



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

January 26, 1995

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review Meeting on **PIPERONYL BUTOXIDE**

FROM: Esther Rinde, Ph.D. *E.R.*
Manager, Carcinogenicity Peer Review
Health Effects Division (7509c)

TO: Addressees

Attached for your review is a package on **Piperonyl Butoxide** ("Pyrethrins" #1) prepared by Dr. John Doherty.

A meeting to consider the carcinogenicity classification of this chemical is scheduled for **Wednesday Feb. 15, 1995, at 10:00 am** in Room 817, CM2.

Addressees

S. Irene
W. Burnam
K. Baetcke
M. Van Gemert
K. Dearfield
H. Pettigrew
B. Fisher
L. Brunsman
E. Doyle
M. Copley
[REDACTED]
R. Hill
Y. Woo
A. Aranda
R. Ross/L. Brénnecke



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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Piperonyl Butoxide

TOX CHEM No.: 670

PC No.: 067501

FROM: John Doherty *[Signature]* 1/26/95
Section IV, Toxicology Branch I
Health Effects Division (7509C)

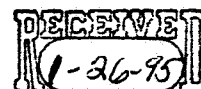
TO: Esther Rinde, Ph.D.
Carcinogenicity Peer Review Manager
Science Analysis Branch
Health Effects Division 7509C

THROUGH: Marion Copley, DVM, Section Head *[Signature]* 1/26/95
Section IV, Toxicology Branch I
Health Effects Division (7509C) *KR 1/26/95*

Attached are Sections C, D, E and F for incorporation into the Peer Review Document on piperonyl butoxide.

The issues of concern are

- liver tumors in rats and mice of both sexes
- thyroid follicular cell tumors in male and female rats
- lymphoma in rats
- hemangioendothelial sarcoma in male mice
- lacrimal gland adenomas in mice



Recycled/Recyclable
Printed with Soy/Canola Ink on paper that
contains at least 50% recycled fiber

Table of Contents

Parts A and B are not included in this document (to be prepared by the Peer Review Committee)	3
C. Background Information	3
D. Evaluation of Carcinogenicity Evidence:	3
Rat Study #1	3
Rat Study #2	6
Rat Study #3	9
Rat Study #4	11
Mouse Study #1	12
Mouse Study #2	14
Mouse Study #3	15
E. Additional Toxicology Data on Piperonyl Butoxide	16
Metabolism	16
Mutagenicity	16
Developmental Toxicity	17
Structure-Activity and Functional Activity Correlations	17
Acute, Subchronic, and Chronic Toxicity Studies	18
F. Weight of Evidence Considerations	18
Attachment 1. Summary Table Carcinogenicity Studies	21
Attachment 2. Reprint "Chronic Toxicity Studies of Piperonyl Butoxide in F344 Rats: Induction of Hepatocellular Carcinoma." by O. Takahashi et al, <i>Fund. Appl. Toxicol.</i> 22:293-303 (1994) and HED DER #019658 for the prepublication monograph of this study.	23
Attachment 3. HED DER for the Bio-Research Study No.: 81690, August 27, 1987 rat chronic feeding/carcinogenicity study with the Crl:CD [®] BR strain rat.	49
Attachment 4. Memo entitled "Piperonyl Butoxide Qualitative Risk Assessment Based on Charles River Sprague-Dawley Crl-CDR Rat and Charles River CD-1 Mouse studies". Prepared by L. Brunsman and dated January 4, 1995.	73
Attachment 5. Copy of the NCI Blue Book Study summary for piperonyl butoxide carcinogenicity studies in F344 strain rats and B6C3F1 strain mice <u>and</u> statistical tables.	85
Attachment 6. Reprint "Lack of evidence of carcinogenicity of technical-grade piperonyl butoxide in F344 rats; Selective induction of ileocecal ulcers". A. Maekawa et al in <i>Fd. Chem. Toxic.</i> 7:675-682 (1985).	93
Attachment 7. HED DER for the Bushy Run Research Center Study No.: 91N0134, August 27, 1993 carcinogenicity study with the CD-1 strain mouse.	101
Attachment 8. Reprint "Letter to the editors "Piperonyl butoxide induces hepatocellular carcinoma in male CD-1 mice" by O. Takahashi et al, in <i>Arch Toxicol.</i> 60:467-469 (1994).	115
Attachment 9. <u>FSTS of piperonyl butoxide.</u>	117
Attachment 10. HED "one liners" for technical grade piperonyl butoxide.	122

Attachment 11: Registrant's 2/13/95 Submission
(proposed future studies)

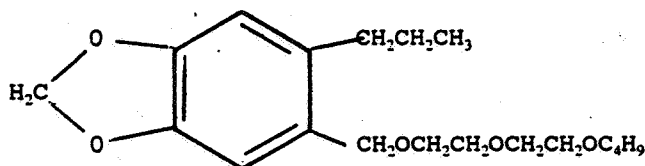
Parts A and B are not included in this document (to be prepared by the Peer Review Committee).

C. Background Information:

Piperonyl butoxide (PBO) is an insecticide synergist that disrupts the degradation of pesticides by inhibiting the mixed function oxidase enzymes primarily found in the liver but also located in other organs. PBO is found in numerous insecticide formulations usually when the active ingredients are pyrethrins and pyrethroids but is also found in some formulations of organophosphates and other chemicals. Since PBO is present in many pesticide formulations, there is potential for inhalation and dermal exposure to applicators and dietary exposure from treatment of RACs.

Following the Data-Call-In Notice, new chronic feeding/carcinogenicity studies with rats and mice have been submitted. Recent publications from the Tokyo Metropolitan Research Laboratory of Public Health report associations between PBO and carcinogenicity in both rats and mice. The NCI has also conducted studies in rats and mice. In addition, a series 82-3 subchronic inhalation toxicity study has also indicated a possible concern based on specific non-neoplastic findings for potential carcinogenicity of the respiratory tract.

The structure of PBO is illustrated as follows:



Structure of piperonyl butoxide (PBO, alpha [2-(2-butoxyethoxy) ethoxy]-4,5-methylenedioxy-2-propyltoluene)).

The Tox Chem (or Caswell) No. of PBO is 670. The Chemical Abstracts Registry Number (CAS No.) is 51-03-6. The PC Number is 067501.

D. Evaluation of Carcinogenicity Evidence:

[Note: The studies are listed by species and the study for each species that most strongly indicates carcinogenic potential is described first. A summary table of these studies is in Attachment 1.]

1. Rat Study #1. Rat Carcinogenicity Study. Tokyo Metropolitan Research Laboratory, as published in Fund. Appl. Toxicol. 22:292-303 (1994). A copy of the paper is attached (Attachment #2). Submitted as a prepublication monograph under MRID No.: 42839601 and 42920201. HED Document No.: 010658

(attached). Note: The DER was based on the prepublication monograph. The actual publication contained more detail and additional information regarding pathology and other aspects of the study.

a. Experimental Design. Four groups of Fischer F344 strain rats 30/sex for the control, 0.6% and 1.2% groups and 33/sex for the 2.4% groups were dosed with PBO based on the percent of PBO in the diet for two years. These dose levels correspond to 0, 547, 1052 or 1877 mg/kg/day in males and 537, 1061 or 2002 mg/kg/day in females.

b. Discussion of Tumor Data. The study was determined to be positive for liver tumors for both males and females. Table 1 below illustrates the neoplastic and non-neoplastic findings from this study. Since the individual animal data were not provided, SAB has not been able to extract the data for an independent statistical assessment.

Table 1 below clearly indicates that based on either the original or Dr. Butler's analysis that there is a test compound related increase in both hepatocellular adenomas and carcinomas. The following criteria were used for diagnosis by the two different pathologists.

Table 1. Liver tumors in F344 rats dosed with piperonyl butoxide. Comparison of original diagnosis (submitted under D192500, no MRID #) and the diagnosis made by Dr. W. H. Butler (MRID # 42920201, page 5).

Lesion	Males				Females			
	Control	0.6%	1.2%	2.4%	Control	0.6%	1.2%	2.4%
<u>Butler</u>	(26) ¹	(23)	(17)	(25)	(25)	(27)	(27)	(26)
Focal hyperplasia ²	2	1	2	3	0	0	13	8
Adenoma ³	0	0	8	13	0	0	1	11
Carcinoma ³	0	0	3	7	0	0	0	5
<u>Original</u>	(25) ¹	(25)	(15)	(25)	(24)	(27)	(25)	(26)
"Liver tumor"	0	1	14	25	0	4	22	26

1. The number in () is the number of animals examined.

2. Focal hyperplasia is not regarded as a tumor but is included to demonstrate possible preneoplastic conditions.

3. More specifically hepatocellular adenoma and hepatocellular carcinoma. Dr Butler did not specifically state that rats with both an adenoma and carcinoma are counted as having carcinoma only.

Original-Based on the classification of Boorman et al (Pathology of the Fischer Rat, Academic Press, New York, 1980? or 1990? - the reference is cited with two different dates). In this diagnosis, nodular lesions were divided into hepatocellular adenoma (including hyperplastic nodules and neoplastic nodule) and hepatocellular carcinoma (cellular atypia, structural atypia,

nucleus/cytoplasm and compression of the adjacent tissue).

Butler-No specific reference was provided but Dr. Butler provided the following comments. Nodular lesions with the presence of abnormal thickened trabeculae often associated with hemorrhage and necrosis are classified as carcinoma. Nodules classified as adenomas have more simple trabecular structure usually 1-2 cells thick with little or no necrosis or hemorrhage. Focal hyperplasia are nodules that differ from adenomas in that the hyperplasia have differentially organized hepatic cords and the presence of residual structures. Dr. Butler also stated that "there are no definitive histological criteria for the differentiation of adenomas (benign neoplasms) and hyperplasia (a reactive proliferation).

No historical control data were provided by the testing laboratory for liver tumors in this strain of rat.

c. Non-neoplastic Lesions. The following non-neoplastic lesions were noted.

-Stomach and cecum, lung and kidneys. These structures were considered associated with increased several lesions based on gross necropsy as in Table 2 below.

Table 2. Non-neoplastic pathology in Fischer 344 rats dosed with piperonyl butoxide.

Lesion	Males				Females			
	Control	0.6%	1.2%	2.4%	Control	0.6%	1.2%	2.4%
Number examined	30	30	30	33	30	30	30	33
Stomach								
Hemorrhages	5	2	6	9	2	2	7	15*
Polyps	0	0	0	2	1	0	0	6
Smooth surface	0	0	2	10*	0	2	5	12*
Cecum								
Enlargement	0	18*	21*	15*	0	7*	13*	9
Hemorrhages	0	7	12*	7	0	6	10*	7
Edema	0	7*	8*	0	0	5	6	3
Hyperplasia	0	1	0	5	0	1	0	4
Lungs								
Whitish spotting	0	0	3*	11*	0	0	0	2
Kidneys								
Black colored	1	5	10*	17*	0	7*	20*	25*
Misshapen	1	1	0	1	0	0	3	8*

* statistically significantly different from control as per study report.

-Liver. The incidence of "focal hyperplasia" in the liver is presented in Table 1 above.

-"Probable essential thrombocythemia" was present in 0, 6 (26%), 3 (20%) and 9 (38%) of the males for the control to high dose groups and in only 1(4%) female in the high dose group.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential. Survival was not affected in a dose dependent manner although many rats dosed with PBO that died had gastrointestinal (cecum) hemorrhage.

Based on the presence of gastrointestinal hemorrhage at all dose levels and decreases in body weight of about 50% in the high dose group when compared to the controls and the prevalence of "probable essential thrombocythemia" in males the high dose group is considered excessive. Based on gastrointestinal effects, the low and mid dose groups may also be considered excessive.

2. Rat Study #2. Rat Carcinogenicity Study. Bio-Research Study No.: 81690, August 27, 1987, MRID No.: 40323701, HED Document No.: 006668. (The DER dated April 15, 1988 is attached as Attachment 3).

a. Experimental Design. Sprague-Dawley Crl-CDR strain rats 60/sex/dose group were dosed as control-1, control-2, 30, 100 or 500 mg/kg/day by the dietary route for 24 months. An interim sacrifice at 1 month of 10/sex for control-1, 15 and 30 mg/kg/day groups was included.

b. Discussion of Tumor Data. There was an increase in thyroid follicular cell adenomas (females) and or adenomas and carcinomas (males) combined based on positive ~~trends~~. Tables 3 (males) and 4 (females, taken from L. Brunsman memo dated Jan. 4, 1995, Attachment 4) illustrate the tumor incidence and present a statistical assessment) of the thyroid data.

Historical control data for thyroid tumors from the testing laboratory have been requested but not provided. The Charles River Breeder's background summary (refer to "Spontaneous Neoplastic Lesions and Selected Non-neoplastic Lesions in the Crl:CD®BR Rat, February, 1992) indicates a range of 1.1 to 25.7% for thyroid follicular adenomas and 1.0 to 6.0% for carcinomas.

*marginal
increase in
thyroid
tumors*

Table 3. Piperonyl Butoxide - Charles River Sprague-Dawley
Crl-CDR Rat StudyMale Thyroid Follicular Cell Tumor Rates* and Exact
Trend Test and Fisher's Exact Test Results (p values)

Dose (mg/kg/day)

	0 ^a	30	100	500
Adenomas (%)	2/115 (2)	1/58 (2)	0/58 (0)	2 ^b /53 (4)
p =	0.212	0.740	0.441 ^a	0.375
Carcinomas (%)	1 ^b /115 (1)	0/58 (0)	0/58 (0)	2/53 (4)
p =	0.090	0.665 ^a	0.665 ^a	0.234
Combined (%)	3/115 (3)	1/58 (2)	0/58 (0)	4/53 (8)
p =	0.040	0.589 ^a	0.291 ^a	0.142

*Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

^aTwo separate control groups were combined for this risk assessment.^bNegative change from control.^cFirst adenoma observed at week 79, dose 500 mg/kg/day.^dFirst carcinoma observed at week 89, dose 0 mg/kg/day.Note: Significance of trend denoted at control.Significance of pair-wise comparison with control denoted at dose
level.If ^a, then p < 0.05. If ^b, then p < 0.01.Table 4. Piperonyl Butoxide - Charles River Sprague-Dawley
Crl-CDR Rat StudyFemale Thyroid Follicular Cell Tumor Rates* and Exact
Trend Test and Fisher's Exact Test Results (p values)

Dose (mg/kg/day)

	0 ^a	30	100	500
Adenomas ^c (%)	1/116 (1)	0/57 (0)	1 ^d /58 (2)	3/58 (5)
p =	0.000	0.671 ^a	0.557	0.109

*Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

^aTwo separate control groups were combined for this risk assessment.^bNegative change from control.^cFirst adenoma observed at week 105, dose 100 mg/kg/day.^dThere were no thyroid follicular cell carcinomas diagnosed.Note: Significance of trend denoted at control.Significance of pair-wise comparison with control denoted at dose
level.If ^a, then p < 0.05. If ^b, then p < 0.01.

Liver tumors were found at a slightly higher incidence in the dosed animals as indicated by the Table 5.

Table 5. Pathological findings in the liver of Cr1:CDBR rats dosed with piperonyl butoxide for 2 years.

Lesion Description	Males					Females				
	C1	C2	30	100	500	C1	C2	30	100	500
Hypertrophy hepatocytes	4	2	1	4	29*	4	2	0	2	47*
Focal mixed cells	1	1	4	1	5	3	3	3	13*	20*
Focal eosinophilic cells	6	6	3	10	12	6	2	5	5	3
Hep. Carcinoma	1	1	0	0	1	0	0	0	1	1
Hep. Adenoma total	0	0	0	0	2	[None reported]				
	1	1	0	0	3	0	0	0	1	1

*statistically significantly different from the controls.
Based on 60 rats/sex/dose group reportedly examined.

female
c. Non-neoplastic Lesions. The liver in all dosed group ⁵ was determined to have increased weight (9% for males and 20% for females in the low dose group). In the group dosed with 100 mg/kg/day and above there were increased hepatic "focal mixed cells". At the 500 mg/kg/day dose level there was hypertrophy of the hepatocytes.

The thyroid was determined to have increased incidence of "pigment in follicles" (80%* of the high dose males vs only 37-45% of the males in the other groups and 73%* of the high dose females vs only 10-17% in the other groups). Hyperplasia in follicular cells was also increased in the high dose in both sexes (35%* in the high dose group vs only 7% to 18% in the other groups and females 18%* in the high dose group and 15%* in the mid dose group vs only 0 to 7% in all other groups). [Note * statistically significant.]

d. Adequacy of Dosing for Assessment of Carcinogenic Potential. Survival was not affected by treatment. Based on only minor (<10%) body weight gain decreases in the first 90 days in the high dose group only, TB-I considers higher doses could have been tolerated. Body weight gains were decreased only slightly at first but in the middle part of the study they were larger. There was, however, a consistent decrease in body weight compared to controls and at termination males were 22% and females were 21% lower in the high dose group.

3. Rat Study #3. Rat Carcinogenicity Study. NCI (Blue Book) Study No.: 120. Frederick Cancer Research Center, Published 1979¹. No DER has been prepared for this study. A copy of the Blue Book will be brought to the Peer Review meeting. The Blue Book Summary is attached (Attachment 5).

a. Experimental Design. Two groups of 50/sex Fischer 344 strain rats were dosed with 5000 or 10000 ppm of piperonyl butoxide (equivalent to approximately 250 or 500 mg/kg/day) for 107 weeks. A concurrent control group of 20/sex untreated rats was also included.

b. Discussion of Tumor Data

-Liver tumors. There were no liver tumors reported in any rats dosed with PBO. A single male control group rat had one incident of "neoplastic nodule".

-Thyroid tumors. Table 6 below illustrates the neoplastic and non-neoplastic findings in the thyroid.

Table 6. Thyroid neoplastic and non-neoplastic pathology in Fischer 344 rats dosed with piperonyl butoxide.

Lesion	Males			Females		
	Control	5000	10000	Control	5000	10000
N	19	49	48	19	48	49
<u>Tumors</u>						
Follic cell adenoma	0	0	2	0	0	0
Follic cell carcinoma	0	1	1	0	0	1
Total	0	1	3			1
C-cell adenoma	1	5	4	4	4	2
C-cell carcinoma	0	1	0	0	2	1
Total	1	6	4	4	6	3
<u>Non-neoplastic</u>						
C-cell hyperplasia	8	10	12	-	-	-
cystic follicles	-	-	-	-	-	1

Table 6 indicates that the rats dosed with PBO have follicular cell adenomas and/or carcinomas whereas the untreated rats do not. This finding is consistent with the results of the BioResearch 1987 study described under Rat Study #2 above. The presence of C-cell tumors in the NCI study was not also observed in the other studies with rats.

-Lymphomas. Three types of malignant lymphomas were noted

¹The test material was described as technical grade piperonyl butoxide from the Niagara Chemical Company, FMC Corporation, Middleport New York. It was demonstrated to be of 88.4% purity and from Lot No.: 5.

in females. Two of these types indicated a possible compound related increase. There was a single incident "malignant lymphoma, mixed type" in the low dose group and none in the control or high dose group. The incidence (number of animals affected/number of animals observed and percentage) of the other two tumor types is indicated as follows for the control, 5000 and 10000 ppm dose groups.

"malignant lymphoma, NOS" 0/20, 1/50(2%) and 6/50 (12%)

"malignant lymphoma, undiffer-type 1/20(5%), 5/50(10%) and 9/50 (18%)

According to the report, malignant lymphomas are a "relatively common tumor in Fischer 344 rats". The Cochran-Armitage trend test was significant ($p < 0.007$) for lymphomas. The Fisher exact test was also significant ($p < 0.020$) for the high dose group. The historical control record for the incidence of lymphomas and leukemias in female rats was reported to be 19/191 or 10%. One group had as much as 7/20 or 35% and another had 6/20 or 30%. The study report assessed the lymphoma data as follows (page 32)

The statistical conclusion suggests that the incidence of lymphomas in female rats may be associated with the administration of piperonyl butoxide; however, this conclusion may be due to the lower than usual incidence in the control group compared with historical data."

It is noted that malignant lymphoma did not appear to be increased in the other three studies with rats.

The statistical report from the NCI Blue Book for liver, the hematopoietic system and thyroid are in Attachment 5

c. Non-neoplastic Lesions. Focal hyperplasia was noted in the liver of all dose groups without an indication of increase with dose. Table 6 above illustrates selected non-neoplastic findings in the thyroid. It should be noted that a compound related increase in follicular hyperplasia was not reported.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential. There was no compound related effect on survival and 80% or greater survived until week 90 of the study. Body weight was decreased (described as "slight") in both sexes for the first 50 weeks. After that time, the weight difference became more evident and a clear dose response was noted. Since no data tables were presented, the percent difference in weight and weight gain cannot be determined. Based on visual inspection of the body weight graphs, it appears that higher doses could have been tolerated. It should be noted that the preliminary study indicated that dose levels of 46,000 ppm but not 31,500 ppm are

potentially lethal to this strain of rat.

4. Rat Study #4. A. Maekawa, H. Onodera, K. Furuta, H. Tanigawa, T. Ogiu and Y. Hayashi. (1985) "Lack of evidence of carcinogenicity of technical-grade piperonyl butoxide in F344 rats: Selective induction of ileocaecal ulcers" As published in *Fd. Chem. Toxic.* 23(7):675-682. No DER has been prepared for this study. A copy of the article is attached (Attachment 6).

a. **Experimental Design.** Three groups of 50/sex Fischer F344/DuCrj strain rats were dosed as controls or with 0.5% or 1.0% technical piperonyl butoxide (98% purity) in their diets for two years. These dose levels correspond to 0, 5000 and 10000 ppm or roughly equivalent to 250 and 500 mg/kg/day of piperonyl butoxide.

b. **Discussion of Tumor Data and Historical Control Data.** The study authors assert that there were no test compound related tumors. TB-I notes, however, that there were 2%, 2% and 6% incidents of "neoplastic nodules" in the liver of males for the control, 0.5% and 1% dose groups respectively and the male high dose group 2% incidents of hepatocellular carcinoma but the control and low dose groups did not have this tumor.

Among the females, there were 2% incidents of "neoplastic nodule" in the 0.5% but none in the control or high dose group. No indications of compound related increases in thyroid tumors were presented. The percent incidence of c-cell adenoma was 21%, 15% and 4% for the control, 0.5% and 1.0% dose groups respectively.

c. **Non-neoplastic lesions.** The study noted that the ileocaecal region of the digestive tract had high incidence of "ulcers", regenerative hyperplasia", ossification and hemorrhage. Males were more severely affected than females. For example, 17 and 24 males in the low and high dose groups developed ulcers but only 1 and 22 females in the low and high dose groups developed ulcers. Ulcers were not reported in the controls.

d. **Adequacy of Dosing for Assessment of Carcinogenic Potential.** Death rates were 16%, 38% and 42% for males and 14%, 22% and 34% for females for the control, 0.5% and 1.0% diets respectively. Body weight was decreased in a dose dependent manner but could not be quantitated with the data available. As indicated above, the ileocaecal portion of the gastrointestinal system developed ulcers and other associated lesions in response to treatment. Because of compound related deaths and the presence of ulcers in the gastrointestinal tract, both dose levels are considered in excess of the MTD.

5. Mouse Study #1. Mouse Carcinogenicity Study. Bushy Run Research Center. Study No.: 91N0134, August 27, 1993, MRID No.: 429037-01, HED Document No.: 010647. (The DER dated October 26, 1993 is attached, Attachment 7).

a. Experimental Design. Five groups of 60/sex CD-1 strain mice were dosed as control-1, control-2, 30, 100 or 300 mg/kg/day for 78 weeks. There were no interim sacrifices.

b. Discussion of Tumor Data. The study was considered positive for liver tumors in males (hepatocellular adenomas and carcinomas) in both the 100 and 300 mg/kg/day dose groups and females (hepatocellular adenomas only) in the 300 mg/kg/day dose group only. There were no carcinomas reported in the females. Tables 7 (males) and 8 (females) taken from the memo by Ms. Lori Brunsman of SAB/HED and dated Jan. 4, 1995 (Attachment 4) illustrate the tumor incidence and present a statistical evaluation of these data.

No historical control data have been provided by the testing laboratory. Charles River Breeding Laboratory summary data (refer to "Spontaneous neoplastic lesions in the Crl:CD-1 [ICR]BR mouse", prepared by Patricia L. Lang, Ph.D.) indicate that the range for adenomas is 0-16.3% in males and 0-2.7% in females and for hepatocellular carcinoma the range is 0-6.0% and 0-0.7% for females based on 8 studies run for 18 months.

c. Non-neoplastic Lesions. Liver weight increases were evident at 100 mg/kg/day (16.2% for males). The 30 mg/kg/day dose in males appeared to be a threshold with an increase of 7.1%. At 300 mg/kg/day there was a minimal body weight decrease in males (6.8% at week 74) but females were not affected. Net gain for the high dose males was 9.6 gms vs 11.4 and 12.4 gms for the controls.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential. Survival was not affected. Since the body weight gain effects were minimal in males (at best 15.7% decrease in the mid dose group and 22.6% decrease in the high dose group) and no effects were noted in females, the dose levels are considered adequate for males. The females, however, are considered to be tested below a pharmacologically active dose.

Table 7. Piperonyl Butoxide - Charles River CD-1 Mouse Study

Male Hepatocellular Tumor Rates* and
Peto's Prevalence Test Results (p values)

	<u>Dose (mg/kg/day)</u>			
	0 ^a	30	100	300
Adenomas (%)	17 ^a /107 (16)	13/57 (23)	22/56 (39)	28/59 (47)
p =	0.000 ^{***}	0.111	0.001 ^{***}	0.000 ^{***}
Carcinomas (%)	4/100 (4)	3/51 (6)	2/54 (4)	7 ^b /53 (13)
p =	0.008 ^{***}	0.260	0.499	0.005 ^{***}
Combined (%)	21/107 (20)	15 ^c /57 (26)	24/56 (43)	30 ^d /59 (51)
P =	0.000 ^{***}	0.116	0.001 ^{***}	0.000 ^{***}

*Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

^aTwo separate control groups were combined for this risk assessment.

^bFirst adenoma observed at week 61, dose 0 mg/kg/day.

^cFirst carcinoma observed at week 69, dose 300 mg/kg/day.

^dOne animal in the 30 mg/kg/day dose group had both an adenoma and a carcinoma.

^eFive animals in the 300 mg/kg/day dose group had both an adenoma and a carcinoma.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted

at dose level.

If ^{*}, then p < 0.05. If ^{***}, then p < 0.01.

Table 8. Piperonyl Butoxide - Charles River CD-1 Mouse Study

Female Hepatocellular Tumor Rates* and Exact Trend
Test and Fisher's Exact Test Results (p values)

	<u>Dose (mg/kg/day)</u>			
	0 ^a	30	100	300
Adenomas ^b (%)	4 ^a /116 (3)	1/58 (2)	1/60 (2)	10/57 (18)
p =	0.000 ^{***}	0.459 ^a	0.444 ^a	0.003 ^{***}

*Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.

^aTwo separate control groups were combined for this risk assessment.

^bNegative change from control.

^cFirst adenoma observed at week 56, dose 0 mg/kg/day.

^dThere were no hepatocellular carcinomas diagnosed.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted
dose level.

If ^{*}, then p < 0.05. If ^{***}, then p < 0.01.

6. Mouse Study #2. Mouse Carcinogenicity Study. O. Takahashi et al in Arch Toxicol 68:467-469 (1994). A copy of the paper as published is attached (Attachment 8). No DER has been prepared for this study.

a. Experimental Design. Male CD-1 strain mice were dosed with PBO as control (52/sex), 0.6% (53/sex) and 1.2% (100/sex) in their diets. These dose levels correspond to 0, 6000 or 12000 ppm or 0, 857 or 1714 mg/kg/day for 12 months.

b. Discussion of Tumor Data. Hepatocellular adenoma and carcinoma was induced in a dose related manner with there being 1.9% 24.5% and 75% incidence of the combined tumor types for the control low and high dose groups respectively. In addition, hemangioendothelial sarcoma was clearly associated with the high dose group and possibly associated with the low dose group. These two tumor types are illustrated in Table 9 below.

Table 9. Neoplastic and non-neoplastic lesion in male CD-1 mice dosed with PBO for 12 months (Takahashi study, 1994).

Lesion	Dose Level, % diet ¹		
	Control	0.6%	1.2%
<u>Non-neoplastic</u> N ¹	52	53	100
Hepatocellular hyperplasia	1 (1.9%)	20 (37.7%)*	8 (8%)
Postnecrotic peliosis	0	13 (24.5%)*	74 (74%)*
<u>neoplastic</u>			
Hepatocellular adenoma	1 (1.9%)	7 (13.2%)*	22 (22%)*
Hepatocellular carcinoma	0	6 (11.3%)*	52 (52%)*
Combined	1 (1.9%)	13 (24.5%)*	75 (74%)*
Hemangioendothelial sarcoma (total large and small)	0	1 (1.9%)	42 (42%)*

1. Total number of mice examined.

* Significantly different, $p < 0.05\%$ by Fisher's exact probability test as provided by the study author.

Specific historical control data were not provided in the publication as presented. However, a reference (Chandra and Firth, Toxicol Lett. 61:67-74 (1992) was made to spontaneous incidence of hepatocellular carcinoma of 24 month old CD-1 strain mice to be 5.7%.

c. Non-neoplastic Lesions. Hepatocellular hyperplasia was a characteristic non-neoplastic finding in the treated mice with 38% of the low dose and 8% of the high dose affected vs only 2% on the controls. The high incidence of adenomas and carcinomas in the high dose obscures the true dose response effect for this lesion. A condition described as "postnecrotic peliosis" (purpura or extravasion of the blood) was also reported in the liver of the dosed mice (see Table 7 above).

d. Adequacy of Dosing for Assessment of Carcinogenic Potential. The high dose group had only 81% survival after one year. The control group had 94% and the low dose group had 98% indicating that survival was adversely affected in the high dose group. Mean terminal body weights of the low and high dose groups were 17% and 29% decreased indicating a dose response effect on body weight. The study authors did not consider that either the increased deaths in the high dose group (which occurred after six months and were attributed to liver tumors) or the body weight decreases indicated that the high dose exceeded the "MTD".

7. Mouse Study #3. Mouse Carcinogenicity Study. NCI (Blue Book) Study No.: 120. Published 1979². No separate DER has been prepared for this study. A copy of the Blue Book will be brought to the Peer Review meeting. The Blue Book study summary is attached (Attachment 5).

a. Experimental Design. Two groups of 50/sex B6C3F1 strain mice were dosed initially as 2500 and 5000 ppm of piperonyl butoxide. A control group of 20/sex mice was also maintained. The initial test diet concentrations were determined to be too toxic and were reduced to 500 and 2000 ppm after 30 weeks. The study was terminated after 112 weeks. The time-weighted average dose was 1,036 and 2,804 ppm for both sexes. These diets correspond to approximately 148 and 298 mg/kg/day.

b. Discussion of Tumor Data.

Liver tumors. Hepatocellular carcinomas were prevalent in the male mice but without evidence of a dose response with there being 10 (50%), 17 (34%) and 20 (40%) for the control, low and high dose groups. Among the females there were 1 (5%), 2 (4%) and 5 (10%) for the control, low and high dose groups strongly indicating a dose response but statistical significance was not attained (p. 109, Table F2, from study report and on page 13A).

Lacrimal gland. In males there was a statistically significant increase trend (Cochrane-Armitage) in adenomas of the lacrimal gland as indicated by there being 0/20, 0/50 4/50 (8%) in the control, low and high dose groups (see page 13B). The results of the Fisher's exact test were not significant. The study authors did not conclude that the increase was due to piperonyl butoxide treatment.

²Refer to footnote number 1 for description of the test material.

c. Non-neoplastic Lesions.

Liver. Nodular hyperplasia in the liver was considered slightly elevated in the males with there being 1 (5%), 3 (6%) and 5 (10%) incidence for the control, low and high dose groups. In females there were no indications of compound related increases in nodular hyperplasia with there being 3 (13%), 7 (15%) and 4 (8%) for the control, low and high dose groups.

Thyroid. In females "cystic follicles" were slightly elevated in the dosed animal with there being 0, 1 (2%) and 5 (11%) for the control, low and high dose groups. In males, "follicular cysts" were present as 0, 1 (2%) and 2 (4%). There was also one incident of "cystic follicles" in the control group.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential. The survival of the mice was not affected by treatment. Figure 3 of the study report (page 34) indicates a dose related effect on body weight. Since no group data were presented, the percent differences in body weight cannot be calculated. Since the initial dose levels had to be reduced, (although there is no detailed description of the reactions to treatment that led to the reduction of dose levels), it cannot be determined that the dose levels were adequate for carcinogenicity assessment.

E. Additional Toxicology Data on Piperonyl Butoxide:

1. Metabolism A study classified as CORE GUIDELINE (MRID No.: 41998401, HED Document No.: 009783, study dated October 3, 1989) has provided useful information on the absorption, excretion and retention of PBO in rats. In essence, most of the material from ¹⁴C labelled piperonyl butoxide was recovered in the feces (46.95% to 54.01%) and lesser amounts in the urine (23.32% to 31.3%) and < 1.5% remained in the tissues. The liver and intestine retained the highest amounts. Eight urinary metabolites were identified that were apparently formed through beta-oxidation and subsequent cleavage of the ether side chain and/or oxidation in the methylene bridge on the benzodioxole ring.

2. Mutagenicity PBO has been tested in several mutagenicity toxicity studies. Studies concluded to be acceptable have been submitted for bacterial mutagenicity, in vitro chromosomal aberrations and sister chromatid exchange. Additional genetic toxicity studies especially in vivo studies are considered desirable.

a) Salmonella assay - (Refer to HED Document No.: 003438 and 007644). Two studies exist (SRS, 1983 and Litton

Bionetics, 1983) which did not indicate that piperonyl butoxide induced chromosome mutations in *Salmonella typhimurium* strains with and without metabolic activation.

7 Same study
b) In vitro cytogenetics in CHO ovary cells. (Refer to HED Document Nos: 003438, 004508, 007644 and 011005 and MRID No.: 43013801 and Acc No.: 257430). Three studies (Litton Bionetics 1983, Arthur D. Little, 1985 and Hazleton, 1993) have been submitted which provide evidence that piperonyl butoxide does not induce chromosome aberrations in CHO cells in vitro. One equivocal study is resolved by the two other negative studies.

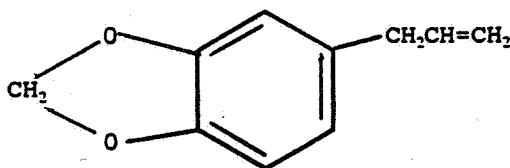
c) In vitro cytogenetics study (Litton Bionetics, 1983) did not indicate sister chromatid exchanges and/or chromosome aberration in CHO cells with and without metabolic activation.

3. Developmental Toxicity

No effects were noted in a rat developmental toxicity study tested at 1000 mg/kg/day. In rabbits, there were some maternal effects (decreased body weight and defecation) at 200 mg/kg/day but no developmental effects at this level (HDT). The rat multi-generation reproduction study was determined to have a NOEL of > 5000 ppm (250 mg/kg/day) for reproductive effects but a NOEL and LEL of 1000 and 5000 ppm for parental effects (decreased body weight) and developmental effects (decreased body weight gain in pups).

4. Structure-Activity and Functional Activity Correlations

a. Piperonyl butoxide is based on the chemical structure of safrole which has been demonstrated to cause tumors of the esophagus (Hagen et al in Toxicol. Appl. Pharmacol. 7:18-24 (1965)) and liver tumors in rats (Trump et al in J. Natl. Cancer Inst. 67:393-406 (1981)). The structure of safrol is as follows:



b. A factor to be considered under this category is the functional effect of piperonyl butoxide as compared with MGK-264 (PC # 057001) and pyrethrins (PC # 069001). MGK-264 like PBO inhibits mixed function oxidases (MFO) and both produce similar non-neoplastic lesions in the liver of rats and mice and hyperplasia and/or tumors in the follicular cells of the thyroid in rats. Pyrethrins are usually formulated with PBO and/or MGK-264 and pyrethrins are metabolized by the MFO. Thus all three chemicals interact with the same physiological system in the

liver. These three chemicals also produce hyperplasia/metaplasia in the upper respiratory tract of rats in subchronic (90-day) inhalation toxicity studies. MGK-264 and pyrethrins are also being presented to the HED Carcinogenicity Peer Review Committee.

5. Acute, Subchronic, and Chronic Toxicity Studies

Acute toxicity. Piperonyl butoxide has acute oral LD₅₀s of 4.7 (4.39-5.03) mg/kg in males and 4.1 (3.53-4.86) mg/kg in females meaning it is not regarded as a very toxic substance. There were no mortalities to rabbits dosed dermally with 2 gm/kg and the LC₅₀ was established to be > 5.9 mg/l. Piperonyl butoxide is Toxicity Category III for eye irritation and toxicity category IV for dermal irritation.

Subchronic Inhalation toxicity. Piperonyl butoxide as well as pyrethrins and MGK-264 have all been indicated to cause hyperplasia and metaplasia in the larynx of rats in 90-day subchronic inhalation toxicity studies. Hyperplasia is in many cases accepted as a preneoplastic condition and that continued exposure would result in tumors in the affected region(s).

F. Weight of Evidence Considerations: The committee will consider the following observations regarding the toxicology data on piperonyl butoxide in a weight of evidence determination of carcinogenic potential.

1. *Rat and mouse carcinogenicity studies.*

Rat Study #1. Fischer F344 strain rats were dosed as control, 0.6%, 1.2% and 2.4% PBO in their diets. The high dose group (equivalent to 1877 mg/kg/day in males and 2002 mg/kg/day in females) was considered to be excessive because of a 50% decrease in weight gain. Gastrointestinal haemorrhagia was noted in the 0.6%, 1.2% and 2.4% test doses but not in the controls. Because of excessive dosing in both sexes in this study, the relevance the tumors occurring in the two highest test dose levels is considered questionable. This study is further compromised since it is a publication from the open literature and no individual animal data are available for review. Thus, HED has not done an independent statistical analysis.

At 24,000 ppm in both males and females, there were increases in liver adenomas and carcinomas and increases in adenomas and carcinomas in males and there was a single incident of an adenoma in females in the 12000 ppm dose level group based on Dr. Butler's assessment. The original pathology report described liver tumors in all dosed groups for each sex but not in the controls.

Rat Study #2. Sprague-Dawley Crl strain rats were dosed as control (two groups), 30, 100 or 500 mg/kg/day by the dietary route for 24 months. Based on only minor (<10%) body weight decreases in the first 90 days in the high dose group only, TB-I considers higher doses could have been tolerated. There was, however, a consistent decrease in body weight reaching 22% for males and 21% for females at termination.

Males and females had a significant increased trend in thyroid follicular cell combined adenomas and/or carcinomas ($p < 0.05$). There were no significant differences in pair-wise comparisons of the dosed groups with the controls.

Rat Study #3. Fischer F344 strain rats were dosed as controls, 5000 or 10000 ppm of PBO for 107 weeks. Since only very minor body weight decreases were noted, it is apparent that higher doses could have been tolerated and this study is considered of limited usefulness in this regard. The study is further compromised because no individual animal data were provided and HED has not done independent statistical analyses.

carcinomas
Males in both treatment groups had thyroid follicular cell adenomas and/or carcinomas. ~~The~~ single incident of an thyroid adenoma in females was in the high dose group. In males the two dosed groups had increased incidence of C-cell adenomas and/or carcinomas. *follicular*

Females had increased incidence of "malignant lymphomas" of two types and both the trend ($p < 0.007$) and pair-wise comparisons ($p < 0.02$) were positive.

Mouse Study #1. CD-1 strain mice were dosed as control (two groups), 30, 100 or 300 mg/kg/day for 78 weeks. Males were considered to be dosed at an adequate dose level since body weight was decreased about 16% in the mid dose group and 23% in the high dose group. Since body weight was only slightly affected in females, this sex is considered to have been tested below a pharmacologically active dose. The female data may be compromised.

females
Male mice had both a significant ($p < 0.01$) positive trend for adenomas and carcinomas and pair wise comparison indicated that the mid ($p < 0.001$) and high dose group ($p < 0.000$) were significant for adenomas. The high dose ($p < 0.005$) was significant for carcinomas. The trend test was also positive ($p < 0.01$) and the mid ($p < 0.001$) and high ($p < 0.000$) dose groups were significant by pair wise comparison for combined adenomas and carcinomas.

Mouse Study #2. CD-1 strain mice (males only) were dosed as control, 0.6% and 1.2% PBO in their diets for one year. Based on decreases in body weights of 17% for the low dose and 29% for the high dose and a slight decrease in survival in the high dose, the

high dose is considered at or in excess of the MTD. The study was obtained from the published literature and no individual animal data are available for independent statistical analysis.

Both the low and high dose groups ($p < 0.05$) had significantly increased incidence of hepatocellular adenoma and carcinoma and combined adenoma and carcinoma.

The males also had significantly increased hemangio-endothelial sarcoma in the high dose group ($p < 0.05$) and an incident in the low dose group but not any control animals were affected.

Mouse Study #3. B6C3F1 strain mice were dosed initially as 2500 and 5000 ppm. These doses were later decreased to 500 and 2000 ppm. The survival was not affected but since undescribed symptoms required decreasing the dose level, it is assumed this high dose was adjusted to adequate testing levels. The study is an NCI Blue Book study and no individual animal data were presented and no independent statistical analysis by HED was done.

Females had an apparent increase in liver tumors that did not reach statistical significance for either trend or pair-wise comparison. There were 1 (5%), 2 (4%) and 5 (10%) incidents of hepatocellular carcinomas.

In males there was a statistically significant increased trend for adenomas in the lacrimal gland but the pair-wise comparison was not significant.

2. PBO is structurally related to safrole a chemical which has been demonstrated to produce esophageal and liver tumors.

3. PBO is not considered mutagenic in several mutagenicity genetic toxicity studies.

Summary Table of Seven Carcinogenicity Studies with Rats or Mice with Piperonyl Butoxide.

Study Identification	Organs of Concern for Neoplasia
<u>Rat Study #1.</u> Tokyo Metropolitan Research Laboratory. in Fund. Appl. Toxicol. 22:292-303 (1994). Fischer F344 strain, 0.6%, 1.2% and 2.4% PBO in diets for two years. > SUPPLEMENTARY	<u>Liver tumors</u> (adenomas and carcinomas) all dose levels. Dose levels considered excessive.
<u>Rat Study #2.</u> Bio-Research # 81690. August 27, 1987. * Sprague-Dawley Crl-CDR strain, control, 30, 100 or 500 mg/kg/day for two years. GUIDELINES	Positive <u>trend</u> for thyroid follicular cell tumors. liver tumors ? Body weight decreases but higher levels could have been tolerated.
<u>Rat Study #3.</u> NCI Blue Book Study. 1979. Fischer F344 strain rats, control 5000 and 10000 ppm for 107 weeks. SUPPLEMENTARY (no DER).	Positive trends and/or pair wise comparisons for <u>Thyroid follicular tumors</u> <u>Lymphomas</u> Doses considered adequate.
<u>Rat Study #4.</u> National Institute Hygienic Sciences, Tokyo, Japan in Fd. Chem. Toxic. 23:675-682 (1985). Fischer F344/DuCrj strain rats, controls, 0.5% and 1.0% for 2 years.	Authors concluded study does not demonstrate carcinogenicity. Ileocaecal ulcers in both treated groups in both sexes. Insufficient data to determine adequacy of dosing.
<u>Mouse Study #1.</u> Bushy Run Research Center, #91N0134, August 27, 1993. * CD-1 strain mice, control 30, 100 or 300 mg/kg/day for 78 weeks. GUIDELINES.	<u>Liver tumors</u> in both sexes. Doses considered adequate in males but females could have tolerated higher levels.
<u>Mouse Study #2.</u> Tokyo Metropolitan Research Institute. AS published in Arch. Toxicol. 68:467-469 (1994). CD-1 strain (males only reported). Control 0.6% and 1.2% in diets for 1 year. SUPPLEMENTARY (no DER).	<u>Liver Tumors</u> in both doses. <u>Hemangioendothelial sarcoma</u> in high and possible low dose group. Higher doses could have been tolerated.

<u>Mouse Study #3.</u> NCI Blue Book Study, 1979. B6C3F1 strain mice. Control, 1036 and 2804 ppm for 112 weeks. SUPPLEMENTARY	<u>Lacrimal gland tumors</u> have increased trend in males. <i>See tumor females?</i>
--	---

Chronic Toxicity Studies of Piperonyl Butoxide in F344 Rats: Induction of Hepatocellular Carcinoma¹

O. TAKAHASHI,² S. OISHI, T. FUJITANI, T. TANAKA, AND M. YONEYAMA

Department of Toxicology, Tokyo Metropolitan Research Laboratory of Public Health, 24-1, Hyakunincho 3-chome, Shinjuku-ku, Tokyo 169, Japan

Received May 20, 1992; accepted July 7, 1993

Chronic Toxicity Studies of Piperonyl Butoxide in F344 Rats: Induction of Hepatocellular Carcinoma. TAKAHASHI, O., OISHI, S., FUJITANI, T., TANAKA, T., AND YONEYAMA, M. (1994). *Fundam. Appl. Toxicol.* 22, 293-303.

Male and female F344 rats (30-33 rats/group) were administered piperonyl butoxide (α -[2-(2-butoxyethoxy)ethoxy]-4,5-methylenedioxy-2-propyltoluene) in the diet at levels of 0 (control), 0.6, 1.2, and 2.4% for nearly 2 years. Beginning at about 40 weeks, 10 rats in the 1.2% treated male group died due to cecal hemorrhages. Piperonyl butoxide induced hepatocellular carcinoma in both sexes in a dose-dependent manner. Hepatocellular carcinoma was found even in the 1.2% treated male group (incidence, 26.7%), and incidences in the 2.4% groups of males and females were 80.0 and 57.7% respectively of all those surviving. Piperonyl butoxide also caused essential thrombocythemia with a dose-response relationship. Hemorrhages in stomach and cecum, anemia, degenerative lesions of alveoli, and nephrotoxicity were also observed related to exposure. These results indicate that piperonyl butoxide is a hepatocarcinogen to the rat. © 1994 Society of Toxicology.

Piperonyl butoxide (α -[2-(2-butoxyethoxy)ethoxy]-4,5-methylenedioxy-2-propyltoluene) is widely used as a synergist for the pyrethrins and related insecticides on a variety of fruit, vegetable, forage, and grain crops. In addition it is used on livestock and in agricultural areas as well as in various domestic pesticide aerosol formulations. Piperonyl butoxide has been allowed as a food additive in Japan since 1955. Its maximum approved use level is 0.024 g/kg (24 ppm) in raw cereals, and the acceptable daily intake (ADI) for humans is 0-0.03 mg/kg body wt (Joint FAO/WHO Meeting, 1973). Pyrethrin formulations produced for stored crops in Japan in 1986 totaled 233 tons.

There have been many toxicological reports on this substance. Liver and kidney damage was found when rats were

given 0.5-3.0% piperonyl butoxide in the diet for 1-13 weeks (Goldstein *et al.*, 1973; Sternberg, 1979; Maekawa *et al.*, 1985; Fujitani *et al.*, 1992). No mutagenic activities have been reported (Ashwood-Smith *et al.*, 1972; Sternberg, 1979; White *et al.*, 1977; Rosenkranz *et al.*, 1990; Nesnow, 1990), nor has carcinogenicity been detected (Cardy *et al.*, 1979; Innes *et al.*, 1969; NCI, 1979; Maekawa *et al.*, 1985). Although teratogenicity has not been reported (Kennedy *et al.*, 1977; Khera *et al.*, 1979), we have recently found teratogenicity (Tanaka *et al.*, 1993a) and developmental toxicities of this compound (Tanaka, 1992, 1993; Tanaka *et al.*, 1992).

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

MATERIALS AND METHODS

Chemicals. Piperonyl butoxide (technical grade; lot Nos. 909002 and 009002) was purchased from Takasago International Corporation (Tokyo). The purities were 94.5 and 94.3% by gas chromatography, and these preparations did not contain safrol or isosafrol.

Diets. CE-2, obtained from CLEA Japan Inc. (Tokyo), was used as a basal diet. After mixing piperonyl butoxide with the powdered diet, pellets were formed and fed to rats. All lots of basal diet were analyzed and determined to be free of contamination by pesticides, PCB, or aflatoxins. Chemicals, detection limits, and analytical methods used were as follows: DDT (<0.05 ppm; ECD-GC), BHC (<0.05 ppm; ECD-GC), aldrin (<0.01 ppm; ECD-GC), dieldrin (<0.01 ppm; ECD-GC), endrin (<0.01 ppm; ECD-GC), heptachlor (<0.01 ppm; ECD-GC), PCB (<0.01 ppm; ECD-GC), parathion (<0.05 ppm; FPD-GC), aflatoxin (<5 ppb; fluorometry).

Animals. Specific pathogen-free male and female Fischer (F344/DuCrj) rats, 4 weeks old, were purchased from Charles River Japan, Inc. (Kanagawa, Japan) and housed individually in stainless steel suspended cages, in an air-conditioned room at a temperature of $25 \pm 1^\circ\text{C}$ and relative humidity of $55 \pm 5\%$, under the barrier system.

Experimental design. After acclimatization for 1 week on the basal diet, male or female rats were divided into four groups of 30, 30, 30, and 33 rats and were given diets containing piperonyl butoxide at levels of 0 (control), 0.6, 1.2, or 2.4%, respectively, and water *ad lib*. for nearly 2 years. The doses are the same as those of the previous subacute test (Fujitani *et al.*, 1992); 2.4% is just equal to 1000 times the maximum approved use level for raw cereals in Japan. In a preliminary experiment we determined food consumption rates four times for 6 rats in each of the dose groups. The averages of those results are 91.1, 91.1, 87.7, and 78.2 g/kg/day in

¹ The views, opinions, and assertions expressed in this article are those of the authors and do not reflect the official policy of the Tokyo Metropolitan Government.

² This study was conducted by these five members equally. Therefore, the order of authors' names is unimportant.

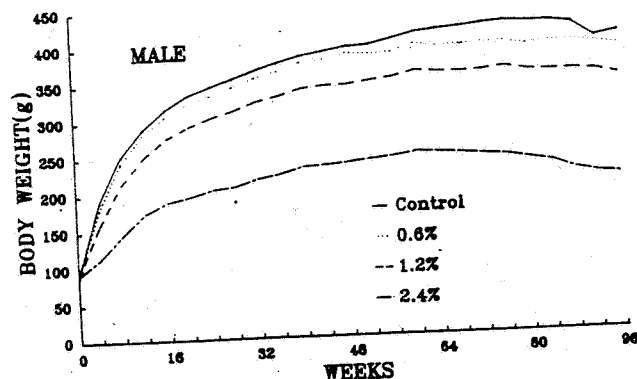


FIG. 1. Mean body weights of male rats given piperonyl butoxide for 95 weeks. Body weights of treated groups were significantly different from the control throughout the experimental period in a dose-dependent manner.

males and 90.1, 89.6, 88.4, and 83.4 g/kg/day in females of 0, 0.6, 1.2, and 2.4% groups, respectively. Calculated intakes of piperonyl butoxide are 547, 1052, and 1877 mg/kg/day for males and 537, 1061, and 2002 mg/kg/day for females of 0.6, 1.2, and 2.4% groups, respectively. During the experimental period, the animals were observed daily, and clinical signs and mortality were recorded. Body weight was measured monthly. Rats that died were necropsied and examined for tumors and nonneoplastic lesions; tumors and sites of lesions were fixed with buffered 4% formaldehyde solution, and a histopathologic examination was conducted. Near the end of the planned 2-year experiment, many male rats given 1.2% piperonyl butoxide and the female controls had died; therefore, the feeding was terminated early. Males and females were killed on Weeks 95 and 96, respectively.

Necropsy. At the termination of administration, all of the surviving rats were killed by deep ether anesthesia. Blood was collected from the posterior vena cava into a plastic syringe containing 3.8% sodium citrate solution (blood:sodium citrate, 9:1, v/v). The citrated blood was used for a hematological study. Separately, the citrated blood was refrigerated and centrifuged, and plasma was separated for plasma biochemistry. Rats were necropsied, and tumors and nonneoplastic lesions were examined. The number and size of hepatic nodules were counted and recorded. Major organs were dissected and weighed.

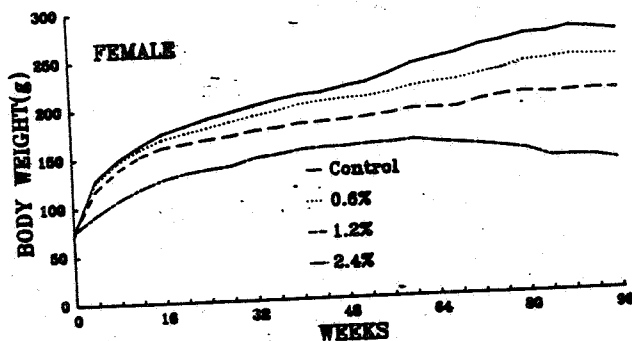


FIG. 2. Mean body weights of female rats given piperonyl butoxide for 96 weeks. Body weights of treated groups were significantly different from the control throughout the experimental period in a dose-dependent manner.

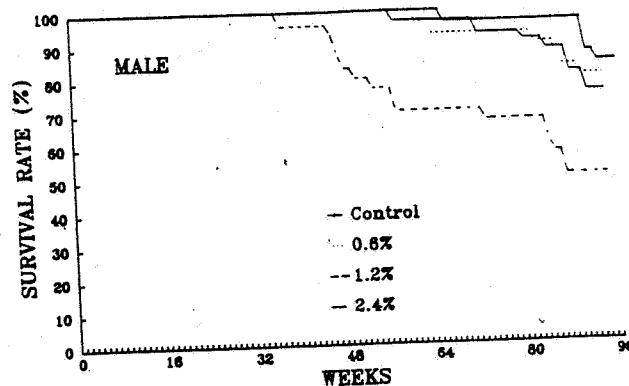


FIG. 3. Survival curves of male rats given piperonyl butoxide for 95 weeks. Mortality of animals treated with 1.2% of the substance was significantly higher than that in other groups after Week 45.

Histopathology. Nodules and major organs (brain, lung, heart, liver, kidney, stomach, intestine, cecum, adrenal gland, spleen, testis, uterus, and ovary) were fixed with buffered 4% formaldehyde solution and sectioned and stained routinely with hematoxylin and eosin. As for livers, two to three sections of the different sites were reviewed by microscopy if nodules were not observed macroscopically. If there were nodules, we reviewed three to five sections of three different major nodules. Judgment and classification of hepatic nodules were done according to the report by Boorman *et al.* (1990) and Maronpot *et al.* (1986). Nodular lesions were divided into focus, hepatocellular focal hyperplasia, hepatocellular adenoma (synonyms for these two lesions include hyperplastic nodule and neoplastic nodule) (Squire and Levitt, 1975), and hepatocellular carcinoma by cellular atypia, structural atypia, nucleus/cytoplasm ratio, and compression of the adjacent tissue. Cholangiofibrosis, cystic cholangioma, and histiocytic sarcoma were also described. Hemangiosarcoma or hemangiosarcoma-like lesions were found but those were not distinguishable.

Hematological studies. Hematological data were obtained by a Systemic E-5000 automated hematology analyzer (Toa Medical Electronics Corporation, Ltd., Kobe, Japan), except for prothrombin and kaolin-activated partial thromboplastin times. The following parameters were measured: particle concentration of white blood cells (WBC), red blood cells (RBC) and platelets (PLT); WBC-small cell count (W-SCC), WBC-small cell ratio (W-SCR); hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH).

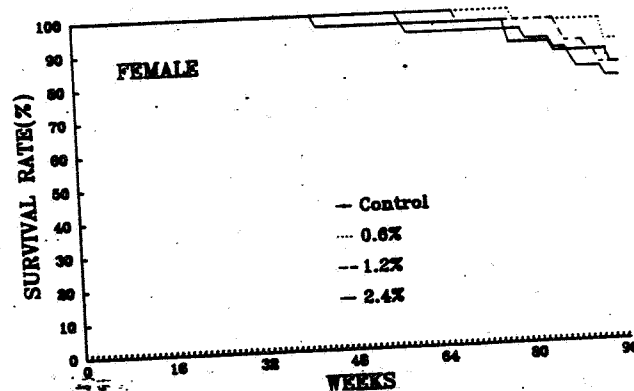


FIG. 4. Survival curves of female rats given piperonyl butoxide for 96 weeks. Mortality levels were not significantly different from each other.

TABLE 1
Hyperplastic and Neoplastic Nodular Lesions of Livers

	Piperonyl butoxide (dietary %)			
	0	0.6	1.2	2.4
Male				
Number of surviving rats	25	23	15	25
Surviving rats bearing nodules (macroscopic) ^a	0	6 ^b (26.1) ^c	14 ^b (93.3)	25 ^b (100)
Mean number of nodules/rat ^d	0	0.4	3.5 ^b	12.7 ^b
Mean number of nodules in size distinction/rat				
1-5 ^a mm diameter	0	0.4	1.5 ^b	8.4 ^b
6-10 mm diameter	0	0	1.7 ^b	2.6 ^b
>11 mm diameter	0	0	0.3	1.8 ^b
Focus (basophilic or clear cell)	0 ^d	11 ^b (47.8)	2 (13.3)	0
Hepatocellular hyperplasia (focal or multifocal)	2 (8.0)	3 (13.0)	2 (13.3)	0
Hepatocellular adenoma ^a	0	0	8 ^b (53.3)	5 ^b (20.0)
Hepatocellular carcinoma ^a	0	0	4 ^b (26.7)	20 ^b (80.0)
Hepatocellular adenoma and carcinoma ^a	0	0	12 ^b (80.0)	25 ^b (100)
Cholangiofibrosis	0	0	0	1 (4.0)
Cholangioma (cystic)	0	0	0	1 (4.0)
Hemangiosarcoma/hemangiosarcoma-like lesion ^a	0	0	2 (13.3)	11 ^b (44.0)
Number of surviving and dead rats	30	30	30	33
Surviving and dead rats bearing nodules (macroscopic) ^a	0	6 ^b (20.0)	18 ^b (60.0)	29 ^b (87.9)
Focus (basophilic or clear cell)	0	11 ^b (36.7)	2 (6.7)	0
Hepatocellular hyperplasia (focal or multifocal)	2 (6.7)	3 (10.0)	5 (16.7)	1 (3.0)
Hepatocellular adenoma ^a	0	0	8 ^b (26.7)	5 ^b (15.2)
Hepatocellular carcinoma ^a	0	0	4 (13.3)	24 ^b (72.7)
Hepatocellular adenoma and carcinoma ^a	0	0	12 ^b (40.0)	29 ^b (87.9)
Cholangiofibrosis	0	0	0	1 (3.0)
Cholangioma (cystic)	0	0	0	1 (3.0)
Histiocytic sarcoma	0	0	0	1 (3.0)
Hemangiosarcoma/hemangiosarcoma-like lesion ^a	0	0	2 (6.7)	15 ^b (45.5)
Female				
Number of surviving rats	24	27	25	26
Surviving rats bearing nodule (macroscopic) ^a	0	1 (3.7)	22 ^b (88.0)	26 (100)
Mean number of nodules/rat ^d	0	0.04	2.2 ^b	14.0 ^b
Mean number of nodules in size distinction/rat				
1-5 ^a mm diameter	0	0.04	2.1 ^b	9.7 ^b
6-10 mm diameter	0	0	0.04	2.8 ^b
>11 mm diameter	0	0	0.04	1.5
Focus (basophilic or clear cell)	1 (4.2)	2 (7.4)	6 (24.0)	0
Hepatocellular hyperplasia (focal or multifocal)	0	0	12 ^b (48.0)	2 (7.7)
Hepatocellular adenoma ^a	0	0	4 (16.0)	9 ^b (34.6)
Hepatocellular carcinoma ^a	0	0	0	15 ^b (57.7)
Hepatocellular adenoma and carcinoma ^a	0	0	4 (16.0)	24 ^b (92.3)
Cholangiofibrosis ^a	0	0	0	3 (11.5)
Cholangioma (cystic) ^a	0	0	0	2 (7.7)
Hemangiosarcoma/hemangiosarcoma-like lesion ^a	0	0	0	8 ^b (30.8)
Number of surviving and dead rats	30	30	30	33
Surviving and dead rats bearing nodules (macroscopic) ^a	0	1 (3.3)	23 ^b (76.7)	31 ^b (93.9)
Focus (basophilic or clear cell)	1 (3.3)	2 (6.7)	6 (20.0)	0
Hepatocellular hyperplasia (focal or multifocal)	0	0	12 ^b (40.0)	2 (6.1)
Hepatocellular adenoma ^a	0	0	4 (13.3)	10 ^b (30.0)
Hepatocellular carcinoma ^a	0	0	0	15 ^b (45.5)
Hepatocellular adenoma and carcinoma ^a	0	0	4 (13.3)	25 ^b (75.8)
Cholangiofibrosis ^a	0	0	0	5 ^b (15.2)
Cholangioma (cystic) ^a	0	0	0	2 (6.1)
Hemangiosarcoma/hemangiosarcoma-like lesion ^a	0	0	0	8 ^b (24.2)

^a The response has a significant dose-related linear trend.

^b Significantly different from the appropriate control (0%) value.

^c The number in the parentheses is the incidence (%).

^d All histologic data are expressed as the number of rats having the lesion.

25

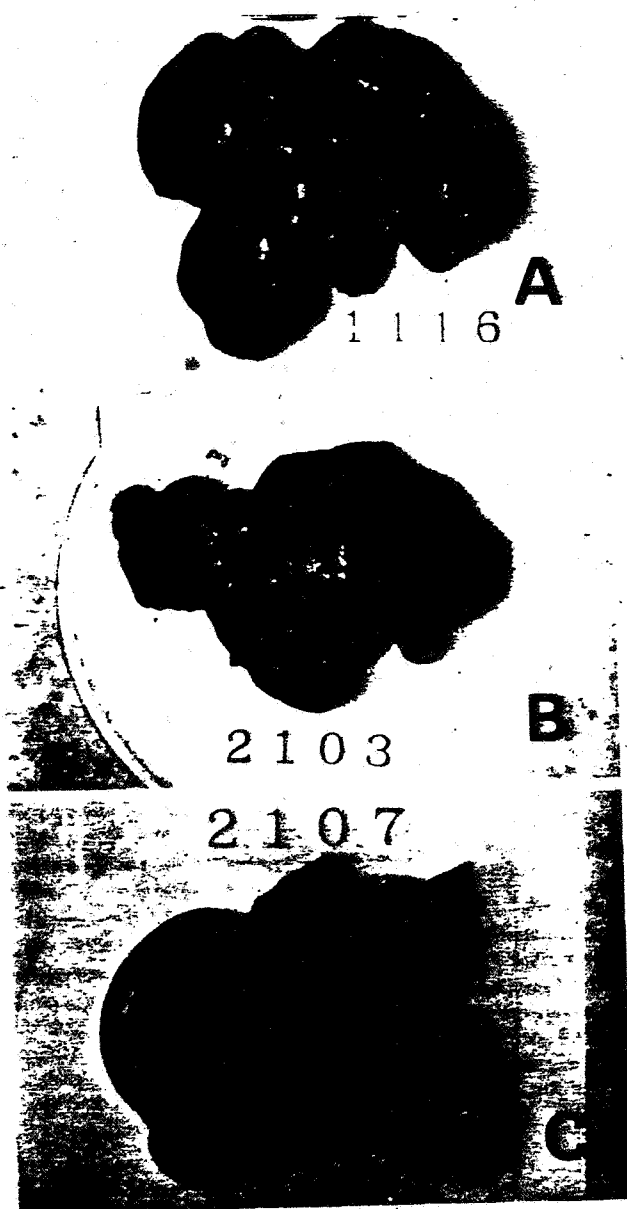


FIG. 5. Liver tumors of the male (A) and female (B and C) rats given 2.4% piperonyl butoxide for 95 and 96 weeks, respectively. (A) Nearly 13 tumors were seen on all lobes. Their colors were gray-white or white. Larger ones were over 8 mm in diameter. A vascular system was observed within the tumor. (B) Many white or muddy yellow tumors (2–30 mm) were seen on all lobes. (C) Many large, medium, and small tumors were seen on all lobes. Large white ones fill the entire lobe.

mean corpuscular hemoglobin concentration (MCHC), RBC distribution width (RDW); mean platelet volume (MPV), PLT distribution width (PDW), platelet-large cell ratio (P-LCR). Citrated blood provided stable data of WBC and PLT counts for at least 6 hr after it was collected. Prothrombin and kaolin-activated partial thromboplastin times (PT and KPTT) were measured by routine methods using simplastin as a thromboplastin and platelin as a partial thromboplastin, for some randomly se-

lected samples. Data are expressed as an index (%) = a mean value of PT or KPTT of control rats/each value of PT or KPTT of a treated rat $\times 100$. Some blood samples were stained with Giemsa solution and examined microscopically. Judgement of leukemia was made by summarizing WBC count, spleen weight, histopathologic study, and the degree of anemia. We looked upon a rat as having probable essential thrombocythemia if PLT counts were over 3,000,000 (the counting limit of this apparatus) and platelet distribution curves had two obvious peaks at 4 femtoliters (fl) (minor and normal peak) and 62 fl (major peak). Essential thrombocythemia is also known as hemorrhagic thrombocythemia and primary thrombocythemia.

Plasma biochemistry. Data were obtained by a Hitachi 705 automatic analyzer (Tokyo). Plasma concentrations of albumin (ALB), total protein (TP), cholesterol (CHO), free cholesterol (F-CHO), esterified cholesterol (E-CHO), phospholipids (PL), triglycerides (TG), glucose (GLC), uric acid (UA), and urea nitrogen (UN); activities of cholinesterase (CHE), γ -glutamyl transpeptidase (γ -GTP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT); and ratios of albumin/globulin (ALB/GLB) and AST/ALT were measured.

Statistics. Variations of results of body weight, organ weight, hematologic parameters, and plasma biochemistry are routinely expressed as the standard deviation. Significance of difference was tested by Bartlett's test, one-way analysis of variance, and Dunnett's or Scheffé's test. If Bartlett's test was significant, data were subjected to the Kruskal-Wallis test and the Scheffé type rank sum test (Gad and Weil, 1982). Mortality data were determined by Kaplan and Meier's test. Pathologic data were evaluated by $C \times R \chi^2$ analysis and Fischer's exact probability test. The Cochran-Armitage test for linear trend in proportion was also conducted for the data on hepatic tumors. In all analyses, the limit for significance was set at $p < 0.05$.

RESULTS

Growth, Clinical Signs of Intoxication, and Mortality

Body weight gains were reduced in piperonyl butoxide-treated groups of both sexes dose dependently during all experimental periods (Figs. 1 and 2). After Week 60, rats of the 2.4% groups of both sexes gradually lost weight.

During the first month, rough hair, lethargy, epistaxis, and a drop in food consumption were observed in the 2.4% group of both sexes. During Weeks 4–16, epistaxis in these same groups and expansion of the abdomen in all piperonyl butoxide-administered groups were found. After Week 20, dirtiness of posterior parts of the abdomen was recognized. Tumors on the skin were found after about 1 year in all groups and deaths began to occur at about Week 40.

Survival curves are shown in Figs. 3 and 4. Mortality in the male 1.2% group was significantly higher than that in other groups. In dead rats, hemorrhages in the cecum were the most profound lesions (see Table 4). Enlargement of the spleen was observed in some expired rats of all groups. Dead rats with hepatic tumors were seen from Week 74, but the cause of death was not hepatic cancer. Most of those rats died from cecum hemorrhages. Leukemia may be another cause of death. In all cases, there was no relationship between deaths and severity of hepatic tumors.

Liver Tumors

Many neoplastic lesions were observed in rats of all groups, with hepatic tumors being the most conspicuously



FIG. 6. Photomicrograph of hepatocellular carcinoma (poorly differentiated) from a male rat given 1.2% piperonyl butoxide for 95 weeks. Anaplastic tumor cells having polychromatic and atypic nuclei and mitotic figures are seen. Increased nucleus/cytoplasm ratios and mitotic figures are also visible. Hematoxylin and eosin stain, $\times 64$.

related to the feeding of piperonyl butoxide. Incidence of hyperplastic and neoplastic nodular lesions is summarized in Table 1; this condition was observed in all piperonyl butoxide-treated groups of both sexes in a dose-dependent manner (Fig. 5). The number of nodules per liver and size of the nodules also increased in a dose-dependent fashion. In the 2.4% groups of both sexes, 13-14 nodules were counted, one with a diameter of over 10 mm.

Nodules occurred in all lobes and were solid and white and whitish gray; many of them occasionally contained blood lakes. Histopathologically, hepatocellular adenomas,

and carcinomas were increased in the treated groups of both sexes in a dose-dependent manner. Carcinomas were observed at more than 1.2% in males or at only 2.4% in females. The incidences of carcinoma in surviving male and female rats of the 2.4% groups were 80.0 and 57.7%. Most carcinomas were the trabecular type characterized by trabeculae multiple thick cell layers (Figs. 6 and 7). In the lower doses, clear cell focus and nodular hyperplasia were significantly increased. Cholangiofibrosis and cystic cholangioma were found in some rats of the 2.4% treated groups of both sexes. Oval cell proliferation was also seen in

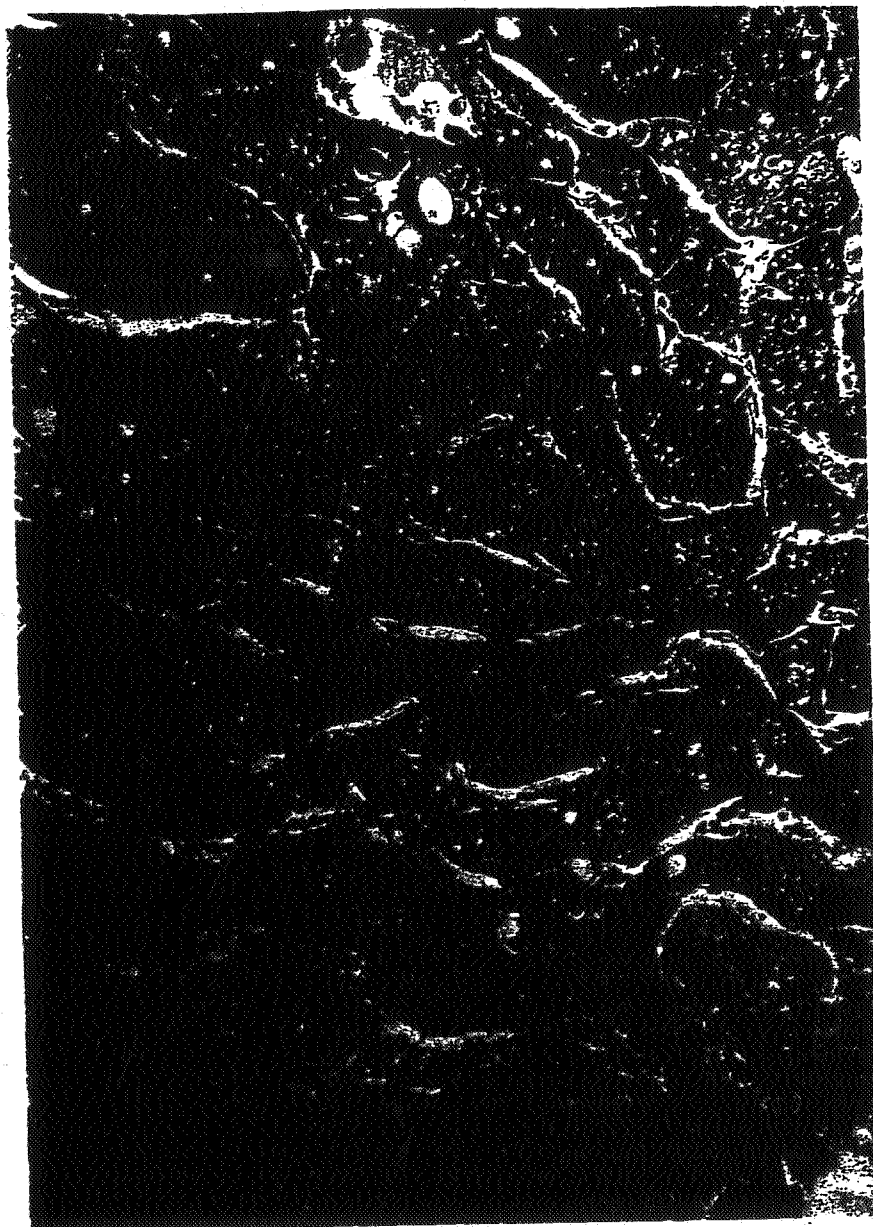


FIG. 7. Photomicrograph of hepatocellular carcinoma (well differentiated) with a trabecular pattern from a male rat given 2.4% piperonyl butoxide for 95 weeks. Hematoxylin and eosin stain, $\times 64$.

the treated groups (data not shown). Hepatocellular carcinoma concurrently contained hemorrhage, necrosis, proliferation of endothelial cells, and hemangiosarcoma or hemangiosarcoma-like lesions,

Essential Thrombocythemia

Probable essential thrombocythemia was observed in treated male rats in a dose-dependent manner (Table 2). In females, only one rat of the 2.4% group had this disease. Large platelets were microscopically detected among erythrocytes.

Common Background Tumors

Pituitary adenoma, testicular tumors, and leukemia were found in control and all treated groups (Table 3).

Nonneoplastic Lesions

In surviving rats, a statistically significant increase in the appearance of glandular stomachs with smooth surfaces was observed in both males and females in the 2.4% exposure group (Table 4). The cecum was enlarged and hemor-

TABLE 2
Essential Thrombocythemia

	Piperonyl butoxide (dietary %)			
	0	0.6	1.2	2.4
Male				
Number of rats examined	24	23	15	24
Essential thrombocythemia ^a	0	6 ^b	3 ^b	9 ^b
Essential thrombocythemia (%)	0	26	20	38
Female				
Number of rats examined	24	27	25	26
Essential thrombocythemia	0	0	0	1
Essential thrombocythemia (%)	0	0	0	4

^a The response has a significant dose-related linear trend.

^b Significantly different from the appropriate control (0%) value.

rhagic, especially in the 0.6 and 1.2% groups; edema of cecum was also found in these groups. In dead male rats, massive hemorrhages of the cecum were observed in 0.6 and 1.2% groups. Histopathologically, erosion of cecal mucosa was observed in piperonyl butoxide-treated rats.

Whitish spots were observed on the surface of lungs in a dose-dependent fashion. Histopathologically, many macrophages and cholesterol clefts were found in alveoli.

A dose-dependent increase in the number of kidneys that were black-colored was observed and this increase was espe-

TABLE 3
Common Background Tumors

	Piperonyl butoxide (dietary %)			
	0	0.6	1.2	2.4
Male				
Number of rats examined	25	23	15	25
Pituitary gland				
Adenoma	4	3	1	1
Testis				
Interstitial cell tumor	23	19	13	15 ^a
Hematopoietic system				
Mononuclear cell leukemia	4 ^b	2	4	1 ^b
Female				
Number of rats examined	24	27	25	26
Pituitary gland				
Adenoma	7	5	4	2
Hematopoietic system				
Mononuclear cell leukemia	2	0	2	2

^a Significantly different from the appropriate control (0%) value.

^b Only 24 rats were examined, because of failure to collect blood.

TABLE 4
Gross Findings of Nonneoplastic Lesions

	Piperonyl butoxide (dietary %)			
	0	0.6	1.2	2.4
Male (surviving)				
Number of rats examined	25	23	15	25
Stomach (glandular)				
Hemorrhages	2	1	2	7
Polyps	0	0	0	2
Smooth surface	0	0	2	10 ^a
Cecum				
Enlargement	0	12 ^a	8 ^a	7 ^a
Hemorrhages	0	2	3	3
Edema	0	7 ^a	8 ^a	0
Hyperplasia	0	1	0	2
Lungs				
Whitish spotting	0	0	3 ^a	11 ^a
Kidneys				
Black-colored	1	5	10 ^a	17 ^a
Misshapen	1	1	0	1
Male (dead)				
Number of dead rats	5	7	15	8
Stomach				
Hemorrhages	3	1	4	2
Cecum				
Enlargement	1	6	10	4
Hemorrhages	0	6 ^a	10 ^a	3
Hyperplasia	0	0	3	2
Female (surviving)				
Number of rats examined	24	27	25	26
Stomach (glandular)				
Hemorrhages	1	1	5	16 ^a
Polyps	1	0	0	6
Smooth surface	0	2	5	12 ^a
Cecum				
Enlargement	0	7 ^a	13 ^a	8 ^a
Hemorrhages	0	5	9 ^a	4
Edema	0	5	6	3
Hyperplasia	0	1	0	3
Lungs				
Whitish spotting	0	0	0	2
Kidneys				
Black-colored	0	7 ^a	20 ^a	25 ^a
Misshapen	0	0	3	8 ^a
Female (dead)				
Number of dead rats	6	3	5	7
Stomach				
Hemorrhages	1	1	2	3
Cecum				
Enlargement	0	0	0	1
Hemorrhages	0	1	1	3
Hyperplasia	0	0	0	1

^a Significantly different from the appropriate control (0%) value.

TABLE 5
Terminal Body and Organ Weights

Weight (g)	Piperonyl butoxide (dietary %)			
	0	0.6	1.2	2.4
Male				
Number of rats	25	23	15	22
Body	406 ± 26 ^a	388 ± 21	347 ± 19 ^b	211 ± 23 ^b
Liver				
Absolute	15.7 ± 2.1	16.5 ± 2.4	19.2 ± 1.7 ^b	23.4 ± 4.7 ^b
Relative	3.87 ± 0.46	4.25 ± 0.52	5.55 ± 0.44 ^b	11.3 ± 2.7 ^b
Kidneys				
Absolute	3.45 ± 0.40	3.53 ± 0.45	3.52 ± 0.28	2.84 ± 0.14 ^b
Heart				
Absolute	1.15 ± 0.13	1.21 ± 0.15	1.16 ± 0.08	0.811 ± 0.080 ^b
Lungs				
Absolute	1.76 ± 0.31	1.64 ± 0.28	1.57 ± 0.16	1.12 ± 0.13 ^b
Spleen				
Absolute	1.40 ± 1.29	1.06 ± 0.58	1.00 ± 0.27	0.468 ± 0.139 ^b
Testes				
Absolute	4.26 ± 1.59	5.43 ± 1.79	4.56 ± 1.17	3.63 ± 1.31
Female				
Number of rats	24	27	25	23
Body	268 ± 26	242 ± 19	207 ± 10 ^b	134 ± 13 ^b
Liver				
Absolute	9.35 ± 1.03	12.1 ± 1.2 ^b	14.1 ± 1.3 ^b	16.0 ± 3.4 ^b
Relative	3.50 ± 0.42	5.04 ± 0.59 ^b	6.82 ± 0.62 ^b	12.0 ± 2.6 ^b
Kidneys				
Absolute	2.18 ± 0.19	2.29 ± 0.18	2.32 ± 0.17	1.81 ± 0.11 ^b
Heart				
Absolute	0.801 ± 0.065	0.850 ± 0.070	0.787 ± 0.061	0.531 ± 0.050 ^b
Lungs				
Absolute	1.17 ± 0.17	1.15 ± 0.08	1.03 ± 0.09 ^b	0.796 ± 0.112 ^b
Spleen				
Absolute	0.744 ± 0.985	0.498 ± 0.105	0.566 ± 0.132	0.463 ± 0.556 ^b
Ovaries				
Absolute	0.121 ± 0.021	0.128 ± 0.024	0.128 ± 0.022	0.0576 ± 0.0129 ^b

^a Mean ± SD.

^b Significantly different from the appropriate control (0%) value.

cially marked in exposed female rats. Misshapen kidneys were numerous in the female 2.4% group. Massive pigmentation (probably hemosiderin) was found in tubular cell cytoplasm in most treated groups. Tubular dilatation, multiple cysts in the cortex and outer medulla, glomeruli with distention of Bowman's space, and interstitial fibrosis were induced in the 2.4% groups. Those changes may be equal to chronic rat nephropathy. The injuries were enhanced by the administration of piperonyl butoxide.

Organ Weights

The effects of piperonyl butoxide on organ weights are shown in Table 5. Absolute and relative liver weights were significantly increased.

Hematology

Treatment of piperonyl butoxide reduced RBC, HGB, and HCT and increased PLT in a dose-dependent manner (Table 6). The values of WBC or PLT in Table 5 are the group means excluding those with leukemia or thrombocytopenia. The prothrombin index was slightly decreased in only the 2.4% groups. Many abnormally shaped erythrocytes (burr or crenated cells, red cell fragments, and anisocytosis) and large platelets were observed in some rats of the 1.2 and 2.4% groups.

Data of W-SCC, W-SCR, RDW-SD, PDW, MPV, and P-LCR are not shown because the table becomes complicated: W-SCC of males and W-SCR of females were significantly decreased in treated groups. RDW-SD were increased in treated groups of both sexes. PDW and MPV

TABLE 6
Hematological Parameters^a

Parameter	Piperonyl butoxide (dietary %)			
	0	0.6	1.2	2.4
Male				
WBC ($\times 10^3/\mu\text{l}$)	4.9 $\pm$ 1.3 (20) ^b	3.9 $\pm$ 1.4 (21)	5.0 $\pm$ 2.3 (11)	4.4 $\pm$ 2.2 (22)
RBC ($\times 10^6/\mu\text{l}$)	7.9 $\pm$ 1.4 (24)	7.3 $\pm$ 1.1 (23)	7.0 $\pm$ 1.5 (15)	6.9 $\pm$ 0.9 (23)
HGB (g/dl)	14 $\pm$ 2 (24)	11 $\pm$ 2 (23) ^f	9.2 $\pm$ 2.3 (15) ^f	8.9 $\pm$ 1.7 (23) ^f
HCT (%)	44 $\pm$ 6 (24)	36 $\pm$ 5 (23) ^f	33 $\pm$ 6 (15) ^f	32 $\pm$ 5 (23) ^f
MCV (fl)	56 $\pm$ 5 (24)	50 $\pm$ 7 (23) ^f	48 $\pm$ 5 (15) ^f	46 $\pm$ 4 (23) ^f
MCH (pg)	18 $\pm$ 1 (24)	15 $\pm$ 3 (23) ^f	13 $\pm$ 2 (15) ^f	13 $\pm$ 1 (23) ^f
MCHC (g/dl)	31 $\pm$ 1 (24)	29 $\pm$ 2 (23) ^f	27 $\pm$ 2 (15) ^f	28 $\pm$ 2 (23) ^f
PLT ($\times 10^3/\mu\text{l}$)	6.4 $\pm$ 1.5 (24)	7.3 $\pm$ 1.4 (17)	8.9 $\pm$ 2.1 (12) ^f	7.8 $\pm$ 2.8 (15)
PT index (%)	100 $\pm$ 3 (19)	103 $\pm$ 4 (18)	104 $\pm$ 5 (15)	95 $\pm$ 5 (19) ^f
KPTT index (%)	100 $\pm$ 8 (17)	96 $\pm$ 15 (16)	91 $\pm$ 11 (12)	91 $\pm$ 9 (14)
Female				
WBC ($\times 10^3/\mu\text{l}$)	2.7 $\pm$ 0.5 (22)	2.9 $\pm$ 1.0 (27)	2.5 $\pm$ 0.7 (23)	2.9 $\pm$ 1.0 (24)
RBC ($\times 10^6/\mu\text{l}$)	7.3 $\pm$ 1.2 (24)	6.9 $\pm$ 0.6 (27)	6.1 $\pm$ 0.6 (25) ^f	6.7 $\pm$ 0.9 (26) ^f
HGB (g/dl)	14 $\pm$ 2 (24)	12 $\pm$ 1 (27) ^f	11 $\pm$ 1 (25) ^f	9.5 $\pm$ 1.7 (26) ^f
HCT (%)	42 $\pm$ 5 (24)	38 $\pm$ 3 (27) ^f	36 $\pm$ 3 (25) ^f	34 $\pm$ 5 (26) ^f
MCV (fl)	58 $\pm$ 6 (24)	55 $\pm$ 3 (27)	59 $\pm$ 3 (25)	50 $\pm$ 5 (26) ^f
MCH (pg)	19 $\pm$ 1 (24)	17 $\pm$ 1 (27) ^f	17 $\pm$ 1 (25) ^f	14 $\pm$ 1 (26) ^f
MCHC (g/dl)	32 $\pm$ 1 (24)	31 $\pm$ 1 (27) ^f	30 $\pm$ 1 (25) ^f	28 $\pm$ 1 (26) ^f
PLT ($\times 10^3/\mu\text{l}$)	5.2 $\pm$ 1.1 (24)	6.4 $\pm$ 1.2 (27) ^f	6.9 $\pm$ 0.9 (25) ^f	6.2 $\pm$ 1.5 (25) ^f
PT index (%)	100 $\pm$ 4 (10)	104 $\pm$ 5 (10)	102 $\pm$ 5 (10)	94 $\pm$ 4 (10) ^f
KPTT index (%)	100 $\pm$ 14 (10)	119 $\pm$ 18 (10)	115 $\pm$ 27 (10)	82 $\pm$ 24 (10)

^a Data of parameters of WBC from leukemic rats and PLT from essential thrombocythemia were excluded in calculating the values in this table.

^b Mean $\pm$ SD (number of rats examined).

^c Significantly different from the appropriate control (0%) value.

were significantly decreased in 2.4% groups of both sexes. P-LCR was decreased in the 0.6 and 2.4% females.

Plasma Biochemistry

γ -GTP and UN were increased, while CHE was decreased in a dose-responsive fashion (Table 7).

DISCUSSION

Hepatocellular Carcinoma

Most conspicuous among the present findings are the hepatic cancers that were observed in the treated animals. Piperonyl butoxide has been considered to be noncarcinogenic (NCI, 1979; Maekawa *et al.*, 1985). Although there are many differences in experimental conditions, the most important factors responsible for these tumor results are the higher dosages used in this study. In the reports of NCI (1979) and Maekawa *et al.* (1985), the dietary concentrations of piperonyl butoxide were 1.0 and 0.5%. In the present experiment, the doses were 2.4, 1.2, and 0.6% (95% purity). NCI (1979) did not find liver tumors. Maekawa *et al.* (1985) observed hepatocellular carcinoma in 2 of 46

male rats given 1.0% piperonyl butoxide (89% purity), while we found hepatocellular carcinoma in 4 of 30 males given 1.2%, and neither incidence was significant, but in surviving rats it was significant in our study (Table 1). Tumors developed at a dosage of 1.2% or more; thus, the induction of hepatocellular carcinoma by piperonyl butoxide may occur initially at doses of at least 1.0–1.2%. One explanation of the large differences in tumor incidences between 1.0 and 1.2% is that the 1% dose is near the foot of a steep dose-response curve. Incidence becomes high in one experiment, but it can be 0% in another test. Then, what are the factors affecting the incidence? They may include feeding conditions, which are very different; no-chip suspended wire cages vs chip-bedded plastic cages; 1 rat/cage vs 3 or 4 or 5 rats/cage; and/or components of basal diets. All previous reports may have failed to find hepatocarcinogenicity of piperonyl butoxide because the doses used were too low. In this report, the incidence of hepatocellular carcinoma was more than 50% in both sexes of the 2.4% groups. The increase in plasma γ -GTP and the decrease in CHE may be in concordance with the induction of hepatic tumors (Schmitt and Schmitt, 1974). While the 1.2% dose is clearly positive, some discussion needs to be given to the 2.4%

TABLE 7
Plasma Biochemical Parameters

Parameter	Piperonyl butoxide (dietary %)			
	0	0.6	1.2	2.4
Male				
Number of rats	24	23	15	20
γ -GTP (IU/liter)	5 $\pm$ 4 ^a	4 $\pm$ 3	9 $\pm$ 4 ^b	38 $\pm$ 23 ^b
CHE (Δ pH)	0.24 $\pm$ 0.10	0.14 $\pm$ 0.08	0.11 $\pm$ 0.10 ^b	0.02 $\pm$ 0.02 ^b
UN (mg/dl)	16.6 $\pm$ 3.1	18.5 $\pm$ 6.4	18.1 $\pm$ 2.6	20.0 $\pm$ 1.8 ^b
AST (IU/liter)	54 $\pm$ 33	40 $\pm$ 11	48 $\pm$ 18	61 $\pm$ 18
ALT (IU/liter)	19 $\pm$ 7	15 $\pm$ 4	18 $\pm$ 8	27 $\pm$ 12
AST/ALT	2.70 $\pm$ 0.74	2.72 $\pm$ 0.64	2.80 $\pm$ 0.96	2.52 $\pm$ 0.96
ALB (g/dl)	1.8 $\pm$ 0.2	2.0 $\pm$ 0.3	1.9 $\pm$ 0.2	2.1 $\pm$ 0.2 ^b
TP (g/dl)	4.7 $\pm$ 0.3	4.7 $\pm$ 0.3	4.6 $\pm$ 0.4	4.6 $\pm$ 0.4
ALB/GLB	0.63 $\pm$ 0.09	0.75 $\pm$ 0.13	0.70 $\pm$ 0.06	0.87 $\pm$ 0.10 ^b
CHO (mg/dl)	110 $\pm$ 36	80 $\pm$ 33 ^b	83 $\pm$ 43 ^b	88 $\pm$ 21
F-CHO (mg/dl)	32 $\pm$ 11	19 $\pm$ 11 ^b	21 $\pm$ 13 ^b	23 $\pm$ 8 ^b
E-CHO (mg/dl)	78 $\pm$ 25	60 $\pm$ 23	62 $\pm$ 30	66 $\pm$ 14
PL (mg/dl)	169 $\pm$ 52	130 $\pm$ 51	134 $\pm$ 61	155 $\pm$ 36
TG (mg/dl)	75 $\pm$ 37	39 $\pm$ 33 ^b	30 $\pm$ 19 ^b	35 $\pm$ 30 ^b
GLC (mg/dl)	184 $\pm$ 33	181 $\pm$ 29	153 $\pm$ 24	92 $\pm$ 11 ^b
UA (mg/dl)	3.3 $\pm$ 1.4	4.3 $\pm$ 1.0	4.2 $\pm$ 0.8	2.1 $\pm$ 0.8 ^b
Female				
Number of rats	24	27	25	23
γ -GTP (IU/liter)	4 $\pm$ 1	4 $\pm$ 1	5 $\pm$ 1	62 $\pm$ 67 ^b
CHE (Δ pH)	0.66 $\pm$ 0.16	0.45 $\pm$ 0.11 ^b	0.30 $\pm$ 0.11 ^b	0.03 $\pm$ 0.03 ^b
UN (mg/dl)	15.0 $\pm$ 1.5	18.1 $\pm$ 2.1 ^b	21.1 $\pm$ 2.1 ^b	27.8 $\pm$ 1.9 ^b
AST (IU/liter)	58 $\pm$ 18	34 $\pm$ 18 ^b	31 $\pm$ 4 ^b	75 $\pm$ 56
ALT (IU/liter)	28 $\pm$ 9	17 $\pm$ 7 ^b	14 $\pm$ 2 ^b	28 $\pm$ 31
AST/ALT	2.09 $\pm$ 0.40	1.96 $\pm$ 0.35	2.19 $\pm$ 0.40	3.13 $\pm$ 0.95 ^b
ALB (g/dl)	2.6 $\pm$ 0.3	2.8 $\pm$ 0.3	2.8 $\pm$ 0.3	2.6 $\pm$ 0.4
TP (g/dl)	5.5 $\pm$ 0.5	5.8 $\pm$ 0.4	5.8 $\pm$ 0.4	5.1 $\pm$ 0.7
ALB/GLB	0.91 $\pm$ 0.10	0.93 $\pm$ 0.11	0.92 $\pm$ 0.09	1.01 $\pm$ 0.15 ^b
CHO (mg/dl)	96 $\pm$ 23	149 $\pm$ 40 ^b	158 $\pm$ 35 ^b	142 $\pm$ 42 ^b
F-CHO (mg/dl)	30 $\pm$ 7	44 $\pm$ 14 ^b	45 $\pm$ 12 ^b	40 $\pm$ 15
E-CHO (mg/dl)	66 $\pm$ 16	105 $\pm$ 26 ^b	113 $\pm$ 24 ^b	104 $\pm$ 27 ^b
PL (mg/dl)	182 $\pm$ 39	266 $\pm$ 71 ^b	276 $\pm$ 55 ^b	244 $\pm$ 65 ^b
TG (mg/dl)	86 $\pm$ 30	65 $\pm$ 29 ^b	58 $\pm$ 19 ^b	53 $\pm$ 25 ^b
GLC (mg/dl)	223 $\pm$ 42	202 $\pm$ 39	159 $\pm$ 16 ^b	83 $\pm$ 14 ^b
UA (mg/dl)	4.6 $\pm$ 1.6	4.8 $\pm$ 1.0	4.1 $\pm$ 0.8	2.4 $\pm$ 0.5 ^b

^a Mean $\pm$ SD.

^b Significantly different from the appropriate control (0%) value.

dose, which many would argue is beyond an acceptable dose given the marked reduction in body weight (this is true for males but not for females). This experiment was initially conducted as a chronic toxicity test and was not intended as a carcinogenicity test. We could not, however, exclude the exposed groups whose body weight gain was statistically reduced.

Piperonyl butoxide is made from safrol by the chemical industry, and the process generates dihydrosafrol and isosafrol. These are both esophageal carcinogens but not hepatocarcinogens (Hagan *et al.*, 1965). When rats were given safrol at a level of 5000 ppm for 2 years, most died during the

period and the incidence of hepatic tumors reached 38% (Long *et al.*, 1963). In addition to the fact that safrol could not be detected chemically in the product used in the present experiment, it cannot be inferred biologically that the carcinogenicity observed, following exposure to piperonyl butoxide is due to contamination of the test agent with safrol, dihydrosafrol, or isosafrol. Piperonyl butoxide is, therefore, based on our observations, a hepatocarcinogen. Hepatocarcinogens can be divided into genotoxic and non-genotoxic agents. Nongenotoxic hepatocarcinogens characteristically cause liver enlargement (Grasso and Hinton, 1991). Based on these characteristics, piperonyl butoxide may be looked upon as a nongenotoxic hepatocarcinogen for the present, since it is nonmutagenic and can cause hepatomegaly (Fujitani *et al.*, 1992). The mechanism of carcinogenicity of such compounds is still unclear.

Increased tumor incidence is induced at 1052 mg/kg/day (1.2%) in the males, and 547 mg/kg/day (0.6%) may be approximately a maximum no-effect level when chemical intakes are calculated from food consumption. The ADI for humans is 0.03 mg/kg/day. The maximum no-effect level is, therefore, about 18,000 times the ADI for humans. Even considering the safety factor, the difference between those levels is very large. Until now the carcinogenicity of piperonyl butoxide has not been reported; however, it has now been so recognized in the present report. Currently, we are using piperonyl butoxide not only as a food additive for rice, wheat, and other grain crops but also as a synergist of pyrethrins postharvest and in domestic insecticides. Even if the use level of piperonyl butoxide remains unchanged, we need to reevaluate the approved levels of all usages on the basis that piperonyl butoxide is a hepatic carcinogen in rats following the dietary exposures reported in this study.

Others

Chronic administration of piperonyl butoxide also induces probable essential thrombocythemia. This benign tumor of platelets has been reported only in case reports of humans and some pets (Iland and Laszlo, 1986; Tablin *et al.*, 1989; Hammer *et al.*, 1990). In the present screening experiment, we were unable to examine large platelets and megakaryocytes by electron microscopy or biochemical techniques, but we considered the disorder caused by piperonyl butoxide as probable essential thrombocythemia because of the enormous increase in platelet number and the changes in platelet particle distribution (Iland and Laszlo, 1986). The induction of essential thrombocythemia was not related to that of mononuclear cell leukemia in the present results.

Testicular adenoma and leukemia did not have dose-related responses and may be spontaneous neoplastic disorders (Boorman *et al.*, 1990). Other toxic effects were seen in lungs, kidneys, stomach, cecum, and blood and were related to the doses of piperonyl butoxide. Degenerative

lesions of pulmonary alveoli, tubular damage in kidneys, hemorrhages in stomach and cecum, and anemia were recognized. In particular, hemorrhages in the cecum were fatal and many rats, especially of the male 1.2% group, died after about 40 weeks. This observation may be similar to that described by Maekawa *et al.* (1985). Hemorrhagic tendency may be related to the properties of platelets, because platelet particle concentrations increased in treated groups, especially in thrombocythemic rats. Early deaths due to cecal hemorrhages may be related with essential thrombocythemia.

ACKNOWLEDGMENTS

The authors thank Dr. W. H. Butler, the Chief Pathologist of BIBRA Toxicology International, and Dr. K. L. Gabriel, Biosearch Inc., for the useful and helpful discussions about our study.

REFERENCES

- Ashwood-Smith, M. J., Trevino, J., and Ring, R. (1972). Mutagenicity of dichlorvos. *Nature* **240**, 418-420.
- Boorman, G. A., Eustis, S. L., Elwell, M. R., Montgomery, C. A., Jr., and MacKenzie, W. F. (Eds.) (1990). *Pathology of the Fischer Rat*. Academic Press, New York.
- Cardy, R. H., Renne, R. A., Warner, J. W., and Cypher, R. L. (1979). *J. Natl. Cancer Inst.* **62**, 569-576.
- Fujitani, T., Ando, H., Fujitani, K., Ikeda, T., Kojima, A., Kubo, Y., Ogata, A., Oishi, S., Takahashi, H., Takahashi, O., and Yoneyama, M. (1992). Sub-acute toxicity of piperonyl butoxide in F344 rats. *Toxicology* **72**, 291-298.
- Gad, S. C., and Weil, C. S. (1982). Statistics for toxicologists. In *Principles and Methods of Toxicology* (A. W. Hayes, Ed.), pp. 237-320. Raven Press, New York.
- Goldstein, J. A., Hickman, P., and Kimbrough, R. D. (1973). Effects of purified and technical piperonyl butoxide on drug-metabolizing enzymes and ultrastructure of rat liver. *Toxicol. Appl. Pharmacol.* **26**, 444-458.
- Grasso, P., and Hinton, R. H. (1991). Evidence for and possible mechanisms of non-genotoxic carcinogens in rodent liver. *Mutat. Res.* **248**, 271-290.
- Hagan, E. C., Jenner, P. M., Jones, W. I., Fitzhugh, O. G., Long, E. L., Brouwer, J. G., and Webb, W. K. (1965). Toxic properties of compounds related to safrole. *Toxicol. Appl. Pharmacol.* **7**, 18-24.
- Hammer, A. S., Couto, C. G., Getzy, D., and Bailey, M. Q. (1990). Essential thrombocythemia in a cat. *J. Vet. Intern. Med.* **4**, 87-91.
- Iland, H. J., and Laszlo, J. (1986). Myeloproliferative disorders: Polycythemia vera, essential thrombocythemia, and idiopathic myelofibrosis/ag-nogenic myeloid metaplasia. In *Hematologic Malignancies* (B. Hoogstraten, Ed.), pp. 31-61. Springer-Verlag, Berlin.
- Innes, J. R. M., Ulland, B. M., Valerio, M. G., Petrucelli, L., Fishbein, L., Hart, E. R., Pallotta, A. J., Bates, R. R., Falk, H. L., Gart, J. J., Klein, M., Mitchell, I., and Peters, J. (1969). Bioassays of pesticides and industrial chemicals for tumorigenicity in mice: A preliminary note. *J. Natl. Cancer Inst.* **42**, 1101-1114.
- Joint FAO/WHO Meeting (1973). *Pesticide Residues in Food*. WHO Tech. Rep. Ser. No. 525. FAO/WHO, Geneva.
- Kennedy, G. L., Jr., Smith, S. H., Kinoshita, F. K., Keplinger, M. L., and Calandra, J. C. (1977). Teratogenic evaluation of piperonyl butoxide in the rat. *Food Cosmet. Toxicol.* **15**, 337-339.
- Khera, K. S., Whalen, C., Angers, G., and Trivett, G. (1979). Assessment of the teratogenic potential of piperonyl butoxide, biphenyl, and phosalone in the rat. *Toxicol. Appl. Pharmacol.* **47**, 353-358.
- Long, E. L., Nelson, A. A., Fitzhugh, O. G., and Hansen, W. H. (1963). Liver tumors produced in rats by feeding safrole. *Arch. Pathol.* **75**, 595-604.
- Maekawa, A., Onodera, H., Furuta, K., Tanigawa, H., Ogiu, T., and Haya-shi, Y. (1985). Lack of evidence of carcinogenicity of technical-grade piperonyl butoxide in F344 rats: Selective induction of ileocaecal ulcers. *Food Chem. Toxicol.* **23**, 675-682.
- Maronpot, R. R., Montgomery, C. A., Jr., Boorman, G. A., and McConnell, E. E. (1986). National Toxicology Program nomenclature for hepatoproliferative lesions of rats. *Toxicol. Pathol.* **14**, 263-273.
- National Cancer Institute (NCI) (1979). *Bioassay of Piperonyl Butoxide for Possible Carcinogenicity*. NCI Tech. Rep. Ser. No. 120: Government Printing Office, Washington, DC.
- Nesnow, S. (1990). ICPEMC working paper 1: A multi-factor ranking scheme for comparing the carcinogenic activity of chemicals. *Mutat. Res.* **239**, 83-115.
- Rosenkranz, H. S., Ennever, F. K., and Klopman, G. (1990). Relationship between carcinogenicity in rodents and the induction of sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells. *Mutagenesis* **5**, 559-571.
- Schmitt, E., and Schmitt, F. W. (1974). The importance of enzymatic analysis in medicine. Diagnosis, control of progress and therapy: Diseases of liver. In *Methods of Enzymatic Analysis* (H. U. Bergmeyer, Ed.), 2nd English ed., Vol. 1, pp. 14-30. Academic Press, New York.
- Squire, R. A., and Levitt, M. H. (1975). Report of a workshop on classification of specific hepatocellular lesions in rats. *Cancer Res.* **35**, 3214-3223.
- Sternberg, S. S. (1979). The carcinogenesis, mutagenesis and teratogenesis of insecticides. Review of studies in animals and man. *Pharmacol. Ther.* **6**, 147-166.
- Tablin, F., Jain, N. C., Mandell, C. P., Hopper, P. E., and Zinkl, J. G. (1989). Ultrastructural analysis of platelets and megakaryocytes from a dog with probable essential thrombocythemia. *Vet. Pathol.* **26**, 289-293.
- Tanaka, T. (1992). Effects of piperonyl butoxide on F₁ generation mice. *Toxicol. Lett.* **60**, 83-90.
- Tanaka, T. (1993). Behavioural effects of piperonyl butoxide in male mice. *Toxicol. Lett.* **69**, 155-161.
- Tanaka, T., Fujitani, T., Takahashi, O., and Oishi, S. (1993). Developmental toxicity evaluation of piperonyl butoxide in CD-1 mice. *Toxicol. Lett.*, in press.
- Tanaka, T., Takahashi, O., and Oishi, S. (1992). Reproductive and neuro-behavioural effects in three generation toxicity study of piperonyl butoxide administered to mice. *Food Chem. Toxicol.* **30**, 1015-1019.
- White, T. J., Goodman, D., Shulgin, A. T., Castagnoli, N. J., Jr., Lee, R., and Petrakis, N. L. (1977). Mutagenic activity of some centrally active aromatic amines in *Salmonella typhimurium*. *Mutat. Res.* **56**, 199-202.



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

ATTACHMENT 2

(10658

Nov 15 1993

11/15/93

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

6(a)2

MEMORANDUM

SUBJECT: EPA ID #067501. Piperonyl butoxide. 6(a)2 submission concerning a literature report of induction of liver tumors in Fischer 344 strain rats.

TOX CHEM No.: 670
PC No.: 067501
Barcode No.: D192500, D193484 and D195273
Submission No.: S443059, S445090 and S448754

FROM: John Doherty, Ph.D., D.A.B.T. 11/12/93
Section IV, Toxicology Branch I
Health Effects Division (7509C)

TO: Linda DeLuise/Jay Ellenberger
Product Manager #50
Special Review and Reregistration Division
(7508W)

THROUGH: Marion Copley, DVM, Section Head
Section IV, Toxicology Branch I
Health Effects Division (7509C) 11/12/93

I. CONCLUSION

The 6(a)2 data from a carcinogenicity study conducted in Japan indicates that Fischer 344 rats dosed with estimated dose levels of 500, 1000 and 1900 mg/kg/day of piperonyl butoxide for two years develop "liver cancers". Induction of liver tumors at lower dose levels in mice has already been recognized by HED and the issue of carcinogenicity classification of piperonyl butoxide will be presented to HED's Carcinogenicity Peer Review Committee sometime in 1994.

There was insufficient information provided in these submissions to cause HED to recommend immediate regulatory action for piperonyl butoxide at this time.

(34)



Recycled/Recyclable
Printed with Soy/Canola Ink on paper that
contains at least 50% recycled fiber

II. Information Submitted and Action Requested

The Piperonyl Butoxide Task Force II has made three successive submissions (refer to letters from John D. Conner, Jr. dated June 4, 1993, July 7, 1993 and September 8, 1993) for 6(a)2 data concerning a report on a rat chronic toxicity study (non-guideline) that was conducted at the Department of Toxicology Tokyo Metropolitan Research Laboratory of Public Health (author O. Takahashi, dated 1993). The original submission (D192500, no MRID No.:) included an English translation (translator not identified) of the study abstract and a summary table of liver tumor data. This submission was considered to be of very limited usefulness for review because of the limited detail provided.

The second submission (D193484, MRID #428396-01) consisted of a pre-publication draft of a paper entitled "Chronic Toxicity Studies of Piperonyl Butoxide in F344 Rats: Induction of Hepatocellular Carcinoma" as authored by O. Takahashi et.al.. This report was obtained when a representative of the Piperonyl Butoxide Task Force visited the testing laboratory. The study is not available in a form meeting the Agency's requirements for submission of data.

The third submission (D195273, MRID #429202-01) consists of a report prepared by the Piperonyl Butoxide Task Force consultant pathologist Dr. W.H. Butler entitled "Chronic toxicity study of Piperonyl Butoxide in 344 rats Induction of Hepatcarcinoma (sic)" and dated June 8-11, 1993. This report reflects Dr. Butler's examination of the liver slides that were available for examination.

III. Toxicology Branch Comments.

1. A summary DER (cursory) was prepared based on the information provided in these three submissions as indicated above. The data are considered SUPPLEMENTARY and not considered a candidate for upgrading since the protocol did not include a sufficient number of animals/sex/dose group as well as several other reasons (refer to DER) to meet acceptable criteria.

2. The study as reported is considered to provide some useful information with regard to the potential for piperonyl butoxide to induce liver tumors at higher dose levels. Table 1 below illustrates the tumor response to treatment as assessed by the original study pathologist and the registrant's consultant, Dr. W.H. Butler.

Table 1 indicates that dietary dose levels of 1.2% and 2.4% of piperonyl butoxide are associated with liver tumors in both sexes and in males there is indicated an increase in malignancy at the 1.2% dietary level. Whereas only the 2.4% dietary level is associated with malignancy in females.

Table 1. Reported incidence of "liver cancer" in "344" rats dosed with piperonyl butoxide for two years. Comparison of readings by the study pathologist and Dr. W.H. Butler, the registrant's consultant.

Test Group	Tumor incidence ¹					
	Males			Females		
	Dose level ³	Original	Butler ²	Dose level ³	Original	Butler
Control	0	0/25	0/26	0	0/24	0/25
0.6%	547	1/25	0/23	537	1/27	0/27
1.2%	1,052	14/15*	11(3)/17	1,061	22/25*	1/27
2.4%	1,877	25/25*	20(7)/25	2,002	26/26*	16(5)/26

* $p < 0.05$ Study report statistics-by Kaplan Meier, Cochrane-Armitage or Chi² test.

1. Data are number of rats diagnosed with "liver cancer"/number of rats examined based on 30 rats per sex initiated on the study. Rats not surviving to termination are not included. There is no definite explanation for why the same denominators are not used for each pathologist.

2. No statistical analysis of the data were presented with Dr. Butler's report. Dr. Butler classified the tumors as adenoma and carcinoma and the total number of rats diagnosed with a carcinoma (the number in parenthesis) in the above table is the incidence of carcinomas.

3. Dose level in mg/kg/day.

3. TB-I notes that the dose levels used for this study are high and induced systemic toxicity particularly decreased body weight and increased liver weight at all dose levels. At all doses there was also hemorrhaging of the cecum which was reported to result in deaths of the males especially in the mid dose group. The systemic toxicity resulting in the two highest test dose groups raises concerns related to excessive toxicity compromising the interpretation of the liver tumor data. This issue will be addressed by the HED Carcinogenicity Peer Review Committee.

4. The data base for piperonyl butoxide includes a previously submitted rat carcinogenicity study (refer to HED document No.: 006668, MRID No.: 403237-01, Bio-Research Ltd Study No.: 81690, August 27, 1987) that tested the Sprague-Dawley Crl-CDR strain rat at dose levels of 0, 30, 100 and 500 mg/kg/day. No evidence of induction of liver carcinogenicity was presented in this study which was classified as CORE GUIDELINE.

A mouse (CD-1) carcinogenicity study (MRID No.: 429037-01, HED Document No.: 019647) which was assessed at dose levels of 30, 100 and 300 mg/kg/day was determined to be positive for induction of liver tumors in both sexes.

The carcinogenicity classification of piperonyl butoxide will be evaluated by the HED Carcinogenicity Peer Review Committee. The rat and mouse studies indicated above as well as mutagenicity and genetic toxicity and other relevant data will be considered. The findings in the study with the Fischer 344 strain rat will also be presented to the committee for consideration.

IV. Studies Reviewed

Study Identification	Material	MRID No.:	Results	Classification
83-1. Chronic feeding-rats Tokyo Metropolitan Research Laboratory of Public Health No study No.: 1993 (no specific study date)	Technical piperonyl butoxide, 94.3 and 94.5% pure, lots 909002 and 009002	428396-01 429202-01	Provisional Conclusions: [Systemic toxicity NOEL and LEL: 537 mg/kg/day. At 537 mg/kg/day: gastrointestinal hemorrhage; increase liver weight; decrease body weight; effect on RBC parameters, possible kidney pathology. At 1,877 (males) and 2,002 (females) mg/kg/day (HDT) possible excessive toxicity as indicated by 50% decrease in body weight. Carcinogenicity potential: positive for liver tumors in both males and females.] <i>Not upgradeable</i> Fischer 344 strain rat. Dose levels tested: 0, 0.6, 1.2 and 2.4% in the diet corresponding to 0, 547, 1,052 and 1,877 mg/kg/day for males and 537, 1,061 and 2,002 mg/kg/day for females.	SUPPLEMENTARY

38

Reviewed by: John Doherty *John Doherty 11/12/93*
Section IV, Toxicology Branch I (7509C)
Secondary reviewer: Marion Copley, DVM
Section IV, Toxicology Branch I (7509C)

010658

DATA EVALUATION REPORT (Informal)

STUDY TYPE: 83-1. Rat chronic feeding-special

MRID NO.: 428396-01 and 429202-01 **TOX. CHEM. NO.:** 670
PC No.: 067501

TEST MATERIAL: Piperonyl butoxide-technical grade, from lot no.: 909002 and 009002 obtained from the Takasago International Corporation, Tokyo, was stated as being 94.5 to 94.3 pure for each lot respectively as determined by gas chromatography.

STUDY NUMBER(S): None

SPONSOR: None specified. Data submitted by the Piperonyl Butoxide Task Force.

TESTING FACILITY: Department of Toxicology, Tokyo Metropolitan Research Laboratory of Public Health, Tokyo, Japan.

TITLE OF REPORT: "Chronic Toxicity Studies of Piperonyl Butoxide in F344 Rats: Induction of Hepatocellular Carcinoma"

AUTHOR(S): O. Takahashi, S. Oishi, T. Fujitani, T. Tanaka and M. Yoneyama. Also a separate report was authored by W.H. Butler

REPORT ISSUED: Study not issued as a laboratory report. Dr. Butler's report issued June 1993.

STUDY DATES: Estimated 1991 to 1993.

CONCLUSIONS: Provisional.

Systemic toxicity NOEL and LEL: < 537 mg/kg/day. At 537 mg/kg/day: gastrointestinal hemorrhage; increase liver weight; decrease body weight; effect on RBC parameters, possible kidney pathology. At 1,877 (males) and 2,002 (females) mg/kg/day (HDT) possible excessive toxicity as indicated by 50% decrease in body weight.

Carcinogenicity potential: positive for liver tumors in both males and females.

Fischer 344 strain rat. Dose levels tested: 0, 0.6, 1.2 and 2.4% in the diet corresponding to 0, 547, 1,052 and 1,877 mg/kg/day for males and 537, 1,061 and 2,002 mg/kg/day for females.

Classification: SUPPLEMENTARY. Study does not satisfy guideline requirement for a series 83-1 or 83-2 study. Very unlikely to be upgraded for chronic feeding (series 82-1) and not upgradeable

for carcinogenicity (series 82-2) assessment since an insufficient number of rats were initiated on the study. The study is in summary form only without supporting individual animal data. Periodic hematology and clinical chemistry assessments were not made. Pathology tables are incomplete. No analytical chemistry data for stability, homogeneity or dietary concentration were presented.

Quality Assurance Statement: Not provided.

Good Laboratory Practice Statement: Not provided.

REVIEW (Cursory)

[Note: The study was presented in the form of a prepublication manuscript (MRID No.: 428396-01) and an appended report on the liver pathology (MRID # 429202-01). As such, the submission does not conform to the Agency guidelines for submission of studies. Since the study shows a positive effect for liver tumors, this DER has abstracted what is considered useful information for the Carcinogenicity Peer Review Committee meeting for piperonyl butoxide. A formal review would require the individual animal data.]

Experimental Constants:

Test Chemical: Piperonyl butoxide-technical grade, from lot Nos.: 909002 and 009002 obtained from the Takasago International Corporation, Tokyo, stated as being 94.5 to 94.3 pure for each lot respectively as determined by gas chromatography. The report stated that the test material did not contain safrol or isosafrol.

Test System: Fischer (F344/DuCrj) specific pathogen free rats were obtained from the Charles River Co. Kanagawa, Japan. They were reported to be 4 weeks old when received and were housed individually.

Basic Experimental Design:

Four groups of male and female rats were dosed as either control (30/sex), 0.6% (30/sex), 1.2% (30/sex) and 2.4% (33/sex) piperonyl butoxide in their diet for a scheduled two years. These dose levels were said to be based on a preliminary study and to represent 1000 times the maximum approved level for the presence of piperonyl butoxide on raw cereals in Japan.

Analytical Chemistry.

No analytical data were included.

Statistics.

The following statistical procedures were reported to be used.

Statistical Test	Parameter Investigated
Bartlett's test for homogeneity One way ANOVA Dunnett's test Scheffe's test If Bartlett's significant: Kruskal-Wallace test Scheffe's test	Body weight Organ weight Hematology parameters Plasma chemistry parameters
Kaplan-Meier's test	Mortality
C x R X ² analysis Fischer's exact test Cochrane-Armitage test for linear trend	Pathology data

Assessments and Results1. Survival and Clinical Signs.

The mid dose male group but not the high dose group showed a marked decrease in survival. A higher rate of deaths in the mid dose group was noted as early as about week 40. Overall, only 50% of the mid dose males survived to termination (96 weeks) whereas based on reading the survival graph, 75% of the high dose, 80% of the low dose and 85% of the control males survived to termination. In the mid dose group, hemorrhages of the cecum were "the most profound lesions" and were thought to contribute to the cause of death. Since the high dose group was not considered by TB-I to be much different with respect to the number of survivors, it is not firmly established that the deaths in the mid dose group were actually compound related. Note: The study authors do not clearly indicate if they believe the increase in mortality is actually due to piperonyl butoxide treatment. Refer also to the section below discussing the pathology of the cecum and stomach.

Based on the graphical data presented, survival among the females was not obviously affected with there being about 78% or greater survival in all groups.

Clinical symptoms were listed as epistaxis (nosebleed), rough hair, lethargy and decreased food consumption in the high dose-groups (both sexes). "Expansion of the abdomen" and "dirtiness of posterior parts" were apparent in all groups dosed with piperonyl butoxide.

CONCLUSION (clinical signs). NOEL and LEL = 1.2% and 2.4%. Presence of clinical reactions. The higher rate of deaths in the mid dose group cannot be explained on the basis of the information provided.

2. Body weight and feed consumption.

Growth curves for each sex were presented. Based on these growth curves, it appears that for both sexes all dose groups were lower at all times (except for the earliest weeks) in a dose dependent manner. For example, the low dose group males had an estimated maximum decrease of about 10% at week 80 and females had an estimated maximum decrease of 12% at week 72. Similarly the mid dose group males had a maximum difference of about 16% at week 84 and females had a maximum difference of about 25% at week 88. The high dose male group had an estimate maximum decrease of roughly 50% from weeks 16 to termination. The female high dose group had an estimated maximum decrease in weight at week 88 of 50%. Based on weight data, the high dose level may be considered excessive for both sexes. The report mentions that there was a drop in food consumption for both sexes but no data were presented.

Based on weight gain and feed consumption (data not presented) the following intake of piperonyl butoxide was reported.

Dose Level	mg/kg/day of piperonyl butoxide	
	Males	Females
0.6%	547	527
1.2%	1,052	1,061
2.4%	1,877	2,002

CONCLUSION (body weight). NOEL < 0.6%. Both males and females have decreased weight.

3. Hematology and clinical chemistry.

Data tables were presented listing the mean values for an apparently comprehensive evaluation of hematology and clinical chemistry assessed using blood samples taken at necropsy (page 6, MRID No.: 428396-01). The tables, however, are not supported by individual animal data. The following possible effects were reported by the study author:

¹The percentage difference in body weight was estimated by visually inspecting the growth curve and approximating the maximum distance between the lines for each dose level and the control group and reading the weight for both the control and treatment group at that time.

- reduced RBC, HGB, HCT, MCV, MCH, MCHC (possibly all dose groups in a dose related manner).
- increased platelets (all groups but dose relationship is questionable).
- decreased prothrombin index (high dose group)
- white blood cell parameters -some parameters increased others decreased in all or the higher dose groups only.
- decreased urea nitrogen, gamma-GTP and cholinesterase

In addition the report states that there were many abnormally shaped erythrocytes (burr or crenated cells, red cell fragments and anisocytosis) and large platelets in the mid and high dose groups.

The above are mentioned in this review without further assessment by TB-I since the individual animal data are not available to support the conclusions.

4. Pathology

The description of the preparation of the tissues for histopathology was very limited mentioning only that they were fixed in buffered 4% formaldehyde solution and stained with hematoxylin and eosin. The following tissues/organs are discussed.

1. Liver. The liver was identified as a target organ for carcinogenicity. Both absolute and relative (in parenthesis) liver weights were increased for both males and females in the low (10% not significant for males, 44% for females), mid (55% for males and 95% for females) and high (192% for males and 243% for females) dose groups. The following data in Table 1 were obtained from the report prepared by Dr. W.H. Butler and compare the earlier report (submitted under barcode D192500, no MRID No.:).

Since there were originally 30 rats/sex/dose, neither the original reading or Dr. Butler's reading accounts for all of the animals on the study. It should be noted that Dr. Butler states that he read only slides that were available to him and the tissues from the rats which died on the study and for which histology was available "could not be identified with certainty".

The draft of the manuscript as prepared by Dr. Takahashi et al (MRID No.: 428396-01) does not contain a table relating the actual number of liver tumors although a reference was made to a "Table 1" in the text of the paper. There is, however, an unnumbered table (copy attached and labelled APPENDIX I) which presents the number and percentage of rats with unspecified (with regard to organ and description) cancers which clearly shows that the mid and high dose males and females have higher rates than

the controls. There seems to be an inconsistency in the data presented in the table (attached) and the text of the paper (page 10) where it is stated that "hepatocellular adenomas were observed in 4/23 males and 1/27 female rats given 0.6% piperonyl butoxide" In the table presented in APPENDIX 1, only a single male rat is indicated as having a cancer.

Table 1. Liver tumors in F344 rats dosed with piperonyl butoxide. Comparison of original diagnosis (submitted under D192500, no MRID #) and the diagnosis made by Dr. W. H. Butler (MRID # 429202-01, page 5).

Lesion	Males				Females			
	Control	0.6%	1.2%	2.4%	Control	0.6%	1.2%	2.4%
<u>Butler</u>	(26) ¹	(23)	(17)	(25)	(25)	(27)	(27)	(26)
Focal hyperplasia ²	2	1	2	3	0	0	13	8
Adenoma ³	0	0	8	13	0	0	1	11
Carcinoma ³	0	0	3	7	0	0	0	5
<u>Original</u>	(25) ¹	(25)	(15)	(25)	(24)	(27)	(25)	(26)
"Liver tumor"	0	1	14	25	0	4	22	26

1. The number in () is the number of animals examined.
2. Focal hyperplasia is not regarded as a tumor but is included to demonstrate possible preneoplastic conditions.
3. More specifically hepatocellular adenoma and hepatocellular carcinoma. Dr Butler did not specifically state that rats with both an adenoma and carcinoma are counted as having carcinoma only.

The above table clearly indicates that based on either the original or Dr. Butler's analysis that there is a test compound related increase in liver tumors or both hepatocellular adenomas and carcinomas. The following criteria were used for diagnosis by the two different pathologists.

Original-Based on the classification of Boorman et al (Pathology of the Fischer Rat, Academic Press, New York, 1980? or 1990? - the reference is cited with two different dates). In this diagnosis, nodular lesions were divided into hepatocellular adenoma (including hyperplastic nodules and neoplastic nodule) and hepatocellular carcinoma (cellular atypia, structural atypia, nucleus/cytoplasm and compression of the adjacent tissue).

Butler-No specific reference was provided but Dr. Butler provided the following comments. Nodular lesions with the presence of abnormal thickened trabeculae often associated with hemorrhage and necrosis are classified as carcinoma. Nodules classified as adenomas have more simple trabecular structure usually 1-2 cells thick with little or no necrosis or hemorrhage. Focal hyperplasia are nodules that differ from adenomas in that the hyperplasia have differentially organized hepatic cords and the presence of residual structures. Dr. Butler also stated that "there are no definitive histological criteria for the differentiation of

adenomas (benign neoplasms) and hyperplasia (a reactive proliferation).

2. Lung. Absolute lung weight (relative not reported) was decreased in the female mid (12%) and high (32%) and male high (36%). Necropsy revealed "whitish spotting" most notably in the mid and high dose group males. No histopathology data were presented to further characterize possible lung injury.

3. Kidneys. Absolute kidney weight (relative not reported) was decreased 17 or 18% for both sexes in the high dose groups and necropsy indicated that there were black colored kidneys in all dose groups. Misshapened kidneys were noted in the mid and high dose group females. No histopathology data were presented. Refer to Table 4 in Appendix 2.

4. Cecum and stomach. Necropsy revealed various descriptions such as hemorrhage and enlargement in all PBO treated groups and some had hyperplasia. Other descriptions included smooth surface, hyperplasia and edema and polyps. It should be noted that hemorrhage of the cecum was indicated by the study author as a probable cause of death for the low and mid dose male groups when it was present. Refer to Table 4 in Appendix 2.

5. Spleen. Absolute spleen weight (relative not reported) was decreased in the high dose male (67%) and female (38%) and lower but not statistically significant weights were noted in the low and mid dose groups. This may be related to the observation that "essential thrombocytopenia was present in 0, 26%, 20% and 38% of the male rats in the control to high dose groups respectively. Only one female high dose group rat had this condition.

6. Heart, testis and ovary weights. The weights of these organs were decreased. Since there were no pathology findings reported TB-I cannot assess these weight effects further.

CONCLUSION (pathology-provisional). NOEL < 0.6%. At 0.6%: liver weight increase; hemorrhage in the gastro-intestinal system and black colored kidneys. At 1.2% liver tumors.

No conclusions are being made with respect to the possibility of effects in the lungs, spleen, heart, kidney or testis which had organ weight differences and/or color changes but no reported supporting histopathology.

CONCLUSION (Study). These data are SUPPLEMENTARY. The study cannot be upgraded to minimum for a series 83-2 rat carcinogenicity study because the protocol did not include a sufficient number of animals/sex at initiation of dosing. The report consists of a series of summary tables only and only data on the liver pathology are available (in a appended report prepared by Dr. Butler). The study supports the following provisional one liner.

Systemic toxicity NOEL and LEL: < 537 mg/kg/day. At 537 mg/kg/day: gastrointestinal hemorrhage; increase liver weight; decrease body weight; effect on RBC parameters, possible kidney pathology. At 1,877 (males) and 2,002 (females) mg/kg/day (HDT) possible excessive toxicity as indicated by 50% decrease in body weight.

Carcinogenicity potential: Positive for liver tumors in both males and females.

Fischer 344 strain rat. Dose levels tested: 0, 0.6, 1.2 and 2.4% in the diet corresponding to 0, 547, 1,052 and 1,877 mg/kg/day for males and 537, 1,061 and 2,002 mg/kg/day for females.

Appendix I

Piperonyl butoxide (dietary %)

0 0.6 1.2 2.4

Male

Number of surviving rats

Surviving rats bearing cancers^a

Surviving rats bearing cancers (%)

Mean number of cancers in size distinction/rat

Diameter (mm)

1-5^a

6-10

>11

Mean number of cancers/rat^a

Number of surviving and dead rats

Surviving and dead rats bearing cancers^a

Surviving and dead rats bearing cancers (%)

Female

Number of surviving rats

Surviving rats bearing cancers^a

Surviving rats bearing cancers (%)

Mean number of cancers in size distinction/rat

Diameter (mm)

1-5^a

6-10

>11

Mean number of cancers/rat^a

Number of surviving and dead rats

Surviving and dead rats bearing cancers^a

Surviving and dead rats bearing cancers (%)

^a The response has a significant dose-related linear trend.

^b Significantly different from the appropriate control (0%) value.

TABLE 4
Gross Findings of Non-neoplastic Lesions

	Piperonyl butoxide (dietary %)			
	0	0.6	1.2	2.4
Male (surviving)				
Number of rats examined	25	23	15	25
Stomach (glandular)				
Hemorrhages	2	1	2	7
Polyps	0	0	0	2
Smooth surface	0	0	2	10 ^a
Cecum				
Enlargement	0	12 ^a	8 ^a	7 ^a
Hemorrhages	0	2	3	3
Edema	0	7 ^a	8 ^a	0
Hyperplasia	0	1	0	2
Lungs				
Whitish spotting	0	0	3 ^a	11 ^a
Kidneys				
Black-colored	1	5	10 ^a	17 ^a
Misshapen	1	1	0	1
Male (dead)				
Number of dead rats	5	7	15	8
Stomach				
Hemorrhages	3	1	4	2
Cecum				
Enlargement	1	6	10	4
Hemorrhages	0	6 ^a	10 ^a	3
Hyperplasia	0	0	3	2
Female (surviving)				
Number of rats examined	24	27	25	26
Stomach (glandular)				
Hemorrhages	1	1	5	16 ^a
Polyps	1	0	0	6
Smooth surface	0	2	5	12 ^a
Cecum				
Enlargement	0	7 ^a	13 ^a	8 ^a
Hemorrhages	0	5	9 ^a	4
Edema	0	5	6	3
Hyperplasia	0	1	0	3
Lungs				
Whitish spotting	0	0	0	2
Kidneys				
Black-colored	0	7 ^a	20 ^a	25 ^a
Misshapen	0	0	3	8 ^a
Female (dead)				
Number of dead rats	6	3	5	7
Stomach				
Hemorrhages	1	1	2	3
Cecum				
Enlargement	0	0	0	1
Hemorrhages	0	1	1	3
Hyperplasia	0	0	0	1

^a Significantly different from the appropriate control (0%) value



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

REVIEWER

APR 28 1988

ATTACHMENT 3

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)

*Budd
4/15/88*

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 of 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 addressed in this memo but will be addressed when the ADI is evaluated by the RfD ADI committee.

Reviewed by: J.D. Doherty
Section II, Tox. Branch (TS-769C)
Secondary reviewer: E.R. Budd
Section II, Tox. Branch (TS-769C)

006668

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 3P3
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 weight and increased hypertrophy of hepatocytes in males and increased hepatic "focal mixed cells" and increased serum cholesterol in females. Increases in "pituitary follicles" and hyperplasia of follicular cells in thyroid in both males and females. Decreased weight 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		Interim Sac. 1 month*	
		male	female	male	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 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). Comparisons 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.

<u>Group</u>	<u>Males</u>	<u>Females</u>
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 comparison 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 desirable 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. Ophthalmological 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 responsible for the ophthalmological 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 dose: with PB and the control groups for these hematology parameters. NOEL > 500 mg/k day.

b. Clinical Chemistry

X

Electrolytes:

- x Calcium*
- x Chloride*
- Magnesium*
- x Phosphorous*
- x Potassium*
- x Sodium*

Enzymes

- x Alkaline phosphatase
- Cholinesterase# (N/A)
- x Creatinine phosphokinase*°
- Lactic acid dehydrogenase
- x Serum alanine aminotransferase (also SGPT)*
- x Serum aspartate aminotransferase (also SGOT)*
- gamma glutamyl transferase
- glutamate dehydrogenase

X

Other:

- x Albumin*
- Blood creatinine*
- x Blood urea nitrogen*
- x Cholesterol*
- Globulins
- x Glucose*
- x Total Bilirubin*
- x Total Serum Protein*
- Triglycerides
- Serum protein electrophoresis
- x Albumin/globulin ration

* 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 possibly 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.

Week	Males			Females		
	Low	Mid	High	Low	Mid	High
25	11(-) ¹	40*(4)	34**(-)	10	8	73***
51	3(-)	39*(-)	34*(-)	11(-)	35(22)	8....
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 comparison, 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 pathological changes since toxic insult to these organs may result in hyperproteinemia.

c. Serum glutamate oxalacetate transaminase (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 creatine 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	Digestive system	X	Cardiovasc./Hemat.	X	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
	Nose°				and masses*
	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 groups.						
Males				Females		
Group	Abs.	Rel ₁	Rel ₂	Abs	Rel ₁	Rel ₂
30 mg/kg/day	8.8	9.6	5.6	20.0 ^{ss}	11.0	18.5 ^{ss}
100 mg/kg/day	9.2	25.5 ^{ss}	10.5	25.2 ^{ss}	27.4 ^{ss}	22.8 ^{ss}
500 mg/kg/day	20.5 ^{ss}	53.2 ^{ss}	24.9 ^{ss}	27.1 ^{ss}	70.9 ^{ss}	29.3 ^{ss}

Abs = based on absolute weight; Rel₁ = based on weight relative to body weight;
Rel₂ = 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).

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 the safrole moiety 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	1
Discoloration	3	3	2	8	7	5	0	1	0	1
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.

C₁ and C₂ 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 reportedly examined.

¹Statistically 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 increased.

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 chronic 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 data showed statistically 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 bilateral) 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 relationship for the incidence of "focal coarsely 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 definitely 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%)

¹Data are incidences/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 relative 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, do not provide 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 consequential effects on circulating hormones. Thus, TB sees little basis for the study reports discussion which tries to relate the subtle changes in the pathological 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.

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

Author

Gerald P. Schoenig, Ph.D.
Toxicology Consultant to
Piperonyl Butoxide Task Force

Completed on:

August 28, 1987

Submitted by

PIPERONYL BUTOXIDE TASK FORCE

c/o Frederick J. Preiss, Ph.D.
McLaughlin Gormley King Co.
8810 Tenth Avenue, N.
Minneapolis, MN 55427

Dated

August 28, 1987

Volume 1 of 1

(68)

25-7

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).

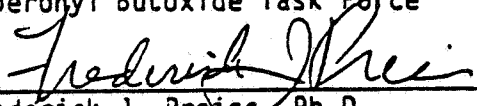
No supplemental claim of confidentiality is made for the information contained in these studies on the basis of FIFRA Section (10) (A) or (B). This document, however, is proprietary to the Piperonyl Butoxide Task Force and is considered to be confidential and trade secret information in all other countries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

(69)

251

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, ocular

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, ocular 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.



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

JAN 4 1995

ATTACHMENT 4

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Piperonyl Butoxide Qualitative Risk Assessment Based On
Charles River Sprague-Dawley Crl-CDR Rat and Charles
River CD-1 Mouse Dietary Studies

Caswell No. 670

TO: John D. Doherty, Toxicologist
Review Section IV
Toxicology Branch I
Health Effects Division (7509C)

FROM: Lori L. Brunsman, Statistician
Statistics Section
Science Analysis Branch
Health Effects Division (7509C)

Lori L. Brunsman

THROUGH: Hugh M. Pettigrew, Section Head
Statistics Section
Science Analysis Branch
Health Effects Division (7509C)

Hugh M. Pettigrew

Summary

This qualitative risk assessment of Piperonyl Butoxide was based upon two chronic carcinogenicity studies conducted in Charles River Sprague-Dawley Crl-CDR rats and CD-1 mice. The rats were fed 0, 30, 100, or 500 mg/kg/day of Piperonyl Butoxide for 105 weeks. The mice were fed 0, 30, 100, or 300 mg/kg/day of Piperonyl Butoxide for 79 weeks.

The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of Piperonyl Butoxide in male or female rats.

Male rats had a significant dose-related increasing trend in thyroid follicular cell combined adenomas and/or carcinomas. There were no significant differences in the pair-wise comparisons of the dosed groups with the controls.

Female rats had a significant dose-related increasing trend in thyroid follicular cell adenomas. There were no significant differences in the pair-wise comparisons of the dosed groups with the controls.

73



Recycled/Recyclable
Printed with Soy/Canola Ink on paper that
contains at least 50% recycled fiber

The statistical evaluation of mortality indicated a significant decreasing trend with increasing doses of Piperonyl Butoxide in male mice. Female mice showed no significant incremental changes in mortality with increasing doses of Piperonyl Butoxide.

Male mice had significant dose-related increasing trends in hepatocellular adenomas, carcinomas, and combined adenomas and/or carcinomas. There were significant differences in the pair-wise comparisons of the 100 mg/kg/day dose group with the controls for hepatocellular adenomas and combined adenomas and/or carcinomas. There were also significant pair-wise comparisons of the 300 mg/kg/day dose group with the controls for hepatocellular adenomas, carcinomas, and combined adenomas and/or carcinomas.

Female mice had a significant dose-related increasing trend and a significant difference in the pair-wise comparison of the 300 mg/kg/day dose group with the controls for hepatocellular adenomas.

Background

A chronic dietary toxicity/carcinogenicity study in Charles River Sprague-Dawley Crl-CDR rats was conducted by Bio-Research, Ltd., Senneville, Quebec, Canada, for the Piperonyl Butoxide Task Force, c/o the McLaughlin Gormley King Company, Minneapolis, Minnesota, and dated August 27, 1987 (Study No. 81690; Accession No. 403237-01).

The study design allocated groups of 60 rats per sex to two separate control groups, which have been combined for this qualitative risk assessment, and to dose levels of 15, 30, 100, and 500 mg/kg/day of Piperonyl Butoxide for 105 weeks. An additional 10 rats per sex of one control group and of the 15 and 30 mg/kg/day dose groups were designated for interim sacrifice at week 5. When it was determined that the rats of the 15 mg/kg/day dose group showed no obvious effects of Piperonyl Butoxide at week 5, the remainder of the group was sacrificed at week 8.

A chronic dietary carcinogenicity study in Charles River CD-1 mice was conducted by the Bushy Run Research Center, Export, Pennsylvania, for the Piperonyl Butoxide Task Force II, c/o McKenna & Cuneo, Washington, DC, and dated August 27, 1993 (Study No. 91N0134; MRID No. 429037-01).

The study design allocated groups of 60 mice per sex to two separate control groups, which have been combined for this qualitative risk assessment, and to dose levels of 30, 100, and 300 mg/kg/day of Piperonyl Butoxide for 79 weeks.

Survival Analyses

The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of Piperonyl Butoxide in male or female rats or female mice. Male mice showed a decreasing trend in mortality with increasing doses of Piperonyl Butoxide. See Tables 1 and 2 for rat mortality test results, and Tables 5 and 6 for mouse mortality test results.

The statistical evaluation of mortality was based upon the Thomas, Breslow and Gart computer program.

Tumor Analyses

Male rats had a significant increasing trend in thyroid follicular cell combined adenomas and/or carcinomas at $p < 0.05$. There were no significant differences in the pair-wise comparisons of the dosed groups with the controls.

Female rats had a significant increasing trend in thyroid follicular cell adenomas at $p < 0.05$. There were no significant differences in the pair-wise comparisons of the dosed groups with the controls.

Male mice had significant increasing trends in hepatocellular adenomas, carcinomas, and combined adenomas and/or carcinomas, all at $p < 0.01$. There were significant differences in the pair-wise comparisons of the 100 mg/kg/day dose group with the controls for hepatocellular adenomas and combined adenomas and/or carcinomas, and significant pair-wise comparisons of the 300 mg/kg/day dose group with the controls for hepatocellular adenomas, carcinomas, and combined adenomas and/or carcinomas, all at $p < 0.01$.

Female mice had a significant dose-related increasing trend, and a significant difference in the pair-wise comparison of the 300 mg/kg/day dose group with the controls, for hepatocellular adenomas, both at $p < 0.01$.

The statistical analyses of the male and female rat and the female mice were based upon the Exact trend test and the Fisher's Exact test for pair-wise comparisons. The statistical analyses of the male mice were based upon Peto's prevalence test since there was a statistically significant negative trend for mortality in male mice with increasing doses of Piperonyl Butoxide. See Tables 3 and 4 for rat tumor analysis results, and Tables 7 and 8 for mouse tumor analysis results.

Table 2. Piperonyl Butoxide - Charles River Sprague-Dawley
Crl-CDR Rat Study

Female Mortality Rates⁺ and Cox or Generalized K/W Test Results

Dose (mg/kg/day)	<u>Weeks</u>						Total
	1-4	5	5-26	27-52	53-78	79-107 ^f	
0 [#]	1/131	10/130	2/120	2/118	17/116	53/99	75/121 (62)
30	2/72	10/70	1/60	2/59	6/57	29/51	40/62 (65)
100	1/61	0/60	0/60	1/60	6/59	19/53	27/61 (44) [*]
500	0/60	0/60	1/60	1/59	7/58	21/51	30/60 (50)

⁺Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[#]Two separate control groups were combined for this risk assessment.

^fFinal sacrifice at week 105.

() Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 1. Piperonyl Butoxide - Charles River Sprague-Dawley
Crl-CDR Rat Study

Male Mortality Rates* and Cox or Generalized K/W Test Results

Dose (mg/kg/day)	<u>Weeks</u>						Total
	1-4	5	5-26	27-52	53-78	79-105 ^f	
0 ^h	2/131	10/129	0/119	4/119	27/115	64/88	97/121 (80)
30	1/70	10/69	0/59	1/59	20/58	30/38	52/60 (87)
100	1/61	0/60	2/60	0/58	15/58	32/43	50/61 (82)
500	5/60	0/55	0/55	2/55	10/53	30/43	47/60 (78)

*Number of animals that died during interval/Number of animals alive at the beginning of the interval.

^hTwo separate control groups were combined for this risk assessment.

^fFinal sacrifice at week 105.

() Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 3. Piperonyl Butoxide - Charles River Sprague-Dawley
Crl-CDR Rat Study

Male Thyroid Follicular Cell Tumor Rates⁺ and Exact
Trend Test and Fisher's Exact Test Results (p values)

	<u>Dose (mg/kg/day)</u>			
	0 [#]	30	100	500
Adenomas (%)	2/115 (2)	1/58 (2)	0/58 (0)	2 ^a /53 (4)
p =	0.212	0.740	0.441 ^a	0.375
Carcinomas (%)	1 ^b /115 (1)	0/58 (0)	0/58 (0)	2/53 (4)
p =	0.090	0.665 ^a	0.665 ^a	0.234
Combined (%)	3/115 (3)	1/58 (2)	0/58 (0)	4/53 (8)
p =	0.040 [*]	0.589 ^a	0.291 ^a	0.142

⁺Number of tumor bearing animals/Number of animals examined,
excluding those that died or were sacrificed before week 53.

[#]Two separate control groups were combined for this risk assessment.

^aNegative change from control.

^{*}First adenoma observed at week 79, dose 500 mg/kg/day.

^bFirst carcinoma observed at week 89, dose 0 mg/kg/day.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted
at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 4. Piperonyl Butoxide - Charles River Sprague-Dawley
Crl-CDR Rat Study

Female Thyroid Follicular Cell Tumor Rates* and Exact
Trend Test and Fisher's Exact Test Results (p values)

	<u>Dose (mg/kg/day)</u>			
	0 [#]	30	100	500
Adenomas [@] (%)	1/116 (1)	0/57 (0)	1 [*] /58 (2)	3/58 (5)
p =	0.029 [*]	0.671 ^a	0.557	0.109

*Number of tumor bearing animals/Number of animals examined;
excluding those that died or were sacrificed before week 53.

[#]Two separate control groups were combined for this risk assessment.

^aNegative change from control.

^{*}First adenoma observed at week 105, dose 100 mg/kg/day.

[@]There were no thyroid follicular cell carcinomas diagnosed.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted
at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 5. Piperonyl Butoxide - Charles River CD-1 Mouse Study
Male Mortality Rates⁺ and Cox or Generalized K/W Test Results

Dose (mg/kg/day)	<u>Weeks</u>				Total
	1-26	27-52	53-68	69-79 ^f	
0 [#]	2/120	5/118	13/113	20/100	40/120 (33) ^m
30	1/60	2/59	6/57	10/51	19/60 (32)
100	0/60	3/60	3/57	10/54	16/60 (27)
300	0/60	0/60	7/60	6/53	13/60 [*] (22)

⁺Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[#]Two separate control groups were combined for this risk assessment.

^mNegative trend.

^fFinal sacrifice at week 79.

() Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 6. Piperonyl Butoxide - Charles River CD-1 Mouse Study
Female Mortality Rates* and Cox or Generalized K/W Test Results

Dose (mg/kg/day)	<u>Weeks</u>				Total
	1-26	27-52	53-65	66-80 ^f	
0 [#]	2/120	2/118	6/116	20/110	30/120 (25)
30	1/60	1/59	4/58	10/54	16/60 (27)
100	0/60	0/60	8/60	15/52	23/60 (38)
300	2/60	1/58	5/57	12/52	20/60 [.] (33)

*Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[#]Two separate control groups were combined for this risk assessment.

^fFinal sacrifice at week 79.

() Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 7. Piperonyl Butoxide - Charles River CD-1 Mouse Study

Male Hepatocellular Tumor Rates⁺ and
Peto's Prevalence Test Results (p values)

		<u>Dose (mg/kg/day)</u>			
		0 [#]	30	100	300
Adenomas (%)	17 ^a /107 (16)	13/57 (23)	22/56 (39)	28/59 (47)	
p =	0.000 ^{**}	0.111	0.001 ^{**}	0.000 ^{**}	
Carcinomas (%)	4/100 (4)	3/51 (6)	2/54 (4)	7 ^b /53 (13)	
p =	0.008 ^{**}	0.260	0.499	0.005 ^{**}	
Combined (%)	21/107 (20)	15 ^c /57 (26)	24/56 (43)	30 ^d /59 (51)	
p =	0.000 ^{**}	0.116	0.001 ^{**}	0.000 ^{**}	

⁺Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[#]Two separate control groups were combined for this risk assessment.

^aFirst adenoma observed at week 61, dose 0 mg/kg/day.

^bFirst carcinoma observed at week 69, dose 300 mg/kg/day.

^cOne animal in the 30 mg/kg/day dose group had both an adenoma and a carcinoma.

^dFive animals in the 300 mg/kg/day dose group had both an adenoma and a carcinoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If ^{*}, then $p < 0.05$. If ^{**}, then $p < 0.01$.

(82)

Table 8. Piperonyl Butoxide - Charles River CD-1 Mouse Study

Female Hepatocellular Tumor Rates⁺ and Exact Trend
Test and Fisher's Exact Test Results (p values)

	<u>Dose (mg/kg/day)</u>			
	0 [#]	30	100	300
Adenomas [@] (%)	4 [*] /116 (3)	1/58 (2)	1/60 (2)	10/57 (18)
p =	0.000 ^{**}	0.459 ^a	0.444 ^a	0.003 ^{**}

⁺Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.

[#]Two separate control groups were combined for this risk assessment.

^aNegative change from control.

^{*}First adenoma observed at week 56, dose 0 mg/kg/day.

[@]There were no hepatocellular carcinomas diagnosed.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If ^{*}, then $p < 0.05$. If ^{**}, then $p < 0.01$.

References

- Armitage, P. (1955) Tests for Linear Trends in Proportions and Frequencies. Biometrics 11, 375-386.
- Cochran, W.G. (1954) Some Methods for Strengthening the Common X² Test. Biometrics 10, 417-451.
- Cox, D.R. (1972) Regression Models and Life Tables (with discussion). J. Royal Stat. Soc. Ser. B. 34, 187-220.
- Gart, J.J., D. Krewski, P.N. Lee, R.E. Tarone, and J. Wahrendorf (1986) The Design and Analysis of Long-Term Animal Experiments. In: Statistical Methods in Cancer Research, Volume III. IARC Scientific Publications No. 79. Lyon, France: International Agency for Research on Cancer, p. 18.
- Peto, R., M. Pike, N. Day, R. Gray, P. Lee, S. Parish, J. Peto, S. Richard, and J. Wahrendorf (1980) Guidelines for Simple, Sensitive, Significant Tests for Carcinogenic Effects in Long-Term Animal Experiments. In: Monographs on the long-term and short-term screening assays for carcinogens: a critical appraisal. IARC Monographs, Supplement 2. Lyon, France: International Agency for Research on Cancer, pp. 311-426.
- Thomas, D.G., N. Breslow, and J.J. Gart (1977) Trend and Homogeneity Analyses of Proportions and Life Table Data. Computers and Biomedical Research 10, 373-381.

NCI Blue Book Piperonyl Butoxide Study
Report 120, 1979

ATTACHMENT

5

SUMMARY

A bioassay of technical-grade piperonyl butoxide for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and B6C3F1 mice.

Groups of 50 rats of each sex were administered piperonyl butoxide in the diet at one of two doses, either 5,000 or 10,000 ppm, for 107 weeks. Matched controls consisted of 20 untreated rats of each sex. All surviving rats were killed at the end of the period of administration of the test chemical.

Groups of 50 mice of each sex were initially administered piperonyl butoxide at one of two doses, either 2,500 or 5,000 ppm. After week 30, the doses for the mice were reduced to 500 and 2,000 ppm, respectively, and administration of the test chemical at the lowered doses was continued for 82 weeks. The time-weighted average doses for the mice were either 1,036 or 2,804 ppm. Matched controls consisted of 20 untreated mice of each sex. All surviving mice were killed at the end of the period of administration of the test chemical.

Mean body weights of dosed groups of rats and mice of each sex were lower than those of corresponding control groups, and the depressions in body weights were dose related. Survival of the rats and mice was unaffected by the piperonyl butoxide and was 80% or greater in all groups at week 90 of the bioassay; thus, sufficient numbers of dosed and control rats and mice of each sex were at risk for the development of late-appearing tumors.

In the female rats, lymphomas occurred at incidences that were dose related ($P = 0.007$); in a direct comparison, the incidence of the tumor in the high-dose group was higher ($P = 0.020$) than that in the control group (controls 1/20, low-dose 7/50, high-dose 15/50). However, the incidence of lymphomas, leukemias, and reticuloses in historical-control female Fischer 344 rats at the same laboratory was 19/191 (10%). These historical-control groups include one with an incidence of animals with lymphoma or leukemia of 7/20 (35%) and another with an incidence of 6/20 (30%). Thus, the incidence of lymphomas in the control female rats of the present bioassay may have been abnormally low, and the occurrence of the higher incidence in the dosed groups cannot be clearly related to administration of piperonyl butoxide.

In the male mice, adenomas of the lacrimal gland occurred at incidences that were dose related ($P = 0.023$), but in direct comparisons the incidences in the individual dosed groups were not significantly higher than that in the control group (controls 0/20, low-dose 0/49, high-dose 4/50); thus, the occurrence of this tumor in the male mice was not clearly related to administration of the test chemical.

It is concluded that under the conditions of this bioassay, piperonyl butoxide was not carcinogenic for Fischer 344 rats or B6C3F1 mice.

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered Piperonyl Butoxide in the Diet (a)

(continued)			
Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma (b)	1/20 (5)	2/47 (4)	5/48 (10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)			
Lower Limit		0.851	2.083
Upper Limit		0.048	0.259
		49.164	96.358
Weeks to First Observed Tumor	112	111	107

109

- (a) Dosed groups received time-weighted average doses of 1,036 or 2,804 ppm.
- (b) Number of tumor-bearing animals/number of animals examined at site (percent).
- (c) Beneath the incidence of tumors in the matched-control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
- (d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.
- (e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.
- (f) The 95% confidence interval of the relative risk between each dosed group and the control group.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Piperonyl Butoxide in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma (b)	1/20 (5)	7/50 (14)	15/50 (30)
P Values (c,d)	P = 0.007	N.S.	P = 0.020
Relative Risk (f)			
Lower Limit		2.800	6.000
Upper Limit		0.403	1.048
		123.407	245.704
Weeks to First Observed Tumor	107	94	73
Pituitary: Chromophobe Adenoma (b)	8/19 (42)	11/48 (23)	14/49 (29)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)			
Lower Limit		0.544	0.679
Upper Limit		0.251	0.336
		1.348	1.606
Weeks to First Observed Tumor	107	107	105

88

Table E1 Analyses of the Incidence of Primary Tumors in Male Rats
Administered Piperonyl Butoxide in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Adenoma or Carcinoma (b)	1/20 (5)	6/49 (12)	4/50 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)			
Lower Limit		2.449	1.600
Upper Limit		0.332	0.175
		110.166	77.169
Weeks to First Observed Tumor	107	104	107
Pancreatic Islets: Islet-cell Adenoma or Carcinoma (b)	1/18 (6)	4/44 (9)	3/48 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)			
Lower Limit		1.636	1.125
Upper Limit		0.181	0.100
		78.690	57.811
Weeks to First Observed Tumor	107	107	107

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats
Administered Piperonyl Butoxide in the Diet (a)

(continued)			
<u>Topography: Morphology</u>	<u>Matched Control</u>	<u>Low Dose</u>	<u>High Dose</u>
Pituitary: Chromophobe Adenoma, Adenoma, NOS or Carcinoma (b)	5/19 (26)	7/49 (14)	7/48 (15)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)			
Lower Limit		0.543	0.554
Upper Limit		0.176	0.180
		1.959	1.997
Weeks to First Observed Tumor	102	104	103
Thyroid: Follicular-cell Adenoma or Carcinoma (b)	0/20 (0)	1/49 (2)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)			
Lower Limit		Infinite	Infinite
Upper Limit		0.023	0.250
		Infinite	Infinite
Weeks to First Observed Tumor	--	107	81

(90)

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered Piperonyl Butoxide in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma (b)	1/20 (5)	2/47 (4)	5/48 (10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)			
Lower Limit		0.851	2.083
Upper Limit		0.048	0.259
		49.164	96.358
Weeks to First Observed Tumor	112	111	107

109

(a) Dosed groups received time-weighted average doses of 1,036 or 2,804 ppm.

(b) Number of tumor-bearing animals/number of animals examined at site (percent).

(c) Beneath the incidence of tumors in the matched-control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.

(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

(f) The 95% confidence interval of the relative risk between each dosed group and the control group.

FA (91)

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice
Administered Piperonyl Butoxide in the Diet (a)

(continued)			
<u>Topography: Morphology</u>	<u>Matched Control</u>	<u>Low Dose</u>	<u>High Dose</u>
Thyroid: Follicular-cell Adenoma (b)	1/20 (5)	3/49 (6)	0/50 (0)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)			
Lower Limit		1.224	0.000
Upper Limit		0.108	0.000
		62.958	7.475
Weeks to First Observed Tumor	112	112	--
Lacrimal Gland: Adenoma, NOS (b)	0/20 (0)	0/49 (0)	4/50 (8)
P Values (c,d)	P = 0.022	N.S.	N.S.
Relative Risk (f)			
Lower Limit		--	Infinite
Upper Limit		--	0.386
		--	Infinite
Weeks to First Observed Tumor	--	--	112

LACK OF EVIDENCE OF CARCINOGENICITY OF TECHNICAL-GRADE PIPERONYL BUTOXIDE IN F344 RATS; SELECTIVE INDUCTION OF ILEOCAECAL ULCERS*

A. MAEKAWA, H. ONODERA, K. FURUTA, H. TANIGAWA, T. OGIU and Y. HAYASHI
Division of Pathology, Biological Safety Research Centre, National Institute of Hygienic Sciences,
Kamiyoga 1-18-1, Setagaya-ku, Tokyo 158, Japan

(Received 20 June 1984; revision received 13 August 1984)

Abstract—The carcinogenicity of technical-grade piperonyl butoxide was studied in F344/DuCrj rats fed a dietary level of 0.5 or 1% for 2 yr. Various tumours were detected in all groups, including the untreated control group, but no significant dose-related increase in the incidence of any tumour was found. Thus, it is concluded that under these experimental conditions piperonyl butoxide was not carcinogenic in F344 rats. Unexpectedly, however, ileocaecal ulcers were found in animals of both sexes in both experimental groups and the incidence was dose related. Further studies are required to establish the mechanism of induction of ileocaecal ulcers by piperonyl butoxide.

INTRODUCTION

Piperonyl butoxide (PB), a pesticide used to enhance the effect of pyrethrin, is known to affect drug-metabolizing enzyme systems. Recently, in a summary of a Japanese co-operative programme, PB was reported to be non-mutagenic to bacteria, cultured mammalian cells and silk worms (Kawachi, Yahagi, Kada *et al.* 1980). An absence of mutagenic activity has also been reported for this chemical by others (Ashwood-Smith, Trevino & Ring, 1972; White, Goodman, Shulgin *et al.* 1977). The carcinogenicity of PB has been tested in mice and rats by several groups, who all reported a lack of evidence that PB was carcinogenic (Cardy, Renne, Warner & Cypher, 1979; Innes, Ulland, Valerio *et al.* 1969; National Cancer Institute, 1979). Accordingly, the International Agency for Research on Cancer recently concluded that PB is unlikely to present a carcinogenic risk to humans (IARC Working Group, 1983).

This paper describes the results of a 2-yr carcinogenicity test in F344/DuCrj rats, in which PB induced ulcers selectively, and at a high incidence, in the ileocaecal mucosa.

EXPERIMENTAL

Animals and maintenance. Specified-pathogen-free Fischer (F344/DuCrj) rats of both sexes were purchased from Charles River Japan, Inc. (Atsugi) and housed in plastic cages in an air-conditioned room maintained at $24 \pm 1^\circ\text{C}$ with a humidity of $55 \pm 5\%$. The basal diet was powdered CRF-1, obtained from

Oriental Yeast Industries Co. (Tokyo), mixed with 2% olive oil.

Test chemical. Technical-grade PB (specific gravity: d_{20}^{20} 1.055; refractive index: n_D^{20} 1.4978) was purchased from Takasago Perfumery Co. Ltd (Hiratsuka). The purity of the preparation used in these studies was about 89%, but the other components were not examined. The batch of basal diet used for the carcinogenicity study was shown to be free of contamination by pesticides, benzo[a]pyrene and aflatoxin, and to contain small but acceptable levels of metals.

Subchronic toxicity study

To establish appropriate dietary levels for the carcinogenicity study, 6-wk-old rats were divided into six groups, each of ten males and ten females. PB was added to the powdered basal diet (mixed with 2% olive oil) to give concentrations of 0 (control), 0.25, 0.5, 1, 2 and 3% and the diets were supplied *ad lib.* to the rats for 13 wk, during which the animals were given tap-water to drink and were housed five/cage. At the end of the experiment, all survivors were killed for a gross and a limited microscopic examination.

Carcinogenicity study

The rats were acclimatized for 1 wk on the basal diet and tap-water prior to the start of the study, during which they were housed three or four males or five females to a cage. Animals were randomly divided into three groups, each consisting of 50 males and 50 females and, from the age of 6 wk, were given 0 (control), 0.5 or 1% PB mixed with powdered basal diet *ad lib.* for 2 yr. The amounts of diet consumed were measured monthly for calculation of the PB intake. Throughout the experiment, rats in each group had free access to tap-water.

During the experimental period, all animals were observed daily, and clinical signs and mortality were

*Part of this work was presented at the 73rd Annual Meeting of the Japanese Pathological Association, held in Tokyo in April 1984. The work was supported by grants-in-aid from the Ministry of Health and Welfare of Japan.

Abbreviations: H&E = Haematoxylin and eosin; PB = piperonyl butoxide.

Table 1. Body and organ weights of F344 rats fed 0-3% piperonyl butoxide in the diet for 13 wk

Dietary level (%)	Mean body weight (g)			Organ weights					
				Liver		Right Kidneys		Left Kidneys	
	Initial	Final	Gain†	g	g/100 g body weight	g	g/100 g body weight	g	g/100 g body weight
Males									
0	118	328	210	—	10.84	3.30	1.09	0.33	1.09
0.25	118	314	196*	(93)	12.68*	4.02**	1.14	0.36*	1.14
0.5	118	312	194*	(92)	12.93**	4.13**	1.20	0.38**	1.18
1	118	300**	182**	(87)	15.26**	5.09**	1.16	0.39**	1.16
2	118	250**	132**	(63)	17.65**	7.03**	1.16	0.46**	1.14
3	117	151**	34**	(16)	16.29**	10.75**	0.77**	0.51**	0.78**
Females									
0	95	171	76	—	5.13	3.00	0.61	0.36	0.62
0.25	96	172	76	(100)	6.23*	3.62*	0.63	0.37	0.66
0.5	95	168	73	(96)	6.90**	4.11**	0.61	0.36	0.63
1	95	167	72	(95)	8.19**	4.89**	0.66*	0.40**	0.67*
2	94	149**	55**	(72)	11.89**	7.94**	0.66	0.44**	0.65
3	96	78**	-18**		10.66**	13.56**	0.49**	0.62**	0.48**

†Mean weight gain over 13 wk, expressed in brackets as a percentage of the control value.

Values are means for groups of ten rats, except those for the 3% male group (nine rats). Those marked with asterisks differ significantly (Student's *t* test) from the control value: **P* < 0.05; ***P* < 0.01.

recorded. Administration of PB was stopped after 104 wk and basal diet without PB was given to animals in all groups from then until wk 110, when all survivors were killed. Moribund or dead animals were given a complete autopsy and the following organs and tissues were examined histologically for development of tumours and non-neoplastic lesions: brain, spinal cord, pituitary gland, salivary glands, thyroid glands (including parathyroids), thymus, heart, lungs (including trachea), liver, pancreas, spleen, kidneys, adrenal glands, tongue and oral cavity, oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, urinary bladder, gonads, accessory genital organs, skin, musculature, mammary glands, lymph nodes, sternum, femur, peripheral nerves, eyes, ear ducts and nasal cavity. All lesions and organs or tissues were fixed with buffered 10% formalin, sectioned and stained routinely with haematoxylin and eosin. Special stains were used when necessary.

RESULTS

Subchronic toxicity study

Only one male, in the 3%-PB group, died during the experimental period. Table 1 shows mean body and organ weights for each group. From wk 1 of the experiment, all groups except the females given 0.25% PB showed lower body-weight gains than the controls and groups of either sex given 2 or 3% PB showed over 10% reduction in weight gain compared with the controls. At autopsy, hepatomegaly was marked in rats of either sex given diet containing 2 or 3% PB. The relative weights of the liver and kidneys increased dose-dependently in the experimental groups of both sexes, and to a considerable degree in the groups fed 2 or 3% PB. Histologically, hepatocyte enlargement and focal necrosis of the liver were prominent in groups given the higher doses of PB. The cytoplasm of the hepatocytes appeared glassy or vacuolated. In the digestive tract, no marked macro- or microscopic changes were observed in any groups, but the caecum was not examined histologically. From these results,

it was concluded that the maximum tolerable dose of PB in the diet was 1%.

Carcinogenicity study

Growth curves and mortality. Figure 1 shows the growth curves for the animals in each group. Throughout the experiment, a dose-dependent effect of PB on growth was apparent in both sexes. By the end of the administration period, the cumulative deaths of rats in the control and 0.5 and 1% groups accounted, respectively, for 16, 38 and 42% of the males, and 14, 22 and 34% of the females. Just before death many of the rats showed signs of anaemia and faecal blood.

PB intake, tumour incidence and mean survival time. The total PB intake, overall benign and malignant tumour incidence and mean survival time are shown in Table 2 for the rats of each group. The first autopsy was at wk 32, when a male rat in the 1%-PB group was killed because it became moribund as a result of the toxic effect of PB. All rats surviving beyond this time were included in the effective numbers (Table 2), except for a few in which advanced autolysis prevented adequate examination. The daily food intake was almost constant throughout the

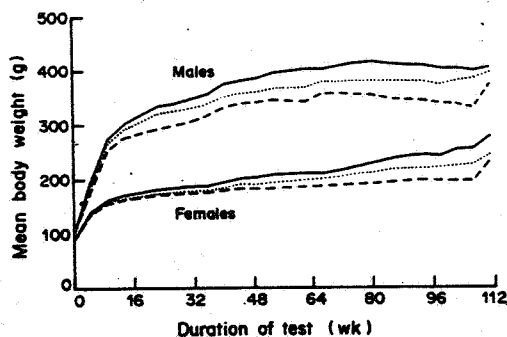


Fig. 1. Mean body weights for male and female F344 rats fed piperonyl butoxide at dietary levels of 0% (—; control), 0.5% (---) or 1.0% (.....) for 104 wk and killed at wk 110.

experime
intakes i
food into
mour inc
in the co
not stati
males, th
1%-PB g

Organ
sites and
are sum
tumours
by thos
thyroid.
lung. In
haemato
were the
in other
but the
mental
inciden
corresp
of hepa
slightly
diet. In
testis, p
in male
lower
0.5%-PB
group,
dence of
was low
statistic
tumour
that d
(Maek
Non
4 sum
in the
(Fig. 2
(Fig. 4
experi
related
the ca
the 1
inflam
some
in the
tumou
group

94

Table 2. Piperonyl butoxide (PB) intake, tumour incidences and mean survival times in F344 rats fed 0.5 or 1% PB in the diet for 2 yr

Dietary level (%)	Effective no. of rats*	Total PB intake in 2 yr (g/rat)	No. of rats with tumours† (% of effective no.)	Mean survival time and range (wk)
Males				
0	48	—	48 (100)	108 (84–110)
0.5	48	52.6	48 (100)	101 (58–110)
1	46	108.7	41 (89)	97 (32–110)
Females				
0	47	—	35 (75)	106 (59–110)
0.5	49	35.4	39 (80)	105 (65–110)
1	49	71.5	30 (61)	103 (65–110)

*Rats surviving beyond wk 32 (from initial groups of 50), except those in which advanced autolysis precluded adequate examination.

†Benign and/or malignant.

experimental period in all groups. The total PB intakes in 2 yr (Table 2) were calculated from the food intake figures. For both sexes, the overall tumour incidence was lower in the 1% PB group than in the control group, although the differences were not statistically significant. For both males and females, the mean survival time was shorter for the 1% PB group than for the other two groups.

Organ distribution and histology of tumours. The sites and histological diagnoses of the tumours found are summarized in Table 3. In the males of all groups, tumours of the testis were the most frequent, followed by those of the haematopoietic organs, adrenals, thyroid, mammary gland, pituitary, pancreas and lung. In females, tumours of the uterus, pituitary, haematopoietic organs, mammary gland and thyroid were the most common. Tumours were also detected in other organs or tissues in all groups of both sexes, but the incidences were low. None of the experimental groups showed a significant increase in the incidence of any specific tumour over that in the corresponding control group, although the incidence of hepatic tumours in the males on 1% PB was slightly higher than in those on 0.5% PB or control diet. In contrast, the incidences of tumours of the testis, pituitary, haematopoietic organs and thyroid in males and of the mammary gland in females were lower in the 1% PB, and generally also in the 0.5% PB, group than in the corresponding control group, showing an inverse dose relation. The incidence of C-cell adenomas in males on the 1% PB diet was low and the difference from the controls was statistically significant. Histologically, almost all the tumours observed in each group were similar to those that develop spontaneously in this strain of rat (Maekawa, Kurokawa, Takahashi *et al.* 1983).

Non-neoplastic lesions in the digestive tract. Table 4 summarizes the incidence of non-neoplastic lesions in the gastro-intestinal tract in each group. Erosions (Fig. 2), ulcers (Fig. 3) and regenerative hyperplasia (Fig. 4) occurred in the ileocaecal mucosa in all the experimental groups and their incidence was dose related. In many rats with ulcers, haemorrhage of the caecum and colon was prominent. Histologically, the main lesions were chronic ulcers with inflammatory-cell infiltration and granulation. In some rats with these ulcers, ossification was detected in the mucosa near the ulcer. Only one ileocaecal tumour was detected, in a male rat in the 0.5% PB group; histologically this was diagnosed as an ade-

nocarcinoma (Fig. 5; Table 3). In this region, however, there was no atypical cell growth, such as is characteristic of preneoplastic lesions, in any rats in the PB-treated groups.

DISCUSSION

PB is known to inhibit drug-metabolizing hepatic microsomal enzymes, but it has a biphasic effect; its immediate effect is to inhibit the enzymes, while its administration over an extended period has been reported to result in increased liver weight, enlargement of hepatocytes, proliferation of smooth endoplasmic reticulum in the cytoplasm of the hepatocytes, and a corresponding increase in the quantities of cytochrome P-450 and other drug-metabolizing enzyme systems (Goldstein, Hickman & Kimbrough, 1973). In the subchronic study reported here, the hepatomegaly and hepatocyte enlargement observed in the higher dose groups were consistent with that report.

Previous studies have not shown that PB is mutagenic or is carcinogenic to mice or rats (IARC Working Group, 1983). In the study reported here, many benign and malignant tumours were detected in each group, including the control group, but no incidences were significantly higher in PB-treated groups than in the controls. This result indicates that, as reported by others, PB is not carcinogenic. On the contrary, the incidences of some tumours, such as interstitial-cell tumours, pituitary tumours, mononuclear cell leukaemias and thyroid C-cell tumours in males and mammary tumours in females, were lower in the groups given the higher dietary level of PB. However, these data may be attributed to the shorter survival time of the rats in these groups.

Since the daily food intake was similar in all groups throughout the experimental period, the reduced body-weight gain in the treated groups is likely to have been due to the toxicity of PB. The early deaths in the high-dose group were attributable to the ileocaecal ulcers induced by PB, although clinical signs, such as anaemia and faecal blood only became apparent shortly before these rats died.

The high and dose-related incidence of erosions, ulcers and regenerative hyperplasia in the ileocaecal mucosa of the PB-treated rats was an unexpected finding. In a carcinogenicity study, Cardy *et al.* (1979) used F344 rats and technical-grade PB at the same dietary levels as in our study, but did not detect lesions of this type. Neither have such lesions been

Left

g/100 g
body weight

0.33
0.36
0.38**
0.39**
0.45**
0.52**

0.36
0.39
0.38
0.40**
0.43**
0.61**

ffer significantly

rable dose of

l shows the
each group.
endent effect
sexes. By the
cumulative
d 1% groups
% of the
sust before
anaemia and

survival time.
id malignant
e are shown
up. The first
n the 1% PB
ribund as a
its surviving
ffective num-
ch advanced
on. The daily
oughout the



96 112

male F344 rats
of 0% (—;
and killed

95

Table 3. Sites and types of tumours in F344 rats fed 0.5 or 1.0% piperonyl butoxide in the diet for 2 yr

Tissue/tumour type	Dietary level (%)... Effective no./group ...	Incidence of tumours (%)†					
		Males			Females		
		0 48	0.5 48	1 46	0 47	0.5 49 ^a	1 49
Genital system							
Testis—interstitial-cell tumour	100	100	87	—	—	—	—
Uterus—endometrial polyp	—	—	—	21	16	27	—
—adenoma	—	—	—	2	2	0	—
—adenocarcinoma	—	—	—	4	4	0	—
Vagina—squamous-cell carcinoma	—	—	—	0	0	2	—
—fibrosarcoma	—	—	—	2	0	2	—
Ovary—granulosa-cell tumour	—	—	—	2	0	0	—
—arrhenoblastoma	—	—	—	0	2	0	—
—granulosa/theca-cell tumour	—	—	—	0	2	0	—
Mammary gland—fibroma	8	4	4	0	0	2	—
—fibroadenoma	4	2	7	13	8	2	—
—adenoma	0	0	0	2	0	0	—
—adenocarcinoma	0	0	0	2	0	0	—
Endocrine system							
Pituitary—chromophobe adenoma	10	6	4	17	20	16	—
—chromophobe carcinoma	0	0	0	0	4	0	—
Thyroid—C-cell adenoma	21	15	4*	6	16	4	—
—C-cell carcinoma	2	0	0	0	0	0	—
—papillary adenocarcinoma	0	0	2	0	0	0	—
Parathyroid—adenoma	0	2	2	0	0	0	—
Adrenal—phaeochromocytoma	13	15	17	4	2	8	—
—malignant phaeochromocytoma	2	8	4	0	0	0	—
—ganglioneuroma	0	0	0	0	2	2	—
Pancreas—insuloma	8	2	4	4	0	0	—
—malignant insuloma	2	0	0	0	0	0	—
—acinar-cell adenoma	0	2	0	0	0	0	—
Haematopoietic system							
Spleen—mononuclear-cell leukaemia	23	19	13	17	12	10	—
Bone marrow—malignant histiocytoma(?)	2	0	0	0	0	0	—
Thymus—thymoma	2	0	0	0	0	2	—
Respiratory system							
Lung—alveolar/bronchiolar adenoma	6	10	2	2	0	0	—
—alveolar/bronchiolar carcinoma	0	0	0	0	2	0	—
Digestive system							
Tongue—papilloma	0	0	2	0	2	2	—
—squamous-cell carcinoma	0	2	0	0	0	0	—
Jejunum—leiomyosarcoma	0	0	0	2	0	0	—
—mucinous adenoma	0	0	2	0	0	0	—
Ileocaecal region—adenocarcinoma	0	2	0	0	0	0	—
Colon—adenomatous polyp	2	0	0	0	0	0	—
Liver—neoplastic nodule	2	2	6	0	2	0	—
—hepatocellular carcinoma	0	0	2	0	0	0	—
Integument, musculo-skeletal system							
Skin—papilloma	0	4	0	0	0	0	—
—trichoeplithelioma	0	2	0	0	0	0	—
—squamous-cell carcinoma	0	2	0	0	0	0	—
Subcutis—fibroma	2	2	0	0	0	0	—
—lipoma	0	2	0	0	0	0	—
—haemangioma	0	2	0	0	0	0	—
—fibrosarcoma	0	0	0	0	2	0	—
—malignant fibrous histiocytoma	0	2	2	0	0	0	—
Preputial/clitoral gland—adenoma	4	2	0	2	2	0	—
—squamous-cell carcinoma	0	0	2	0	0	0	—
Ear duct—papilloma	0	0	0	2	0	0	—
—squamous-cell carcinoma	2	0	0	0	2	0	—
—fibroma	0	0	0	0	2	0	—
Nervous system							
Brain/spinal cord—astrocytoma	0	2	2	0	0	0	—
—granular-cell tumour	2	0	0	0	0	0	—
Peripheral nerve—neurinoma	0	2	0	0	2	0	—
Cardiovascular system							
Heart—myxoma	2	0	2	0	0	0	—
Others							
Pleura—mesothelioma	2	0	0	0	0	0	—
Peritoneum—mesothelioma	2	0	4	0	0	0	—

†Number of rats affected, expressed as a percentage of the effective number in the group. The percentage marked with an asterisk differs significantly from the control value ($P < 0.05$ by chi-square test).

found in any other previous long-term studies on the effect of PB in rats and mice (Epstein, Joshi, Andrea *et al.* 1967; National Cancer Institute, 1979). Thus, this is the first report that PB induces ulcers selec-

tively in the ileocaecal mucosa of rats. Some anti-inflammatory drugs are known to induce gastrointestinal ulcers (Shriver, White, Sandor & Rosenthale, 1975), but following oral administration

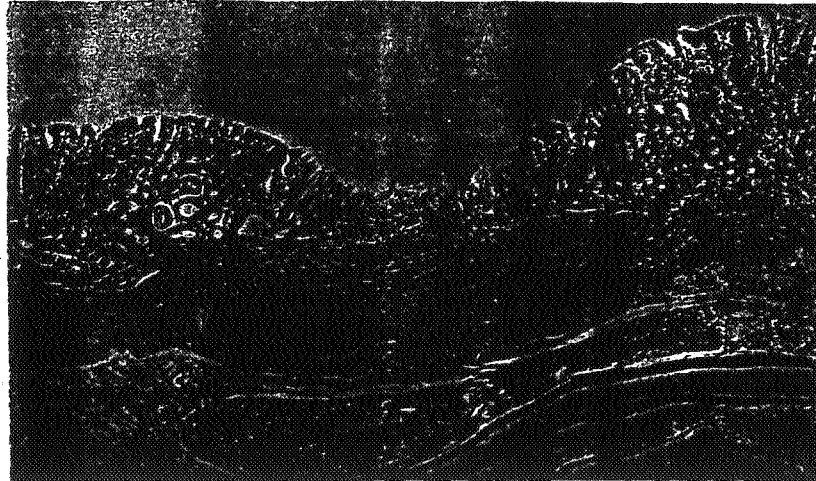


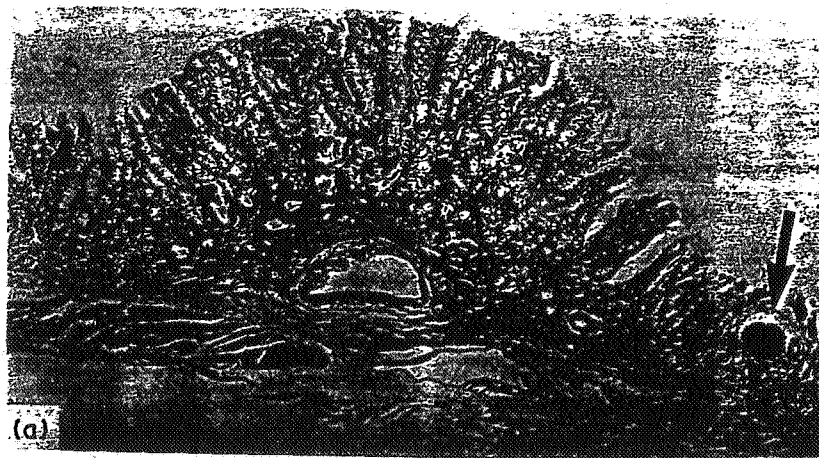
Fig. 2. Erosion of the ileocaecal mucosa in a 110-wk-old male rat fed 1% piperonyl butoxide in the diet for 104 wk. H&E $\times 10$.



Fig. 3. Ileocaecal ulcers from 110-wk-old male rats fed (a) 0.5% and (b) 1.0% piperonyl butoxide in the diet for 104 wk. H&E $\times 8$ (a) and $\times 2$ (b).

ed with

3. Some anti-
induce gastro-
Sandor &
ministration



(a)

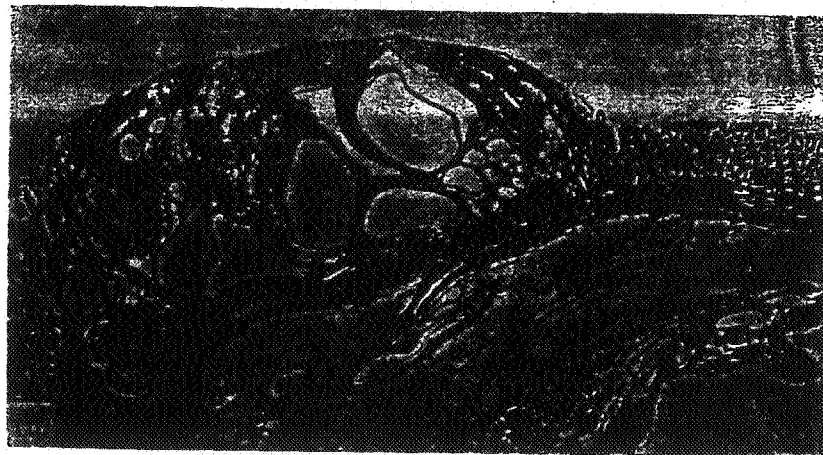


Fig. 4. Regenerative hyperplasia of the ileocaecal mucosa of a 110-wk-old male rat fed 0.5% piperonyl butoxide in the diet for 104 wk: (a) showing loss of the muscle layer and some ossification (arrowed) in the mucosa near the lesion (H&E $\times 13$); (b) showing prominent dilatation of the glands without cellular atypia (H&E $\times 5$).

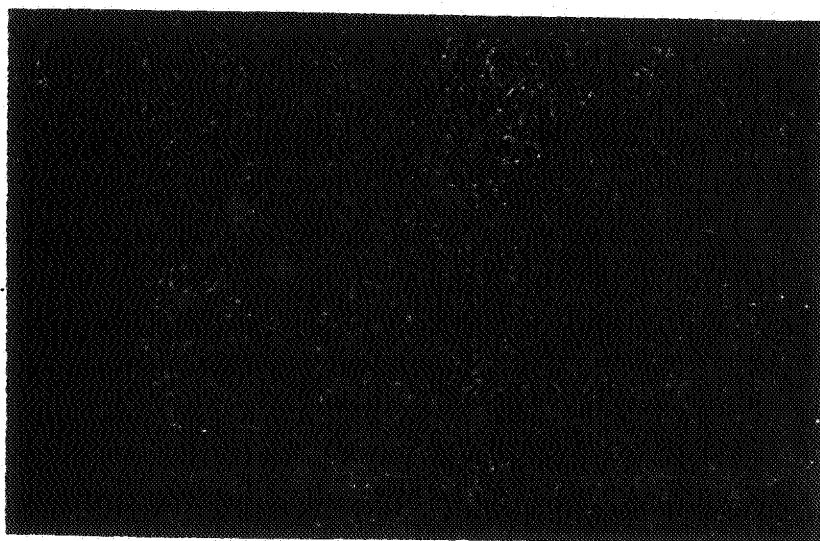


Fig. 5. Ileocaecal adenocarcinoma in an 80-wk-old male rat fed 0.5% piperonyl butoxide in the diet for 74 wk, showing carcinoma cells infiltrating the stromal tissue and marked cellular and structural atypia. (H&E $\times 25$).

98

Table 4. Non-neoplastic lesions in the digestive tract of rats fed 0, 5 or 1% piperonyl butoxide in the diet for 2 yr

No. of rats with lesions in the:									
Dietary level (%)	Effective no. of rats†	Forestomach		Glandular stomach	Ileocaecal region				Caecum/colon
		Hyperplasia	Ulcers	Erosion	Erosion	Ulcers	Regenerative hyperplasia	Ossification	Haemorrhage
Males									
0	48	0	0	0	0	0	0	0	0
0.5	48	0	3	2	1	17**	6*	7*	5
1	46	1	1	1	3	24**	10**	9**	8**
Females									
0	47	0	0	0	0	0	0	0	0
0.5	49	0	0	0	0	1	0	0	0
1	49	0	0	1	1	22**	2	0	6*

†Rats surviving beyond wk 32 (from initial groups of 50), except those in which advanced autolysis precluded adequate examination. Numbers marked with asterisks differ significantly (chi-square test) from the control figure: * $P < 0.05$; ** $P < 0.01$.

of these drugs, the ulcers are generally found in the stomach and/or small intestine. In our study, however, the ulcers were restricted to the ileocaecal mucosa, and no erosions or ulcers with a dose-related incidence were observed in other parts of the gastrointestinal mucosa in the experimental groups. The induction time of ulcers attributable to PB was also characteristic; ulcers usually appear soon after oral administration of high doses of anti-inflammatory drugs, but the ulcers in PB-treated rats were observed at a late stage of the experiment. Sarles, Dove & Moore (1949) reported earlier that signs of acute piperonyl butoxide toxicity in laboratory animals included anorexia, vomiting, diarrhoea, haemorrhagic enteritis and bloody discharge from the nose and eyes. In our subchronic toxicity study, however, no marked change was observed in the digestive tract of any treated groups, and in an acute toxicity study, we detected no ulcers in the small intestine including the ileocaecal mucosa. These results indicate that the ulcers induced by PB differ from those induced by anti-inflammatory drugs, with respect to their location and induction time.

The mechanism of the formation of ileocaecal ulcers by PB is not clear. Since PB did not induce ulcers in the stomach, the mechanism may not be related to the direct action of PB. Moreover, since Kimura, Deguchi & Murata (1983) reported that only 2.4% of a dose of PB given orally was excreted in the faeces in 24 hr, it is unlikely that PB given orally remains in the ileocaecum long enough to induce ulcers directly. The PB used in this study was of technical grade, and its purity was about 89%, but its major contaminants were not examined. Generally, technical-grade PB contains many compounds related to PB (Albro, Fishbein & Fawkers, 1972) and the toxic effects of these contaminants on the intestinal mucosa are unknown. On the other hand, there are reports that oral administration of indomethacin, an inhibitor of prostaglandin synthesis, causes intestinal ulcers in rats and that the intestinal flora may play a role in the development of these ulcers (Benoni, Cuzzolin, Raimondi & Velo, 1981; Kent, Cardelli & Stamler, 1969). Studies are needed on the effect of PB or its metabolites on the intestinal flora, to determine the mechanism of ulcer formation by PB, as the PB-induced ulcers are observed in the lower digestive tract.

In this study, regenerative hyperplasia of the mu-

cosa was observed in the ileocaecal region. However, precancerous change or carcinoma *in situ* was not observed in any rats in the PB-treated groups, although one adenocarcinoma was detected in a male given 0.5% PB. This adenocarcinoma may have been connected with the ileocaecal ulcers, because this type of tumour does not occur spontaneously. Cardy *et al.* (1979) also reported one unusual neoplasm in the caecum of a rat treated with PB. Neither tumour seems likely to be related to a direct action of PB or its metabolites, however, because of the low incidence, the lack of any dose-related occurrence and the absence of precancerous lesions in this region.

On the other hand, the chemical structure of PB is similar to that of safrole, which is known to be a hepatocarcinogen. Epstein *et al.* (1967) reported that PB increased both the hepatotoxic and carcinogenic effects of Freon 112 in Swiss mice. Our study suggests that PB has no hepatocarcinogenic activity in F344 rats, since it did not increase the incidence of hepatic tumours.

From the above results, we conclude that PB showed no carcinogenic activity in F344/DuCrj rats when given continuously in the diet for 2 yr. However, an unexpected finding was that PB induced ulcers selectively and dose-dependently in the ileocaecal mucosa.

REFERENCES

- Albro P. W., Fishbein L. & Fawkers J. (1972). Purification and characterization of pesticidal synergists. I. Piperonyl butoxide. *J. Chromat.* **65**, 521.
- Ashwood-Smith M. J., Trevino J. & Ring R. (1972). Mutagenicity of dichlorvos. *Nature, Lond.* **240**, 418.
- Benoni G., Cuzzolin L., Raimondi M. G. & Velo G. P. (1981). Indomethacin-induced intestinal lesions and fecal flora. In *Side-effects of Antiinflammatory/Analgesic Drugs* (Advances in Inflammation Research, Vol. 6). Edited by K. D. Rainsford & G. P. Velo. p. 103. Raven Press, New York.
- Cardy R. H., Renne R. A., Warner J. W. & Cypher R. L. (1979). Carcinogenesis bioassay of technical-grade piperonyl butoxide in F344 rats. *J. natn. Cancer Inst.* **62**, 569.
- Epstein S. S., Joshi S., Andrea J., Clapp P., Falk H. & Mantel N. (1967). Synergistic toxicity and carcinogenicity of 'Freons' and piperonyl butoxide. *Nature, Lond.* **214**, 526.
- Goldstein J. A., Hickman P. & Kimbrough R. D. (1973). Effects of purified and technical piperonyl butoxide on

- drug-metabolizing enzymes and ultrastructure of rat liver. *Toxic. appl. Pharmac.* **26**, 444.
- IARC Working Group (1983). *Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*. Vol. 30. *Miscellaneous Pesticides*. p. 183. International Agency for Research on Cancer, Lyon.
- Innes J. R. M., Ulland B. M., Valerio M. G., Petrucelli L., Fishbein L., Hart E. R., Pallotta A. J., Bates R. R., Falk H. L., Gart J. J., Klein M., Mitchell I. & Peters J. (1969). Bioassays of pesticides and industrial chemicals for tumorigenicity in mice: A preliminary note. *J. natn. Cancer Inst.* **42**, 1101.
- Kawachi T., Yahagi T., Kada T., Tajima Y., Ishidate M., Sasaki M. & Sugiyama T. (1980). Cooperative programme on short-term assays for carcinogenicity in Japan. In *Molecular and Cellular Aspects of Carcinogen Screening Tests*. Edited by R. Montesano, H. Bartsch & L. Tomatis. IARC Scient. Publ. no. 27, p. 323. International Agency for Research on Cancer, Lyon.
- Kent T. H., Cardelli R. M. & Stamler F. W. (1969). Small intestinal ulcers and intestinal flora in rats given indomethacin. *Am. J. Path.* **54**, 237.
- Kimura R., Deguchi H. & Murata T. (1983). Absorption, distribution and excretion of piperonyl butoxide in rats. (In Japanese.) *J. Fd Hyg. Soc., Japan* **24**, 319.
- Maekawa A., Kurokawa Y., Takahashi M., Kokubo T., Ogiu T., Onodera H., Tanigawa H., Ohno Y., Furukawa F. & Hayashi Y. (1983). Spontaneous tumors in F-344/DuCrj rats. *Gann* **74**, 365.
- National Cancer Institute (1979). Bioassay of piperonyl butoxide for possible carcinogenicity. NCI Tech. Rep. Ser. No. 120. Government Printing Office, Washington, DC.
- Sarles M. P., Dove W. E. & Moore D. H. (1949). Acute toxicity and irritation tests on animals with the new insecticide piperonyl butoxide. *Am. J. trop. Med.* **29**, 151.
- Shriver D. A., White C. B., Sandor A. & Rosenthale M. E. (1975). A profile of the rat gastrointestinal toxicity of drugs used to treat inflammatory diseases. *Toxic. appl. Pharmac.* **32**, 73.
- White T. J., Goodman D., Shulgin A. T., Castagnoli N. J., Jr, Lee R. & Petrakis N. L. (1977). Mutagenic activity of some centrally active aromatic amines in *Salmonella typhimurium*. *Mutation Res.* **56**, 199.

Photo
the tv
expos
toxicit
compa
elicite
ultrav
intens
shorte
spond
chemi
(time)
below
Altho
nature
two or
photo.
logica
(induc
of pho
longer
genera
not ne
inducin
Seve
photo
chemi
Harbe
1970;
1960
treatn
subse
which
blackl
disadv
other

100



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

10-29-93

C10647

ATTACHMENT 7

6(a)2

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Id# 067501. Piperonyl butoxide. Review of a series 83-2 mouse carcinogenicity study. Evidence for liver carcinogenicity.

TOX CHEM No.: 670
PC No.: 067501
Barcode No.: D194633
Submission No.: S447069

FROM: John Doherty, Ph.D. *John Doherty* 10/26/93
Section IV, Toxicology Branch I
Health Effects Division (H7509C)

TO: Linda DeLuise/Jay Ellenberger
Product Manager #50
Special Review and Reregistration Division
(H7508W)

THROUGH: Marion Copley, DVM, Section Head *Marion Copley*
Section IV, Toxicology Branch I
Health Effects Division (H7509C) 10/26/93 KB 10/29/93

I. CONCLUSION

The series 83-2 mouse carcinogenicity study with piperonyl butoxide was reviewed and determined to be CORE GUIDELINE. No additional mouse carcinogenicity data are required at this time. The study demonstrated increased incidence of liver hepatocellular adenomas in males and females and hepatocellular carcinomas in males. This information together with other relevant data will be presented to the Health Effects Division (HED) Carcinogenicity Peer Review Committee to determine the carcinogenicity classification of piperonyl butoxide.

Toxicology Branch-I recommends that new registrations for this chemical which result in significant increases in exposure should be not approved until the carcinogenicity classification of piperonyl butoxide is determined. No regulatory action is needed at this time regarding current registrations of piperonyl butoxide.

161



Recycled/Recyclable
Printed with Soy/Canola Ink on paper
contains at least 50% recycled paper

II. Action Requested

The McKenna & Cuneo Law Firm on behalf of their client the Piperonyl Butoxide Task Force II has submitted a series 83-2 carcinogenicity study in mice as a part of the reregistration requirements for piperonyl butoxide (PBO). This study has been reviewed by Toxicology Branch I (TB-I) and the following comments apply.

III. Toxicology Branch Comments

1. The study was determined to be CORE GUIDELINE. A copy of the DER is attached.
2. The study demonstrates that dose and compound related increases in hepatocellular adenomas in males and females and hepatocellular carcinomas in males result from dosing with piperonyl butoxide. The issue of the carcinogenicity classification of PBO will be reviewed by the HED Carcinogenicity Peer Review Committee. No date has been set for this peer review at this time.
3. The liver pathology data used by Toxicology Branch-I (TB-I) in preparing the DER and determine the carcinogenic potential of PBO was the consensus of a Peer Review that included the study pathologist Dr. Patricia E. Losco and the sponsor's consultant pathologist Dr. Charles Firth and monitored by Dr. Edward H. Fowler (the Associate Director of the Laboratory).

The registrant has also prepared a document entitled "Submission of Pathology Reviews from the BRRC Mouse Oncogenicity Study and a Review of Long-Term Studies with Piperonyl Butoxide" dated September 30, 1993 and prepared by the Piperonyl Butoxide Task Force II¹. This document contains a report on the reading of liver slides by Dr. W. Ray Brown (Veterinary Pathologist) and a comparison of Dr. Brown's assessment with the consensus assessment mentioned above and with the readings of selected slides by still another pathologist Br. W.H. Butler. The report also discusses how the tumors may arise by secondary (non-genetic) mechanisms. cursory review of this document indicates some differences in the tumor counts but the qualitative conclusion that the study is positive for liver tumors does not change.

TB-I will reassess the differences in the actual tumor

¹ This document was sent directly to HED and as of this date does not have an MRID No.. It will be resubmitted through channels and reviewed at a later time.

counts obtained by the different pathologist and present the report to the HED Carcinogenicity Peer Review Committee when piperonyl butoxide is reviewed by the Committee.

4. The GLP statement as signed by the study sponsor (Dr. Frederick Preiss) was provided under separate cover (MRID No.: 429780-01). In this statement some 17 deviations to GLP standards were indicated. TB-I acknowledges these deviations but does not consider any of these of sufficient magnitude to compromise the conclusions of the study. The laboratory, however, should be advised to correct these deviations for future experiments.

IV. Studies Reviewed

Study Identification	Material	MRID No.:	Results	Classification
83-2. Carcinogenicity study - mice. Bushy Run Research Center, Study No.: 91NO134, August 27, 1993	Technical Piperonyl butoxide, lot No.: FEP-100, 90.78% purity.	429037-01 (3 volumes plus suppl.) 429780-01 (GLP report)	Strain: CD-1 mouse. Dose levels tested: Control (two groups), 30, 100 or 300 mg/kg/day. NOEL and LEL (Systemic Effects): 30 and 100 mg/kg/day. At 100 mg/kg/day and above: liver weight increase (threshold level = 30 mg/kg/day for males). At 300 mg/kg/day: minimal body weight gain decreases in males. Females: LEL > 300 mg/kg/day for body weight. Carcinogenic potential: Considered positive for liver tumors in both males (hepatocellular adenomas and carcinomas) and females (hepatocellular adenomas only).	GUIDELINE

104

Reviewed by: John Doherty, Ph.D.
Section IV, Tox. Branch (H7509C)
Secondary reviewer: Marion Copley, DVM
Section IV, Tox. Branch (H7509C)

[83-2, Piperonyl butoxide-mice/1993]

10/26/93

Marion Copley
10/26/93

DATA EVALUATION REPORT

STUDY TYPE: 83-2. Carcinogenicity-mice **TOX. CHEM. NO.:** 613
PC Number: 067501
MRID NUMBER: 429037-01 (3 volumes plus supplement) and 429780-01
(GLP report).

TEST MATERIAL: Piperonyl butoxide, Lot # FEP-100 12/12/89.
90.78% purity.

STUDY NUMBER(S): 91N0134

SPONSOR: Piperonyl Butoxide Task Force II

TESTING FACILITY: Bushy Run Research Center (BRRC)

TITLE OF REPORT: "Chronic Dietary Oncogenicity Study with
Piperonyl Butoxide in CD-1 Mice".

AUTHOR(S): S.J. Hermansky and C.L. Wagner

REPORT ISSUED: August 27, 1993

CONCLUSIONS:

Strain: CD-1 mouse. Dose levels tested: Control (two groups), 30, 100 or 300 mg/kg/day.

NOEL and LEL (Systemic Effects): 30 and 100 mg/kg/day. At 100 mg/kg/day and above: liver weight increase (threshold level = 30 mg/kg/day for males). At 300 mg/kg/day: minimal body weight gain decreases in males. Females: LEL > 300 mg/kg/day for body weight.

Carcinogenic potential: Treatment related increases in liver tumors in both males (hepatocellular adenomas and carcinomas) and females (hepatocellular adenomas only).

Classification: core-GUIDELINE. The study satisfies the requirement for a series 83-2 carcinogenicity study in mice.

Special Review Criteria (40 CFR 154.7): Demonstration of liver carcinogenicity.

Quality Assurance Statement: Provided.

Good Laboratory Practice Statement: Provided as per MRID No.: Several deviations from GLP standards were indicated but these are not considered by TB-I to alter the conclusions of the study.

105

REVIEW

C 010647

Experimental Constants:

Test Material: Piperonyl butoxide (5-[[2-(2-butoxyethoxy)ethoxy]methyl-6-propyl-1,3-benzodioxole or alpha-[2-(2-butoxyethoxy)-4,5-methylenedioxy-2-propyltoluene. It was stated as being of 90.39% purity as determined by the sponsor. Described as a yellow, slightly viscous liquid and to be from Lot No. FEP-100 12/12/89.

Test Animals: CD-1 strain mice obtained from the Charles River Laboratories (Portage, Michigan). They were described as being of 32 days old on receipt. They were determined by the testing laboratory to be free of diseases. During the study, the animals were housed one per cage and were fed with Purina Rodent Chow #5002.

Basic Experimental Design:

The study consisted of five groups of 60/sex. There were two separate control groups and three test dose groups which were dosed at dose levels of 30, 100 and 300 mg/kg/day. The test dose levels were achieved by monitoring the body weight and feed consumption of the previous weeks and adjusting the dietary with the appropriate dose level of PBO in ppm. There were no interim sacrifices or other satellite groups. The dose levels selected were based on a preliminary range finding study (described briefly in the study report) in which there was decreased body weight (males more severely affected than females), liver weight increase, hepatocellular necrosis and marked hepatocellular hypertrophy at 1000 mg/kg/day.

for 78 weeks

Analytical Chemistry:

Assessments of stability, homogeneity and concentration of the test material in the prepared diets were assessed and are summarized as follows. The procedures and results of these assessments are located in Appendix I of the study report (pages 81-97. PBO was determined in the feed by means of a HPLC.

1. Stability: Studies were conducted on dietary preparations of 38 and 3800 ppm. The samples were assayed after preparation and on days 7, 14 and 21 following storage at room temperature in either open hoppers (7 and 14 days) or polyethylene sealed containers (all intervals). From 95.6 to 99.1 and from 94.8 to 100.7 percent of the nominal concentrations for the 38 and 3800 ppm groups, respectively, were detected following standing indicating that the PBO is stable under the experimental conditions.

2. Homogeneity: Studies were conducted on dietary preparations of 38 and 3800 ppm. Three samples were reportedly taken from the top, middle and bottom of the mixing bowl. The mean for these for the 38 ppm preparation samples (all 9) was 37.7 ± 2.3 ppm and the percentage range was from 93.4 to 108.3 with a coefficient of variation of 6.3. The mean range for the 3800 ppm samples was 3601 ± 30 ppm and the percentage range was 92.9 to 95.5% with a coefficient of variation of 0.8. These data indicates that acceptable homogeneity was achieved for the preparation assessed.

106

3. Concentration: Data for dietary preparations prepared at weeks 1, 2, 3, 4 and for each fourth week thereafter were presented. Data for both the male and female diets were presented separately. With only two reported exceptions, the dietary concentrations were from 90.3 to 108.8 percent of the target dose level. Two preparations were only 87.5 to 85.2 percent of the target. These data indicate that the target dose levels were achieved throughout the study.

Statistics. The following statistical procedures were used.

Statistical Test	Parameters Investigated
Levene's test for equality of variances	Quantitative continuous variables (body weight, feed consumption, hematology etc.
Analysis of variance (ANOVA)	
t-tests	used when F value of the ANOVA was significant
pooled t-test for pairwise comparisons	when Levene's test indicated similar variances, and when the ANOVA was significant
separate variance t-test for pairwise comparisons	when Levene's test indicated heterogeneous variances - were compared by ANOVA for unequal variances.
Kruskal-Wallis test followed by Mann-Whitney U-test.	Nonparametric data
Fisher's Exact test	Incidence data
Life Tables analysis	Incidence data

Control groups were compared with each other and each dose group was compared with each control group.

ADDITIONAL METHODS AND RESULTS

1. Clinical reactions and survival. The mice were assessed twice daily for mortality and signs of clinical reactions.

The study report asserts that no clinical reactions to treatment resulted and that survival was not affected by treatment.

There were 44, 36, 41, 44 and 47 males and 41, 49, 44, 37 and 40 females which survived the full 78 weeks of exposure until termination by sacrifice for the control-1, control-2, 30, 100 and 300 mg/kg/day groups. Thus all groups had greater than 50% survival and the mean survival time in days was similar for all experimental groups. .

2. Body weight and feed consumption. Body weight and feed consumption were assessed weekly. The test dose levels were kept constant at 30, 100 and 300 mg/kg/day by monitoring the body weight and feed consumption and adjusting the level (in ppm) of PBO in the feed.

The study report asserts that only small decreases in absolute body weight and body weight gain in high dose male and female groups resulted. Male absolute weight was statistically significantly reduced (about 6.8%, $p < 0.01$ at week 74 and about 5.9%, $p < 0.01$ at termination). Body weight gain for males both the mid and high dose groups was statistically significantly reduced for most of the study when compared to the second control group only. The high dose group occasionally reached statistically significant reductions in weight gain when compared to the first control group. Over the course of the study the males gained 11.4, 12.4, 12.7, 11.6, and 9.6 grams (the standard deviations were about 33% for all groups). The high dose group was statistically lower when compared to either control group (-15.7%, $p < 0.05$ and -22.6% $p < 0.1$).

Female body weight and weight gain rarely showed statistical differences. At week 4, all groups were 28.7 to 28.9 gms in weight. Over the course of the study, all groups gained from 12.1 to 12.8 gams with there being no indication of a compound related effect.

The study report asserts that no biologically significant effect on mean feed consumption was observed in any dose group of males and females. Occasionally decreases in feed consumption were noted in the high dose group but these differences were small (under 5%).

CONCLUSION (body weight effects). Based on the small effects on body weight gain in the males, TB-I considers that an adequate dose level was assessed. In females, however, there were no effects. Neither sex is considered to have been assessed at too high a dose that elicits competing toxicity.

3. Hematology. Blood was sampled at one year and just prior to sacrifice. At one year only the controls (both) and high dose groups were assessed. At termination all groups were assessed. The CHECKED (X) parameters were assessed based on the presence of data reports.

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	Reticulocyte count
Blood Clotting Measurements	(Thromboplastin time)
(Clotting time)	(Prothrombin time)

The study report asserts that no compound related effects were noted. TB-I concurs that this conclusion is supported by the data presented.

Sacrifice and Pathology The survivors were sacrificed at week 78 by anesthesia with methoxyflurane and terminated by severing the brachial vessels to permit exsanguination. The mice were subjected to necropsy, organ weight analysis and histopathology (no special staining techniques were employed).

4. Organ weight analysis. The following organs were assessed for absolute weight and weight relative to body and brain.

liver	heart
kidneys	spleen
brain including stem	testes

Only the liver was determined to have weight deviations associated with treatment. Table 1 below illustrates the liver to body weight ratio for both males and females for all animals surviving to termination and for the males not having tumors or obvious masses¹.

Table 1. Relative liver weight at termination in male and female mice dosed with PBO.

Test Group	Males				Females All animals
	All animals N	Ratio	Without tumors N	Ratio	
Control-1	44	6.06	37	5.82	5.82
Control-2	36	5.87	30	5.58	5.77
30 mg/kg/day	41	6.49ns (7.1%)	30	5.64	5.78
100 mg/kg/day	44	7.04* (16.2%)	23	5.88	6.31** (8.4%)
300 mg/kg/day	47	10.13** (67.2%)	19	8.15 ns (40%)	6.96** (19.6%)

Data are the mean relative weight and the standard deviation was from 20 to 42% for these means for males and about 11% to 22% for females. Number in () is the percentage increase when compared to control group 1.

* p < 0.05, ** p < 0.01 relative to either of the control groups but not always for both groups. ns = increased but not significantly. It was not clear from the study if statistics were done on the animals without masses.

The above table indicates that the liver weight for mid and high dose group males and females is increased. The low dose male group is increased slightly (7.1% and 10.6%) but neither the absolute (6.7% or 10%) or the weight relative to brain (6.9% or 10%) was statistically significantly increased (first control and second control).

¹Data for absolute weight and weight relative to brain weight demonstrated essentially the same pattern and are not illustrated in this DER.

control comparisons).

CONCLUSION (organ weight). NOEL and LEL = 30 and 100 mg/kg/day. At 100 mg/kg/day and above liver weight increased in both sexes. [The lowest dose level for males is also slightly increased but not significantly indicating a possible threshold level.]

[Note: TB-I finds the assessment in the absence of masses of interest but the effect is being based on all animals available.]

5. Tissue and organ pathology. Based on inspection of the data presented the following tissues and organs were reportedly examined.

<u>Digestive system</u>	<u>Cardiovasc./Hemat.</u>	<u>Neurologic</u>
x Tongue (0-1)	x Aorta	x Brain
x Salivary glands	x Heart	x Sciatic 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 (thymic reg)	<u>Glandular</u>
x Ileum	<u>Urogenital</u>	x Adrenals
x Cecum	x Kidneys	x Lacrimal gland
x Colon	x Urinary bladder	x Mammary gland (females only)
x Rectum	x Testes	x Parathyroids
x Liver*	x Epididymides	x Thyroids
x Gall bladder	x Prostate	<u>Other</u>
x Pancreas	x Seminal vesicle	x Bone (sternum/femur)
<u>Respiratory</u>	x Ovaries	x Skeletal muscle
x Trachea	x Uterus	x Skin
x Lung	x Cervix	
x Nose (0-1)	x Vagina	
Pharynx		
x Larynx (0-2)		

Reports of lesions were also noted in the following organs/tissues (number examined in each dose group):

adipose tissue (0-4 males, 0-3 females); mesentery/"omatum" (more correctly omentum) (0-1 males, 0-3 females); subcutis (0-1 males, 0-2 females); ears (1-7 males, 0-6 females); nares (0-1 males 0-1 females); paws/feet (0-1 males, 0-1 females); tail (0-4 males, 0-3 females); bone/joint (0-1 males); Harderian gland (0-3 males); vasa deferentia (0-1); coagulating gland (0-2); penis (0-8); ureter (2-6 males); urethra (0-1 males); peritoneum (0-1 females); bone vertebrae (0-1 females); diaphragm (0-1 females)

The protocol called for examination of all the animals in the two control and the high dose groups except for the liver, lung, kidneys and for any gross lesion which were examined for all dose groups. Routinely the tissues were reportedly paraffin embedded, sectioned at approximately 5 microns and stained with hematoxylin and eosin. No mention was made of any other stain used for these studies.

Individual organ discussions and other pathology considerations.

A. Liver. The study report concluded that the liver was a target organ with respect to increased weight and increases in non-neoplastic and neoplastic lesions. Table 2 below illustrates the increases in non-neoplastic and neoplastic lesions in the liver based on the report submitted under MRID #429037-01. The data reported below for liver tumors is the consensus of a Peer Review that included the study pathologist Dr. Patricia E. Losco and the consultant pathologist Dr. Charles Firth and monitored by Dr. Edward H. Fowler (the Associate Director of the Laboratory).

Table 2. Liver non-neoplastic and neoplastic lesions in CD-1 mice dosed with piperonyl butoxide.

Lesion	Males					Females				
	C-1	C-2	30	100	300	C-1	C-2	10	100	300
<u>Non-neo-plastic</u>										
Hemorrhage	2	1	3	2	13**	3	3	1	4	7
Hypertrophy	6	11	11	16*	43**	0	4	0	1	9*
Hyperplasia	0	2	1	2	5	0	0	0	1	4
<u>Neo-plastic</u> ¹										
Adenoma ³	9	8	13	22**	28**	2	2	1	1	10*
(%)	(15%)	(13%)	(22%)	(37%)	(47%)	(3.3%)	(3.3%)	(1.7%)	(1.7%)	(16.7%)
Hist Control	(mean = 13.6%, range = 8.7-21.7%)					(Mean = 0.8%), range 0-1.7%)				
Carcinoma	2	2	3	2	7	None diagnosed.				
	(1.7%)	(1.7%)	(5%)	(1.7%)	(11.7%)					
Hist Control	(mean = 2.4%; range = 0-5%)					0%, none reported.				
.....										
Total ²	11	10	15	24**	30**	2	2	1	1	10**
	(18.3%)	(16.7%)	(25%)	(40%)	(50%)	(3.3%)	(3.3%)	(1.7%)	(1.7%)	(16.7%)

All groups had livers from 60 animals reportedly examined. Non-Neoplastic and neoplastic data for males are from Appendix 3 Table 15 page 194-195 and for females are from Appendix 3 Table 19 page 240-241.

* p < 0.05 and ** p < 0.01, study report statistics by Fisher' Exact Test.

Historical control data are based on 8 studies of from 59 to 70 CD-1 strain mice. All studies were from the Bushy Run Research Center and were conducted over the years 1983 to 1990. Refer to attachment to study report entitled "Summary of the Bushy Run Research Center (BRRC) Neoplasm Historical Control Database in CD-1 Mice".

1. More specifically hepatocellular adenoma and hepatocellular carcinoma.
2. Total number of animals affected with either a carcinoma, adenoma or both.
3. Animals with adenomas that may or may not have carcinoma.

The above table indicates that both males in the mid and high dose and females in the high dose groups have statistically significant increases in hepatocellular adenomas. The low dose group males (22%) also has a possible increase in adenomas in excess of the concurrent control groups (15 and 13%). The control groups (both 3.3%), for the females have a slightly higher rate of incidence than the historical controls (range 0-1.7%). This, however, does not affect the conclusion that the high dose

group (16.7%) has a compound related increase in liver adenomas.

The high dose group males have an obvious increase in hepatocellular carcinomas that does not reach statistical significance but is clearly in excess of the historical control range for the CD-1 strain mouse.

The non-neoplastic lesions in the liver (hypertrophy, hyperplasia and hemorrhage) are considered by TB-I to be expected findings in mice with liver tumors. The hypertrophy is most likely a response to the increased microsomal enzyme production induced by the inhibition of the microsomal enzymes by PBO. The hemorrhages are possibly related to the physical presence of the tumors.

Some additional comments on the character of the liver tumors that are considered related to speculation on their origin are presented in APPENDIX I of this DER.

B. Thyroid. The rat carcinogenicity study (refer to HED Document No.: 006668 and MRID No.: 403237-01, BioResearch Ltd., Study No.: 81690, August 27, 1987) indicated that there were increases in "pigment in follicles" and hyperplasia of follicular cells in both males and females.

In this study, only the two control and the high dose groups were examined. There was no evidence of any compound related increase in any lesion type in either males or females. The condition "pigment in follicles" and the lesion hyperplasia of follicular cells were not mentioned among the thyroid findings for either sex.

CONCLUSION (pathology): NOEL and LEL for liver non-neoplastic pathology is 30 and 100 mg/kg/day. Indications of carcinogenic potential is evident by test compound related increases liver hepatocellular adenomas in both sexes and hepatocellular carcinomas in males.

CONCLUSION: ^(Study) This study is classified as CORE GUIDELINE. The following one liner is supported.

NOEL and LEL (Systemic Effects): 30 and 100 mg/kg/day. At 100 mg/kg/day and above: liver weight increase for both sexes (threshold level = 30 mg/kg/day for males). At 300 mg/kg/day: minimal body weight gain decreases in males. Females: LEL > 300 mg/kg/day for body weight.

Carcinogenic potential: Treatment related increases in liver tumors in both males (hepatocellular adenomas and carcinomas) and females (hepatocellular adenomas only).

Strain: CD-1 mouse. Dose levels tested: Control (two groups), 30, 100 or 300 mg/kg/day.

Note: The dose levels were considered adequate although there was no systemic effect in females other than liver weight effects. The presence of liver tumors in this sex in the high dose group precludes the need for an additional study at higher dose levels.

Appendix I.

Additional Information Included in this DER for possible future reference.

The study report contains some information on the character of the liver tumors (page 15, in Appendix 3, page 124 of the study report) where it states:

"The appearance of many hepatocellular adenomas in PBO treated animals in this study, particularly in the high dose group, was characteristic for most animals, and was different from the common appearance of spontaneous adenomas. Adenomas in PBO treated mice were frequently composed of large, polyhedral, densely-packed cells with abundant, granular, eosinophilic cytoplasm. Many adenoma cells had enlarged pleomorphic nuclei, and some had eosinophilic cytoplasmic inclusions. Adenomas in PBO treated mice, particularly in males, were generally large, often encompassing most of the affected lobes. Multiple tumors were common, more so in males, and a few males had malignant as well as benign liver tumors".

Among the males, the tumors were further categorized along the lines of staining characteristics as follows for the controls (two), low, mid and high dose groups:

Basophilic adenomas	7, 6, 10, 15 and 10.
Eosinophilic adenomas	4, 1, 3, 8 and 19.
Mixed type adenomas	0, 1, 2, 2 and 5.

[Note: some tumors were classified as both basophilic and eosinophilic such that the these numbers represent the number of adenomas of each type found at each dose level.]

The female high dose group had 9 incidents of the eosinophilic adenomas to indicate that compound related increase in adenomas is of the eosinophilic type. An increase in eosinophilic appearance of cells has previously been associated with an increase in the smooth endoplasmic reticulum in the cytoplasm (Burger and Herdon, Amer. J. Pathol. 48:793-803 (1966)).

TB-I considers the above discussion to be of possible importance in characterizing the origin of the liver tumors induced by PBO. This information may be taken into consideration in determining the carcinogenicity classification of PBO and other chemicals which are known to affect the microsomal oxidation system.

ATTACHMENT

8

Letter to the editors

Piperonyl butoxide induces hepatocellular carcinoma in male CD-1 mice

O. Takahashi, S. Oishi, T. Fujitani, T. Tanaka, M. Yoneyama

Department of Toxicology, Tokyo Metropolitan Research Laboratory of Public Health, 24-1, Hyakunincho 3-chome, Shinjuku-ku, 169, Japan

Received: 18 January 1994/Accepted: 8 March 1994

Abstract. Male CD-1 mice in groups of 52, 53 or 100 were administered piperonyl butoxide (α -[2-(2-butoxyethoxy)ethoxy]-4,5-methylenedioxy-2-propyltoluene) in the diet at levels of 0 (control), 0.6 and 1.2% for 12 months. Hepatocellular carcinoma was induced in treated groups in a dose-dependent manner but not in the control group. The incidences of hepatocellular carcinoma were 11.3 and 52.0% in mice given 0.6 and 1.2% piperonyl butoxide, indicating that piperonyl butoxide can cause hepatocellular carcinoma in mice as it is known to do in rats.

Key words: Piperonyl butoxide – Hepatocellular carcinoma – Hemangiosarcoma – Carcinogenesis – Mice

Introduction

Piperonyl butoxide (α -[2-(2-butoxyethoxy)ethoxy]-4,5-methylenedioxy-2-propyltoluene) is widely used as a synergist for pyrethrins and related insecticides on a variety of fruit, vegetable, forage, and grain crops. It is also used on livestock and in agricultural areas as well as in various domestic pesticide aerosol formulations. Piperonyl butoxide has been a legal food additive in Japan since 1955. Its maximum approved use level is 0.024 g/kg (24 ppm) in raw cereals, and acceptable daily intake (ADI) for humans is 0–0.03 mg/kg body weight (Joint FAO/WHO Meeting 1973). Pyrethrin formulations produced for stored crops in Japan in 1986 totalled 233 tons.

This is an abridged report of the research conducted. We plan to report detailed results of the chronic toxicity study of male mice together with the data on females at a future time. This study was conducted equally by the five authors named, so that the order used here is of no consequence.

Correspondence to: O. Takahashi

We initially detected the hepatocarcinogenicity of piperonyl butoxide in Fischer rats and its teratogenicity in mice (Takahashi et al. 1994; Tanaka et al. 1994).

We now describe only the hepatocarcinogenicity of this chemical in male mice which were included in a chronic toxicity study conducted to learn the species differences of the toxicity of this compound.

Materials and methods

Piperonyl butoxide (technical grade) purchased from Takasago International Corporation (Tokyo) was used. The purity was 94.3% by gas-liquid chromatography, and this preparation contained neither safrol nor isosafrol. Specific pathogen-free male Crj:CD-1 (ICR) mice (4 weeks old) obtained from Charles River Japan, Inc. (Kanagawa, Japan) were housed individually in chip-bedded polycarbonate plastic cages, in an air conditioned room at a temperature of $25 \pm 1^\circ\text{C}$ and relative humidity of $55 \pm 5\%$. After acclimatization for 1 week on a basal diet (CE-2 from CLEA Japan Inc., Tokyo), mice were allocated to three groups of 52, 53 and 100 each and were given basal diets containing piperonyl butoxide at levels of 0 (control), 0.6 or 1.2%, respectively, and water ad lib for 12 months. The dietary levels were the same as those of the chronic test for rats (Takahashi et al. 1994), except that the earlier study also used 2.4%. We found in our preliminary experiment that this level was acutely toxic to mice and that all those given 2.4% piperonyl butoxide died within 1 week, so this level was not used. During the experimental period, the animals were observed daily, and clinical signs and mortality were recorded. Mice that died were necropsied and examined for tumors and non-neoplastic lesions; tumors and sites of lesions were fixed with buffered 4% formaldehyde solution, and a histopathologic examination was conducted. At the termination of administration, all of the surviving mice were killed by deep ether anesthesia. After blood collection, mice were necropsied and tumors and non-neoplastic lesions were examined. Livers were fixed with buffered 4% formaldehyde solution and sectioned and stained routinely with hematoxylin and eosin. Diagnosis of hepatic nodular lesions was done using conventional criteria: focal and multifocal hyperplasias are nodular lesions comprising normal or hypertrophic hepatocytes with less distorted hepatic lobular architecture containing portal triads and a central vein and accompanied by periportal fibrosis, oval cell proliferation, bile duct proliferation and hepatocellular degeneration; hepatocellular adenomas do not have normal hepatic lobular architecture and occasionally show a single trabecular structure; hepatocellular carcinomas have a distinctly thick-

(15)

Table 1. Hyperplastic and neoplastic lesions of the liver of male CD-1 mice administered piperonyl butoxide in the diet for 12 months

	Piperonyl butoxide (dietary %)		
	Control	0.6%	1.2%
<i>Surviving mice</i>			
Number of mice	49	52	81
Hepatocellular hyperplasia	1 (2.0) ^a	20 ^b (38.5)	8 (9.9)
Hepatocellular adenoma ^c	1 (2.0)	7 ^b (13.5)	21 ^b (25.9)
Hepatocellular carcinoma ^c	0 (0.0)	6 ^b (11.5)	43 ^b (53.1)
Hepatocellular adenoma and carcinoma ^c	1 (2.0)	13 ^b (25.0)	64 ^b (79.0)
Postnecrotic peliosis	0 (0.0)	0 (0.0)	16 ^b (19.8)
Hemangioendothelial sarcoma (small)	0 (0.0)	0 (0.0)	24 ^b (29.6)
Hemangioendothelial sarcoma (large)	0 (0.0)	1 (1.9)	12 ^b (14.8)
<i>Dead mice</i>			
Number of mice	3	1	19
Hepatocellular hyperplasia	0 (0.0)	0 (0.0)	0 (0.0)
Hepatocellular adenoma	0 (0.0)	0 (0.0)	1 (5.3)
Hepatocellular carcinoma	0 (0.0)	0 (0.0)	9 (47.4)
Hepatocellular adenoma and carcinoma	0 (0.0)	0 (0.0)	10 (52.6)
Postnecrotic peliosis	0 (0.0)	0 (0.0)	1 (5.3)
Hemangioendothelial sarcoma (small)	0 (0.0)	0 (0.0)	0 (0.0)
Hemangioendothelial sarcoma (large)	0 (0.0)	0 (0.0)	6 (31.6)
<i>Surviving and dead mice</i>			
Number of mice	52	53	100
Hepatocellular hyperplasia	1 (1.9)	20 ^b (37.7)	8 (8.0)
Hepatocellular adenoma ^c	1 (1.9)	7 ^b (13.2)	22 ^b (22.0)
Hepatocellular carcinoma ^c	0 (0.0)	6 ^b (11.3)	52 ^b (52.0)
Hepatocellular adenoma and carcinoma ^c	1 (1.9)	13 ^b (24.5)	74 ^b (74.0)
Postnecrotic peliosis	0 (0.0)	0 (0.0)	17 ^b (17.0)
Hemangioendothelial sarcoma (small)	0 (0.0)	0 (0.0)	24 ^b (24.0)
Hemangioendothelial sarcoma (large)	0 (0.0)	1 (1.9)	18 ^b (18.0)

^a Numbers in parentheses are incidence (%)

^b Significantly different ($P < 0.05$) from the control (0%) value by Fisher's exact probability test

^c The response has a significant dose-related linear trend

Frith 1992). Therefore, the present results are clearly significant. By the way, 1.2% used is equal to 500 times the maximum approved use level for raw cereals in Japan.

NCI (1979) reported negative results of carcinogenicity of piperonyl butoxide at up to 2804 ppm (0.28%) for about 2 years (5000 ppm for 30 weeks and 2000 ppm for a further 82 weeks) using B6C3F1 mice. In that experiment the doses were lowered in the midst of the experiment, probably due to the high lethality or toxicity. Furthermore, the incidence of spontaneous hepatocellular carcinomas of B6C3F1 mice was very high (10 out of 20 male mice of the control). We therefore do not consider this study to be adequate. In fact, when we used B6C3F1 mice in a preliminary study, this strain could not tolerate even a 1.2% level of piperonyl butoxide. This is one of the reasons we used CD-1 mice in preference to B6C3F1 mice. With regard to the NCI study, it should be noted that a lower dose was used than in the present study. The absence of an effect may correspond to the nonlinearity suggested by the disproportionate increase in liver neoplasms between the low and the high doses in the present study.

In conclusion, the present results clearly indicate that piperonyl butoxide can induce hepatocellular carcinoma in mice as well as in rats as reported earlier (Takahashi et al. 1994).

References

- Chandra M, Frith CH (1992) Spontaneous neoplasms in aged CD-1 mice. *Toxicol Lett* 61: 67-74
- Gad SC, Weil CS (1989) Statistics for toxicologists. In: Hayes AW (ed) Principles and methods of toxicology. Raven Press, New York. pp 435-483
- Joint FAO/WHO Meeting (1973) Pesticide residues in food (WHO Tech Rep Ser No. 525). FAO/WHO, Geneva
- National Cancer Institute (NCI) (1979) Bioassay of piperonyl butoxide for possible carcinogenicity (NCI Tech Rep Ser No. 120). Government Printing Office, Washington, DC
- Takahashi O, Oishi S, Fujitani T, Tanaka T, Yoneyama M (1994) Chronic toxicity studies of piperonyl butoxide in F344 rats: induction of hepatocellular carcinoma. *Fundam Appl Toxicol* 22: 293-303
- Tanaka T, Fujitani T, Takahashi O, Oishi S (1994) Developmental toxicity evaluation of piperonyl butoxide in CD-1 mice. *Toxicol Lett* 71: 123-129
- Umemura T, Tokumoto K, Sirma H, Gebhardt R, Poirier MC, Williams GM (1993) Dose response effects of 2-acetylaminofluorene on DNA damage, cytotoxicity, cell proliferation and neoplastic conversion in rat liver. *Cancer Lett* 73: 1-10
- Williams GM, Gebhardt R, Sirma H, Stenback F (1993) Non-linearity of neoplastic conversion induced in rat liver by low exposures to diethylnitrosamine. *Carcinogenesis* 14: 2149-2156

(116)

NON-ACUTE TOX PROFILE FOR: Piperonyl butoxide

TOX NO. - 670

STUDY	SPECIES	YR	GR	SYS MEL	SYS LEL	ONCO MEL	ONCO LEL	MAT MEL	MAT LEL	REPROD/		REPROD/
										DEV	NEL	DEVLEL
Developmental Toxicity Study	rat	76	I			N/A	N/A					N/A
Developmental Toxicity Study	rat	79	M			N/A	N/A	> 500 mg/kg				N/A
Developmental Toxicity Study	rat	91	G			N/A	N/A	200 mg/kg	500 mg/kg			N/A
Developmental Toxicity Study	rabbit	86	G			N/A	N/A	50 mg/kg	100 mg/kg			N/A
Reproduction-2 generation	rat	86	G			N/A	N/A	1000 ppm	5000 ppm			N/A
Reproduction-3 generation	rat	79	S			N/A	N/A			1000 ppm		N/A
Carcinogenic	mice	93	G	30 mg/kg	100 mg/kg	mg/kg	mg/kg	N/A	N/A	N/A	N/A	N/A
Carcinogenic	rat	79	I	?	5000 ppm			N/A	N/A	N/A	N/A	N/A
Carcinogenic	mice	79	I	?	1036 ppm			N/A	N/A	N/A	N/A	N/A
Carcinogenic	mice	92	S					N/A	N/A	N/A	N/A	N/A
Feeding/carcinogenic-2 year	rat	52	I	1000 ppm	10000 ppm			N/A	N/A	N/A	N/A	N/A
Feeding/carcinogenic-2 year	rat	87	G	?	30 mg/kg	> 500 mg/kg		N/A	N/A	N/A	N/A	N/A
Feeding/carcinogenic-2 year	rat	79	S	1000 ppm	10000 ppm			N/A	N/A	N/A	N/A	N/A
Chronic Feeding	rat	93	S	?	537 mg/kg			N/A	N/A	N/A	N/A	N/A
Feeding-1 year	dog	52	I	700 ppm	3000 ppm			N/A	N/A	N/A	N/A	N/A
Feeding-1 year	dog	93	G	3.9 mg/kg	16.5 mg/kg			N/A	N/A	N/A	N/A	N/A
Dermal-3 week	rabbit	?	S			N/A	N/A	N/A	N/A	N/A	N/A	N/A
Dermal-3 week	rabbit	92	M	> 1000 mg/kg		N/A	N/A	N/A	N/A	N/A	N/A	N/A
Mutagenic-gene mutation	bacteri	?	I	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Mutagenic-Ames	salmon	83	A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Mutagenic- in vitro cytogen.	mammali	83	I	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

ATTACHMENT 10

112
122

NON-ACUTE TOX PROFILE FOR: Piperonyl butoxide

TOX NO. - 670

STUDY	SPECIES	YR	GR	SYS NEL	SYS LEL	ONCO NEL	ONCO LEL	MAT NEL	MAT LEL	REPROD/ DEV NEL	REPROD/ DEV LEL	MUTA
Mutagenic-chromosomal	CHO ass	85	A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	?
Mut- Chrom. aberr. in vitro	CHO cel	93	A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	--
Mutagenic- cytogenetic	CHO cel	83	A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	-
Pharmacokinetics	rat	85	S									
Metabolism	rat	89	G									
Dom. animal safety env. exp.	cat	87	U									
Dom. animal safety env. exp.	dog	87	U									
Dom. animal safety env. exp.	dogs &	90	U									
Dom. animal safety env. exp.	cat (ki	91	A									
Dom. animal safety env. exp.	horses	93	A									
Dom. animal safety env. exp.	cat (ki	92	U									
Dom. animal safety env. exp.	dog (pu	92	U									
Dom. animal safety env. exp.	dog	89	A									
Dom. animal safety env. exp.	cat (ki	92	U									
Dom. animal safety env. exp.	dog (pu	92	U									
Dom. animal safety env. exp.	cats	93										
Dom. animal safety env. exp.	dog	93	S									

EPA CANNOT CURRENTLY GUARANTEE THE ACCURACY OR
VALIDITY OF THE INFORMATION INCLUDED IN THE TOXICOLOGY
ONE-LINER DUE TO COMPUTER PROGRAMMING ERRORS

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDES/HED/TB-1
TOX ONELINERS

PAGE 1
CASWELL#: 670
CAS-REG#: 51-03-6

P.C. CODE 067501- Piperonyl butoxide FILE LAST PRINTED: 11/21/94

CITATION	MATERIAL	ACCESSION/ HWID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
Carcinogenic Species: mice Bushy Run Res. Center 91N0134; 08/27/93	Piperonyl butoxide tech. 90.78%; lot# FEP-100	429037-01	Strain: CD-1 mouse. Dose levels tested: Control (two groups), 30, 100 or 300 mg/kg/day. NOEL and LOEL (systemic effects): 30 and 100 mg/kg/day. At 100 mg/kg/d and above: liver weight increase (threshold level = 30 mg/kg/day for males). At 300 mg/kg/day: minimal body weight gain decreases in males. Females: LEL > 300 mg/kg/day for body weight. Carcinogenic potential: considered positive for liver tumors in both males (hepatocellular adenomas and carcinomas) and females (hepatocellular adenomas only).		Guideline 010647
83-2(a) Carcinogenic Species: rat National Cancer Inst. 51-03-6; 1979	Piperonyl butoxide		Levels tested: 5000 and 10,000 ppm. Negative oncogen Systemic NOEL < 5000 ppm (decr. body wt.). Onco NOEL > 5000 ppm		004507
83-2(b) Carcinogenic Species: mice National Cancer Inst. 51-03-6; 1979	Piperonyl butoxide		Levels tested: 2,500 and 5000 ppm (after 30 weeks dose reduced to 500 & 2000 ppm.) Average levels: 1,036 & 2804 ppm. Negative oncogen Syst. NOEL < 1036 ppm (decr. body wt.) Onco LOEL > 2000 ppm.		004507
83-2(b) Carcinogenic Species: mice Bushy Run Res. Center not provided; 09/09/92	Piperonyl butoxide	7777	Preliminary report indicates increase in liver masses in males based on necropsy. Liver weights also increased in males & females in CD-1 str. Doses levels: 0, 30, 100 & 300 mg/kg/day.		Supplementary 009847
83-1(a) and 83-2(a) Feeding/carcinogenic-2 year Species: rat Am. J. Trop. Med 1, 862 1952	Piperonyl butoxide		Levels tested: 100, 1000, 10,000 and 25,000 Systemic NOEL = 1000 ppm. Systemic LEL = 10,000 ppm. Hepatic cell hypertrophy, decr. reprod. eff., organ wt. changes, & wt. retardation.		004503
83-1(a) and 83-2(a) Feeding/carcinogenic-2 year Species: rat Bioresearch Labs, Quebec, Can. 81690; 8/27/87	Piperonyl butoxide 86%	403230-01	Systemic NOEL < 30 mg/kg/day; liver weight decreases in females and trend for decreases in males. At 100 mg/kg/day - there were increases in liver weights, increases in hepatic increased cholesterol levels (all in females). At 500 mg/kg/day (HDT), there were increased liver weights and hypertrophy of hepatocytes and increases in plasia of follicular cells of the thyroid in both sexes. All females there was increased cholesterol levels and sexes showed decreases in body weight. Oncogenic NOEL > 500 mg/kg/day (HDT). Oncogenicity: Negative at HDT. Levels tested: 0, 30, 100, 500 mg/kg/day in Sprague dawley Crl-CDR str.		Guideline 006668

119

**U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDES/HED/TB-1
TOX ONELINERS**

**PAGE 2
CASWELL#: 670
CAS-REG#: 51-03-6**

CITATION	MATERIAL	ACCESSION/ NRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
P.C. CODE 067501- Piperonyl butoxide		FILE LAST PRINTED: 11/21/94			
83-1(a) and 83-2(a) Feeding/carcinogenic-2 year Species: rat Tox. Appl. Pharm. 47:353 1979	Piperonyl butoxide tech. 80%		Fed levels of: 0, 100, 1000, 10,000 and 25,000 ppm. NOEL = 1000 ppm. LEL = 10,000 ppm - body weight gains. Gross pathology - liver, kidneys and adrenals. Too few animals and observations precludes this study being totally acceptable.		Supplementary 007673
83-1(a) Chronic Feeding Species: rat Tokyo Met. Res Lab of Pub Hlth 1993	Piperonyl butoxide tech., 94.3 and 94.5% pure, lots 909002 and 009002	428396-01 429202-01	Provisional conclusions: Systemic toxicity NOEL and LOEL < 537 mg/kg/d. At 537 mg/kg/day: gastrointestinal hemorrhage, increased liver weight; decr. body weight; effect on RBC parameters, possible kidney pathology. At 1,877 (M) and 2002 (F) mg/kg/d (HDT) possible excessive toxicity as indicated by 50% decrease in body weight. Carcinogenic potential: positive for liver tumors in both males & fe- males. Fischer 344 strain rat. Dose levels tested: 0, 0.6, 1.2, and 2.4% in the diet corresponding to 0, 547, 1,052 and 1877 mg/kg/d (M) and 537, 1,061 and 2,002 mg/kg/day (F).		Supplementary 010658
83-1(b) Feeding-1 year Species: dog Am. J. Trop. Med 1, 862 1952	Piperonyl butoxide		Levels tested: 80, 700, 3000, and 11000 ppm. Systemic NOEL = 700 ppm. Systemic LEL = 3000 ppm. (body weight and liver weight changes)		004503
83-1(b) Feeding-1 year Species: dog Internl. Res. and Develop. Co 542-005; 09/1993	Piperonyl butoxide tech. 90.78%	429260-02 429260-01	NOEL and LEL = 100 and 600 ppm (2.9 and 15.5 mg/kg/d in males). At 15.5 mg/kg/d: decr. body wt. gain and trends for incr. alkaline phos- phatase (60% in males) and relative liver wt. (13% males & 19% females). At 2000 ppm (52.8 mg/kg/d in males); definite increases (> 500%) in alkaline phosphatase and relative liver wt. (52% in males and 86% in females). Beagle dogs. Dose levels tested: 0, 100, 600 or 2000 ppm corresponding to 2.9, 15.5 or 52.8 mg/kg/d (M); and 2.8, 16.3 or 71.0 mg/kg/d (F).		Guideline 010885
83-3(a) Developmental Toxicity Study Species: rat IBT 8533-08317; 6/10/76	Piperonyl butoxide (Tech)	00083623	IBT Invalid Clement Associates Contract No. 68-01-5824 Accepted by EPA 4/1/82		Invalid 002415
83-3(a) Developmental Toxicity Study Species: rat Tox. Appl. Pharm. 47:353 1979	Piperonyl butoxide Tech. 80%	05008254	Doses: 62.5, 125, 250 and 500 mg/kg from days 6-15 of gestation did not adversely effect maternal or fetal weight, and the incidences of minor skeletal variants or gross malformations. NOEL > 500 mg/kg/day.		Minimum 007673

EPA CANNOT CURRENTLY GUARANTEE THE ACCURACY OR
VALIDITY OF THE INFORMATION INCLUDED IN THE TOXICOLOGY
ONE-LINER DUE TO COMPUTER PROGRAMMING ERRORS

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDES/HED/TB-1
TOX ONELINERS

PAGE 3
CASWELL#: 670
CAS-REG#: 51-03-6

P.C. CODE 007501- Piperonyl butoxide

FILE LAST PRINTED: 11/21/94

ACCESSION/
NO.

ADDITIONAL

RESULTS

TOX
CAT

COREGRADE/
DOCUMENT#

CITATION

83-3(a) Developmental Toxicity Study Species: rat Bushy Run Res. Center 54-566; 12/20/91	Piperonyl butoxide 90.78% pure; lot FEP-100	423808-01	Doses administered: 0, 200, 500 and 1000 mg/kg/day, administered by gavage in deionized water to pregnant Sprague-Dawley CD rats from days 6-15 of gestation, inclusive. Maternal NOEL: 200 mg/kg/d. Maternal LOEL = 500 mg/kg/d, based on stat. sig. reduction in body wt. gain during treatment & food consumption during days 6-9 of treatment & increased incidence of urogenital red discharge and perineal encrustation. At 1000 mg/kg/day, in addition to depressed body weight gain and feed consumption, the incidence of urogenital wetness, urine stains, red discharge & perineal encrustation increased. Developmental NOEL => 1,000 mg/kg/day (HDT).	Guideline 010108
83-3(b) Developmental Toxicity Study Species: rabbit Internatl. Res. and Develop. Co 542-002; 2/7/86	Piperonyl butoxide technical grade (Task Force Committee Sample) Ident: FEG32 Purity: 100%	261926 00157157	Maternal NOEL = 50 mg/kg/day, Maternal LEL = 100 mg/kg/day (decrease body weight gain) At 200 mg/kg/day, more definite decrease in body weight gain, also an indefinite effect described as decreased defecation. Developmental NOEL = 200 mg/kg/day (HDT). Doses: 0, 50, 100, and 200 mg/kg/day by gavage on days 7 - 19 of gestation, NZ white rabbits.	Guideline 007174
83-4 Reproduction-2 generation Species: rat Bioresearch Inc. 81689; 7/1/86	Piperonyl butoxide Tech.	263635- 263640 00161118	Levels tested: 0, 300, 1000 & 5000 ppm in Sprague-Dawley str. Reprod. NOEL > 5000 ppm (HDT). Maternal NOEL = 1000 ppm. Maternal LEL = 5000 ppm (decreased body wt. gain 12%). Develop. NOEL = 1000 ppm. Develop. LEL = 5000 ppm. Day 4: up to 19% decreased body wt. gain in pups.	Guideline 006414
83-4 Reproduction-3 generation Species: rat Tox. Appl. Pharm. 47:353 1979	Piperonyl butoxide tech. 80%		Fed levels: 0, 100, 1000, 10,000 and 25,000 ppm. Reprod. NOEL = 1000 ppm. Reprod. LEL = 10,000 ppm. Liver effects at 25,000 ppm. Body weight decreased at 10,000 and 25,000 ppm. Gross pathology - liver, kidneys and adrenals. Too few animals and observations precludes study being totally acceptable.	Supplementary 007673
82-2 Dermal-3 week Species: rabbit	Piperonyl butoxide tech. pyrethrin	242923	4/4 animals showed dry skin w/clearing in 2/4 by day 12.	Supplementary
82-2 Dermal-3 week Species: rabbit IRDC - Mattawan, Mich. 542-007; 02/11/92	Piperonyl butoxide 89.1%	422182-01	Doses: 0, 100, 300 and 1000 mg/kg/day in NZW rabbits. Systemic NOEL => 1000 mg/kg/day (limit dose). Local (Dermal) NOEL - not established - very slight erythema, edema & fissuring at the 100 mg/kg/day dose; in addition desquamation at the mid and high dose. Histologically - acanthosis, hyperkeratosis and chronic inflammation of the epidermis at all doses. Dermal LOEL <= 100 mg/kg/day - a mild irritant.	Minimum 009607

EPA CANNOT CURRENTLY GUARANTEE THE ACCURACY OR
VALIDITY OF THE INFORMATION INCLUDED IN THE TOXICOLOGY
ONE-LINER DUE TO COMPUTER PROGRAMMING ERRORS

121

**U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDES/HED/TB-1
TOX ONELINERS**

**PAGE 4
CASWELL#: 670
CAS-REG#: 51-03-6**

P.C. CODE	067501- Piperonyl butoxide	FILE LAST PRINTED: 11/21/94	CITATION	MATERIAL	ACCESSION/ NRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
84-2(a)	Mutagenic-gene mutation Species: bacterie SRS International 9/26/83		Piperonyl butoxide tech. (unstated purity)		001434A 001434A	Negative for reversions in <i>S. typhimurium</i> , with and without activation up to levels of lethality or precipitation (contract to NTP/NIEHS). Sponsor must provide information on test material, positive controls, and statistical treatment of data. (Under contract to NTP/NIEHS).		Prov. Acceptable 003438
84-2(a)	Mutagenic-Ames Species: salmonella Litton Bionetics Inc. P80 Task Force; 3/24/83		Piperonyl butoxide tech.			Negative.		Acceptable 007644
84-2(b)	Mutagenic- in vitro cytogen. Species: mammalian cells Litton Bionetics Inc. 9/26/83		Piperonyl butoxide tech. (unstated purity)			Negative for both sister-chromatid exchanges & chromosome aberrations in CHO cells, with/without activ. up to toxicity levels or precipitation. Sponsor must provide information on test material, positive controls, and statistical treatment of data). (Under contract to NTP/NIEHS).		Prov. Acceptable 003438
84-2(b)	Mutagenic-chromosomal Species: CHO assay Arthur D. Little Inc. ADL-53906; 8/1/85		Piperonyl butoxide tech.		257430 0014767 0014613	Weakly mutagenic under only the nonactivated condition. However, when re-examined, this effect was considered an equivocal response. Addendum to final report - noticed variation not biologically significant		Acceptable 004508
84-2(b)	Mut- Chrom. aberr. In vitro Species: CHO cells Hazleton 14413-1-437C; 09/20/93		Piperonyl butoxide tech. 90.78%; lot# FEP-100		430138-01	Chinese Hamster Ovary (CHO) cell were exposed in the absence of S-9 metabolic activation system in vitro to dose levels of 15 to 30 ug/mL of P80 for 10 or 20 hrs & in the presence of metab. activ. (rat (liver S-9) to dose levels of 12-120 ug/mL for 2 hrs & 10, 20 or 30 hrs were allowed for cell cycles. The positive controls (mitomycin C in the absence of metab. activ. & cyclophosphamide in the presence of metab. activ.) produced their expected/positive results of incr. chromosome aberrations. No evidence of chromosome aberrations were indicated in cell exposed to P80 including dose levels that were cytotoxic (20-30 ug/mL in the absence of activ. & 90-120 ug/mL in the presence of activation).		Acceptable 011005
84-4	Mutagenic- cytogenetic Species: CHO cells Litton Bionetics Inc. P80 Task Force; 3/24/83		Piperonyl butoxide tech.			Negative. Provisionally acceptable subject to sponsors providing: 1.) Source & purity of material 2.) Formal statistical analysis of data.		Acceptable 007644

EPA CANNOT CURRENTLY GUARANTEE THE ACCURACY OR
VALIDITY OF THE INFORMATION INCLUDED IN THE TOXICOLOGY
ONE-LINER DUE TO COMPUTER PROGRAMMING ERRORS

EPA CANNOT CURRENTLY GUARANTEE THE ACCURACY OR
VALIDITY OF THE INFORMATION INCLUDED IN THE TOXICOLOGY
ONE-LINER DUE TO COMPUTER PROGRAMMING ERRORS

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDES/HED/TB-1
TOX ONELINERS

PAGE 5
CASWELL#: 670
CAS-REG#: 51-03-6

P.C. CODE 067501- Piperonyl butoxide FILE LAST PRINTED: 11/21/94

CITATION	MATERIAL	ACCESSION/ NRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
85-1 Pharmacokinetics Species: rat Burroughs Wellcome 10/18/85	Piperonyl butoxide-C14 (labelled in the [2-(2-butoxyethoxy)methyl side chain.	260122 00152568	PB is slowly absorbed from the GI tract and blood levels peak after 4 to 6 hrs. Urinary and fecal excretion peaked between 12 to 24 & 24 to 48 hrs respectively. No evidence of specific tissue retention was presented. No attempts were made to determine identity or structure of metabolites.		Supplementary 006609
85-1 Metabolism Species: rat Biol. Testing Cen.; Irvine, Ca 9/18/85; 10/03/89	14C-piperonyl butoxide (99.1% radiochemical purity)	419987-01 419984-01	After 36-48 hrs, most of the 14C from piperonyl butoxide was recovered in the feces (46.95 to 54.01%) & lesser amounts were recovered in the urine (23-31.3%). After 7 days, recovery was 92.86% to 100.05% and < 1.5% remained in the tissues. The highest levels were in the small intestine and the liver. Eight metabolites were identified in the urine, five of which were also identified in the feces. These eight metabolites were formed through beta oxidation and subsequent cleavage of the ether side chain and/or oxidation of the methylene bridge on the benzodioxole ring. Only trace amounts of parent piperonyl butoxide were present in the urine. The percentage of parent compound in the feces was highest in the high dose group (23.3-30.6%), followed by the single low dose group (9.7-11%), and the lowest in the repeated low dose group (2.2-3.6%).		Guideline 009783
86-1 Dom. animal safety env. exp. Species: cat Prof. Lab. Res. Services Inc 12/22/87	Seargent's Foam N' Comb (Pyrethrins and Piperonyl butoxide)	406707-02	No effects on kittens (7 -10 days old) at the label usage rate. Not tested at 3 - 5X the recommended usage rate.		Unacceptable 007257
86-1 Dom. animal safety env. exp. Species: dog Res Service Inc 8612 & 86-094; 12/22/87	Seargent's Foam N' Comb (Pyrethrins and Piperonyl butoxide)	406707-01	No effects in puppies (6-15 days old) at the label usage rate. Not tested at 3-5X the recommended usage rate.		Unacceptable 007257
86-1 Dom. animal safety env. exp. Species: dogs & cats Fementa Res. Cen.; #2229 9/7/90	Fenoxycarb 1%; Permethrin .15%; Pip. butox .50%; Caswell 613 1.0%; Caswell 025A 0.1%; Casw 400, 0.2%	416374-01	Groups of cats & dogs (each consisting of 1 adult male, 1 adult female, 3 male & 3 female kittens/puppies) were sprayed with the proposed formulation or a 4X conc. of its active ingredients or the vehicle (nonactive) components on days 0, 7 & 14 of a 21 day study. For dogs, individual applications (of either the vehicle, 1X or 4X formulations) ranged from 11.12 to 25.76 grams; for cats the corresponding values were 3.25 to 17.6 gms. In terms of the active ingred. Fenoxycarb single doses ranged from 0.0018 to 0.0100 g/kg at 1X, and from 0.0067 to 0.0415 g/kg at 4X. Possible symptoms observed only in a few dogs exposed to 1X & 4X formulations (and only in periods immediately after spraying): slight serous ocular discharge, panting, slight erythema on the lower abdomen. For cats only depression (highest level recorded defined as 'moderately depressed, lies down mostly, will stand') appeared to be correlated with exposure to the actives (observed only in a few kittens of the 1X & 4X groups on days 0-2, 7 & 14). Occurrence of matted hair in 4X cats & kittens, along		Unacceptable 008238

123

with observation of 'oily' hair, particularly for a few days after spraying on day 14, suggests grooming behavior differences. While no significant adverse effects in cats and/or dogs were observed as a result of normal use-application of the proposed product, a point of toxicological concern is with 'inerts' in this formulation. Application of the 4X concentrate resulted in an exposure to 'inerts' equivalent to only that occurring with a 1X exposure. No information provided as to rate of delivery of this product (from a pump-sprayer). Comment in report (p. 13) as to 'the inherent inaccuracy of spray application' is also a point of concern. Not acceptable without additional information.

EPA CANNOT CURRENTLY GUARANTEE THE ACCURACY OR
VALIDITY OF THE INFORMATION INCLUDED IN THE TOXICOLOGY
ONE-LINER DUE TO COMPUTER PROGRAMMING ERRORS

INFORMATION SUPPORTING AN UNREGISTERED PRODUCT IS NOT INCLUDED

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDES/HED/TB-1
TOX ONELINERS

PAGE 6
CASWELL#: 670
CAS-REG#: 51-03-6

P.C. CODE 067501 - Piperonyl butoxide FILE LAST PRINTED: 11/21/94

SYNOPSIS	MATERIAL	ACCESSION/ NRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
86-1 Dom. animal safety env. exp. Species: cat (kittens) 3/6/91 2239	Fenoxycarb .1%; Allethrin .1%; Permethrin .15%; Pi peronyl but .5%; cas 613 1%; Cas 400 .2%; other 97%		Six kittens (14-17 weeks old, weighing between 2.6 & 3.8 lbs) were sprayed 4 times with the formulation, with sufficient time between spraying for the formulation to dry. Total time elapsed between 1st and 4th spray for any one kitten was no more than 2 hrs & 15 min. Individual single applications ranged from 3.02 to 6.52 gms; cumulative applications ranged from 13.02 to 24.59 gms. One kitten (subsequently diagnosed to have a respiratory tract infection) was slightly depressed following the 4th spraying. The minimum rectal temperatures observed in 5/6 kittens (exception was the kitten with the infection) were observed on the day following spraying, but were still within normal range. A 'chalky' hair coat appearance was observed in 4/6 kittens following the 4th spraying, but was no longer detectable the second day after treatment. No significant toxicological effects were observed in any of the kittens; this study, with the previously reviewed studies, demonstrates that there is a reasonably adequate margin of safety (4X) associated with the normal use of this product in kittens. Acceptable when combined with previously reviewed material.		Acceptable 008304
86-1 Dom. animal safety env. exp. Species: horses Bio-Life Associates 132-001-22; 01/29/93	Farnam Crash Fly Spray (Cypermethrin 0.6%; Py- rethrin 0.8%; Piperonyl butoxide 6.4%)	426579-01	Repeated application (10 daily doses) of 2X the recommended usage rate for Farnam Crash Fly Spray Concentrate result in dermal irritation or other reactions in horses. Test species: Mixed bred horses. Dose levels: 2X label dose applied on daily basis (label rate is once every 5-7 days).		Acceptable 010166
86-1 Dom. animal safety env. exp. Species: dog Kansas State Univ. 11/14/89	Pyrethrins 0.14%; Tetra- methrin 0.063%; Pip but. 1%; Fenoxycarb 0.15%; N-octyl bicyclohep. 1.00%	422439-01	Groups of 4M, 4F dogs received a single 1X, 3X, or 10X normal-use application of the product. There was a control group consisting of 3M and 3F which were sprayed with a placebo formulation. Following spraying, animals were observed for 14 days. There were no symptoms of toxicity, even in the 10X group, & there were no indications of any effects on such parameters as body weight, food consumption, hematology, clinical chemistry or urinalysis.		Acceptable 010222

EPA CANNOT CURRENTLY GUARANTEE THE ACCURACY OR
VALIDITY OF THE INFORMATION INCLUDED IN THE TOXICOLOGY
ONE-LINER DUE TO COMPUTER PROGRAMMING ERRORS

125

INFORMATION SUPPORTING AN UNREGISTERED PRODUCT IS NOT INCLUDED

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDES/HED/TB-1
TOX ONELINERS

PAGE 7
CASWELL#: 670
CAS-REG#: 51-03-6

P.C. CODE 067501- Piperonyl butoxide FILE LAST PRINTED: 11/21/94

TOX COREGRADE/
CAT DOCUMENT#

RESULTS

ACCESSION/
NRID NO.

MATERIAL

CITATION

86-1 Dose, animal safety env. exp. Species: cats P.A.C.E. International 20-434-0793; 09/27/93	Sergeant's Skip-Flea and Tick Shampoo for dogs (0.1% Permethrin; 0.5% piperonyl butoxide)	429696-02	Groups of six (3/sex) adult cats (American short hair and Persian cross) were dosed at a rate of 5 gm/kg or 25 gm/kg of Sergeant's Skip-Flea and Tick Shampoo for dogs and compared with an untreated group for a period of nine days. There were no reactions to treatment noted. The study demonstrates a safety factor of 5 fold based on a single application rate of the product of 5 gm/kg of adult cat.	010818
Special-Air concentration Species: Boyle Midway Inc. 90-441; 6/9/82	Piperonyl butoxide 127 mg DDVP 10 mg; pyrethrine 52 mg	247795	Conc. of actives was 2.7 mg/m³ at 11 hrs and 3.6 mg/m³ at 15 hrs	Supplementary 002498
81-1 Acute oral LD50 Species: rat Cosmopolitan Safety Eval. 01530; 8/6/79	Butacide Tech presumably piperonyl butoxide 89.5%	246018	LD50 (M) = 4.7 (4.39-5.03) g/kg. LD50 (F) = 4.1 (3.53-4.76) g/kg LD50 (combined) = 4.3 (3.94-4.69) g/kg	3 Minimum 001226
81-1 Acute oral LD50 Species: rat Stillmeadow Inc. 5666-88; 11/28/88	Butacide 8 E.C., 91% a.i.	415115-02	LD50 > 500 mg/kg (combined)	3 Guideline 008824

EPA CANNOT CURRENTLY GUARANTEE THE ACCURACY OR
VALIDITY OF THE INFORMATION INCLUDED IN THE TOXICOLOGY
ONE-LINER DUE TO COMPUTER PROGRAMMING ERRORS

126

**U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDES/HED/TB-1
TOX ONELINERS**

**PAGE 8
CASWELL#: 670
CAS-REG#: 51-03-6**

CITATION	MATERIAL	ACCESSION/ PKTD NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
81-2 Acute Dermal LD50 Species: rabbit Cosmopolitan Safety Eval. 0153C; 8/9/79	Butacide presumably piperonyl butoxide 89.5%	246018	No mortalities LD50 > 2g/kg	3	Minimum 001226
81-2 Acute Dermal LD50 Species: rabbit Stillmeadow Inc. 5687-88; 11/28/88	Butacide 8 E.C., 91% a.i.	415115-03	LD50 > 2020 mg/kg.	3	Guideline 008824
81-3 Acute Inhalation LC50 Species: rat Stillmeadow Inc. 5691-88; 12/09/88	Butacide 8 E.C., 91% a.i.	415115-04	LC50 > 4.72 mg/L.	3	Guideline 008824
81-3 Acute Inhalation LC50 Species: rat Bio/dynamics Inc. 91-8350; 08/16/91	Piperonyl butoxide 90.7% pure	419900-01	LC50 > 5.9 mg/L (piperonyl butoxide) for 4 hr exposure. All rats survived. Symptoms included lacrimation, salivation, nasal discharge, ano-genital staining & labored breathing in some animals for one week. Tox. category IV. Sprague-Dawley rat. Level tested - 5.9 mg/L (15% of particles < 1 micron in diameter.	4	Acceptable 009529
81-4 Primary eye irritation Species: rabbit Cosmopolitan Safety Eval. 0153A; 8/9/79	Butacide Tech presumably piperonyl butoxide (tech) 89.5%	246018	slight irritation in 2/6 unwashed, 1/3 washed eyes, totally cleared by 7 days	3	Minimum 001226
81-4 Primary eye irritation Species: rabbit Stillmeadow Inc. 5688-88; 11/14/88	Butacide 8 E.C., 91% a.i.	415115-05	All eye involvement and/or irritation cleared in 7 days.	3	Guideline 008824
81-5 Primary dermal irritation Species: rabbit Cosmopolitan Safety Eval. 0153B; 7/27/79	Butacide Tech 89.5% presumably piperonyl but oxide	246018	PIS = 0.44. No edema; only erythema. Sites all scored zero at 72 hrs	4	Minimum 001226

EPA CANNOT CURRENTLY GUARANTEE THE ACCURACY OR
VALIDITY OF THE INFORMATION INCLUDED IN THE TOXICOLOGY
ONE-LINER DUE TO COMPUTER PROGRAMMING ERRORS

127

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDES/HED/TB-1
TOX ONELINERS

P.C. CODE 067501- Piperonyl butoxide FILE LAST PRINTED: 11/21/94

CITATION	MATERIAL	ACCESSION/ NRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
81-5 Primary dermal irritation Species: rabbit Stillmeadow Inc. 5689-88; 11/29/88	Butacide 8 E.C., 91% a.i.	415115-06	It is a moderate irritant to rabbits.	3	Minimum 008824
81-6 Dermal sensitization Species: guinea pig	Piperonyl butoxide	242923	Not a sensitizer		Supplementary
81-6 Dermal sensitization Species: guinea pigs Stillmeadow Inc. 5690-88; 12/22/88	Butacide 8 E.C., 91% a.i.	415115-07	It did not sensitize guinea pigs.		Guideline 008824

EPA CANNOT CURRENTLY GUARANTEE THE ACCURACY OR
VALIDITY OF THE INFORMATION INCLUDED IN THE TOXICOLOGY
ONE-LINER DUE TO COMPUTER PROGRAMMING ERRORS

128

Mechanistic Data for Piperonyl Butoxide (PBO) Oncogenic Hazard Identification and Risk Assessment¹

Executive Summary

PBO has a long history of safe use and a complete toxicological data base. PBO was not genotoxic in a comprehensive battery of tests conducted by the Task Force and has been assayed in several oncogenicity studies in rats and mice. An increase in benign liver tumors was observed in the mouse oncogenicity study conducted in response to an EPA Data Call In. In that study, eosinophilic hepatadenomas were observed in CD-1 mice at dose levels of 100 (males only) and 300 mg/kg/day (males and females). There is a growing recognition that not all chemically-induced mouse liver tumors are of equal regulatory concern and that knowledge of the underlying biology of a chemical-induced mouse liver tumor is important to both the hazard identification and risk assessment. The goal of this research is to provide information that permits a more rational and scientific understanding of the potential carcinogenic hazard of PBO under realistic conditions of exposure. This discussion of supplemental data concerning the mechanism of tumor formation was undertaken as a result of the December 2 meeting of the PBO Task Force with EPA technical and regulatory management staff². Sufficient data are now available to show that PBO has little or no potential for oncogenic risk in humans.

¹Prepared by PBO Task Force II. Member companies are AgrEvo, S.C. Johnson, McLaughlin, Gormley King Company, Prentiss, Takasago and Endura.

²EPA participants: P. Fenner-Crisp, S. Irene, K. Baetke, L. True, J. Ellenberger, and A. Dixon.

I. Introduction

The value of mechanistic information in the regulation of potential human carcinogens is being increasingly recognized by regulatory Agencies and scientific review bodies in the US and other countries. Mechanistic understanding has played an important role at the US EPA in regulating certain chemicals that have the potential to induce tumors in rodents.

Knowledge that a carcinogen acts through an unequivocally genotoxic mode has led to great caution in the extrapolation from rodents to humans. In other cases in which the mechanism does not involve direct genotoxicity, such as the α -2u globulin-induced renal tumors in male rats, rodent follicular cell thyroid tumors, and rat bladder tumors resulting from stones or microcrystal-induced irritation, the understanding of mechanism has clearly reduced the level of concern for human exposure to the rodent oncogens. The draft revision to the EPA Guidelines for Carcinogen Risk Assessment (EPA, 1994) describes the utility of understanding the mode of action:

"The purpose of analyzing the mode of action is to make a reasoned judgment about the ways agents appear to be producing carcinogenic effects. This judgment affects the ways of characterizing hazards to humans and means of evaluating potential dose-response relationships. Given the gaps in the general understanding of carcinogenesis, these analyses and judgments do not lay out detailed molecular mechanisms. Nevertheless, commonly available data support general views as to physical, chemical, and biological factors that appear to be influencing the carcinogenic process and are acknowledged by scientists as playing a role. For hazard characterization, these analyses address the relevance of the animal tumor model to humans and the conditions of expression of potential hazard...For dose-response assessment, these analyses help to guide the development of biologically based models for low-dose extrapolation."

The importance of biological information relevant to the understanding of the oncogenic

process has also been emphasized by the National Toxicology Program (NTP) Board of Scientific Counselors. NTP is now incorporating mechanistic studies, including some of the studies discussed in this paper, into their cancer bioassays. With respect to the generation of mechanistic information, the NTP Carcinogenesis Working Group of the Board of Scientific Counselors concluded:

"The NTP places too much emphasis on testing per se [i.e., too much emphasis on hazard identification] and not enough emphasis on providing mechanistic insight required for a realistic interpretation of the significance of the test results with regard to human health. This should change. (National Toxicology Program, 1992, cited in Goodman, 1993)"

Mouse liver tumors are a common finding in oncogenicity bioassays and may be associated with either genotoxic or nongenotoxic etiology (Ashby and Tennant, 1988). Their relevance to human risk assessment has been the subject of continuing discussion (see, for example, reports of the fifth mouse liver tumor workshops sponsored by the International Life Sciences Institute). As research has progressed on the biological nature of liver tumors induced under different conditions in various strains of mice, it has become increasingly clear that not all mouse liver tumors should be of equal concern from a regulatory standpoint (see Example 3 in the recent draft EPA Guidelines for Carcinogen Risk Assessment). Mechanistic studies now permit better characterization of the nature of mouse liver tumors and a more rational and scientific understanding of potential oncogenic hazard. These data can be used to confirm a lack of oncogenic risk to humans.

II. Background

PBO is used as a synergist to enhance the insecticidal properties of the pyrethroids and other pesticides and has a long history of safe use. The use of PBO greatly reduces the amount of active ingredient that must be used for insecticidal purposes. Between 1982 and 1986 PBO was subject to Re-registration and Data Call In (DCI) by the US EPA. A Task Force was formed in order to conduct the studies requested by the EPA.

Although PBO had been subjected to extensive oncogenicity testing, additional oncogenicity testing in rats and mice was requested by EPA. A well-conducted study in the rat, performed in response to the EPA DCI, showed no increase in neoplasia at any site (Graham, 1992). In the mouse, the most recent study available (conducted in response to the EPA DCI) found eosinophilic nodules that have been classified as benign liver tumors in both males (100 and 300 mg/kg/day) and females (300 mg/kg/day) (Hermansky and Wahner, 1993).

The mouse oncogenicity study (Hermansky and Wahner, 1993) showing the increase in benign liver tumors was conducted in response to the EPA Data Call In notice and was run at a dose level approximately twice that previously employed. The high dose level was associated with reduced weight gain, and dramatic increases in liver weight, consistent with that seen in earlier chronic and subchronic studies. The increased liver weight is the result of the demonstrated ability of PBO to serve as an inducer of microsomal mixed function oxidase and to increase hepatic enzyme synthesis (including P-450 isozymes) following repeated exposure (Wilkinson et al., 1984). Necrosis and hemorrhage of the liver was observed at the high dose level. At termination of the mouse oncogenicity study after 78 weeks there was a statistically significant increase in liver eosinophilic adenomas/hyperplastic nodules in males at 100 mg/kg/day and in males and females at 300 mg/kg/day (1, 0, 0, 9, 17 in males and 0, 1, 0, 0, 9 in females for the two control groups, and the 30, 100 and 300 mg/kg/day groups, respectively). The incidence of neoplasia is as follows:

Table. Summary of neoplastic findings in the CD-1 Mouse Liver

Dose level	Control 1	Control 2	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day
Adenoma (♂)	8	7	13	21	25
Carcinoma(♂)	3	3	2	2	5
Adenoma (♀)	2	2	1	1	10
Carcinoma(♀)	0	0	0	0	0
Total examined	60	60	60	60	60

PBO was not genotoxic in a comprehensive battery of studies conducted by the Task Force. PBO was examined for mutagenic activity in the Ames test and was found to be negative (Lawlor, 1991). PBO did not induce chromosomal aberrations in CHO cells under both activation and non-activation conditions (Tu, 1986) and did not increase unscheduled DNA synthesis in rat liver cells in culture (McKeon, 1986) or in human liver slices (Price et al., 1994). A mouse dominant lethal assay also confirmed the lack of genotoxicity (Epstein et al, 1979).

In summary, PBO induced hypertrophy of hepatocytes and increased cell turnover, eventually resulting in an increased incidence of eosinophilic adenomas in a susceptible strain of mouse. This response was predictable based upon the pharmacological properties of the compound. PBO induced liver adenomas in one study in the mouse (NOEL= 30 mg/kg/day) but showed no increase in tumors in the rat in studies considered by the EPA as scientifically acceptable. PBO is not genotoxic in a battery of studies conducted by the Task Force including the mouse micronucleus study, CHO chromosomal aberration study, the Ames assay and in unscheduled DNA synthesis studies. The tumor type that has been associated with PBO, eosinophilic hepatadenomas, commonly occurs with nongenotoxic oncogens in some strains

of mice. The relevance of this tumor for human risk assessment has been widely questioned and it has typically been of low concern at regulatory agencies. At the recent ILSI mouse liver tumor workshop, Drs. K. Baetke and R. Engler of EPA presented a number of factors that effect the level of concern for a mouse liver oncogen. As applied to PBO, these factors lead to a low level of concern:

1. The incidence of tumors is increased only in a strain with a moderately-high background rate.
2. Only benign tumors are increased.
3. PBO is not genotoxic.
4. A clear positive response is observed only at dose level with persistent enzyme induction and liver cell hypertrophy.
5. There is a clear threshold for the liver cell hypertrophy and the oncogenic response.
6. Only a single site in a single species shows an oncogenic response.
7. There are no indications of early onset.

Nevertheless, to assure the EPA that PBO is unlikely to be a human oncogen under realistic conditions of exposure and that a threshold-based approach is most appropriate for risk assessment, the Task Force has undertaken a number of additional initiatives that are not typically required by the EPA. These studies have been conducted to show that PBO induces mouse liver tumors via the sequence of enzyme induction, liver cell hypertrophy and proliferation in susceptible mice.

III. Task Force Initiatives to Characterize PBO Hazard and Risk

Group A

- 1. Unscheduled DNA synthesis in human liver**
- 2. Cell proliferation studies in rats and mice**
- 3. Enzyme induction studies**
- 4. Comparative metabolism and pharmacokinetic studies
(with expert summary)**
- 5. Comprehensive exposure assessment**

Comment:

All of the items in Group A are either completed, underway or are planned for the near future. It is expected that all of the work described in Group A can be submitted to the Agency in the next 6 months. Initiatives in this group are those that the Task Force believes are of greatest utility in determining the risk that PBO may pose for humans.

Rationale:

1. Central to an understanding of the mechanism of toxicity of PBO is making a determination of whether or not PBO has the ability to directly damage DNA. A study of unscheduled DNA synthesis (UDS) in human liver from 5 healthy humans was recently undertaken by the Task Force (Price et al., 1994). No increase in UDS was found in human tissue. This negative genotoxicity finding is consistent with other Task Force studies. This study will be submitted to the Agency by April 15.

2. The importance of increased cell proliferation in the carcinogenic process is now being better understood and may be incorporated into more sophisticated risk assessment models

such as the Moolgavkaar-Knudsen model (Moolgavkaar et al., 1986). The Task Force is undertaking cell proliferation studies in the mouse. To date, study results in the mouse indicate that PBO causes a transient but significant increase in cell proliferation in the liver (Phillips et al., 1994). These studies will be completed, summarized and submitted to the Agency by April 15.

3. PBO is known to be both an inhibitor and an inducer of cytochrome P-450 isozymes (Wilkinson et al., 1984). Characterization of the pattern and dose-response for induction in various species will allow better correlation of enzyme induction/cell proliferation/ oncogenicity. These studies are underway and will be submitted by April 15.

4. In the rat PBO is metabolized via oxidation and subsequent cleavage of the ether side chain or methylene bridge on the benzodioxole ring and is rapidly eliminated from the body. It is now understood that species differences in response to toxicants are due to a very large extent to the concentration of the proximate toxicant at the target site. These studies will investigate *in vitro* PBO metabolism in excess donor human liver from transplant material. The results from that study will be compared with the metabolism of PBO in rats and mice. Pharmacokinetic studies will be conducted in rats, mice and humans (if possible). The rat metabolism and pharmacokinetic studies have been completed and submitted to the Agency. Metabolism studies in the mouse have been recently completed and an overview of all metabolism/ pharmacokinetic studies is in preparation. This information will be submitted to the Agency within 4 months.

5. The ability to predict the oncogenic hazard posed under realistic conditions of exposure is of paramount importance for regulatory purposes. The Task Force proposes to collect and analyze information characterizing exposure by all routes. This information will facilitate prediction of oncogenic hazard and risk assessment. This work will be completed within 6 months.

Group B

- 1. Reversibility study in the mouse**
- 2. Oncogene activation**
- 3. Alterations in cell-cell communication**
- 4. DNA hypomethylation**
- 5. Transplantation studies**

Comment:

These long-term studies have been undertaken for other mouse liver tumor oncogens. They would determine whether PBO-induced lesions are reversible, whether they are likely to progress and whether they have the potential to be transplanted. The PBO Task Force would consider undertaking these studies only if EPA concluded that they could potentially allow reclassification of PBO-induced lesions as nonneoplastic.

Rationale:

1. Reversibility studies (also called "stop exposure" studies) have been conducted for phenobarbital and a number of chemicals tested by the National Toxicology Program (Maronpot et al., 1987). For phenobarbital, hepatadenomas were shown not to progress to carcinomas upon the cessation of treatment and the number of eosinophilic nodules decreased in animals returned to control diet after 60 weeks of exposure and sacrificed at 80 weeks (Evans et al., 1992).

2. It has been suggested that oncogene activation studies (and study of inactivated tumor suppressor genes) may provide a greater understanding of the underlying nature of mouse liver neoplasia (Reynolds et al., 1987). Activated proto-oncogenes, particularly the H- ras

oncogene at codon 61, have been found in spontaneously occurring and genotoxic carcinogen-induced tumors (Chen et al, 1993). A decreased incidence of H-ras oncogene activation has been observed in mouse liver tumors induced by the mouse liver oncogens phenobarbital (Pedrick et al., 1994) and oxazepam (Cunningham et al., 1994). The relevance of these DNA alterations (and their absence in some murine hepatic tumors) is unknown at the present time.

3. Alterations of cell-cell communication are found in mouse liver tumors induced by nongenotoxic agents and may be a necessary precursor to tumor development (Goodman et al., (1993)).

4. Alterations in DNA methylation is a common finding in mouse liver tumors induced by nongenotoxic agents. Ray et al., (1994) has suggested that this may be a necessary precursor of tumor development.

5. Tumor transplantation studies into nude mice are a measure of the aggressiveness of a tumor i.e., those tumors that are transplanted and continue to grow are more highly neoplastic than those that fail. The ability of transplanted tumors to grow in agar media could also be examined. Eosinophilic mouse liver tumors associated with phenobarbital have been found to be less transplantable than those induced by genotoxic mouse liver carcinogens (Pedrick et al., 1994).

References

Ashby, J. and R.W. Tennant (1988). Chemical structure, Salmonella mutagenicity and extent of carcinogenicity as indicators of genotoxic carcinogenesis among 222 chemicals tested in rodents by the U.S. NCI/NTP. *Mutation Research* 204:17-115.

Chen et al., (1993). Dose-dependent ras mutation spectra in N-nitrosodiethylamine induced mouse liver tumors and 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone induced mouse lung tumors. *Carcinogenesis* 14:1603-1608.

Cunningham, M.L., Maronpot, R.R., Thompson, M. and J.R. Bucher (1994). Early responses of the liver of B6C3F1 mice to the hepatocarcinogen oxazepam. *Tox Appl Pharm* 124:31-38.

EPA (1994). Revisions to Guidelines for Carcinogen Risk Assessment, EPA, Washington, D.C., August 1994.

Epstein, S.S., et al., *Toxicology and Applied Pharmacology* 23: 288-325.

Evans, J.G., Collins, M.A., Lake, B.G., and Butler, W.H. (1992). The history and development of hepatic nodules and carcinoma in C3H/He and C57BL/6 mice following chronic phenobarbitone administration. *Tox Path* 20:585-594.

Goodman, J.I. (1993). A rational approach to risk assessment requires the use of biological information: An analysis of the National Toxicology Program (NTP), final report of the advisory review by the NTP Board of Scientific Counselors, *Reg Tox Pharm* 6/93.

Goodman, J.I., Ward, J.M., Popp, J.A., Klaunig, J.E., and T.R. Fox (1991). Mouse liver tumorigenesis: mechanisms and relevance. *Fund. Appl. Toxicol.* 5:66-78.

Graham, C. (1987). 24 month dietary toxicity and carcinogenicity study of piperonyl butoxide in the albino rat. BioResearch Laboratories, Ref. 816990.

Hemalatha, M. (1991). CHO chromosome aberrations. Hazleton Laboratories, 14413-0-437C.

Hermansky, S.J. and Wahner, C.L. (1993). Chronic dietary oncogenicity study with piperonyl butoxide in CD-1 mice. Bushy Run Research Center, Report no. 91NO134.

Lawlor, T.E. (1991). Mutagenicity test on piperonyl butoxide in the Salmonella/mammalian microsome reverse mutation assay (Ames test) with confirmatory assay. Hazleton Laboratories, 14413-0-401R.

Maronpot, R.R., Haseman, J.K., Boorman, G.A., Eustis, S.E., Rao, G.N., and Huff, J.E. (1987). Liver lesions in B6C3F1 Mice: the National Toxicology Program, Experience and Position. Arch. Tox, Suppl. 10, 10-26.

McKeon, M.E. (1986). Genotoxicity test on piperonyl butoxide in the assay for unscheduled DNA synthesis in rat liver primary cell cultures with a confirmatory assay. Hazleton Laboratories, 14413-0-447R.

Moolgavkar, S.H. (1983). Model for human carcinogenesis: action of environmental agents. Environ. Hlth. Perspect. 50: 285-291.

Pedrick, M.S., Rumsby, P.C., Wirght, V., Philmore, H.E., Butler, W.H., and J.G.Evans (1994). Growth characteristics and Ha-Ras mutations of cell cultures isolated from chemically induced mouse liver tumors. Carcinogenesis 15:1847-1852.

Price, R. J., Beamond, J.A., Glynn-Jones, G.D., Osimitz, T., Gabriel, K.L., Preiss, F.J., Butler, W.H., and B.G. Lake (abstract, in press). Effect of piperonyl butoxide (PBO) on Unscheduled DNA Synthesis in precision-cut human liver slices. Toxicol Appl. Pharm.

Phillips. et al., (abstract, in press). Effect of piperonyl butoxide (PBO) on cell replication and xenobiotic metabolism in mouse liver. Fund. Appl. Pharm.

Ray, J.S., Harbison, M.L., McClain, R.M. and Goodman, J.I. (1994). Alterations in the methylation status and expression of the raf oncogene in phenobarbital-induced and spontaneous B6C3F1 mouse liver tumors. Molecular Carcinogenesis 9:155-166, 1994.

Reynolds, S.H., Stowers, S.J., Patterson, R.M., Maronpot, R.R., Aaronson, S.A. and M.W. Anderson (1987). Activated oncogenes in B6C3F1 mouse liver tumors: Implications for risk assessment. Science 237:1309-1316.

Tu, A.S. (1986). Evaluation of piperonyl butoxide in the CHO/HGPRT mutation assay with and without metabolic activation. AD Little, Ref 1-1683, amended report no. 53906.

Wilkinson, C.F., Murray, M. and C.B. Marcus (1984). Interactions of methylenedioxyphenyl compounds with cytochrome P-450 and effects on microsomal oxidation. In "Reviews in Biochemical Toxicology", Hodgson, E., Bend, J.R., and Philpot, R.M. eds., Elsevier, New York, pp.27-64.