Bull. Environm. Contam. Toxicol. 19, 692-696 (1978)

Effects of Feeding a Polybrominated Biphenyl Flame Retardant (fireMaster BP-6) to Male Rats

Susan J. Harris[1], Helene C. Cecil[2], and Joel Bitman[1]

[1]U.S. Department of Agriculture, Agricultural Research Service, Animal Physiology and Genetics Institute, Beltsville, Md. 20705, [2]Send correspondence to: U.S. Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, APGI, Avian Physiology Laboratory, Building 262, Beltsville, Md. 20705

The accidental contamination of cattle and poultry feed in Michigan with fireMaster BP-6[1/] has caused much concern. BP-6, a mixture of polybrominated biphenyls (PBBs) containing an average of 6 bromine atoms in each biphenyl molecule, has been used as a flame retardant for resins and synthetic fibers. The PBBs closely resemble the polychlorinated biphenyls (PCBs) and are environmentally persistent.

In the Michigan incident, fireMaster BP-6 was accidently added to cattle feed instead of the nutritive supplement, magnesium oxide. Residues in the feed mill then contaminated chicken feed (CARTER, 1976). Concentrations of BP-6 in the cattle feed were estimated to be as high as 4,000 p.p.m. Farmers reported that for cattle fed the contaminated feed, feed intake and milk production were lower than normal. BP-6 also depressed reproduction (JACKSON and HALBERT, 1974) and was fatal to calves and local rodents. Cows in early pregnancy returned to estrus and those in later pregnancy gave birth to stillborn calves.

Little research has been published on the toxicity of BP-6. In preliminary studies, we found that oral injection of 100 mg BP-6/Kg body weight reduced sleeping times of male Japanese quail to one-half those of controls (CECIL et al. 1975); these results indicate an alteration in liver metabolism. In adult White Leghorn hens fed 20 p.p.m. BP-6 for 9 weeks, food consumption, egg production, and progeny growth were reduced significantly (LILLIE et al. 1974). The U.S. Food and Drug Administration established 0.3 p.p.m. as the tolerance for BP-6 in human food. Our objective was to determine in male rats the effects of 0, 50, 100, 150, and 200 p.p.m. BP-6 on food consumption, growth, and liver chemistry and to determine the residues of BP-6 in the body tissues.

1/ Michigan Chemical Corporation. Trade names are used only to provide specific information. Mention of a trade name does not constitue a guarantee or warranty of the product by the U. S. Department of Agriculture nor an endorsement by the Department over products not mentioned.

0007-4861/78/0019-0692 $01.00
© 1978 Springer-Verlag New York Inc.

Materials and Methods

Four month-old-male Sprague Dawley rats (484 to 606 g body weight) were randomly assigned to 5 groups and fed ad libitum ground rat chow containing 0, 50, 100, 150, and 200 p.p.m. of BP-6. Daily food consumption and weekly body weight gains were measured. After 10 weeks the rats were decapitated and adrenals, spleens, kidneys, testes, seminal vesicles, and livers were weighed. Liver glucose was determined by the glucose oxidase procedure (Worthington Biochemical Corp., Freehold, N. J. 07728). Glycogen was determined by the anthrone method of SEIFTER et al. (1950). Liver lipid was determined gravimetrically with a dried acetone:ethanol extract. Liver nucleic acids were extracted by the method of SCHNEIDER (1945). DNA was determined by measuring the deoxyribose content according to the Dische diphenylamine method of VOLKIN and COHN (1954). RNA was determined by ribose estimation according to the orcinol procedure of BROWN (1946).

BP-6 residues were determined in abdominal fat and liver tissue. Tissue samples were ground with sodium sulfate and extracted with petroleum ether. Lipid content was determined gravimetrically on a dried aliquot of the petroleum ether. BP-6 residues in the petroleum ether extracts were determined by gas liquid chromatography after florisil cleanup of the extract (FRIES et al., 1973). In this paper, single factor analysis of variance and Dunnett's test for multiple comparisons were used to determine statistical significance.

Results and Discussion

TABLE 1

Effects of Feeding 0, 50, 100, 150, and 200 p.p.m. BP-6 to 4-Month-Old Male Rats for 10 Weeks.

Dietary BP-6 (p.p.m.)	n	Daily food intake (g)	Initial body weight (g ± SE)	Body weight gain (g ± SE)
0	5	27	538 ± 17	55.6 ± 7.6
50	6	26	527 ± 10	65.2 ± 10.0
100	5	27	538 ± 19	69.2 ± 8.3
150	5	26	543 ± 17	47.8 ± 5.6
200	6	26	532 ± 15	27.8 ± 7.6

n = number of rats per treatment group.
SE = standard error of the mean.

TABLE 2

Effects on Livers of 4-Month-Old Male Rats Fed 0, 50, 100, 150, and 200 p.p.m. BP-6 for 10 weeks

Dietary BP-6 (p.p.m.)	n	Liver weight g ± SE	Fat %	mg glycogen / g ww ± SE	mg glucose / g ww ± SE	mg RNA / g ww ± SE	mg DNA / g ww ± SE
0	5	18.08 ± 0.41	7.45	60.6 ± 7.9	3.9 ± 0.7	15.0 ± 1.0	2.8 ± 0.2
50	6	23.48[a] ± 1.28	10.90	28.5[a] ± 6.6	2.6[a] ± 0.5	11.6[a] ± 0.2	2.3 ± 0.1
100	5	27.27[a] ± 0.51	9.32	38.9[a] ± 6.4	4.5 ± 0.3	11.8[a] ± 0.6	2.2 ± 0.1
150	5	29.82[a] ± 1.34	9.76	29.1[a] ± 5.0	2.6[a] ± 0.3	11.9[a] ± 0.3	2.2 ± 0.2
200	6	25.50[a] ± 0.87	11.54	34.8[a] ± 0.8	1.8[a] ± 0.4	12.3[a] ± 0.7	2.2 ± 0.2

[a] $P<0.05$ control vs treatment, Dunnett's test.
n = number of rats per treatment group.
SE = standard error of the mean.

Daily food intake of the males fed BP-6 did not differ significantly between treated and control fed males (Table 1). Gain in body weight, however, was lower, but not significantly so in rats fed 150 and 200 p.p.m. BP-6 than in the controls (47.8 g and 27.8 g, respectively in comparison with 55.6 g for the control). If, however, the rats fed 150 and 200 p.p.m. BP-6 were considered as one group, the weight gain would significantly differ ($P<0.05$) from that of the control. Subsequently we found that 600 p.p.m. of BP-6 greatly reduced food consumption and body weight of adult female rats (unpublished).

Organ weights (adrenals, 56 mg; spleen, 0.65 g; kidneys, 3.7 g; testes, 4.1 g; and seminal vesicles, 0.8 g) did not differ between control and treated rats. Liver weights, however, were significantly higher in all BP-6 treatment groups than in the control group (Table 2). The fat content of liver was 7.5% for controls and 11.5% for the rats fed 200 p.p.m. BP-6. In all treatment groups, the concentration of glycogen per unit of liver weight was significantly reduced. However, because liver weights increased, glycogen contents of the whole livers were similar. Liver glucose results were inconsistent, but indicated that glucose decreased both per unit of liver weight and per total liver (Table 2).

To determine whether BP-6 increased liver weight by increasing the number of liver cells or the growth of existing cells, we determined DNA and RNA in liver. The concentration of DNA in liver did not differ significantly between control and treated rats, but the concentration of RNA was significantly lower in treated than in control rats (Table 2). Thus the differences in liver weight were not due to increase in the number or size of liver cells but rather to increased amounts of fat, water, or other constituents.

TABLE 3

Accumulation of BP-6 in Liver and Abdominal Fat of Rats Fed Several Levels of BP-6 for 10 Weeks

Dietary BP-6 (p.p.m.)	n	Liver μg BP-6/g liver	Abdominal fat μg BP-6/g lipid
0	5	N.D.	31
50	6	55	864
100	5	107	3460
150	5	295	3574
200	6	245	3242

n = number of rats per treatment group.
N.D. = not detectable.

BP-6 accumulated in the liver and fat tissue of the rats (Table 3) with BP-6 concentrations 7 to 19 times higher in abdominal fat than in liver. Concentrations in abdominal fat were as high in rats fed 100 p.p.m. BP-6 as for those fed 150 and 200 p.p.m.; accumulation of BP-6 in the liver reached a plateau at a dietary level of 150 p.p.m. These data show that BP-6 concentrates in the liver and body fat as do the widely studied polychlorinated biphenyls (PCBs). In our study the levels of BP-6 that accumulated in rat tissues were higher than the levels of PCBs that accumulated when PCBs were fed (KIMBROUGH, 1974).

Young male rats fed 50 to 200 p.p.m. BP-6 showed no overt symptoms of toxicity. The effects of BP-6 on several liver parameters were, however, suggestive of the well-described hepatotoxic effects of chlorinated hydrocarbons. Additional studies are needed to determine the acute and chronic toxicity levels of BP-6 in rats.

References

BROWN, A. H.: Arch. Biochem. 11, 269 (1946).

CARTER, L. J.: Science 192, 240 (1976).

CECIL, H. C., S. J. HARRIS and J. BITMAN: Arch. Environ. Cont. Toxicol. 3, 183 (1975).

FRIES, G. F., G. S. MARROW, JR. and C. H. GORDON: J. Agr. Food. Chem. 21, 117 (1973).

JACKSON, T. F. and F. L. HALBERT: J. Am. Vet. Med. Assn. 165, 437 (1974).

KIMBROUGH, R. D.: Critical Rev. Toxicol. 2, 445 (1974).

LILLIE, R. J., H. C. CECIL, J. BITMAN and G. F. FRIES: Poultry Sci. 53, 726 (1974).

SCHNEIDER, W. C.: J. Biol. Chem. 161, 293 (1945).

SEIFTER, S., S. DAYTON, B. NOVIC, and E. MUNTWYLER: Arch. Biochem. Biophys. 25, 191 (1950).

VOLKIN, E. and W. E. COHN: Methods of Biochemical Analysis. Vol. I. D. Glick, Ed., Academic Press, New York, 287 (1954).