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SUBACUTE TOXICITY STUDY OF PIPERONYL BUTOXIDE IN MALE MICE: HISTOLOGICAL EXAMINATION OF MAJOR ORGANS

By

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ABSTRACT

Male CD-1 mice (20 mice/group) were administered piperonyl butoxide in the diet at levels of 0 (control), 0.1, 0.3, and 0.6% for *4* weeks (5 days/ week). Piperonyl butoxide induced essential hepatotoxicity, renal and brain toxicity with a dose-response relationship. Oedema and disturbance of hepatic architecture in liver, marked widening of interstitial spaces, marked dilatation of renal tubules with flattened epithelium in kidneys, and patches of increased eosinophilia in the nuropls in brain were observed in the high treated group (0.6%) of piperonyl butoxide. These results indicated that piperonyl butoxide caused essential histological changes in liver, kidney, and brain of male mice.

INTRODUCTION

Piperonyl butoxide (α-[2-(2-butoxyethoxy) ethoxy}-4,5-methylenedioxy-2-propyltoluene) is a commonly used synergist for naturally

derived pyrethrins. Piperonyl butoxide (PBO) is registered for applications on a variety of crops (including fruits and vegetables), livestock, lawn and turf, mosquitoes, agricultural premises, food-processing plants, dairies, pets, and residential dwellings. Nearly *50%* of the amount of PBO produced is distributed for use in indoors residential products. As a result, humans could potentially be exposed to low levels of PBO from dietary and residential sources (Philips *et al.*, 1997).

A number of studies have examined the chronic effects of PBO in the mouse and rat. In a 2-year study, PBO treatment did not result in any liver lesions in male CD-1 mice fed 30 mg/kg/day PBO or in female mice fed 30 and 100 mg/kg/day PBO (Philips *et al.*, 1997). However, PBO treatment resulted in increases in eosinophilic liver nodules in male CD-1 mice at dose levels of 100 and 300 mg/kg/day and in female mice at a dose level of 300 mg/kg/day (Butler, 1996). At dietary levels of 0.6 and 1.2% (850 and 2000 mg/kg/day PBO, respectively), where mortality and/or marked effects on body weight were observed, PBO was reported to produce hepatocellular adenoma and carcinoma in male CD-1 mice (Takahashi *et al.*, 1997). Liver and kidney damage was found in rats were given 0.5-3.0% piperonyl butoxide in the diet for 1-13 weeks (Glodstein *et al*, 1973, Maekawa *et al.*, *1985*, Fujitani *et al.*, 1992).

We now describe a subacute toxicity study of piperonyl butoxide in male mice as a follow-up on the previous subacute test (Fujitani *et al.*, 1992).

MATERIAL AND METHODS

Test Material:

Piperonyl butoxide (α-[2-(2-butoxyethoxy) ethoxy}-4,5-methylenedioxy-2-propyltoluene) was obtained from the EPA (Environmental Protection Agency, R.T.P., N.C. USA). The purity of the chemical was 98.00 % (Lot: 121-112A).

Test Species and Husbandry:

Male ICR (CD-1) mice, approximately 10 weeks old, were obtained from the High Institute of Public Health, Alexandria University, Alexandria, Egypt. All mice were examined for health status and acclimated to the laboratory environment for 2 weeks prior to use. The animal room was designed to maintain temperature at 25^{0}C, relative humidity at approximately 50 % and a 12 hr light: 12 hr dark photoperiod. All animals were housed in stainless- steel cages and given standard diet and water *ad Libitum* throughout the study.

Experimental design:

Piperonyl butoxide was administered in the diet to 60 male mice (20 mice/ group) at dietary levels of 0.1, 0.3, and 0.6 % for 4 weeks *(5* days/week). The 20 mice in the control were given the basal diet (sterilizable diet, the High Institute of Public Health, Alexandria, Egypt). Feed consumption was recorded daily for each mice every week.

Necropsy and Histological Examination:

At the termination of administration, all of the surviving mice were killed by deep ether anesthesia. Body, liver, brain, and kidneys weights were recorded. These organs were fixed with buffered 4% formaldehyde solution, sectioned, stained routinely with hematoxylin and eosin and examined by light microscope.

Statistical Analysis:

Statistical analysis were performed with the Statistical Analysis System SPSS (Noursis, 1994). Organ weights were subjected to analysis of covariance using the final body weight as the covariant. Average food intake was assessed with Bonferroni multiple comparison tests after Kruskal-Wallis test. A P-value of less or equal to 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Food and chemical intake:

While average food intake was increased in the 0.1, and 0.3% treated groups. It was normal in the 0.6% treated group compared to the control. Therefore, piperonyl butoxide was taken in proportion to dietary levels in the treated weeks (Table 1).

Clinical signs of toxicity:

Clinical signs of toxicity were observed in the treated groups. Abnormal behaviour and mortality were observed in the treated males during the course of the experiment. Mortality was observed in two males in the 0.3% treated group and four males in the 0.6% treated group. The activity of males was increased in all treated groups especially in the 0.6% treated group compared to the control. Males moved long distances rapidly.

Body and organ weights:

Body and organ weights at termination are given in Table (2). No significant effect in male body weights in any of piperonyl butoxide treated groups, were noted. Therefore, there was no significant differences were found between relative and absolute organ weights. Kidneys and liver weights were increased in all the treated groups. No effects in the brain weights in any of the treated groups, were noted. A possible explanation for these findings is that PBO caused hepatocellular adenoma and carcinoma in male CD-1 mice (Takahashi *et al.*, 1994). Besides the kidneys of treated rats (0.6% PBO) showed atrophy of epithelium in the proximal convoluted tubules which might cause increase of kidneys weight (Butler *et al.*, 1998).

Histological Examination:

Liver:

Histological changes in liver are shown in Fig.(1). Dose-related hepatotoxic signs as mild widening of intercellular spaces, congestion of

central veins, and cellular infiltration around the portal tract were shown in the low dose group (0.1%). On increasing the dose at 0.3%, localized area of cellular infiltration, some cells are degenerated and vacuolated with dark shrunken nuclei, and the hepatic architecture as diffuse affection of hepatocytes in the fannay degenerative changes, interstitial and disturbance of hepatic architecture were observed in the high dose group (0.6% PBO).

Although the irritation of the hepatocytes noticed in the present study, may be an early step in the process of carcinogenesis, piperonyl butoxide has been considered to be noncarcinogenesis (Maekawa *et al., 1985).* There are many differences in experimental conditions, the most important factors responsible for the hepatotoxicity are the higher dosages and long-term exposure used (Takahashi *et al.*, 1994). The histological findings in the present study were in consistent with the published data suggesting that liver damage was found when rats were given 0.5-3.0% piperonyl butoxide in the diet for 1-13 weeks (Goldstein *et* at., 1973, Maekawa *et al.*, 1985, Fujitani *et al.*, 1992). The mechanism (s) of hepatotoxicity by PBO and interstitial oedema remains to be fully elucidated (Grasso and Hinton, 1991, Grasso *et al.*, 1991, Phillips *et al.*, 1997). However, the mutagenic and promotional effects of PBO are clearly important and oedema formation may involve the promotion of particular populations of hepatocytes through differential effects on cell replication, growth factors, intercellular communication, etc. (Grasso and Hinton, 1991, Grasso *et al.*, 1991, Jirtle, 1994, Anderson *et al.*, 1995).

Kidneys:

The histological changes in kidney are shown in Fig. (2). The present renal findings as mild dilatation of renal tubules with mild flattening of the epithelial linnings, loss of brush border of the proximal tubules were observed in the 0.1% treated group. Some tubules showing lumen filled with exfoliated cells and cell debris were shown in the same dose group. On increasing the dose, widening of interstitial and perivascular spaces with marked mononuclear cellular infiltration (interstitial and perivascular) were

observed at 0.3% PBO. In the same treated group (0.3%), the renal tubules were dilated with low linning epithelial covering, and the tubular lumen were filled with cellular debris. In the high treated group 0.6%, marked widening of interstitial spaces, marked dilatation of renal tubules with flattened epithelium, loss of cellular details and brush borders, and some renal corpuscles showing litiro were observed. These findings were in consistent with the published results showed that PBO caused degenerative lesions of pulmonary, and alveoli, and tubular damage in kidneys (Takahashi *et al.*, 1994).

Brain:

The histological changes in the brain are shown in Fig.(3). The present histological results of the brain showed brain affection as disturbance in normal appearance of cortical layers at 0.1% PBO. Degenerative changes in the form of cellular vacillation were observed in the treated group 0.3%. Besides, the nuclei were dark and shrunken in the same dose and there was disarrangement of cortical cells. In the high treated group 0.6%, patches of increased eosinophilia in the nuropls. These findings are in consistent with the published results which showed that piperonyl butoxide was concentrated in the brain (Tanaka *et al.*, 1994).

The present study indicated that liver, kidneys, and brain damage can be associated with PBO exposure at doses of 0.1% and greater. This could be due to the toxicity of piperonyl butoxide, which was directed primarily to the liver, kidney, and brain. The toxicity of piperonyl butoxide is attributed to its an unmetabolized form, together with its slow rate of elimination from the body, its ability to inhibit microsomal enzymes and thereby enhance toxicity of other chemicals (Fishbein *et al.*, 1969, Epstein *et al*, 1967).

Under the condition of this study and at the tested levels, piperonyl butoxide induced liver, kidneys, and brain damage in male mice.

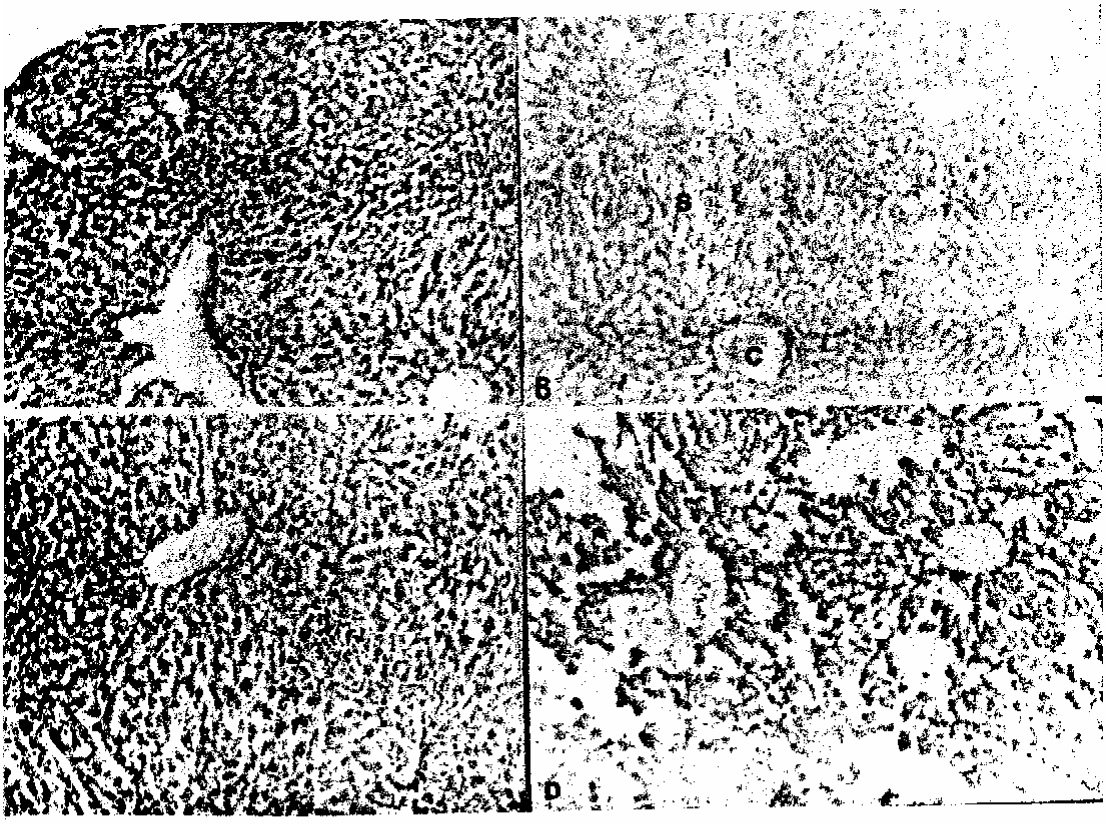

Fig. 1: Photomicrographs of liver from (A) control mouse, (B) from mouse treated with 0.1% PBO showing mild widening of intercellular spaces (S), congestion of central veins (C), and cellular infiltration (I) around the portal tract, (C) from mouse treated with 0.3% PBO showing location area of cellular infiltration (I), some cells are degenerated and vacuolated with dark shrunken nuclei (V), and (D) from mouse treated with 0.6% PBO showing diffuse affection of hepatocytes and intestinal oedema. The hepatic architecture is loss (H & E (X 100)).

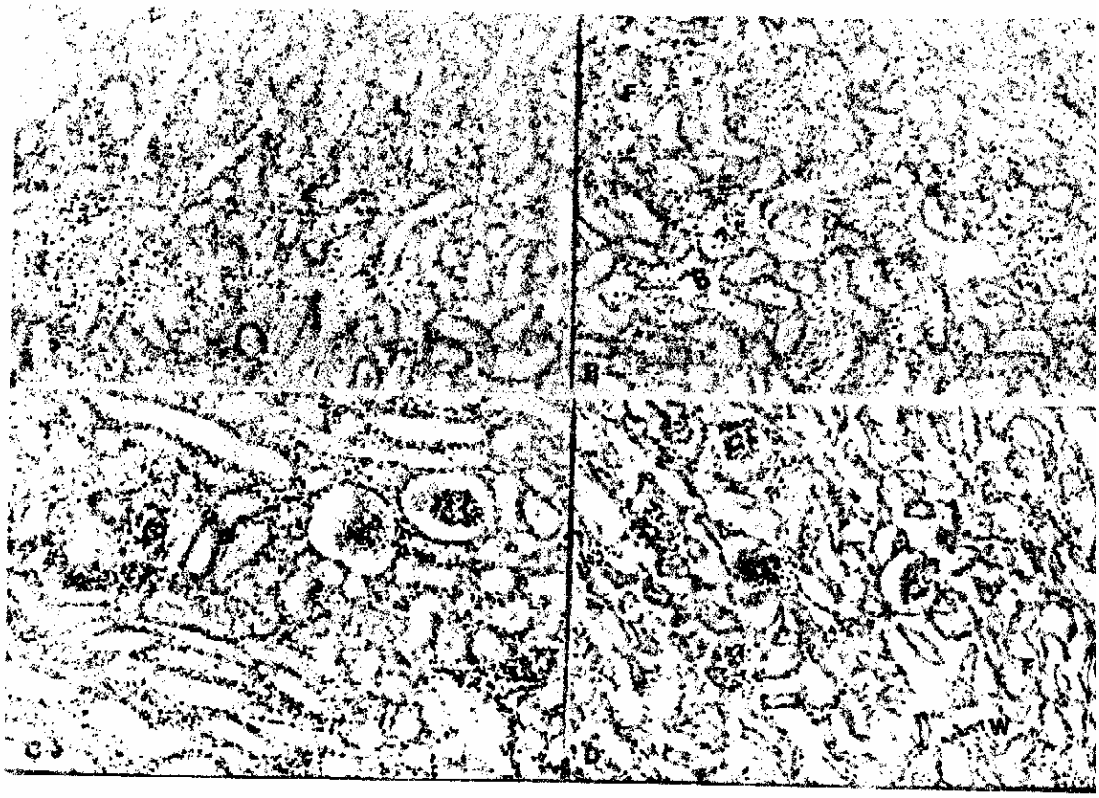

Fig. 2: Photomicrographs of kidney from (A) control mouse, (B) from mouse treated with 0.1% PBO showing mild dilation of renal tubules with mild flattening of the epithelial lining (F) and some tubules showing lumen filled with exfoliated cells and cell debris (B), (C) from mouse treated with 0.3% PBO showing widening of interstitial and perivascular spaces with marked mononuclear cellular infiltration (C), and (D) from mouse treated with 0.6% PBO showing marked widening of interstitial spaces (W), marked dilation of renal tubules with flattered epithelium (F), and some renal corpuscles showing obliterated Bowman's space (G) (H & E (X 100)).

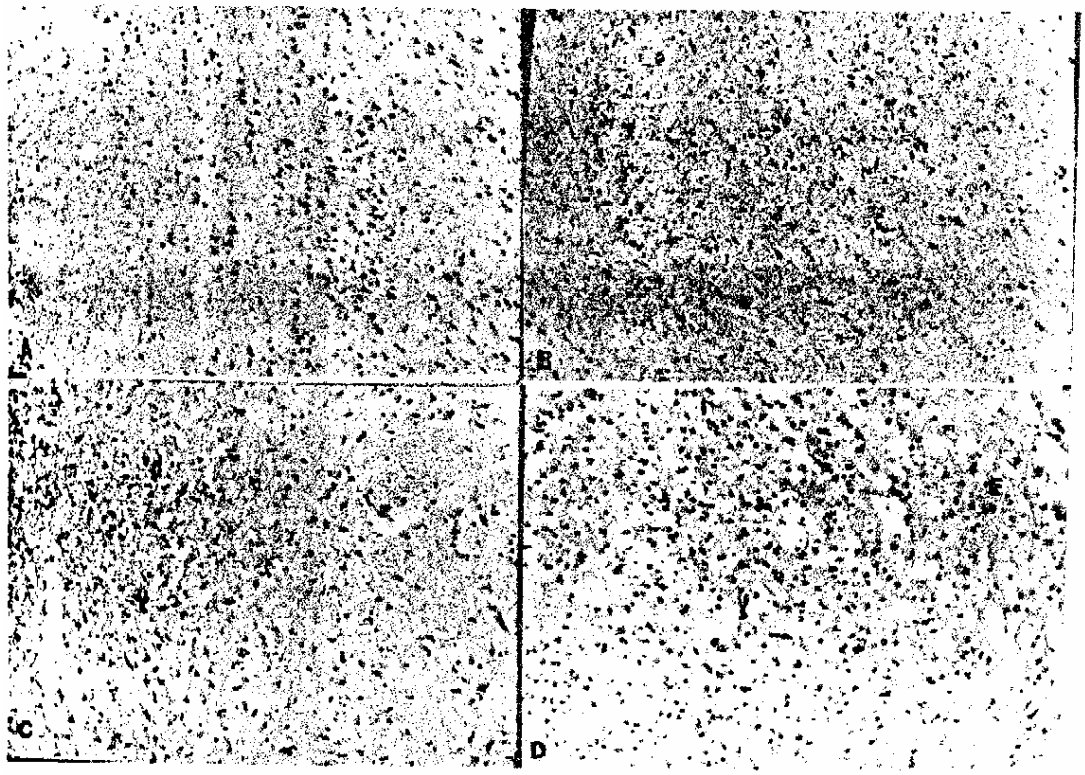

Fig. 3: Photomicrographs of brain from (A) control, (B) from mouse treated with 0.1% PBO showing more or less the normal appearance of cortical layers, (C) from mouse treated with 0.3% PBO showing degenerative changes in the form of cellular vacuolation (D), and there is disarrangement of cortical cells, and (D) from mouse treated with 0.6% PBO showing vacuolated cells (V), and patches of increased eosinophilia in the nuropls (E) (H & E (X 100)

Table (1): Average Food and Chemical Intake of Male Mice Treated with Piperonyl Butoxide

	Dose Levels (%)			
	0	0.1	0.3	0.6
	Food intake (g/kg/day)			
Weeks of treatmenta [a]				
1	264.61 ± 1.19	284.98 ± 4.15*	279.82 ± 5.16*	261.94 ± 3.11
2	255.87 ± 2.10	285.55 ± 2.70*	280.10 ± 3.01*	253.26 ± 4.22
3	255.60 ± 3.17	285.55 ± 1.77*	270.99 ± 2.7*	256.51 ± 5.00
4	265.70 ± 2.81	285.55 ± 3.70*	284.66 ± 4.90*	271.44 ± 2.99
	Chemical intake (mg/kg/day)			
Weeks of treatment [a]				
1	-	284.98 ± 5.70	839.46 ± 2.70	1571.64± 1.70
2	-	285.98 ± 2.70	840.30 ± 5.11	1519.56± 2.77
3	-	285.55 ± 3.15	812.97 ± 2.17	1539.06± 5.11
4	-	285.55 ± 1.70	853.98 ± 1.80	1628.64± 3.00

[a] Data are presented as mean ± S.D.

* Significant different from control at $P \leq 0.05$.

Table (2):Body and Organ Weights in Male Mice Treated with Piperonyl Butoxide[a]

	Dose levels (%)			
	0	0.1	0.3	0.6
Body weight (g)	36.62 ± 0.15	35.02 ± 0.15	35.13 ± 0.70	36.84 ± 0.20
Absolute weight [b]				
Liver	1.50 ± 0.14	2.45 ± 0.22*	2.35 ± 0.14*	2.99 ± 0.13*
Kidneys	0.45 ± 0.04	0.54 ± 0.05*	0.55 ± 0.08*	0.59 ± 0.06*
Brain	0.33 ± 0.04	0.33 ± 0.06	0.33 ± 0.04	0.36 ± 0.06
Epididymis	0.06 ± 0.08	0.04 ± 0.01*	0.04 ± 0.00*	0.04 ± 0.00*
Testes	0.27 ± 0.02	0.15 ± 0.21**	0.14 ± 0.79**	0.15 ± 0.0**
Relative weight [c]				
Liver	4.10 ± 0.54	7.00 ± 0.90*	10.93 ± 0.54*	9.58 ± 0.44*
Kidneys	1.23 ± 0.15	1.54 ± 0.22*	1.53 ± 0.34*	1.60 ± 0.20*
Brain	0.90 ± 0.15	0.94 ± 0.20	0.94 ± 0.16	0.98 ± 0.27
Epididymis	0.16 ± 0.02	0.11 ± 0.02*	0.11 ± 0.12*	0.11 ± 0.01*
Testes	0.74 ± 0.05	0.43 ± 0.10**	0.40 ± 0.18**	0.41 ± 0.03**

[a] Twenty mice per group were examined. Data are presented as mean ± SD.

[b] Organ weights (g)/ body weight.

[c] Organ weights/ 100g body weight.

* Significant different from control value at $P \leq 0.05$.

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الملخص العربي

دراسة السمية التحت حادة لمركب البيرونيل بيوتوكسيد فى ذكور فئران التجارب : دراسة هستولوجية فى الاعضاء الاساسية.

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تم إعطاء ذكور فئران التجارب CD-1 (٢٠ مجموعة) جرعات من مركب البيبرونيل بيوتكسيد (١,. ٣٤,.٦٤,.%) لمدة (٤_٢٠يوم)
أوضحت النتائج ان مركب البيبرونيل بيوتيكسيد يحدث سمية علي الكبد والكلي والمخ يحدث هذا التأثير كنتيجة لـــ A dose response relationship . أحدث الـ PBO تضخم كبدي وإزالة للترتيب الخلوي الوجود في خلايا الكبد وكذلك توسيع في الفراغان الموجودة بين الخلايا الكلوية .كما احدث تمدد واضح في الأنابيب الكلوية مع تسطح في خلايا الابيثليم الكلوية .أما في خلايا المخ ،فقد احدث الPBO زيادة في خلايا Eosinophilia الموجودة في nuropls. كم هذة التغيرات الهستولوجية وجدت علي كل التركيزات المستخدمة ولكن التأثير الواضح كان علي الجرعة ٦و%PBO. أوضحت هذه النتائج أن مركب PBOيحدث تغييرات هستولوجية معنوية في الكبد والكلي والمخ لذكور فئران التجارب.