Contrib. Toxicol. 21,815-821 (1979)

Subchronic Effects of Piperonyl Butoxide on Carcinogen Metabolism in Hamster Liver

Marvin A. Friedman

Department of Pharmacology and MCV/VCU Cancer Center, Medical College of Virginia, Health Sciences Division, Virginia Commonwealth University, Richmond, Va. 23298

Piperonyl butoxide is a widely used pesticidal synergist which has been studied extensively as an inhibitor of mammalian mixed function oxidase (ANDERS 1968, FRIEDMAN et al. 1972, PHILPOTT and HODGSON 1971, FRANKLIN 1972). To this end, the acute inhibitory effects of piperonyl butoxide on several liver mixed function oxygenases have been determined in vivo and in vitro. Piperonyl butoxide binds to microsomal cytochromes inducing a spectral change not characterizable either as Type I or Type II (PHILPOTT and Hodgson 1971, 1972, FRANKLIN 1972). In contrast to the acute effects, in chronic experiments, piperonyl butoxide appears to have stimulatory effects on liver mixed function oxidase activities (KAMIENSKI and MURPHY 1971, GOLDSTEIN et al. 1973, WAGSTAFF and SHORT 1971).

Among the many substrates of these enzymes are chemical carcinogens. Both activation and deactivation of carcinogens are mediated via mixed function oxidases. For example, the involvement of cytochrome P-448 and arylhydrocarbon hydrocarbon hydroxylase in the carcinogenicity of 3,4-benzpyrene is now beginning to clarify (NEBERT et al. 1972). Similarly microsomal N-hydroxylation of acetylaminofluorene appears to be a necessary step in its activation to a carcinogen while microsomal ring hydroxylation is involved in preparation for excretion of this chemical (MILLER 1970). The situation is not as clear with reference to dimethylnitrosamine. There appear to be soluble enzymes which are as active in metabolism of dimethylnitrosamine as microsomes (CZYGAN et al. 1973). There is also a high affinity and a low affinity enzyme involved in dimethylnitrosamine metabolism which are under separate genetic control (ARCOS et al. 1976).

It is the purpose of the present communication to detail the effects of acute and subchronic exposure to piperonyl butoxide on the activities of each of these enzymes in hamster liver.

0007-4861/79/0021-0815 $01.40

MATERIALS AND METHODS

Syrian golden hamsters were obtained from Sprague Dawley, Inc. and used in all studies. These animals were housed in cages equipped with automatic watering devices and HEPA filtered air systems. Hamsters had constant access to Purina Chow throughout these studies. Piperonyl butoxide used in these studies was practical grade. The diet was prepared by mixing Purina Chow with 1% piperonyl butoxide and subsequent pelleting.

Hamsters were killed by decapitation, livers removed and homogenized in 0.25M sucrose, buffered with 0.1M phosphate (pH 7.4). Samples were centrifuged at 9000 x g for 15 min to prepare S-9. An aliquot of S-9 was centrifuged at 100,000 x g for an hour to prepare microsomes for arylhydrocarbon hydroxylase activity. Aminopyrene demethylase (FRIEDMAN et al. 1972), aryl-hydrocarbon hydroxylase (FRIEDMAN 1976) and dimethyl-nitrosamine demethylase (FRIEDMAN and COUCH 1976) assays have been described previously. In the case of acetylaminofluorene, the incubation contained in a 3 mL volume, 0.24 µMole NADP, 2 µMole ATP, 6 µMole glucose-6-phosphate, 120 µMole nicotinamide, 200 µMole KCl, 0.3 mL enzyme and 1 µCi acetylaminofluorene-^{14}C (1 µCi/50 µg). The solution was incubated for 1 h and the reaction stopped by addition of 38% KOH. Unhydroxylated acetylaminofluorene was extracted into 1:1 petroleum ether:diethyl ether and radioactivity determined by a liquid scintillation spectrometer. CPM were subtracted from a 0 time incubation mix to determine the fraction of acetylaminofluorene hydroxylated.

RESULTS

EFFECTS OF PIPERONYL BUTOXIDE ON AMINOPYRINE DEMETHYLASE ACTIVITY - The activity of hamster hepatic aminopyrine demethylase at various times up to 3 mo after feeding a diet 1% in piperonyl butoxide is shown in Table 1. There was no consistent inductive or inhibitory effect observed at any time up to 2 mo after treatment. At 7 days after treatment there was a 34% inhibition of aminopyrine demethylase activity while there was a 1.49 fold induction at 91 days both of which are statistically significant at the $p<0.05$ level.

EFFECTS OF PIPERONYL BUTOXIDE ON ARYLHYDROCARBON HYDROXYLASE ACTIVITY - The results of subchronic feeding of piperonyl butoxide on liver arylhydrocarbon hydroxylase activity as shown in Table 2. After one day diet consumption, there was a precipitous decrease in enzyme activity to 36% of control which continued as 64% of control on day two. At 7 days and later,

TABLE 1

EFFECTS OF DIETARY PIPERONYL BUTOXIDE ON HAMSTER LIVER AMINOPYRINE DEMETHYLASE ACTIVITY

TIME OF FEEDING (DAYS)	N	AMINOPYRINE DEMETHYLASE ACTIVITY[1] CONTROL	1% PIPERONYL BUTOXIDE
1	5	62.8 ± 4.4	62.4 ± 2.9
2	5	60.2 ± 6.4	71.0 ± 9.4
7	5	65.2 ± 4.7	43.1 ± 4.6
14	5	57.9 ± 7.5	65.8 ± 3.6
28	10	68.8 ± 20.9	66.0 ± 4.6
56	8	77.6 ± 9.9	62.7 ± 7.2
91	3	31.6 ± 1.9	47.0 ± 5.4

[1]EXPRESSED AS μMOLES CH_2O/MG PROT/HR

Groups of hamsters were fed Purina Chow or Purina Chow containing 1% piperonyl butoxide for the time intervals indicated above. Hamsters were sacrificed and Aminopyrine Demethylase Activity determined on the post-mitochondrial supernatant.

TABLE 2

EFFECTS OF FEEDING PIPERONYL BUTOXIDE ON HAMSTER LIVER MICROSOMAL ARYLHYDROCARBON HYDROXYLASE ACTIVITY

TIME OF FEEDING (DAYS)	N	ARYLHYDROCARBON HYDROXYLASE ACTIVITY[1] CONTROL	1% PIPERONYL BUTOXIDE
1	5	0.72 ± 0.07	0.26 ± 0.04
2	5	1.21 ± 0.10	0.77 ± 0.12
7	10	1.12 ± 0.10	1.29 ± 0.05
14	10	0.93 ± 0.10	1.27 ± 0.14
28	10	0.81 ± 0.13	1.03 ± 0.11
56	8	1.63 ± 0.14	1.62 ± 0.34
91	3	2.84 ± 0.47	2.52 ± 0.88

[1]n-MOLES 3-HYDROXYBENZPYRENE/μg PROTEIN/HR

Groups of hamsters were fed Purina Chow or Purina Chow containing 1% piperonyl butoxide for the time intergals specified above. These hamsters were sacrificed by decapitation and hepatic arylhydrocarbon hydroxylase activity determined on the microsomal preparation.

TABLE 3

EFFECTS OF DIETARY PIPERONYL BUTOXIDE ON HAMSTER LIVER DMN DEMETHYLASE ACTIVITY

TIME OF FEEDING (DAYS)	N	DMN DEMETHYLASE ACTIVITY[1] CONTROL	1% PIPERONYL BUTOXIDE
1	5	79.5 ± 11.2	72.1 ± 06.0
2	5	43.8 ± 05.7	64.2 ± 14.4
7	10	38.2 ± 09.3	64.5 ± 07.5
14	5	70.0 ± 14.8	82.6 ± 07.6
28	10	64.3 ± 06.8	85.9 ± 13.5
56	8	47.0 ± 0.51	73.4 ± 16.0
91	3	18.1 ± 03.1	26.9 ± 01.6

[1] μMOLES CH_2O/MG PROTEIN/HR

Groups of hamsters were fed Purina Chow or Purina Chow containing 1% piperonyl butoxide for the time intervals indicated above. Hamsters were sacrificed and DMN demethylase activity determined on the post-mitochondrial supernatant.

TABLE 4

EFFECTS OF FEEDING PIPERONYL BUTOXIDE ON HAMSTER LIVER ACETYLAMINOFLUORENE HYDROXYLATION

TIME OF FEEDING (DAYS)	N	ACETYLAMINOFLUORENE HYDROXYLATION[1] CONTROL	1% PIPERONYL BUTOXIDE
1	5	0.84 ± 0.04	0.78 ± 0.02
2	5	0.82 ± 0.04	1.11 ± 0.11
7	10	0.88 ± 0.05	0.86 ± 0.10
14	5	0.49 ± 0.13	0.54 ± 0.04
28	10	0.49 ± 0.06	0.51 ± 0.03
56	4	0.66 ± 0.08	0.65 ± 0.09
91	3	0.35 ± 0.01	0.52 ± 0.02

[1] μMOLES AAF HYDROXYLATED/MG PROTEIN/HR

Groups of hamsters were fed Purina Chow or Purina Chow containing 1% piperonyl butoxide for the time intervals indicated above. Hamsters were sacrificed and acetylaminofluorene hydroxylation determined on the post-mitochondrial supernatant.

there appeared to be little change in enzyme activity. It is noteworthy that the arylhydrocarbon hydroxylase activity of the piperonyl butoxide animals was consistently above control levels throughout the rest of the first month.

EFFECTS OF DIETARY PIPERONYL BUTOXIDE ON DMN DEMETHYLASE ACTIVITY - As can be seen in Table 3, there were no acute inhibitory effects of piperonyl butoxide on dimethylnitrosamine demethylase. From day 2 through 91, there appeared to be a stimulation of enzyme activity. This stimulation was 1.47, 1.69, 1.18, 1.34, 1.56, and 1.49 fold at 2, 7, 14, 48, 56, and 91 days. It appears that there was a biphasic stimulation of DMN demethylase with an early component in the first week and a subchronic component at 2 and 3 months.

EFFECTS OF DIETARY PIPERONYL BUTOXIDE ON ACETYLAMINOFLUORENE HYDROXYLATION - Data presented in Table 4 show that feeding piperonyl butoxide had no consistent effect on the hydroxylation of acetylaminofluorene. There was, however, a 1.35 fold stimulation of hydroxylation observed at day 2 and a 1.49 fold stimulation at day 91, both of which were statistically significant.

DISCUSSION

Data presented here show that piperonyl butoxide had no subchronic inhibitory effects on the aminopyrine demethylase, acetylaminofluorene hydroxylase or DMN demethylase. There was a transient, but marked inhibition of arylhydrocarbon hydroxylase activity. In marked contrast DMN demethylase activities were markedly stimulated by piperonyl butoxide with an early and late component to the induction. The magnitude of both stimulations were similar and were on the order of 50%.

When rats were fed a 1% PB diet, there was a marked induction of mixed function oxidase activity which was not reflected in the hamsters studied here (GOLDSTEIN et al. 1973). In rats, cytochrome P-450, hexobarbital oxidase and aniline hydroxylase were increased 2.42, 2.69, and 1.72 fold, respectively, at one week and 2.1, 1.65, and 1.88 fold, respectively, at 8 weeks. The differences between these species in response to PB is not clear. The mechanism for the highly specific induction of DMN demethylase in hamsters is also not clear. It is clear that there are both microsomal and nonmicrosomal components of this enzyme which may account for some of the differences. Although they were not statistically significant, acetylaminofluorene hydroxylase and arylhydrocarbon hydroxylase

tend to be increased over controls. It is important to note that acetylaminofluorene metabolism represents the sum of at least 2 enzymes, an N-hydroxylase and a ring hydroxylase which may respond differently to piperonyl butoxide. PB acutely inhibits each of the enzymes assayed in these studies. Single acute administration of PB inhibits aminopyrine demethylase activity in a dose dependent fashion for periods up to 24 h (FRIEDMAN et al. 1972, ANDERS 1968). Although acutely this is a non-competitive inhibition, at 16 h after treatment there are changes in both the Km and Vm. Similarly, PB induces a non-competitive dose dependent inhibition of DMN demethylase (FRIEDMAN and SANDERS 1976). By 48 h after PB treatment there was a 60% stimulation of DMN demethylase activity. Similarly, PB acutely inhibited rat liver methylcholanthrene metabolism (LEVINE 1972). However, in subacute studies, PB had little effect on rat liver or kidney arylhydrocarbon hydroxylase activity at 7, 14, or 21 days. PB increased arylhydrocarbon hydroxylase in intestinal mucosa by 1120%, 500%, and 615%, respectively, and in lung by 250%, 220%, and 11%, respectively. In the case of acetylaminofluorene in rats, PB acutely decreases overall metabolism of acetylaminofluorene but not N-hydroxyacetylaminofluorene (LEVINE 1971).

It may be noted from Tables 3 and 4 that the control mixed function oxidase activity in hamsters tended to drop markedly in the second and third months of the test. Similarly, aminopyrine demethylase activity also decreased in the third month. This may represent an age related phenomenon, the mechanism of which is not clear. It may be important to note that Syrian hamsters have a much shorter life-span than rats and mice and this three month span represents a greater portion of its life-span than a corresponding time period in rats or mice.

The practical relevance of these observations lies in the obvious ability of hamsters to adapt to piperonyl butoxide exposure and adequately regulate their liver enzyme systems. However, since complex regulatory systems are involved, it would be over-extending our observations to imply that piperonyl butoxide would have no effects on carcinogenesis in chronic experiments. The response of mixed function oxidases to piperonyl butoxide in carcinogen fed animals must also be determined in order to more fully understand and evaluate the significance of environmental exposure to piperonyl butoxide.

REFERENCES

ANDERS, M.W.: Biochem. Pharmacol. 17, 2367 (1968).

ARCOS, J.C., D.L. DAVIES, C.E.L. BROWN, and M.F. ARGUS: Pharmacologist 18, 211 (1976).

CZYGAN, P., H. GRIEM, A.J. GARRO, F. HUTTERER, F. SCHAFFNER, H. POPPER, O. ROSENTHAL, AND D.Y. COOPER: Canc. Res. 33, 2983 (1973).

FRANKLIN, M.R.: Biochem. Pharmacol. 21, 3287 (1972).

FRIEDMAN, M.A., E.J. GREENE, R. CSILLAG, and S.S. EPSTEIN: Toxicol. Appl. Pharmacol. 21, 419 (1972).

FRIEDMAN, M.A.: Res. Comm. in Chem. Path. and Pharmacol. 15, 541 (1976).

FRIEDMAN, M.A. and D.B. COUCH: Biochem. Pharmacol. 25, 2709 (1976).

GOLDSTEIN, J.A., R. HICKMAN, and R.D. KIMBROUGH: Toxicol. Appl. Pharmacol. 26, 444 (1973).

KAMIENSKI, F.X. and S.D. MURPHY: Toxicol. Appl. Pharmacol. 18, 883 (1971).

MILLER, J.A.: Cancer Res. 30, 559 (1970).

NEBERT, D.W., W.F. BENEDICT, J.E. GIELEN, F. OESCH, and J.W. DALY: Molec. Pharmacol. 8, 374 (1972).

PHILPOTT, R.M. and E. HODGSON: Chem. Biol. Interactions 4, 185 (1971).

PHILPOTT, R.M. and E. HODGSON: Mol. Pharmacol. 8, 204 (1972).

WAGSTAFF, D.J. and C.R. SHORT: Toxicol. Appl. Pharmacol. 19, 54 (1971).