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Potentiation of Methylmercury Toxicity by Piperonyl Butoxide

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Piperonyl butoxide (PB) is a widely used pesticidal synergist which has been studied extensively as an inhibiter 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 PB on several liver mixed function oxygenases have been determined in vivo and in vitro. PB binds to microsomal cytochromes inducing a spectral change not characterizable either as Type I or Type II (PHILPOTT and HODGSON, 1971; FRANKLIN, 1972). In contrast to the acute effects, in chronic experiments PB appears to have stimulatory effects on liver mixed function oxidase activities (KAMIENSKI and MURPHY, 1971; WAGSTAFF and SHORT, 1971).

Methylmercury is an environmental pollutant having been associated with disasters in Minamata, Niigata, Iraq and Sweden. Analysis of data from these disasters have produced estimates of risk associated blood levels of methylmercury. The biological half life of methylmercury in people is 70 days with the vast majority excreted unchanged (ABORG et al, 1969). The metabolic fate of methylmercury is unclear except that inorganic mercury is produced. In rats the biological half life is 14 days (CLARKSON, 1972).

It is the purpose of the studies presented here to determine the effects of an inhibitor of mixed function oxidase activity on methylmercury toxicology.

MATERIALS AND METHODS

Male Sprague Dawley rats weighing 100 to 115 grams were used in these studies. These rats were fed ad libitum a semisynthetic diet described previously (FRIEDMAN et al, in press). Rats were housed 2 per cage in clean air cages equipped with automatic watering and self flushing. Methylmercury chloride was dissolved in DMSO, bound to casein and added to diets as the casein preparation at levels of 0, 20 and 40 ppm. Each experimental group consisted of 8 rats. Rats were observed daily and weighed weekly. Each animal was observed for neurotoxicity manifesting itself as crossed hind legs. Feeding studies were carried out for 12 weeks.

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RESULTS

The effects of a diet 1% in piperonyl butoxide on rat body weight is shown in Figure 1. The growth rate in the 1% PB fed animals was less than control throughout the study. Although the initial body weights were similar, the PB group weighed 87 grams less than controls at the end of the study.

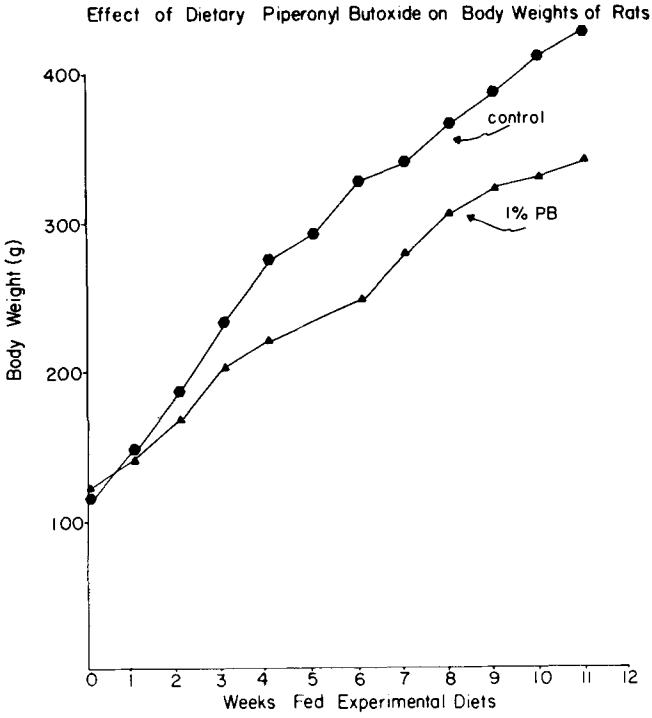

FIGURE 1 Groups of 8 rats were fed an experimental diet containing 1% piperonyl butoxide and body weight determined weekly.

The body weights of rats fed 0, 0.5 and 1.0% PB in combination with 20 ppm methylmercury are shown in Figure 2. There was a dose dependent decrease in body weight through the study. In week 9 of the study methylmercury effects on body weight became apparent as the controls began to lose weight. Rats fed 0.5% PB lost weight at a far more rapid rate. There appeared to be 2 periods of weight loss in rats fed 1% PB. The first started at week 4 and the latter at week 8.

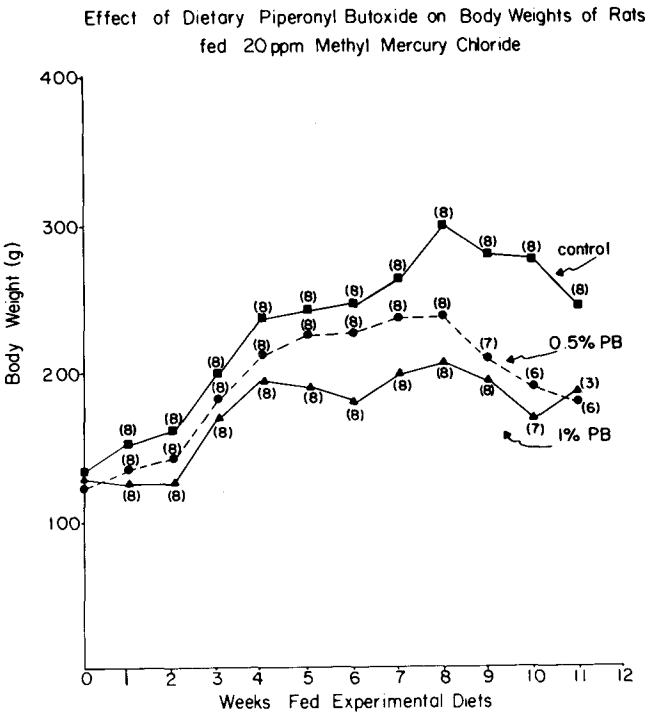

FIGURE 2. Groups of 8 rats were fed 20 ppm methylmercury chloride containing experimental diet which also contained either 0, 0.5 or 1% piperonyl butoxide and body weights determined weekly.

Body weights of rats fed 0, 0.5 and 1% PB in combination with 40 ppm methylmercury are presented in Figure 3. Control rats gained weight through week 3 and then had a precipitous weight loss ending in death. Rats fed 0.5% PB lost weight throughout the study. In the case of rats fed 1% PB, there was a precipitous weight loss in the second week followed by a weight gain up to week 4. At this point there was precipitous weight loss followed by death.

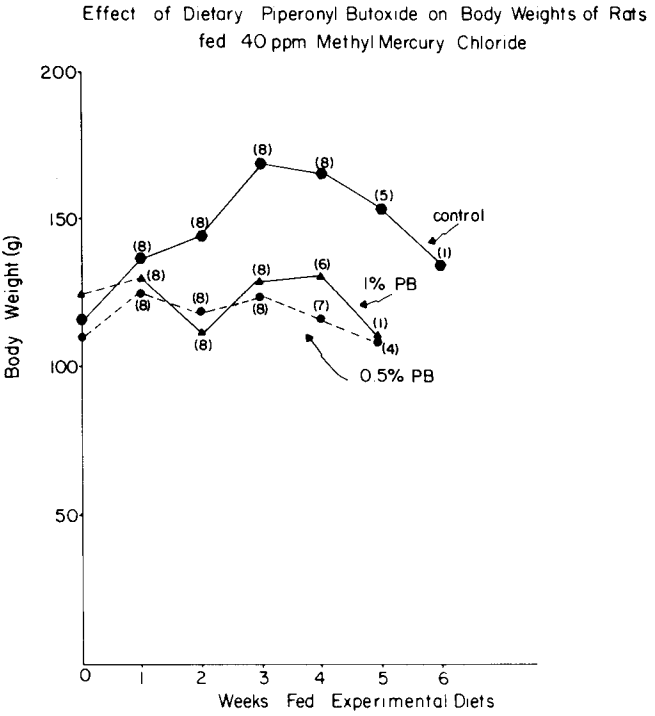

FIGURE 3. *Groups of 8 rats were fed a 40 ppm methylmercury chloride containing experimental diet which also contained either 0, 0.5 or 1% piperonyl butoxide and body weights determined weekly.*

The influence of dietary PB on mortality of experimental rats fed methylmercury is shown in Figure 4. The mortality pattern associated with 1% PB diet with 40 ppm methylmercury is similar to that of the 40 ppm comtrol except that there was a week difference in time of onset of toxicity. The rats fed 0.5% PB 40 ppm methylmercury died sooner than controls but later than the 1% PB counterparts. The dose response to 20 ppm methylmercury was not as clear. The only mortality observed in controls was in week 11 when 63% of the rats died. The rats fed 1% PB showed parallel mortality only initiating 1-2 weeks earlier. In the case of 0.5% PB single animals died in week 9 and 10 with the majority dying in week 11. There was still, however, some apparent dose response.

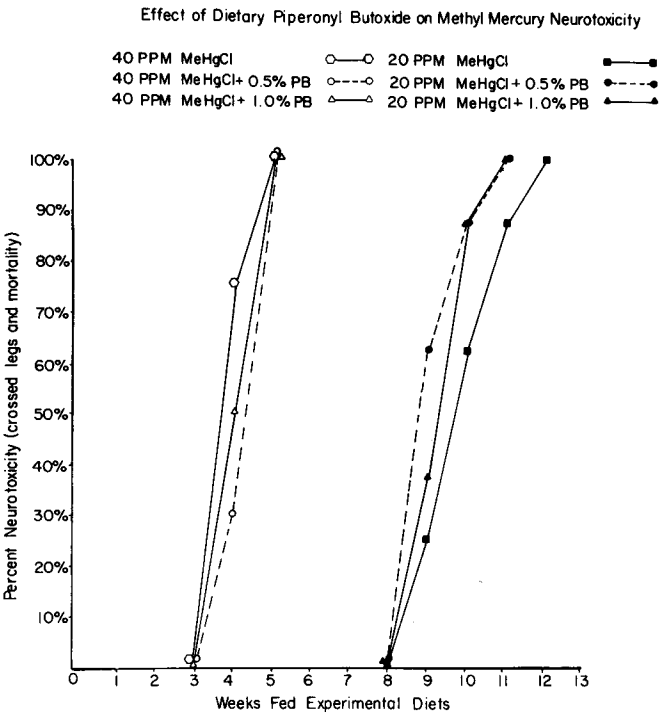

FIGURE 4. Mortality in rats fed 20 or 40 ppm methylmercury chloride in combination with 0, 0.5 or 1% piperonyl butoxide.

The appearence of neurotoxicity is shown in Figure 5. In week 4 there was a clearly dose dependent, highly reproducible increase in neurotoxicity. At this time 6, 4, or 2 rats showed neurotoxicity when fed 1, 0.5 or 0% PB, respectively. At 20 ppm, there was a dose response which closely parelleled the effects observed at 40 ppm. At week 9, 5, 3 or 2 rats were affected at 1, 0.5 and 0% PB, respectively, while at week 10, 7, 7 and 5 were affected at the corresponding doses.

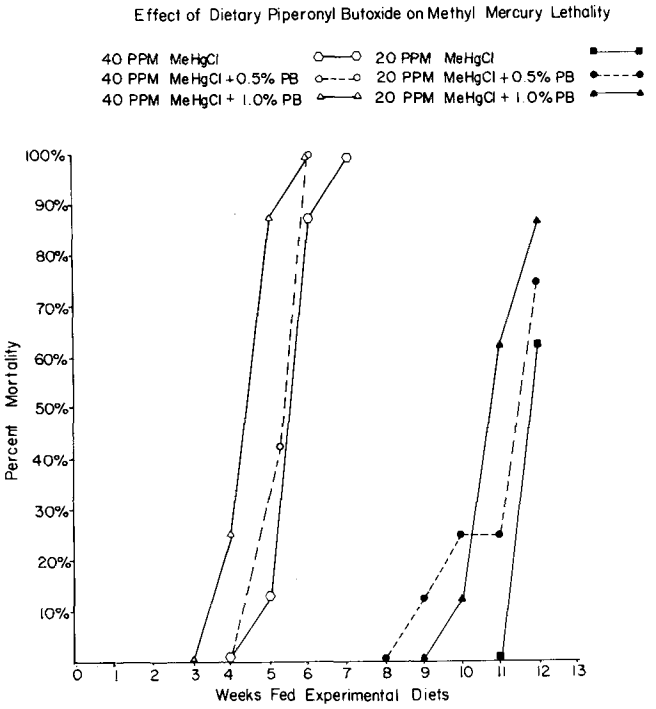

FIGURE 5. Neurotoxicity in rats fed 20 or 40 ppm methylmercury chloride in combination with 0, 0.5 or 1% piperonyl butoxide.

DISCUSSION

Data presented here are clear and consistent that PB synergises methylmercury poisoning in a dose dependent fashion. All three measurements, namely body weight, mortality, and neurotoxicity, responded in identical fashions. The magnitude of the effect appears to be 1 week both at 20 and 40 ppm methylmercury. This difference is not related to increased methylmercury intake as there was if anything decreased food and methylmercury intake in the PB fed animals. The rats fed 1% PB did not grow as fast as their corresponding controls.

The mechanism of action of PB in these studies is not clear. Although it acutely inhibits liver mixed function

oxidases, chronically it appears to increase the activity (KAMIENSKI and MURPHY, 1971). It is, therefore, possible that the 1 week delay may represent the only time PB inhibits the mixed function oxidase. It is currently not known whether the action of mixed function oxidases on methylmercury increases or decreases its toxicity. It appears likely from pharmacokinetic studies that the liver demethylates mercury and by extrapolation one might expect liver mixed function oxidases to detoxify methylmercury (NORSETH and CLARKSON, 1970).

Dietary methylmercury, itself, influences hepatic mixed function oxidase activity. Feeding quail doses of dietary methylmercury as low as 4 ppm caused an increase in pentobarbital sleeping time (GOLDSMITH and SOARES, 1975). Similarly, single ip injection of 5 mg/kg methylmercury chloride caused a decrease in rat liver cytochrome P-450 and an inhibition of hepatic mixed function oxidase activity quantitatively similar to the loss of P-450 (ALVARES et al, 1972). These observations raise the possibility that the PB activity is short-lived because the methylmercury itself produces the same effects.

However, it is clear from these data that inclusion of a non-metallic, reasonably non-toxic chemical into a methylmercury contaminated diet did increase the metal toxicity. Therefore, one can raise questions about human diets containing other non-metallic contaminants. It appears that levels of other materials bear directly on the toxicology of methylmercury. We are currently evaluating other environmental constituents and nutritional deficits.

SUMMARY

Methylmercury (MeHg) is an extremely potent neurotoxin about 25% of which is degraded in vivo to inorganic mercury. Piperonyl butoxide (PB) is a widely used pesticidal synergist which inhibits many mammalian detoxification reactions. In a preliminary experiment with the high doses of PB and MeHg, PB induced a 12% decrease in mean survival time and a 20% decrease in mean latency time to neurotoxicity. The weight loss in PB-MeHg group was far greater than the control MeHg group. In a dose response experiment, mean survival times in rats fed 40 ppm MeHg-Cl were 5.75, 5.3, and 5.0 weeks at 0, 0.5, and 1% PB, respectively. By the ninth week 25% of rats fed 20 ppm MeHg-Cl showed neurotoxicity and 63% of the 0.5% PB fed showed neurotoxicity with some mortality. In experiments at 20 ppm MeHg-Cl both PB fed groups weighed considerably less than corresponding controls.

ACKNOWLEDGEMENTS

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