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ACUTE TOXICITY IN GUINEA PIGS AND RABBITS OF SOOT FROM A POLYCHLORINATED BIPHENYL-CONTAINING TRANSFORMER FIRE

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Acute Toxicity in Guinea Pigs and Rabbits of Soot from a Polychlorinated Biphenyl-Containing Transformer Fire. Silkworth, J.B., McMartin, D.M., DeCaprio, A.P., Rej, R., Kumar, S., and Kaminsky, L.S. (1982). Toxicol. Appl. Pharmacol. 00,00-00.

A fire involving a polychlorinated biphenyl (PCB)-containing transformer extensively contaminated the State Office Building in Binghamton, New York with a soot-like material containing 1 ppm 2,3,7,8-tetrachlorodibenzo-p-dioxin, 50 ppm 2,3,7,8-tetrachlorodibenzofuran and high concentrations of numerous other polychlorinated dibenzodioxins, dibenzofurans, and PCBs. The oral LD50 values of the soot and of its benzene extract in female guinea pigs in 0.75% aqueous methyl cellulose were 410 mg soot/kg and 327 mg soot equivalent/kg, respectively. Soot (dose range 1-500 mg/kg) decreased WBC counts, platelet counts, and percent neutrophils, and increased percent lymphocytes in females at 100 mg/kg. Serum triglycerides were elevated in males at 100 and 500 mg/kg and in females at 500 mg/kg. Alkaline phosphatase and γ-glutamyl transferase were lowered in females at 500 and 100 mg/kg, respectively. Histopathology revealed dose-related goblet cell hyperplasia of the pancreatic duct and salivary gland duct metaplasia in males. Body weight loss was observed in both sexes at 500 mg soot/kg. Thymus weight decreased in both sexes at 100 and 500 mg/kg, and kidney weights decreased in males at 100 and 500 mg/kg. Dermal application of soot to rabbits for 24 hr caused no overt toxicity, although hepatic centrilobular hypertrophy was observed in both sexes. Similar application of soot extract caused a local serous inflammation in addition to hepatic

centrilobular hypertrophy. The oral LD50s for 2,3,7,8-TCDD in guinea pigs in aqueous methyl cellulose or corn oil were 19 and 2.5 µg/kg, respectively. It was concluded that the soot matrix alters dermal but not oral toxicity of its components, that the toxic effects were consistent with those reported following exposure to dibenzodioxins and dibenzofurans, and that the aqueous vehicle markedly diminished the acute toxicity of 2,3,7,8-TCDD relative to that in corn oil vehicle.

On February 5, 1981, a transformer was involved in a fire at the State Office Building in Binghamton, New York. The transformer contained a dielectric fluid (Pyranol) which was composed of the polychlorinated biphenyl (PCB) mixture, Aroclor 1254 (65%) and chlorinated benzenes (35%) together with some trace additives. The fire resulted in extensive contamination of the building with a soot-like material.

Chemical analysis of two soot samples collected in the stairwells of the 3rd and 4th floors of the building indicated that the highly toxic compounds 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) and 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF) were present at concentrations of 2.9, 2.8 and 273, 124 ppm respectively (Smith et al., 1981a). Based on PCB pyrolysis experiments which indicate the relative contribution of 2,3,7,8-TCDF to the total polychlorinated dibenzofurans (Buser et al., 1978) it was estimated that the soot possibly contained approximately 0.5% polychlorinated dibenzofurans.

Both 2,3,7,8-TCDF and 2,3,7,8-TCDD are highly toxic compounds and their toxicity has recently been reviewed (Huff et al., 1980). 2,3,7,8-TCDD is lethal at very low concentrations in certain species (oral LD50 in guinea pigs is 2 µg/kg, in mice 284 µg/kg). Other effects observed include thymus involution, spleen weight reduction, bone marrow hypoplasia, liver megalocytosis, bile duct hyperplasia, testicular degeneration, renal pelvis hyperplasia, adrenal cortical atrophy, hemorrhage and cutaneous lesions. The most commonly observed dermal effect of both compounds in primates is the formation of acneform lesions. Both

compounds produce general debilitation and wasting. 2,3,7,8-TCDD is also a potent teratogen in mice and a carcinogen in rats. Environmental disasters in Italy and Japan have resulted in human exposures to both 2,3,7,8-TCDD and 2,3,7,8-TCDF and have provided some insight into the human toxicity of these compounds (see references in Huff et al., 1980).

In many environmental contamination accidents the identification and quantitation of the individual compounds of known toxicity may be sufficient to permit risk assessments of human exposure to be made. However, because of the complexity of the contamination of these soot samples, additional animal toxicology studies with the soot were essential for such assessments to be accurate. Since the soot contains many PCB, dibenzodioxin and dibenzofuran and biphenylene isomers and congeners in addition to unknown components, the potential for synergistic or inhibitory interactions of the toxicity of any component by one or many of the other components is unknown. Also, the binding affinity for the various chemicals to the soot matrix is probably variable and is unknown. It is possible that soot particles could be excreted after ingestion without releasing the tightly bound toxic materials which would substantially diminish the toxicity. It has been demonstrated, for example, that activated carbon, when administered with 2,3,7,8-TCDD, almost completely prevented the uptake of the dioxin by rats (Poiger and Schlatter, 1980). On the other hand, chemical extraction procedures may not remove all of the toxic compounds from the soot matrix. This would result in a low estimate of the total toxicity of the chemicals present

in the soot. Animal toxicity studies of the crude soot will not resolve all of these questions but should provide a clear indication of the overall toxicity of the soot taking these factors into account.

There are two major aspects of potential human exposure to the soot and its components: acute exposure of personnel during clean-up operations and chronic exposure during any subsequent occupation of the building. The potential pathways of human exposure to the soot, in both cases, are dermal absorption, ingestion or inhalation of soot particles and volatilized components.

Only acute oral and dermal exposures were addressed in the present study. The results provided information on such exposure and provided a basis for dosing during any subsequent subchronic and chronic studies. In view of the complex mixture of xenobiotics present, the soot was treated as a single entity for the purposes of the study.

Acute oral exposure was tested in guinea pigs, a species known to be sensitive to 2,3,7,8-TCDF (Moore et al., 1979), probably the most hazardous toxic component of the soot because of the relatively large quantities present. To model the potential human exposure by ingestion, the soot was administered as a powder suspended in an aqueous vehicle containing 0.75% methyl cellulose to facilitate administration. A similar system has recently been demonstrated to be a safe vehicle in toxicological studies (Fritz and Becker, 1981). Acute dermal exposure was tested with rabbits which are susceptible to dermal lesions following direct exposure to polychlorinated dibenzodioxins (Huff

et al., 1980). The potential of the soot matrix to diminish bioavailability of the xenobiotics was also investigated by comparing the toxicity of the soot itself to that of a benzene extract of the soot.

METHODS

Male and female New Zealand White rabbits (3.5 kg) were used (H.A.R.E., Hewitt, NJ). Male and female Hartley guinea pigs (500-600 g) were obtained from a colony maintained in this Division. All animals were acclimated on arrival to a 12-hr light cycle for at least a week. Room temperature was maintained at 22-24°C, and relative humidity at 40-60%. Guinea pigs were housed two per standard rat cage and were identified by individual body markings. They were fed Certified Guinea Pig Chow 5026 (Purina, St. Louis, MO) and tap water ad libitum. Rabbits were housed one per standard rabbit cage and were fed Certified Rabbit Chow No. 5322 (Purina, St. Louis, MO) and tap water ad libitum.

Contaminated soot was collected from the stairwells of the 3rd and 4th floors of the State Office Building in Binghamton, New York with a vacuum cleaner fitted with clean collection bags. It was then sieved through #40 wire mesh to remove gross inert contamination. An extract of 5.39 g of the soot was prepared by Soxhlet extraction with benzene for 16 hr and the volume of benzene was reduced to 5 ml by heating the solution. 2,3,7,8-TCDD was obtained from the Dow Chemical Co. (Midland, MI) as a solution in toluene. The soot was analyzed as previously reported (Smith et al., 1981b).

Several investigations were performed and are described individually.

a) Acute Oral Toxicity of Soot in Guinea Pigs:

The soot was suspended in aqueous 0.75% methyl cellulose and administered by gavage to groups of 6 male and 6 female guinea pigs at doses of 1, 10, 100 or 500 mg soot/kg body weight in volumes of 0.5 ml/100 g body weight. Lower doses of soot were administered in a single dose and the top dose was administered in three aliquots at approximately 1-hr intervals. Animals were fasted overnight prior to dosing. Control groups received either 0.5 ml aqueous methyl cellulose/100 g, 500 mg activated carbon/kg in 0.5 ml methyl cellulose/100 g or were untreated.

All animals were observed daily for overt toxicity and behavioral alterations. Body weights were recorded 5 days per week and food consumption was monitored weekly. All animals, which survived the 42 day observation period were anesthetized with an overdose of carbon dioxide, exsanguinated and necropsied. At necropsy major organs were weighed and 32 organs and tissues from each animal were fixed in 10% neutral buffered formalin. Tissues were processed by standard paraffin embedding and sections were stained with hematoxylin and eosin. Hematocrit, hemoglobin, RBC and WBC count and differential cell counts were determined. Serum was collected and analyzed for aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transferase, alkaline phosphatase, lactate dehydrogenase, and triglycerides.

b) LD50 of Soot and Soot Extract in Guinea Pigs:

Female guinea pigs in groups of six were administered a single oral dose of soot of 250, 500, 750, 1000 or 1250 mg/kg suspended in 0.75% aqueous methyl cellulose, or a benzene extract of the soot at doses equivalent to 4, 20, 100, 500 or 1000 mg soot/kg suspended in the same vehicle. In the latter case the benzene concentration was adjusted to 18% at all dose levels including the methyl cellulose control. The volume of all doses was 0.5 ml/100 g. The extract doses were administered in a single aliquot while the soot doses were administered in two to four aliquots over a maximum period of 6 hr. A soot control group received 1250 mg activated carbon/kg in the aqueous vehicle. Body weights were determined three times weekly and animals were observed daily. All animals which survived the 42 day observation period were sacrificed. LD50 values were calculated using a modification of the method of Bliss (Carmines et al., 1980).

c) LD50 of 2,3,7,8-TCDD in Guinea Pigs:

Female guinea pigs in groups of six were administered 2,3,7,8-TCDD by a single gavage administration at doses of 0.1, 0.5, 2.5, 12.5 and 20 µg/kg as a solution in corn oil (0.5 ml/100 µg) or in suspension in 0.75% aqueous methyl cellulose (0.5 ml/100 µg). Body weights were determined twice weekly and animals were observed daily. All surviving animals were killed on day 42 of exposure.

d) Dermal Exposure of Soot and Soot Extract to Rabbits:

The effect of dermal exposure to soot was determined by applying 500 mg soot/kg to 64.5 cm^2 of the shaved, unabraded,

dorsal surface of three male and three female New Zealand White rabbits for a 24 hr period. Active carbon, 500 mg/kg, was applied to one male and one female rabbit as controls. The soot and carbon were moistened with sterile saline and held in place with large adhesive bandages for 24 hr after which the patch was removed and the area washed free of loose soot or carbon particles. Disk collars prevented ingestion of soot and were left on until there were no visible traces of soot (14 days).

The relative dermal toxicity of the extractable components of the soot was determined by spreading an extract of the soot in benzene at a dose equivalent to 500 mg soot/kg over a 64.5 cm^2 area of dorsal skin of one male and one female rabbit. The area was protected as described above. Benzene was applied to one male and one female rabbit as controls. All rabbits were observed daily and weighed twice weekly. All rabbits were sacrificed on day 67 using carbon dioxide and were examined for gross pathology. Liver, thymus, kidney and samples of skin from exposed and non-exposed areas were saved for histopathology.

RESULTS

a) Acute Oral Toxicity of Soot in Guinea Pigs:

Male and female guinea pigs were necropsied 42 days after a single oral administration of Binghamton soot in 0.75% aqueous methyl cellulose. At doses of 1 and 10 mg soot/kg there was no consistent significant dose-related alteration from control values in body weight gain, liver, spleen, kidney, thymus or adrenal weights, hematology values or serum chemistry values (Fig. 1a,b, Tables 1-3). However, significant changes were observed in groups which received 100 and 500 mg soot/kg. Thirty-three percent mortality was recorded in the females administered 500 mg/kg (Fig. 1b). Decreased weight gain occurred in both sexes at 100 mg/kg and the body weights of both sexes at 500 mg/kg were significantly below control values (Fig. 1a,b, Table 1). The absolute thymus weight and the thymus weight relative to brain weight were significantly diminished from the control values in males at the 500 mg soot/kg dose and showed dose-related decrease in females administered 100 and 500 mg soot/kg. The absolute and relative kidney weights were decreased in a dose-related manner in males administered 100 and 500 mg soot/kg. The combined adrenal weights were significantly reduced only in males administered 100 mg soot/kg (Table 1).

Hematology values from male and female guinea pigs administered either 1, 10 or 500 mg soot/kg in addition to males administered 100 mg soot/kg were unaltered from control values (Table 2). However the 100 mg soot/kg dose in females resulted in significant decrease in the WBC count, platelet count and the percent of neutrophils in whole blood and an increase in the

percent of lymphocytes in whole blood.

Serum chemistry values are shown in Table 3. There were no significant alterations from control values in male guinea pigs administered 1, 10, 100 or 500 mg soot/kg in aspartate aminotransferase, γ-glutamyl transferase, alanine aminotransferase, alkaline phosphatase or lactate dehydrogenase. However, triglycerides were significantly elevated in a dose-related manner in the 100 and 500 mg soot/kg groups. Female guinea pigs showed a dose-related elevation in serum aspartate aminotransferase at 100 and 500 mg soot/kg, a decrease in γ-glutamyl transferase at 500 mg soot/kg, a decrease in serum alkaline phosphatase at 100 mg soot/kg and an increase in serum triglycerides at 500 mg soot/kg.

Histologic examination of 32 tissues from both sexes of control guinea pigs or guinea pigs administered either 1, 10, 100 or 500 mg Binghamton soot/kg indicated that the soot affected only the pancreas and salivary gland in a clearly dose-related manner and that the observed microscopic lesions occurred only in the males (Tables 4a,b,c). Goblet cell hyperplasia of pancreatic interlobular ducts was observed in all males administered 500 mg soot/kg and in one of the six males administered 100 mg soot/kg. Normal duct epithelium was replaced by a single layer made up primarily of mucin-secreting cells which resulted in an epithelium of twice normal thickness. Metaplasia of salivary gland interlobular duct epithelium was observed in 3 of 6 males administered 500 mg soot/kg. The epithelium was thickened by proliferating epithelial cells which appeared similar to those pre-

sent in the stratum spinosum of skin. Goblet cells, which are present in normal columnar epithelium of the duct, were present in the superficial layer of many of these thickenings. There were no lesions in any animals which indicated cause of death.

Fatty infiltration of the liver was present in two animals administered 500 mg soot/kg. These large cytoplasmic vacuoles were located centrilobularly in one male and periportally in one female.

Microscopic lesions which occurred in many control and treatment groups and showed a tendency to be more frequent and/or severe in treatment groups included bile duct hyperplasia, hepatocellular cytoplasmic inclusions, vacuolation of the adrenal cortex and focal lacrimal adenitis (Tables 4a,b,c).

Bile duct hyperplasia was observed in 3 of 4 control groups and in all treatment groups. A grade of +1 was assigned to livers with one to a few foci of bile ducts per 25 x microscopic field proliferating 100-200 μm outward from the portal area. Fibroplasia was present in some of these foci. A grade of +2 represented 5-15 areas similar to or larger than those graded +1. Lesions graded +2 were observed in females administered 10 and 100 mg soot/kg and males administered 500 mg soot/kg. Hyaline eosinsophilic spheres and rings were found in hepatocellular cytoplasm of animals from every control and treatment group.

Vacuolation of adrenal cortical cells was present only in female guinea pigs. Cytoplasmic vacuoles ranged in size from that equal to the nucleus to nearly that of the total cytoplasmic area. The size and shape of the vacuoles were compatible with having been lipid filled. A grade of +1 was assigned to those

adrenals in which about 11-30% of the cortical cells contained vacuoles. A grade of +2 was assigned to adrenals in which about 31-50% of cortical cells contained vacuoles. Only one female guinea pig from the 500 mg soot/kg received a +2 grade.

Focal inflammation of the lacrimal gland was observed in every male control and treatment group but only in one female administered 500 mg soot/kg. Lacrimal glands graded +1 contained a few foci containing lymphocytes and a few neutrophils located in and around acini. Degeneration of a few acinar cells was indicated by nuclear pyknosis, karyorrhexis and cytoplasmic vacuolation. The most severe (+2) lesion was found in one male administered 500 mg soot/kg in which 10-50% of the gland was involved.

Microscopic lesions for which there were no indications of a relationship to treatment were observed in both sexes in both control and treatment groups. These lesions included hepatic focal coagulative necrosis, hepatic mononuclear cell foci, focal interstitial nephritis and renal mineral foci.

Focal coagulative necrosis of hepatocytes occupied areas from less than 0.2 mm to over 1 cm in diameter. Inflammatory reactions were absent from early lesions but later lesions possessed a basophilic rim of Kupfer cells and other macrophages, a few lymphocytes, degenerating hepatocytes and fibroplasia. Proliferating bile ducts were also present in the rim when necrotic tissue was adjacent to a portal area. A few small (100-150 μm diameter) foci of lymphocytes and a few macrophages were observed in many livers and were not associated with degenerative

changes of liver tissue.

Focal interstitial nephritis consisted of one to several foci of inflammatory cells, primarily lymphocytes and a few neutrophils, located between and occasionally in cortical tubules. A few cortical tubule cells were undergoing degenerative changes as indicated by nuclear pyknosis and karyorrhexis. Many kidneys also contained one to a few mineral foci beneath the pelvic epithelium or in the adjacent renal pelvis. These foci, which measured about 0.2 mm, were often surrounded by a fibrous capsule.

b) LD50 of Soot and Soot Extract in Guinea Pigs:

A single oral dose of either 250 or 500 mg soot/kg to female guinea pigs resulted in a less than normal rate of weight gain after an initial 10-day period of weight loss of 12 and 15%, respectively, of starting weights (Figure 1c). Deaths occurred as early as day 12 and as late as day 42 in these groups. Doses of 750, 1000 or 1250 mg soot/kg produced losses of 27, 37 and 34%, respectively, of initial body weight during the study. Deaths occurred as early as day 9 and all animals in each of these dose groups were dead by day 27. The 42 day LD50 for soot was calculated to be 410 mg soot/kg (Carmines et al., 1980).

Female guinea pigs administered an extract of the soot equivalent to 4 mg soot/kg gained weight at the control rate during the entire observation period (Figure 1d). Doses of either 20 or 100 mg soot equivalent extract/kg resulted in slower rate of weight gain than in controls although there were no deaths at these doses. Animals administered either 500 or 1000

mg soot equivalent extract lost up to 31 or 36%, respectively, of their initial body weight during the study. The cumulative mortality was 80% and 100%, respectively, in the 500 and 1000 mg soot equivalent/kg groups. Deaths occurred as early as day 7 and as late as day 21 and were preceded by gradual weight loss. The 42 day LD50 of the soot extract was calculated to be equivalent to 327 mg soot/kg.

c) LD50 of 2,3,7,8-TCDD in Guinea Pigs:

Female guinea pigs administered a single dose of either 0.1 or 0.5 µg 2,3,7,8-TCDD/kg in corn oil gained weight at the control rate (Figure 1e). Animals administered 2.5 µg 2,3,7,8-TCDD/kg gained weight at a lower rate than controls after an initial weight loss of 7%. Deaths were not observed until day 32 and by the end of the 42 day observation period the cumulative mortality was 50%. Groups administered either 12.5 or 20.0 µg 2,3,7,8-TCDD/kg lost 34 and 33%, respectively, of their initial body weight and had reached 100% cumulative mortality by days 16 and 9 respectively. The 42 day LD50 of 2,3,7,8-TCDD in corn oil was calculated to be 2.5 µg/kg.

Female guinea pigs administered either 0.1, 0.5, 2.5 or 12.5 µg 2,3,7,8-TCDD/kg in 0.75% methyl cellulose gained weight at the same rate as controls. However, animals administered 20.0 µg 2,3,7,8-TCDD/kg in methyl cellulose lost up to 15% of their initial body weight by day 12. The first death in this group occurred on day 12 and the cumulative mortality reached 67% by day 42. The 42 day LD50 of 2,3,7,8-TCDD in methyl cellulose was calculated to be 19 µg/kg.

d) Dermal Exposure of Soot and Soot Extract to Rabbits:

Rabbits dermally exposed to soot for a 24 hr period exhibited no signs of overt toxicity or weight loss during the 65 day observation period. Histologic examination of thymus, kidney and skin from exposed and unexposed areas showed no lesions. However, hypertrophy of centrilobular hepatocytes involving 25-75% of the hepatic lobule was observed in 2 of the 3 exposed males ... and 1 of the 3 exposed females. Large round vacuoles, indicative of fatty infiltration, were also observed in approximately 25% of the hepatocytes of the female with centrilobular hepatocyte hypertrophy. Rabbits exposed to active carbon were in excellent condition throughout the study and were histologically normal.

The male and female rabbits which received a single dermal application of an extract of the soot equivalent to 500 mg soot/kg each developed a mild inflammatory reaction at the application site which first appeared on day 4 (Figure 2). The lesion developed into a serous inflammatory reaction of moderate intensity and 2-3 mm in thickness. The reaction reached and maintained its maximum severity during days 14-34 after application. Complete healing had occurred by day 41. Microscopic evaluation of skin taken from the reaction site on day 67 appeared similar to control tissues. Thymus and kidney tissue from the male and the female rabbits exposed to the extract were normal. However, although the liver from the male appeared normal, centrilobular hypertrophy which involved 51-75% of the hepatic lobule was observed in the female. There was no weight loss in animals exposed to the soot extract. Control animals which received a dermal application of benzene were in excellent condition

throughout the observation period and were histologically normal.

DISCUSSION

The results of these studies have provided a basis for the assessment of the acute toxicity of the Binghamton soot by relating its toxicity to that of the known components of the soot and by addressing the question of the bioavailability of its toxic components.

Chemical analysis of the soot sample used in these studies indicated a composition of approximately 1 ppm 2,3,7,8-TCDD, 50 ppm 2,3,7,8-TCDF and 0.5% PCBs in addition to many chlorinated dibenzodioxin and dibenzofuran congeners. However, the carbon-like matrix of the soot presented the possibility that the toxic components of the soot may be tightly bound, and may prevent absorption and result in an observed toxicity lower than expected. This concept is supported by previous reports that a charcoal matrix almost completely prevented absorption of 2,3,7,8-TCDD (Poiger and Schlatler, 1980). The toxicological findings of this study, in which guinea pigs were administered a single oral dose of soot, included mortality, decreased weight gain, immediate and precipitous weight loss after which there were no cases of survival, thymic atrophy, decreases in kidney and adrenal weights, decreased platelet and WBC counts and an increase in serum aspartate aminotransferase. Pathology findings indicated that glandular and duct epithelium were primary target tissues. These findings are consistent with the findings of McConnell et al. (1978) in which guinea pigs were administered

various purified chlorinated dibenzo-p-dioxin congeners, and with the findings of Moore et al. (1979) in which guinea pigs were administered 2,3,7,8-TCDF and indicate that the soot toxicity is due to its chlorinated dibenzo components.

The oral LD50s of the soot and an extract of the soot, 410 mg soot/kg and 327 mg soot equivalent/kg, respectively, indicate that the carbon-like matrix has only a minimal role in decreasing soot toxicity. The method used for extraction of the soot has been demonstrated to be the most effective in the extraction of chlorinated dibenzodioxins and dibenzofurans from fly ash (Kooke et al., 1981).

However, our dermal studies with rabbits, although the numbers of animals were not large enough for statistical comparisons, indicated that the soot matrix prevented a local inflammatory reaction even though enough absorption occurred to produce liver pathology similar to that produced in rabbits exposed to the extract. The contrasting absence of hepatocellular hypertrophy in our guinea pigs exposed to either soot or soot extract represents a species difference in the toxic response.

The aqueous vehicle used in the study with guinea pigs was chosen because it is toxicologically innocuous (Fritz and Becker, 1981), provides a stable suspension of the soot thus facilitating homogenous dosing, and is more likely to model potential human oral exposure than would an oil based vehicle. However, previously reported acute toxicities of 2,3,7,8-TCDD and 2,3,7,8-TCDF (see Huff et al., 1980; McConnell et al., 1978) have used oil vehicles. Therefore, to compare soot toxicity with chlorinated dibenzodioxin and dibenzofuran toxicities, an assessment of

the role of the vehicle was necessary. Our data with 2,3,7,8-TCDD (LD50 = 19 μg/kg) indicate that the aqueous vehicle diminishes acute oral toxicity in guinea pigs by a factor of approximately 8 relative to that in corn oil (LD50 = 2.5 μg/kg). If similar factors apply to other chlorinated dibenzodioxins and dibenzofurans, an acute oral exposure to the soot in an aqueous vehicle would be less hazardous than in an oil vehicle. The oral LD50 of 2,3,7,8-TCDD in corn oil in our study was consistent with the literature value (Huff et al., 1980).

Based on the chemical analyses of the soot (Smith et al., 1981b), the LD50 dose of soot administered to the guinea pigs contained approximately 0.4 μg 2,3,7,8-TCDD/kg and 21 μg 2,3,7,8-TCDF/kg. Therefore, since the LD50 of 2,3,7,8-TCDD in the aqueous vehicle is 19 μg/kg, the lethal effects of the soot did not arise from the 2,3,7,8-TCDD but rather from the 2,3,7,8-TCDF and/or other pollutants. Two analyses of a sample of soot which differed from that used in this study, but which was collected from the same building, revealed relatively high quantities of a wide variety of other chlorinated dibenzodioxins and dibenzofurans (Stalling, 1981; Rappe, 1981). The LD50 of 2,3,7,8-TCDF in guinea pigs has been reported to be 5-10 μg/kg (Huff et al., 1980). If it is assumed that the acute toxicity of 2,3,7,8-TCDF is also diminished by a factor of 8 when administered in aqueous vehicle then a major fraction of toxicity of the soot must arise from components other than the 2,3,7,8- chlorinated compounds.

In summary, our findings indicate that: the soot matrix does not significantly alter the oral toxicity of the soot but

does influence its dermal toxicity; the toxic effects produced by the soot are consistent with previously reported effects produced by dibenzodioxins and dibenzofurans in guinea pigs; the amounts of 2,3,7,8-TCDD and 2,3,7,8-TCDF in the soot are, alone, insufficient to account for the observed toxicity and therefore other dibenzodioxins, dibenzofurans and/or other contaminants are involved; and the aqueous vehicle used in these studies diminished 2,3,7,8-TCDD toxicity.

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TABLE 1
BODY AND ORGAN WEIGHTS FROM GUINEA PIGS 6 WEEKS AFTER A SINGLE ORAL ADMINISTRATION OF BINGHAMTON SOOT IN 0.75% METHYL CELLULOSE[a]

Parameter	Sex	Controls			Binghamton Soot mg/kg			
		Untreated	Vehicle	Active Carbon[b] 500 mg/kg	1	10	100	500
Body Wt (g)	M	728±15	655±33	732±17	739±24	737±21	649±34	522±40[e]
	F	--	--	692±44	698±25	667±24	641±12	570±29[c]
Body Wt Change During Study(g)	M	+202	+213	+196	+200	+186	+100	-3
	F	--	--	+115	+126	+78	+45	-31
Brain Wt(g)	M	3.75±0.05	3.72±0.08	3.87±0.050	3.91±0.12	3.75±0.06	3.65±0.05[c]	3.74±0.08
	F	--	--	3.75±0.04	3.79±0.12	3.93±0.18	3.68±0.03	3.61±0.06
Liver Wt(g)	M	31.00±1.60	36.00±3.31	29.63±1.65	32.01±3.43	34.61±1.65	30.08±2.48	27.85±2.24
	F	--	--	30.60±3.76	30.13±3.21	34.01±3.80	28.12±1.86	22.71±0.41
Liver Wt/ Brain Wt (%)	M	8.28±0.41	9.75±0.97	7.67±0.35	8.24±0.94	9.22±0.38	8.29±0.79	7.42±0.50
	F	--	--	8.17±1.02	7.91±0.63	8.68±0.96	7.64±0.52	6.296±0.06
Spleen Wt (g)	M	0.580±0.025[c]	0.628±0.043	0.758±0.061	0.688±0.027	0.726±0.050	0.650±0.034	0.853±0.084
	F	--	--	0.749±0.108	0.941±0.066	1.120±0.103[c]	1.016±0.073	0.818±0.107
Spleen Wt/ Brain Wt (%)	M	0.155±0.007[c]	0.170±0.012	0.196±0.015	0.176±0.007	0.194±0.014	0.179±0.011	0.227±0.020
	F	--	--	0.20±0.03	0.285±0.030	0.284±0.019[c]	0.276±0.020	0.227±0.030

[a] Data expressed as mean ± standard error. n = 4-6 per group.
[b] Active carbon in 0.75% aqueous methyl cellulose served as the control group for statistical evaluation using the Student's t-test.
[c] $p \leq 0.05$
[d] $p \leq 0.02$
[e] $p \leq 0.01$
[f] $p \leq 0.001$

TABLE I (Cont'd)
BODY AND ORGAN WEIGHTS FROM GUINEA PIGS 6 WEEKS AFTER A SINGLE ORAL ADMINISTRATION OF BINGHAMTON SOOT IN 0.75% METHYL CELLULOSE[a]

Parameter	Sex	Controls			Binghamton Soot mg/kg			
		Untreated	Vehicle	Active Carbon[b] 500 mg/kg	1	10	100	500
Thymus Wt (g)	M	0.527±0.029	0.433±0.034	0.598±0.122	0.431±0.033	0.457±0.014	0.327±0.028	0.250±0.038[c]
	F	--	--	0.711±0.058	0.693±0.064	0.584±0.058	0.527±0.015[d]	0.368±0.018[f]
Thymus Wt/ Brain Wt (%)	M	0.141±0.009	0.117±0.009	0.154±0.030	0.112±0.009	0.122±0.003	0.090±0.009	0.066±0.010[c]
	F	--	--	0.190±0.02	0.183±0.013	0.151±0.018	0.143±0.004[c]	0.102±0.006[e]
Combined Kidney Wts (g)	M	5.129±0.157	4.810±0.365	5.153±0.249	4.923±0.244	5.480±0.243	4.218±0.151[e]	3.955±0.217[e]
	F	--	--	4.982±0.442	5.170±0.428	4.658±0.311	4.223±0.178	3.671±0.526
Kidney Wt/ Brain Wt (%)	M	1.371±0.049	1.296±0.096	1.334±0.053	1.267±0.077	1.462±0.059	1.159±0.05[c]	1.056±0.045[e]
	F	--	--	1.330±0.120	1.363±0.090	1.204±0.107	1.147±0.049	1.018±0.015
Combined Adrenal Wts (g)	M	0.256±0.012	0.236±0.023	0.283±0.012	0.262±0.010	0.272±0.010	0.249±0.009[c]	0.237±0.017
	F	--	--	0.282±0.018	0.295±0.019	0.287±0.022	0.276±0.020	0.305±0.035
Adrenal Wt/ Brain Wt	M	0.069±0.004	0.064±0.006	0.074±0.004	0.099±0.033	0.073±0.002	0.068±0.002	0.063±0.005
	F	--	--	0.075±0.005	0.078±0.008	0.074±0.008	0.075±0.005	0.084±0.008

[a] Data expressed as mean ± standard error, n = 4-6 per group.
[b] Active carbon in 0.75% aqueous methyl cellulose served as the control group for statistical evaluation using the Student's t-test.
[c] $p \leq 0.05$
[d] $p \leq 0.02$
[e] $p \leq 0.01$
[f] $p \leq 0.001$

TABLE 2
HEMATOLOGY VALUES FROM GUINEA PIGS 6 WEEKS AFTER A SINGLE ORAL ADMINISTRATION OF BINGHAMTON SOOT IN 0.75% METHYL CELLULOSE[a]

Parameter	Sex	Controls			Binghamton Soot mg/kg			
		Untreated	Vehicle	Active Carbon Control[b] 500 mg/kg	1	10	100	500
PCV(%)	M	49±1	50±1	45±1	47±1	46±1	47±1	41±2
	F	--	--	44±2	46±1	44±3	43±2	41±2
WBC ($x10^6$/cc)	M	4.9±0.8	5.5±0.5	4.5±0.3	5.5±0.7	5.0±0.4	5.8±1.1	4.5±0.5
	F	--	--	10±1.0	7.9±1.8	9.2±2.1	6.6±0.8[c]	7.2±1.4
RBC ($x10^9$/cc)	M	4.2±0.3	4.8±0.2	5.1±0.2	5.2±0.2	4.8±0.3	5.3±2.5	4.5±0.4
	F	--	--	5.2±0.5	5.5±0.2	4.6±0.2	4.9±0.3	5.2±0.2
Platelet ($x10^7$/cc)	M	42±24	50±26	8±3	8±3	9±5	11±4	18±7
	F	--	--	34±4.7	24±3.8	23±13	14±3.3[d]	23±9.5
Lymphocytes %	M	73±6	78±2	73±4	67±7	65±1	72±5	71±3
	F	--	--	64±6	65±6	73±6	80±2[c]	66±11
Neutrophils %	M	26±6	21±2	26±4	31±8	35±2	28±5	27±4
	F	--	--	34±6	34±6	23±5	20±2[c]	33±11
Eosinophils %	M	0.6±0.2	0.7±0.5	0.3±0.2	1.0±0.7	0.2±0.2	0.5±0.2	0.7±0.4
	F	--	--	2.5±0.6	2.3±0.8	4.6±1.9	0.8±0.3	1.4±0.4
Monocytes	M	0.3±0.2	0.5±0.2	0.3±0.2	0.8±0.5	0.5±0.3	0.3±0.2	0.7±0.2
	F	--	--	0.5±0.3	0.8±0.2	0.3±0.2	0.3±0.2	0.8±0.2

[a] Data expressed as mean ± standard error. n = 4-6 per group.
[b] Active carbon in 0.75% aqueous methyl cellulose served as the control group for statistical evaluation using the Student's t-test.
[c] $p \leq 0.05$
[d] $p \leq 0.02$
[e] $p \leq 0.01$
[f] $p < 0.001$

TABLE 3

SERUM CHEMISTRY VALUES FROM GUINEA PIGS 6 WEEKS AFTER A SINGLE ORAL ADMINISTRATION OF BINGHAMTON SOOT IN 0.75% METHYL CELLULOSE[a]

Parameter	Sex	Controls			Binghamton Soot mg/kg			
		Untreated	Vehicle	Active Carbon[b] 500 mg/kg	1	10	100	500
Aspartate Aminotransferase (μM/min/L)	M	41.6±4.8	68.8±11	53.2±5.4	46.7±6.0	53.8±11.6	53±9	44.5±3.0
	F	--	--	37.3±3.1	44.2±8.1	60.6±11.6	51.5±3.5[d]	60.8±9.3[c]
γ-Glutamyl Transferase (μM/min/L)	M	12.4±1.1	14.8±2.1	13.1±1.0	13.5±1.6	14.1±0.7	13.5±0.7	10.9±1.3
	F	--	--	19.0±1.7	18.0±1.1	13.8±0.9	13.9±1.5	11.7±1.0[e]
Alanine Aminotransferase (μM/min/L)	M	29.7±1.0	25.3±2.1	35.3±4.6	30.7±3.1	35.5±3.1	27.8±1.8	26±3
	F	--	--	23.2±1.5	24.5±2.0	24.6±2.2	23±2	20±2
Alkaline Phosphatase (μM/min/L)	M	160±12	163±28	126±20	123±13	155±8	107±6	111±9
	F	--	--	126±21	93±12	77±7	72±7[c]	85±13
Lactate Dehydrogenase (μM/min/L)	M	590±33	562±17	567±48	741±99	620±87	486±62	476±98
	F	--	--	600±38	638±65	571±37	567±87	539±30
Triglycerides (mg/dL)	M	101±22	138±24	87±5	113±39	114±32	166±34[c]	282±38[f]
	F	--	--	101±17	114±28	77±21	120±29	168±10[d]

[a] Data expressed as mean ± standard error. n = 4-6 per group.

[b] Active carbon in 0.75% aqueous methyl cellulose served as the control group for statistical evaluation using the Student's t-test.

[c] $p \leq 0.05$

[d] $p \leq 0.02$

[e] $p \leq 0.01$

[f] $p \leq 0.001$

TABLE 4a

MICROSCOPIC LESIONS IN CONTROL GUINEA PIGS

Lesion	Male, Untreated				Male, MC[a]				Male, Carbon[b]+ MC				Female, Carbon + MC			
	# animals with lesion	# tissue examined	% animals with lesion	average grade of lesion	# animals with lesion	# tissue examined	% animals with lesion	average grade of lesion	# animals with lesion	# tissue examined	% animals with lesion	average grade of lesion	# animals with lesion	# tissue examined	% animals with lesion	average grade of lesion
Pancreas;metaplasia of duct epithelium	0	6	0		0	6	0		0	6	0		0	6	0	
Salivary gland;squamous metaplasia of duct epithelium	0	6	0		0	6	0		0	6	0		0	6	0	
Liver;fatty infiltration	0	6	0		0	6	0		0	6	0		0	6	0	
Liver;bile duct hyperplasia	0	6	0	0	2	6	33	1.0±0	2	6	33	1.0±0	3	6	50	1.0±0
Adrenal;cortical vacuolation	0	6	0		0	6	0		0	6	0		3	6	50	1.0±0
Lacrimal gland;focal adenitis	3	6	50	1	3	6	50	1	1	6	17	1	0	6	0	
Liver;focal necrosis	3	6	50		3	6	50		4	6	67		1	6	17	
Liver;mononuclear cell foci	1	6	17		5	6	67		2	6	33		3	6	50	
Kidney;focal interstitial nephritis	2	6	33		5	6	83		2	6	33		4	6	67	
Kidney;mineral foci	2	6	33		1	6	17		2	6	33		0	6	0	

[a] 0.75% methyl cellulose, 0.5 ml/100 g body weight
[b] Active carbon, 500 mg/kg body weight

TABLE 4b

MICROSCOPIC LESIONS IN MALE GUINEA PIGS ADMINISTERED BINGHAMTON SOOT[a]

Lesion	1 mg/kg				10 mg/kg				100 mg/kg				500 mg/kg			
	# animals with lesion	# tissue examined	% animals with lesion	average grade of lesion	# animals with lesion	# tissue examined	% animals with lesion	average grade of lesion	# animals with lesion	# tissue examined	% animals with lesion	average grade of lesion	# animals with lesion	# tissue examined	% animals with lesion	average grade of lesion
Pancreas;metaplasia of duct epithelium	0	7	0		0	6	0		1	6	17		6	6	100	
Salivary gland;squamous metaplasia of duct epithelium	0	7	0		0	6	0		0	6	0		3	6	50	
Liver;fatty infiltration	0	7	0		0	6	0		0	6	0		1	6	17	1
Liver;bile duct hyperplasia	1	7	14	1	3	6	50	1 ±0	2	6	33	1 ±0	3	6	50	1.7±0.6
Adrenal;cortical vacuolation	0	7	0		0	6	0		0	6	0		0	6	0	
Lacrimal gland;focal adenitis	2	7	29	1 ±0	2	6	33	1 ±0	2	6	33	1 ±0	5	6	83	1.2±0.5
Liver;focal necrosis	2	7	29		4	6	67		1	6	17		2	6	33	
Liver;mononuclear cell foci	5	7	71		2	6	33		0	6	0		3	6	50	
Kidney;focal interstitial nephritis	3	7	43		2	6	33		4	6	67		6	6	100	
Kidney;mineral foci	3	7	43		2	6	33		3	6	50		3	6	50	

[a] Soot administered orally in 0.75% aqueous methyl cellulose.

TABLE 4c

MICROSCOPIC LESIONS IN FEMALE GUINEA PIGS ADMINISTERED BINGHAMTON SOOT[a]

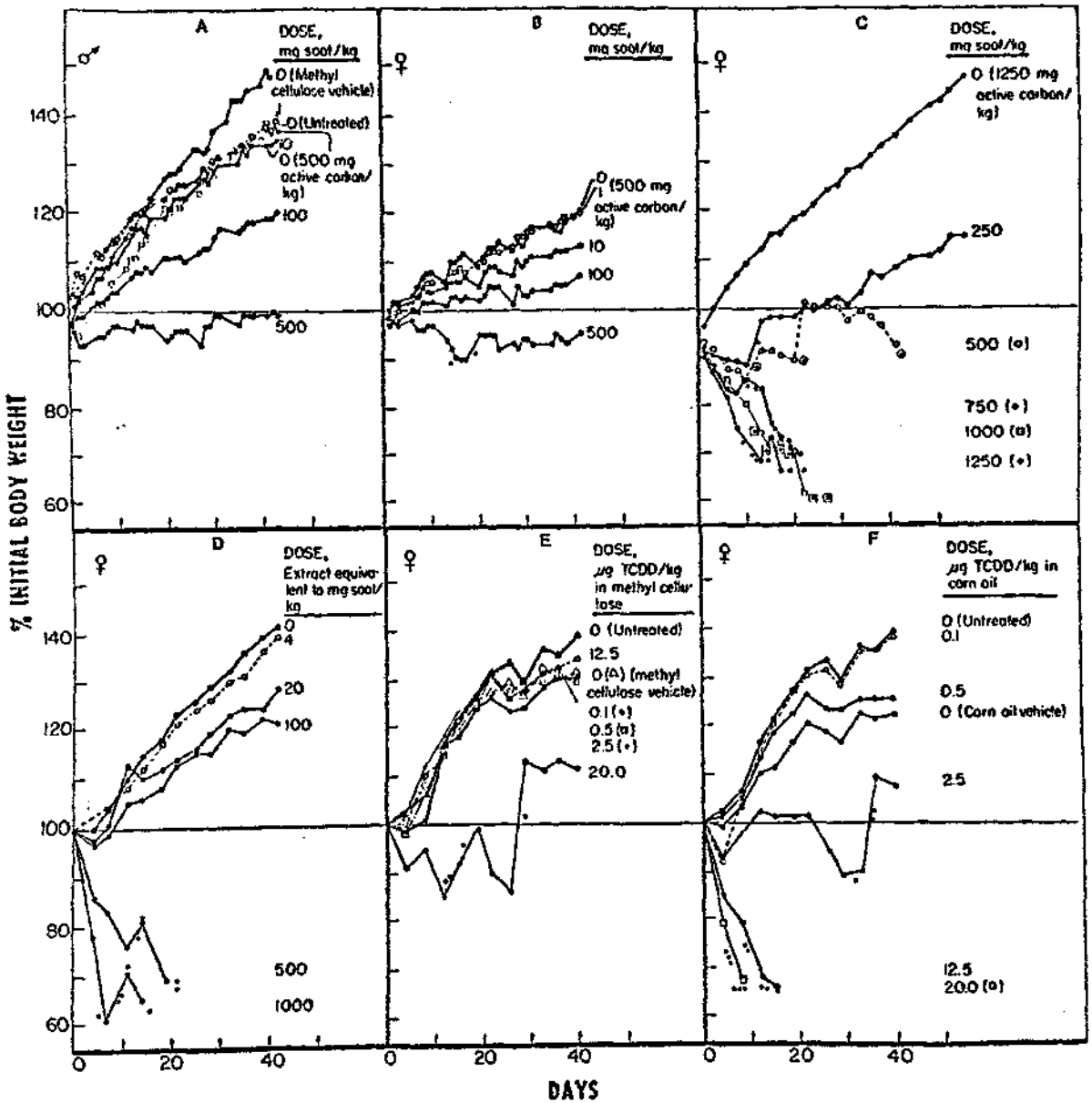
Lesion	1 mg/kg				10 mg/kg				100 mg/kg				500 mg/kg			
	# animals with lesion	# tissue examined	% animals with lesion	average grade of lesion	# animals with lesion	# tissue examined	% animals with lesion	average grade of lesion	# animals with lesion	# tissue examined	% animals with lesion	average grade of lesion	# animals with lesion	# tissue examined	% animals with lesion	average grade of lesion
Pancreas;metaplasia of duct epithelium	0	5	0		0	6	0		0	6	0		0	5	0	
Salivary gland;squamous metaplasia of duct epithelium	0	5	0		0	5	0		0	5	0		0	5	0	
Liver;fatty infiltration	0	5	0		0	6	0		0	6	0		1	5	20	2
Liver;bile duct hyperplasia	3	5	60	1 ±0	3	6	50	1.7±0.6	6	6	100	1.5±0.7	2	5	40	1 ±0
Adrenal;cortical vacuolation	2	5	40	1 ±0	3	6	50	1 ±0	3	6	50	1 ±0	5	5	100	1.2±0.5
Lacrimal gland;focal adenitis	0	5	0		0	6	0		0	5	0		1	5	20	1
Liver;focal necrosis	1	5	20		1	6	17		1	6	17		2	5	40	
Liver;mononuclear cell foci	3	5	60		3	6	50		3	6	50		2	5	40	
Kidney;focal interstitial nephritis	4	5	80	1	6	6	100	1	5	6	83	1	3	5	60	1
Kidney;mineral foci	2	5	40		0	6	0		2	6	33		1	5	20	

[a] Soot administered orally in 0.75% aqueous methyl cellulose.

LEGENDS

Fig. 1. Body weight changes in male and female guinea pigs administered single, oral doses (Day 0) of Binghamton soot (A-C) or soot extract (D) in 0.75% aqueous methyl cellulose, or 2,3,7,8-TCDD in either corn oil (E) or methyl cellulose (F). Asterisks indicate individual mortalities during the observation period.

Fig. 2. Female rabbit 32 days after dermal application of 1.4 ml benzene extract equivalent to 500 mg Binghamton soot/kg, showing a serous inflammation at the application site.


A
DOSE, mg soot/kg
♂
0 (Methyl cellulose vehicle)
0 (Untreated)
0 (500 mg active carbon/kg)
100
500
B
DOSE, mg soot/kg
♀
0 (500 mg active carbon/kg)
10
100
500
C
DOSE, mg soot/kg
♀
0 (1250 mg active carbon/kg)
250
500 (o)
750 (•)
1000 (▫)
1250 (•)
D
DOSE, Extract equivalent to mg soot/kg
♀
0
4
20
100
500
1000
E
DOSE, µg TCDD/kg in methyl cellulose
♀
0 (Untreated)
12.5
0 (A) (methyl cellulose vehicle)
0.1 (•)
0.5 (▫)
2.5 (•)
20.0
F
DOSE, µg TCDD/kg in corn oil
♀
0 (Untreated)
0.1
0.5
0 (Corn oil vehicle)
2.5
12.5
20.0 (o)
% INITIAL BODY WEIGHT
DAYS
60
80
100
120
140
0
20
40