinguish in the paraffin H&E sections. The vacuoles usually exhibited an irregularly dark stained outline and pale homogeneous contents. A small number of vacuoles contained RBCs in their lumina. The vacuoles varied considerably in size, some being small and nearly circular, others larger than the round nuclear profile and irregularly shaped. Vacuoles were observed in all groups including the controls, but had a higher incidence and were the predominant alteration in the lowest dose group (0.2 ppm). The two highest dose groups had approximately the same incidence as the controls. The females in the lowest dose group (0.2 ppm) had on the average 10.8 more effected hepatocytes per field than the control females; the 0.2 ppm males had on the average 4.5 more than the control males. The 1.9 ppm group females were not statistically different from the control females, but the males were statistically different (see table 1). In addition, females in the 0.2 ppm group had a statistically increased number of effected hepatocytes with respect to the males in that group. See Table 1 for a summary of the statistical analysis.

CMAs, a predominant alteration in the highest 2 dose groups, were rarely observed in the 2 lower dose groups and were absent from the controls. The statistical analysis showed that the data was well fitted by the model

$$Y_i = \beta_0 + \beta_1 X_i + \beta_2 X_i^2$$

where Y = number of effected hepatocytes per field

X = dose

i = 1, ... , 5 corresponds to the dose groups.

The value of the parameters and their 95% confidence intervals are given in table 2.

Glycogen was observed in excess of that in the controls in the 1.9, 9.3 a 46.3 ppm groups, but not in the 0.2 ppm group.

Adenofibrosis (bile duct proliferation) and focal cell necrosis were occasionally observed, but neither showed any correlation between incidence and dose. Nine guinea pigs had focal cell necrosis and 6 had adenofibrosis. Neither lesion was observed in the controls.

Electron Microscopy

The ultrastructural analysis supported the light microscopy and provided higher resolution images of the structures described (see Turner and Collins, 1982 attached). Cytoplasmic vacuoles were the prominent structure in the lowest dose group. The dark outline observed by light microscopy was shown to be layers of parallel membranes which appear to condense from the smooth endoplasmic reticulum (SER). The vacuoles frequently contained highly stained condensed membrane fragments similar to those found in the sinusoids and bile canaliculi. The 2 highest doses showed extensive cytoplasmic alterations including large single or multiple CMAs, marked proliferation of the SER, accumulation of glycogen and displacement of mitochondria peripherally to the region of the plasma membrane. Glycogen bodies were also conspicuous in these 2 dose groups. Details of the ultrastructure are similar to those in the attached off print (Turner and Collins, 1982).

Discussion

The only alteration observed in the control group was cytoplasmic vacuolation which occurred at low incidence. Since no other alterations except one animal with a small amount of iron were observed in this group, an adequate control was established.

Several alterations, that were absent from or present at substantially lower levels in the controls, were observed in the experimental animals and were dependent on dose levels. The CMAs were absent in the controls, rare in the lower two dose groups (0.2 and 1.9 ppm), but numerous in the higher two groups (9.3 and 46.3 ppm). The proliferation of the SER and margination of

organelles particularly mitochondria was also marked in the higher two doses. The cytoplasmic vacuoles were most numerous in the lowest dose group (0.2 ppm) but occurred in similar numbers in the highest 2 dose groups and controls. These trends were confirmed by the preliminary quantitative analysis based on the number of hepatocytes per field which contained one or more vacuoles or CMAs. The analysis showed that the number of hepatocytes per field with vacuoles in the lowest dose group was statistically increased with respect to the controls, and that the number with CMAs was statistically greater in the highest two doses. The vacuoles did not follow the classical dose response of an increase with a higher dose. However, the vacuoles appeared to be part of a system of proliferating membranes including SER and CMAs which qualitatively appears to increase with dose. In addition this system suggests a possible means of membrane excretion.

The occurrence of vacuoles at low doses, and proliferated SER and CMAs at higher doses is a trend previously observed for DDT (Ortega et al., 1957; Ortega, 1966). Rats fed 5 ppm DDT for 2 to 9 months showed vacuolation of the cytoplasm sometimes with included RBCs and a lumen which stained similar to the sinusoids. The vacuoles were observed to have a distinct boundary that showed a staining reaction similar to that reported here and were sometimes closely associated with lipospheres (CMAs) (Ortega et al., 1957). At higher dietary levels of 100 to 2500 ppm DDT for 1 to 6 months Ortega (1966) reported CMAs at all levels and the majority of animals demonstrated them at or above 400 ppm. Vacuoles were not a prominent alteration at these levels. At intermediate levels of DDT Jonsson et al. (1981) reported both vacuoles, and CMAs. Furthermore clinical signs of toxicity were significantly manifested only at 2500 ppm and liver weights were not significantly increased below 400 ppm (Ortega et al., 1957; Ortega, 1966). In the current experiment DeCaprio et al. (1983) reported decreased body weight gain for the two higher dose groups (9.3 and 46.3), altered relative organ weights in the 1.9, 9.3 and 46.3 ppm groups

and no alterations in the 0.2 ppm group. Thus, the vacuoles were the most prominent alteration at a dose level nearly 10 times lower than that required to produce any other alteration. It should also be noted that the average consumption of BSOB soot in this group (0.2 ppm) was 0.3% of the acute oral LD₅₀ (DeCaprio et al., 1983). Thus, vacuole formation was induced at a low dose level of BSOB soot and follows a pattern similar to that observed by others for DDT.

The idea that this proliferating membrane system is a general mechanism of detoxification is supported by the observation of similar vacuoles with and without internal membrane fragments as a result of a variety of toxins being fed to a number of species: PCBs and polychlorinated quaterphenyls in cynomolgus monkeys (Hori et al., 1982), bile acids in rabbits (Schaffner and Javitt, 1966), and hamsters Miyai et al., 1982), bile salts in rats (Miyai et al., 1977), endotoxin in dogs (Boler and Bibighaus, 1967; Boler et al., 1969), and acetaminophen in mice (Walker et al., 1980). RBCs were observed in vacuoles produced by acetaminophen (Walker et al., 1980). Some of the vacuoles produced by endotoxin and bile acids had halo-like regions surrounding them similar to those reported here (Bolar and Bibighaus, 1967; Boler et al. 1969; and Schaffner and Javitt, 1966). In addition, membrane fragments in the lumina of the vacuoles were reported by a number of workers (Boler and Bibighaus, 1967; Boler et al., 1969; Miyai et al., 1977; Walker et al., 1980; and Miyai et al., 1982).

The cytoplasmic vacuoles and a few CMAs were observed at a dose level at which no other alterations clinical (DeCaprio et al., 1983) or morphological were observed. Thus it is important to ask whether these changes at the level of incidence observed are pathologic or whether they represent a physiologic mechanism. Unfortunately there is no direct evidence in our experiment with which to address this point. However, similar changes were reversible when

rats were removed from low levels of DDT (Ortega et al., 1957). Vacuoles resulting from acute tauroolithocholate intoxication in the hamster were reversible (Schaffner and Javitt, 1966) as were CMAs in rats intoxicated with thiohydantoin (Herdson et al., 1964). Thus it would appear logical that the level of alteration in the 0.2 and 1.9 ppm groups may be reversible. The origin of the vacuoles is also left in doubt by the present study, but they probably arise from large dilated saccules of SER around which proliferated SER condenses. However, the possibility that they are formed from an invagination of the bile canalicular, sinusoidal or lateral plasma membranes cannot be ruled out.

Moderate iron deposition was observed in 6 of 20 guinea pigs in the lowest dose group (0.2 ppm). One animal in the control group and one in the 1.9 ppm group showed a small amount of finely stippled pigment. No explanation for the retention nor its occurrence only in this group is offered at this time. Due to the porphyrinogenic effect of 2,3,7,8-TCDD (Strik et al., 1980) and the role of iron in 2,3,7,8-TCDD toxicity (Jones and Sweeney, 1982) further study of this observation is underway.

Focal cell necrosis was present in all experimental groups but showed no relationship to dose either in terms of severity, or number of effected animals. Bile duct proliferation or adenofibrosis was also present in all groups and did not show any relationship to dose. However, more females than males showed the lesion, a result consistent with previous observations (Kimbrough et al., 1972; Turner and Collins, 1983). Neither of these changes were observed in the controls.

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Table 1
 Statistical Analysis of the Number of
 Hepatocytes per Field with Cytoplasmic Vacuoles

<u>Comparison</u>	<u>Estimated Differences</u>	<u>Simultaneous Lower Bound</u>	<u>95% Confidence Interval Upper Bound</u>
0.2 ppm males vs control males	4.533	0.958	6.138
1.9 ppm males vs control males	3.795	0.179	7.411
0.2 ppm females vs control females	10.80	7.261	14.34
1.9 ppm females vs control females	1.608	-2.004	5.220
0.2 ppm females vs 0.2 ppm males	7.017	3.478	10.56

Table 2

Model Parameters for the Number of Hepatocytes

with CMAs per Field

<u>Parameter</u>	<u>Estimate</u>	<u>95% Confidence Interval</u>
β_0	0.01024	$0.0000 \leq \beta_0 \leq 0.0388$
β_1	8.084	$6.901 \leq \beta_1 \leq 9.261$
β_2	-2.597	$-3.027 \leq \beta_2 \leq -2.167$

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Attachments for BSOB Expert Advisory Panel meeting, June 7, 1983

1. Determination of TCDFs and TCDDs in air samples from the sixteenth floor of the Binghamton State Office Building, March 16, 1983.
2. Progress Report: evaluation of in vitro assays for the detection of "dioxin-like" activity in the Binghamton State Office Building, May, 1983.
3. Liver morphology in guinea pigs fed soot from the Binghamton State Office Building continuously for ninety days, May 16, 1983.
4. Subchronic oral toxicity in guinea pigs of soot from a polychlorinated biphenyl-containing transformer fire; *Tox & Applied Pharmacol*, 68, 1983.
5. Toxicology center protocol; acute and chronic toxicity testing, March 7, 1983.
6. Status report for the Binghamton State Office Building medical surveillance program. Fitzgerald et al, May, 1983.
7. Liver morphology in guinea pigs administered either pyrolysis products of a polychlorinated biphenyl transformer fluid or 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Tox & Applied Pharmacol*, 67, 417-429, 1983.
8. Determination of tetra-hexa CDFs and tetra-CDDs in air samples from the 11, 14, 16 and 17th floors of the Binghamton State Office Building, May 16, 1983.
9. Statement by New York State Department of Labor.