REVIEWER

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

APR 28 1988

006668

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Reg. No.: 4816-72 - Piperonyl Butoxide: Review of a chronic feeding/oncogenicity study submitted by the Piperonyl Butoxide Task Force.

TOX CHEM No.: 670
TOX PROJECT No.: 8-0259
Record No.: 208305

FROM: John Doherty 4/15/88
Toxicology Branch
Hazard Evaluation Division (TS-769)

TO: Phil Hutton
Product Manager #17
Registration Division (TS-767)

and

Geraldine Werdig
Chief
DATA-CALL-IN Section
Registration Division (TS-767)

THRU: Edwin Budd
Section HEad
Toxicology Branch
Hazard Evaluation Division (TS-769)

Mr. John D. Conner, Jr. of the law firm McKenna, Conner and Cuneo acting on behalf of their client the Piperonyl Butoxide Task Force has submitted a chronic feeding/oncogenicity study to meet the toxicity data requirement to support registrations and tolerances for piperonyl butoxide. Refer to the letter from Mr. Connor addressed to Ms. Geraldine Werdig of EPA dated August 31, 1987.

This study was reviewed by Toxicology Branch (TB) and the following comments apply.

Toxicology Branch Comments.

1. The study was reviewed and determined to be CORE GUIDELINES.

2. The following "one liner" applies.

NOEL (absolute) < 30 mg/kg/day. At this level there are increases in the weight of the liver for females and a trend for males.

NOEL (toxicity) = 30 mg/kg/day.

LEL = 100 mg/kg/day. At this level there are increases in liver weight, increases in cholesterol levels, increased hepatic "focal mixed cells". All in females. Trend for increased liver weight in males.

at 500 mg/kg/day there were increase liver weights and increased hypertrophy of hepatocytes in males and females. Increased hepatic "focal mixed cells" and increased serum cholesterol in females. Increases in "pigment in follicles" and hyperplasia if follicular cells of the thyroid in both sexes. Decreased body weight gains in both sexes.

No unequivocal evidence that PB induced tumors in rats was generated by this study.

Levels tested: 0, 30, 100, and 500 mg/kg/day.

2. As indicated above there were effects noted at the lowest dose levels tested. These were signs of liver weight increases particularly in females with evidence of a trend for this effect in males. At the lowest dose level, these weight increases were not associated with pathological changes. The relevance of these findings with regard to setting the ADI will not be addresses in this memo but will be addressed when the ADI is evaluated by the RfD ADI committee.

Reviewed by: J.D. Doher 4/15/88
Section II, Tox. Branch (TS-769C)
Secondary reviewer: E.R. Budd 4/15/88
Section II, Tox. Branch (TS-769C)

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DATA EVALUATION REPORT

STUDY TYPE: 83-1: Chronic toxicity rat and
83-3: Oncogenicity rat

TOX. CHEM. NO.: 670

ACCESSION NUMBER: 403237-01 (nine volumes)

MRID NO.: Not provided.

TEST MATERIAL: Piperonyl butoxide provided by the Fairfield American Co. Described as a yellow oily liquid and Ref # FEG - 32.

SYNONYMS: PB

STUDY NUMBER(S): 81690

SPONSOR: Piperonyl Butoxide Task Force

TESTING FACILITY: Bio-Research Ltd., 87 Senneville Road, Senneville, Quebec H9X 3R3 CANADA

TITLE OF REPORT: 24-month dietary toxicity study and carcinogenicity study of piperonyl butoxide in the albino rat.

AUTHOR(S): Caroline Graham

REPORT ISSUED: August 27, 1987.

CONCLUSIONS: NOEL (absolute) < 30 mg/kg/day. At this level there are increases in the weight of liver for females and a trend for males.

NOEL (toxicity) = 30 mg/kg/day.

LEL = 100 mg/kg/day. At this level there are increases in liver weight, increased cholesterol levels, increased hepatic "focal mixed cells". All in females.

at 500 mg/kg/day there were increased liver weights and increased hypertrophy of hepatocytes in males and female. Increased hepatic "focal mixed cells" and increased serum cholesterol in females. Increases in "pigment in follicles" and hyperplasia of follicular cells of the thyroid in both males and females. Decreased body weigh gain in both sexes.

Levels tested 0, 30, 100 and 500 mg/kg/day.

Classification: core-GUIDELINES

Special Review Criteria (40 CFR 154.7): N/A.

Quality Assurance Statement:

A statement (pages 77 and 78) signed by two individuals whose signatures were illegible attesting that multiple inspections were made from the period starting in April 11, 1984 until August 10-27, 1987.

A. MATERIALS:

1. Test compound: piperonyl butoxide, Description: yellow oily liquid, Batch: Ref #FEG 32 obtained from the Fairfield American Co., Purity: 88% (87.67 to 89.71%), list of contaminants: not provided. The test material was stored at room temperature away from the light.

2. Test animals: Species: rats, Strain: Sprague-Dawley Crl-CDR, Age: about 50 days at start of test diet feeding, Weight: about 245 to 373 for males and 152 to 198 for females, Source: Charles River, Kingston, New York.

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned by a computer based random number generator to the following test groups:

Test Group	Dose in diet mg/kg/day	Main Study 24 months male	Main Study 24 months female	Interim Sac. 1 month* male	Interim Sac. 1 month* female
1 Control	0	60	60	10	10
2 Control	0	60	60	-	-
3 Low (LDT)	15	60	60	10	10
4 Low (LDT)	30	60	60	10	10
5 Mid (MDT)	100	60	60	-	-
6 High (HDT)	500	60	60	-	-

*These rats were sacrificed after 4 weeks and when it was determined that there was no obvious effects of PB, the remainder of the rats in group 3 (15 mg/kg/day) were terminated at week 8.

2. Diet preparation

Diet was prepared weekly and stored at room temperature but shielded from light. Samples of treated food were analyzed for stability over a period of 5 weeks and for concentration each time the diets were prepared. Top middle and bottom samples were taken. More than 270 analyses were reported and most of these were reported to be near 100% of the expected target level for the dose level. There were some outliers (i.e. 31.4% of the target was achieved for the low dose group males during week one) but the precision in preparing the diets was otherwise acceptable (usually between 90 and 110% of the desired level).

3. Animals received food (Purina Lab Chow 5002) and water ad libitum.

4. Statistics - Statistical analysis of the data was the work of Mr. Ian McMillan (apparently a consultant to the testing laboratory and residing in Guelph Ontario). The numerous statistical tests used by Mr. McMillan are discussed in a 7 page report (pages 59-65). Comparisions were made with either/or each of the two control groups. Where appropriate the statistical tests were adjusted to include only those rats at risk.

C. METHODS AND RESULTS:

1. Observations

Animals were inspected Twice daily for signs of toxicity and mortality. In the later weeks of the study apparently 4 daily inspections were made. A detailed examination (palpation) was reported as being made weekly.

Toxicity: No clinical signs of PB intoxication were reported as developing.

Some of the rats in all of the test groups were reported to have displayed evidence of a viral infection (sialodacryoadentitis) during weeks 24-25 and 28 and again during weeks 63-67. The affected rats recovered after losing (or failing to gain weight) as well as showing the typical signs of this viral infection (swelling of the ventral cervical region and ocular discharge or abnormal respiratory sounds). Neither the severity or the duration of this apparent viral infection was reported as being increased in the rats dosed with PB.

Mortality (survival)

There was no evidence presented that treatment with PB resulted in increased deaths in any of the test groups as indicated in the following table showing the number of survivors and percentage dying.

Group	Males	Females
1 Control - 1	11/(81%)	27(55%)
2 Control - 2	13/(78%)	19(68%)
4 low (30 mg/kg/day)	8/(87%)	22(63%)
5 mid (100 " " ")	11/(81%)	34(43%)
6 high (500 " " ")	13/(78%)	30(50%)

*number of survivors/(percent deaths). No group has less than 50% survival prior to weeks 85-88 for males and 93-96 for females.

2. Body weight

Animals were weighed weekly for first 14 weeks, then biweekly thereafter.

[Note: Control groups I and II appeared similar and the statistical comparision for the treated rats were made against group I only.]

No consistent significant effects on the weight gain in the rats in the low or mid dose groups were evident. The rats in the high dose groups (500 mg/kg/day) both sexes were reported to have decreases in body weight gain.

Among the males body weight gain was 3-4% less than control group I (statistically significant by the students t test) for the first 10 weeks of the study. After that time the body weight differences increased to 8% at week 20, 10% at week 40, 13% at week 70, 17% at week 90 and 22% at week 100. At termination (or week 104) the high dose groups was 21% lower in body weight.

Among the females, the initial weight difference was larger being 6% at week 5 and 9% at week 9. This increment became larger being 13% at week 20, 17% at week 40, 23% at week 70, 28% at week 90 and at termination the difference was 21%.

On the basis of a sustained weight decrement in excess of 10% (at least after week 40 for males and after about week 12 for females), the high dose group is considered to be within the limits for the Maximum Tolerated Dose. TB notes however, that it would have been desireable for the earlier stages (< 90 days of the study) to have shown larger (i.e. 10%) the decrease in weight gain.

A NOEL of 100 mg/kg/day is assigned for this aspect of the study.

3. Food consumption and compound intake

Consumption was determined and mean weekly diet consumption was calculated. Efficiency and compound intake were calculated from the consumption and body weight gain data. Dietary concentrations of the test material were adjusted for weekly body weight changes for the first 15 weeks and every two weeks thereafter to obtain the desired levels in mg/kg body weight/day.

The high dose groups (both males and females) showed "slight" decreases in food intake that on some occasions were statistically significant.

The mean achieved intake of PB for the test groups was 99-100% as shown in the following table.

	Nominal Level (mg/kg/day)	Achieved Level (in mg/kg/day) Males	Females
Group 4	30	29.8	29.9
Group 5	100	99.2	99.7
Group 6	500	495.4	497.6

4. Ophthalmalogical examinations

Performed on all surviving rats at week 99 with a funduscope (indirect ophthalmoscope) and a biomicroscope (slit lamp).

No changes attributable to PB treatment were reported. Only a low incidence of normal age related changes were noted. A. Leith, M.D. was the person responible for the ophthalmalogical examination.

5. Blood was collected before treatment and at weeks 25,51,79 and 97/98 for males/females for hematology and clinical analysis from 10 animals per sex. The blood was taken from the orbital sinus under light ether anesthesia The rats were fasted overnight prior to removal of the blood except for week 97/98 when they were fasted 10-12 hours prior to collection.

The CHECKED (X) parameters were examined.

a. Hematology

X		X	
x	Hematocrit (HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*	x	Mean corpuscular HGB conc.(MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpuscular volume (MCV)
x	Platelet count*	x	Reticulocyte count
	Blood Clotting Measurements		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic and chronic studies

No consistent of significant differences were reported between rats dosed with PB and the control groups for these hematology parameters. NOEL > 500 mg/kg/day.

b. Clinical Chemistry

X	Electrolytes:	X	Other:
x	Calcium*	x	Albumin*
x	Chloride*		Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
x	Phosphorous*	x	Cholesterol*
x	Potassium*		Globulins
x	Sodium*	x	Glucose*
	Enzymes	x	Total Bilirubin*
x	Alkaline phosphatase	x	Total Serum Protein*
	Cholinesterase# (N/A)		Triglycerides
x	Creatinine phosphokinase*°		Serum protein electrophoresis
	Lactic acid dehydrogenase	x	Albumin/globulin ration
x	Serum alanine aminotransferase (also SGPT)*		
x	Serum aspartate aminotransferase (also SGOT)*		
	gamma glutamyl transferase		
	glutamate dehydrogenase		

* Required for subchronic and chronic studies
\# Should be required for OP
° Not required for subchronic studies

Of these parameters cholesterol, BUN, and total protein were recognized by the testing laboratory as being possibily affected by the test material. In addition some of the serum enzyme levels were reported as being reduced.

a. The following table summarizes the data for cholesterol analysis at the several times analyses were made.

	Males			Females		
Week	Low	Mid	High	Low	Mid	High
25	11(-)[1]	40*(4)	34**(-)	10	8	73***
51	3(-)	39*(-)	34*(-)	11(-)	35(22)	89***(71)
79	10(-)	41(-)	32(-)	13	15	80***
97/98	18(-)	38(-)	46(5)	25	93*	128**

1. The data are in % increase over control group 1 and the number in ( ) is the % increase over control group 2.
* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

The number in ( ) is the % increase over control group 2. The study report compared control group I with the test groups. When a - is present in the ( ) the value obtained for the test group was less than the value reported for control group II. When there is no ( ), both control group I and control group II were very similar and it was not necessary to separately compare the groups.

The high dose group females were consistently higher at all assay times. The mid dose group females was higher at termination. There were some increases noted among the males but no consistent statistically significant effects were noted especially when the data are compared with control group II.

Increased cholesterol levels are an indication of possible liver, kidney, thyroid or heart toxicity (refer to J. Whalan "Clinical Pathology Data in Laboratory Animals, April, 1987). These organs will be further specifically discussed in this review in the histopathology and organ weight sections.

The test laboratory asserted a NOEL of 30 mg/kg/day based on increased cholesterol levels in females.

b. The females in the high dose group also showed higher levels of total serum protein at each time interval tested. Depending upon which control group was used for comparision, as much as 13% elevation was attained. The small magnitude of the increase in total protein and the fact that males are not also affected do not provide a strong basis for associating PB with this increase. The liver, kidneys and GI tract will, however, be especially examined for patological changes since toxic insult to these organs may result in hyperproteinimia.

c. Serum glutamate oxalacetate transmainase (SGOT) was reported as being depressed in both males and females at weeks 97/98. The males were decreased about 37% and 54% and the females about 22% and 30% for the mid and high dose groups respectively. The females were also reported to be slightly lower at weeks 25 and 51 but not at week 79.

The males receiving 500 mg/kg/day were also "slightly" lower in serum glutamate pyruvate transaminase at each sampling time. Occasionally the next lower dose group for males and the high dose group females were also lower for this enzyme.

TB does not currently associate any clinical significance for a decrease in the serum levels of this either SGOT or SGPT. Their decreases in this study are, however, noted.

d. The males in the high dose group were reported as having decreased creatinine phosphokinase levels at week 97 (-63%) but because of the large standard errors and absence of a similar effect in other groups, this finding is not considered a definite effect of PB treatment. Similarly, the high dose group females had elevated BUN (21%) which also not considered a definite response to treatment.

NOEL for clinical chemistry is set at 30 mg/kg/day, LEL = 100 mg/kg/day increased cholesterol levels in females. Other clinical chemistry findings are not considered as definite responses to PB treatment.

6. Urinalysis

Urine was collected from fasted animals at weeks 25, 51, 79 and 98/99. The CHECKED (X) parameters were examined.

X		X	
x	Appearance*	x	Glucose*
x	Volume*	x	Ketones*
x	Specific gravity*		Bilirubin*
x	pH		Blood*
x	Sediment (microscopic)*	x	Nitrate (nitrite)
x	Protein*	x	Urobilinogen
		x	Hemoglobin

* Required for chronic studies
° Not required for subchronic studies

No consistent or statistically significant differences were reported between the rats dosed with PB and the control groups for these urinalysis parameters. NOEL > 500 mg/kg/day.

7. Sacrifice and Pathology -
All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
x	Tongue	x	.Aorta*	xx	.Brain*†
x	.Salivary glands*	x	.Heart*	x	Periph. nerve*#
x	.Esophagus*	x	.Bone marrow*	x	Spinal cord (3 levels)*#
x	.Stomach*	x	.Lymph nodes*	x	.Pituitary*
x	.Duodenum*	x	.Spleen*	x	Eyes (optic n.)*#
x	.Jejunum*	x	.Thymus*		Glandular
x	.Ileum*		Urogenital	xx	.Adrenals*
x	.Cecum*	xx	.Kidneys*†		Lacrimal gland#
x	.Colon*	x	.Urinary bladder*	x	Mammary gland*#
x	.Rectum*	xx	.Testes*†	x	.Parathyroids*††
xx	.Liver*†	x	Epididymides	x	.Thyroids*††
	Gall bladder*#	x	Prostate		Other
x	.Pancreas*	x	Seminal vesicle	x	Bone*#
	Respiratory	xx	Ovaries*†	x	Skeletal muscle*#
x	.Trachea*	x	.Uterus*	x	Skin*#
x	.Lung*				All gross lesions and masses*
	Nose°				
	Pharynx°				
	Larynx°				

* Required for subchronic and chronic studies
° Required for chronic inhalation
In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
† Organ weights required in subchronic and chronic studies
†† Organ weight required for non-rodent studies

a. Organ weight

The following table depicts the results of weighting the liver from the rats at terminal sacrifice.

Liver weight as percent increase when compared to the mean of both control grooups.

	Males			Females		
Group	Abs.	Rel_1	Rel_2	Abs	Rel_1	Rel_2
30 mg/kg/day	8.8	9.6	5.6	20.0ss	11.0	18.5ss
100 mg/kg/day	9.2	25.5ss	10.5	25.2ss	27.4ss	22.8ss
500 mg/kg/day	20.5ss	53.2ss	24.9ss	27.1ss	70.9ss	29.3ss

Abs = based on absolute weight; Rel_1 = based on weight relative to body weight; Rel_2 = based on weight relative to brain weight.

ss = shown to be statistically significant by the testing laboratory when compared to groups 1 and 2.

This table shows that male liver weight is definitely affected in the high dose test groups and possibly also in the low and mid dose groups where the trend is evident although there was no consistency in attaining statistical significance. Among the females, the increases were statistically significant for all dose groups.

These data support a NOEL of < 30 mg/kg/day. The increase in liver weights in the low dose group, however, is not necessarily a toxic response to PB.

Testis weight relative to body weight for the high dose group was elevated 16% when compared to group 2 control but was not significantly elevated when compared to group 1 control. There were no statistically significant increases or decreases in testis weight when expressed as either absolute or relative to brain weight. The absence of consistent noticeable effects on testis weight is somewhat surprising because this organ is reported as being atrophied (see below under pathology discussion).

Histopathology

The pathology report was prepared by George J. Losos, DVM, PhD, Vice President Division of Pathology of the testing laboratory. The report states that complete histological examination was undertaken on animals in the two control groups and for the high dose group. Histological examination of the rats in the low and intermediate dose groups was completed for the rats dying during the study. The liver, kidney, lung, thyroid, testis, epididymis, ovary, and any observed abnormalities were examined for the rats killed at termination of the study. The usual procedures of fixing the organs/tissues in 10% formalin and staining with hematoxylin and eosin were used. Microscopic lesions were graded for severity. Individual animal pathology sheets with both gross and microscopic findings are presented.

Individual Organ Discussions:

1. Liver Status.

The liver would be expected to show signs of response to PB insult because this chemical effects (inhibits) the metabolizing systems found in this organ. This effect might then result in increased proliferation in the liver resulting in a larger size and other observable changes. Other indications for special discussion of the liver in this review are that PB was shown to possibly affect the serum levels of cholesterol and the liver weights were increased. Furthermore the liver of mice and rats has been suggested as being a neoplastic target organ for for the safrole moeity which is included in the structure of PB.

The following table depicts the total incidences of gross lesions in both male and female rats which show possible test chemical related effects.

Lesion Description	Males					Females				
	C_1	C_2	L	M	H	C_1	C_2	L	M	H
Area Pale	4	5	11	8	21	5	5	9	12	31
Mass	1	0	0	1	4	1	0	2	2	5
Enlargement	1	2	4	7	14	0	1	2	1	2
Prominent lobular Architecture	5	1	6	4	7	0	4	4	5	7
Area Raised	0	4	3	5	3	1	3	3	8	8
Discoloration	3	3	2	8	7	5	0	1	0	3
Area Dark	18	18	13	13	8	22	19	24	27	11

Note: There were 60 rats of each sex per dose group reported as being examined.

C_1 and C_2 are control groups I and II. L, M and H are the low, mid and high dose groups respectively.

Evidence for PB effects in the liver based on grossly observable lesions is most pronounced for the lesions described as "area pale" and "mass". In females even the low dose group may have higher incidences than the controls for the "pale" condition. . The next four lesions listed in the above table show evidence for one sex only. The lesion described as "dark area" shows an inverse relationship with the presence of PB in the diet.

The following table depicts the total incidences of microscopic lesions in the both males and females which either showed indications of a response to PB treatment or are otherwise of interest to the review.

Lesion Description	Males					Females				
	C_1	C_2	L	M	H	C_1	C_2	L	M	H
Hyper. Hepatocytes	4	2	1	4	29[1]	4	2	0	2	47[1]
Focal Mixed Cells	1	1	4	1	5	3	3	3	13[1]	20[1]
Focal Eosinophilic cells	6	6	3	10	12	6	2	5	5	3
B. Duct Hyperplasia	20	26	8	11	11	21	24	20	26	7
Vac. Hepatocytes	14	15	19	23	21	20	17	14	7	9
Liver Tumors										
Hepatocellular Carcinoma	1	1	0	0	1	0	0	0	1	1
Adenoma	0	0	0	0	2		[None reported]			
(Total Liver Tumors)	1	1	0	0	3	0	0	0	1	1

Note: There were 60 rats per sex per dose reportly examined.
[1]Statistically significantly increased (see p. 62 of the study report).

Microscopically there were increased incidences of hyperplasia of hepatocytes in the high dose groups of both sexes. There were increased incidences of focal mixed cells in the mid and high dose female groups but only in the high dose group males. The apparent increase in focal eosinophilic cells in the mid and high dose group males is small and also not noted in the females. Bile duct hyperplasia shows an inverse relationship with there being fewer incidences in the high dose test group for both sexes. There is also a possible inverse relationship noted for vacuolation of hepatocytes for the females. Focal basophilic cells also showed a negative trend (data not shown).

Based on microscopic changes in the liver, NOELs of 30 mg/kg/day in females of 100 mg/kg/day in males are supported. The liver weight increases noted in the low dose females are not corroborated by histopathological findings. The three incidences (total adenoma and carcinoma) of liver tumors in the high dose group males are not statistically significant. The liver tumors were reported to be found in rats at or near the termination of the study and were not considered life threatening. The frequency in the high dose group males (5%) for combined adenomas and carcinomas was very near the historical control for this strain of rat (up to 4.5% based on the analysis of over 500 male rats).

2. Kidney Status

Increased serum cholesterol is an indication of possible kidney damage. There were also some indications (but not definitely dose dependent) increases in kidney weight.

Inspection of the gross necropsy data tables did not indicate evidence for a dose related increase in kidney lesions in either males or females. Inspection of the histopathological findings data reveals that both the males and females have high frequencies (88-97% for males and 53-90% for females) of chronic interstitial glomerulonephritis. There was no evidence of a dose related effect in males but among the females there were higher frequencies in a progressive order (73, 85 and 90% for the low, mid and high dose groups versus 53 and 63% for the two control groups). Because of the high background of this spontaneously occurring condition, it is not convincing that the increased incidences were a direct result of PB in the diet. It is noted, however, that the incidence in all females groups when compared to control group I and for the mid and high dose groups when compared to control group II can be demonstrated to be statistically significant. Severity was also said to be increaaed.

Among the females there was an inverse relationship between PB in the diet and development of calculi (34% in the control versus 5% in the high dose group.

Among the males, there were a total of 59 cysts noted at gross necropsy, but only 1 cyst (in a control rat) was confirmed histologically. Many of the rats with cysts noted at necropsy were diagnosed as having chronic interstitial glomerulonephritis.

There were a total of three incidences of kidney tumors reported. Two incidences of adenocarcinoma (one each in the control males and females). There was also a single adenoma reported in the mid dose group males.

In summary, there was no evidence that PB clearly affected the kidney. A possible increase in chronis interstitial glomerulonephritis is noted in females, however.

3. Male Reproductive System Status (testis, seminal vesicles, epididymis, prostate)

Gross pathology revealed that there were 10, 12, 11, 14, and 23 rats for which the testis were described as "small". Microscopically there were 25, 25, 26, 33, and 33 rats for which the testis were reported in the total incidence table as having "atrophy" for the two control, low, mid and high dose test groups. Both the pathologist's (Dr. Losos) and statistician's (Mr. McMillan) reports show that there were 11, 9, 20, 28, and 26 incidences of "bilateral atrophy" among the test groups for the two controls, low, mid and high dose test groups. These dats showed statisitcally significant increases for all three treatment groups when compared to either of the two control groups. There was also noted a tendency towards increased severity of this lesion as the dose level increased.

[Note: The data tables showing incidences of atrophy reported the total number of rats with either bilateral or unilateral atrophy but not the incidences of bilateral atrophy alone. Thus, TB could not confirm the statistics without an animal by animal accounting of testis pathology. This reviewer also noticed

that the total incidences for "atrophy" for the mid dose group which was reported as 33 on page A237 does not equal the sum of the incidences among the rats dying during the study on page A187 (25) and on page A212 for the rats sacrificed at termination (0).]

Other findings in the testis included that the high dose group was highest (4 in 60 rats or 6.7%) in incidence of hyperplasia of interstitial cells (versus 0.8% in the combined control groups) and for interstitial cell tumors (4 in 60 rats or 6.7%) incidences versus 3.3% for the combined controls.

The gross necropsy report indicated that the seminal vesicles were smaller. There were 3, 4, 9, 10 and 12 incidences for 60 rats per group for this condition for the controls, low, mid and high dose groups. There were no microscopic correlates for this condition reported. Neither the prostate or epididymis were reported to have dose dependent lesions.

In conclusion for the male reproductive system, although the incidence of "bilateral atrophy" can be shown to be statistically significant, the total incidence of atrophy (unilateral plus bilatal) was not shown to be statistically significant. Testis weight was paradoxically increased (about 16%) for the high dose group rather than a decrease as would be expected to be correlated with atrophy of the testis. Another contributing factor is that the multi-generation reproduction study (refer to review by J. Doherty dated October 30, 1987) did not indicate any effect of PB treatment on the male reproductive function. Thus, TB does not consider that the data presented provide conclusive evidence that PB effects the testis or other aspect of the male reproductive system in this study.

4. Adrenal Status

The adrenal gland of males showed an apparent inverse relationhship for the incidence of "focal coursely vacuolated enlarged cortical cells" with there being 20, 26, 18, 15 and 7 incidences reported for the two controls (60 and 60), low (60), mid (56) and high (60) dose groups respectively (number of rats examined). Among the females, there were 17-19 reported for each group thus not showing any relationship to the presence of PB in the diet. The mid and high dose group males can be shown to be statistically significantly different from control group 2. This observation of decreased incidence among the males for the mid and high dose males is not considered by TB to be definetly a result of PB in the diet but is mentioned here because the adrenal gland is an exocrine gland and the study report maintains that PB may affect circulating hormones (see discussion below). Although gross necropsy revealed that there were occasionally higher incidences of adrenal enlargement among the females and female adrenal weight was higher, there were no histological correlates. It should be noted that because of the wide variation and standard deviation in adrenal weight it is somewhat doubtful that the female high dose group was actually higher.

The incidence of tumors (total adenoma plus carcinoma) in the males for the adrenal was seven and five of these were in the control rats. Among the females the total incidence of tumors was six and four of these were in the control rats. There is thus no evidence that PB induced neoplasms in this organ.

5. Thyroid Status

The study report notes that "marginally higher incidences of thyroid enlargement among the males from all dose groups which were found dead or killed for humane reasons". Inspection of the total incidence table for gross lesions reveals that there were 1, 0, 4, 3 and 4 incidences of this condition among the males and 1, 0, 1, 5 and 2 incidences among the females for the controls, low, mid and high dose test groups. Microscopically there were noted higher incidences of "pigment in follicles" for the high dose group (48 of 60 rats or 80%) versus 37-45% for all of the other male groups and the high dose female groups (44 of 60 rats or 73%) versus 10-17% for the other females groups. It is rather apparent that the thyroid is in some way affected as indicated by the increased incidence of "pigment in follicles" in the high dose groups of both sexes.

"Hyperplasia of follicles" in the high dose group was also statistically significantly increased for the males (21 incidences versus 4 or 11 incidences for the controls, 60 rats per group) and females (11 incidences versus 0 or 4 incidences of 60 rats per group) when compared with either control group. The mid dose female group (9 incidences) was also statistically significantly higher when compared to the control groups 1 (0 incidence). Another finding was that para-follicular cells showed an apparent dose related decrease.

There were four types of primary thyroid tumors noted for a total incidence of 16 among both sexes. Five of these were in the control groups. Follicular cell adenomas among females were more frequent in the high dose group (3 of 60 rats) than in any other group (0 or 1) but statistical significance was not attained. It is noted, however, that the 5% frequency reported for the high dose group exceeds the historical control data for this strain of rat (0-1.8%). Among the males there were 2 incidences (3.33%) in the high dose group of this tumor type versus 0 or 1 in the other groups but the range in males for this tumor type in historical controls is 0-5.3%.

In conclusion for the thyroid gland, it appears that the thyroid is affected as evidenced by increases in "pigment in follicles" and hyperplasia of follicular cells (NOEL = 100 mg/kg/day). An increase in follicular cell adenomas among the females in the high dose group is also noted but the available data do not provide a convincing case for a specific neoplastic response to PB.

6. Female Reproductive System Status.

Gross necropsy revealed the following incidence of "enlargement" of the ovary 0, 1, 1, 0 and 4 for the controls, low, mid and high dose groups. There were no effects noted on weight gain. Microscopically there was no evidence of dose related lesions although there were slightly less cysts in the rats dosed with PB.

In the mammary gland there were less incidences of "hyperplasia of

acini (65%) in the high dose group than in the controls (83.3%). With regard to female mammary tumors, the following table illustrates the findings for adenocarcinoma and fibroadenoma.

Dose Group	Adenocarcinoma	Fibroadenoma
Control-1	8/60(13.3%)[1]	11/60(18.3%)
Control-2	12/60(20.0%)	17/60(28.3%)
Low (30 mg/kg/day)	10/55(18.2%)	19/55(34.6%)
Mid (100 mg/kg/day)	10/46(21.7%)	18/46(39.1%)
High (500 mg/kg/day)	11/60(18.3%)	8/60(13.3%)

[1]Data are incidenes/number of rats (as %)

It is noted here that the low and mid dose groups have higher incidence of fibroadenoma but the high dose group has the lowest incidence. The lack of dose response in this case precludes a relationship between dietary PB and induction of mammary gland tumors. [Note: Historical control incidence data for these tumor types were not provided.]

7. Pituitary Status

Many of the females (82.1 to 89.8%) and males (56.6 to 71.6%) had tumors of the pars anterior but there was no evidence for increased incidence realtive to dietary PB.

8. Gastro-Intestinal System Status (abdominal cavity, cecum, colon, digesta, duodenum, ileum, ingesta, jejunum, rectum and stomach).

There were no abnormalities of sufficient frequency to indicate a dose related effect of PB in these tissues or organs.

9. Heart Status.

There were many rats affected with "fibrosis" in the heart. Among the males there were 37, 37, 36, 35 and 32 incidences for the controls (60), low (54), mid (53) and high (60) dose groups respectively (number of rats examined). Among the females there was a suggestion of an inverse relationship with there being 26, 18, 13, 6, and 7 affected hearts for the controls (60), low (39), mid (28) and high (60) dose groups respectively. These data, however, fo not provide a basis to conclude that PB treatment affected this organ.

10. Lymphosarcomas.

There were 0, 1, 0, 1 and 3 male rats reported to have systemic lymphosarcoma. The high dose groups did not attain statistical significance and the 5% frequency for this group was said to be within the range of historical control data (up to 7.2%). There were only two incidences of lymphosarcomas among the females for this condition and these were in the controls.

D. DISCUSSION:

The study report maintains that the primary action of PB is to induce the formation of liver cytochrome metabolizing systems which would result in increased levels of enzymes involved with degrading xenobiotics as well as endogenous circulating substances such as hormones. The study report also maintains that since these enzyme systems would be elevated there would be expected to be lower levels of circulating hormones. The effect on the hormones would in turn result in effects in both the target and originating organs for these hormones.

This contention is somewhat paradoxical. The primary action of PB is to inhibit the enzymes involved in drug metabolism and circulating hormones. This would result in elevation of the circulating hormones. The combined effects of increased levels of drug metabolizing enzymes plus the inhibition of these same enzymes by PB would not be expected to cause consequental effects on circulating hormones. Thus, TB sees little basis for the study reports discussion which tries to relate the subtle changes in the pathologcal findings on endocrine and hormone sensitive organs to the liver effects of PB. The multi-generation reproduction study (refer to review by J. Doherty dated October 30, 1987) did not provide any indications of altered hormonal function at dose levels up to and including a dietary level of 5000 ppm (estimated 250 mg/kg/day).

The testing laboratories speculation that dietary PB alters circulating hormone levels would have to be proved by quantitating these hormones if rats dosed with PB.

E. CONCLUSION: CORE Classification of this study is GUIDELINES. The following one liner applies.

NOEL (absolute) < 30 mg/kg/day. At this level there are increases in liver weight of females (20% based on absolute weights), a trend for increased liver weight is noted for males.

NOEL (toxicity) = 30 mg/kg/day

LEL = 100 mg/kg/day. At this level there are increases in the weight of liver (females, 25%), increased cholesterol levels (females), increased hepatic "focal mixed cells" (females) and a continuation of a trend for increased liver weight in males.

At 500 mg/kg/day there were increased liver weights in males and females, increased hypertrophy of hepatocytes in males and females, increased hepatic "focal mixed cells" in females, increased cholesterol in females, increases in "pigment in follicles" and hyperplasia of follicular cells of the thyroid in both males and females. Decreased body weight gain was noted for both sexes.

No unequivocal evidence that PB induced tumors on rats was generated by this study.

Levels tested: 0, 30, 100 and 500 mg/kg/day.

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PIPERONYL BUTOXIDE SUBMISSION

Discussion of Findings in a 24-Month Dietary Toxicity and Carcinogenicity Study of Piperonyl Butoxide in the Albino Rat

Data Requirement
Guidelines 83-5

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Completed on:
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No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10(d) (1) (A), (B), or (C).

No supplemental claim of confidentiality is made for the information contained in these studies on the basis of FIFRA Section (10) (A) or (B). This document, however, is proprietary to the Piperonyl Butoxide Task Force and is considered to be confidential and trade secret information in all other countries and for all purposes other than those enunciated in FIFRA Sections 3 and 10.

Information contained in these studies should not be reviewed, abstracted or used by persons other than EPA without the expressed written consent of the Piperonyl Butoxide Task Force except as required to carry out the requirements of FIFRA.

Sponsor: Piperonyl Butoxide Task Force

Sponsor's Agent: Frederick J. Preiss, Ph.D.

Title: Chairman, Piperonyl Butoxide Task Force

Date: August 28, 1987

DISCUSSION OF FINDINGS IN A 24-MONTH DIETARY TOXICITY AND CARCINOGENICITY STUDY OF PIPERONYL BUTOXIDE IN THE ALBINO RAT

This study was conducted at Bio-Research Laboratories Ltd. for the Piperonyl Butoxide Task Force. It was conducted in accordance with the U. S. Environmental Protection Agency's Pesticide Assessment Guidelines, Guideline Reference 83-5. It was also conducted in accordance with the U. S. Environmental Protection Agency's Good Laboratory Practice Standards (40 CFR, Part 160).

The study initially consisted of four treatment groups and two control groups. The dosage levels in the treatment groups were 15, 30, 100 and 500 mg/kg/day. A fourth treatment group was included as a time saving measure because the no-effect level for minor changes in liver cell morphology was not clearly defined in a preliminary dose range finding study. Extra animals were included in one control group and in the two lower dose groups (15 and 30 mg/kg/day) so that an interim sacrifice could be conducted after four weeks of the chronic study. The highest dosage level without any morphological change(s) in the liver was scheduled to be continued in the chronic study and the

other group was scheduled to be terminated. No morphological changes were observed at either dosage level in rats sacrificed at the four-week interim sacrifice so the 30 mg/kg/day dosage level was selected as the low-dosage level for the chronic study.

The study was conducted in accordance with the protocol and the Laboratory's Standard Operating Procedures. The conduct of the study was monitored by the Laboratory's Quality Assurance Unit and independently by me as a representative of the Sponsor. No deviations from protocol or standard operating procedures occurred during the conduct of this study which would have adversely influenced the outcome or the interpretation of the results.

At the highest dosage level (500 mg/kg/day), a number of treatment related changes were observed. Most, if not all, of these changes appear to be the result of the induction of the liver microsomal enzyme system, a well documented property of piperonyl butoxide. These changes included effects on body weight, food consumption, clinical pathology, organ weight and gross and microscopic pathology. No clinical signs, occular

changes, hematologic changes or effects on mortality or tumor incidence were observed.

No body weight or food consumption effects were observed in the mid-dose group (100 mg/kg/day), but some minor changes in clinical pathology, organ weight and gross and microscopic pathology were observed. Again no clinical signs, occular changes, hematologic changes or effects on mortality and tumor incidence were observed.

At the lowest dosage level (30 mg/kg/day), no definitive treatment related effects were observed.

Based upon the results of this study, the dosage levels evaluated clearly satisfy the maximum tolerated dose requirements for a chronic toxicity/oncogenicity study and no evidence of oncogenicity was observed. All other changes appear to be adaptive rather than toxic in nature. The no-effect level for these adaptive changes is 30 mg/kg/day.