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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Piperonyl Butoxide

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Review Section IV
Toxicology Branch I
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and
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Registration Division (7505C)
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Product Manager #53
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THROUGH: Stephanie R. Irene Ph.D.
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The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on February 15, 1995 to discuss and evaluate the weight-of-the-evidence on piperonyl butoxide (PBO) with particular reference to its carcinogenic potential. The CPRC concluded that PBO should be classified as a Group C - possible human carcinogen - and recommended that for the purpose of risk characterization, the Reference Dose (RfD) and Margin of Exposure (MOE) approaches should be used for quantitation of human risk. The Group C classification was based primarily on statistically significant increases in hepatocellular tumors in both sexes of the CD-1 mouse (adenomas, carcinomas and combined adenomas/carcinomas in males and adenomas in females). The decision to use the RfD and MOE approaches rather than the Q1* approach to risk assessment was based on only slight increases in thyroid follicular cell tumors in rats, increases in liver tumors only at excessive doses in rats and in other mouse studies, and generally low concern for mutagenicity.

SUMMARY

There were 4 studies in the rat and 3 studies in the mouse conducted with PBO, briefly summarized below (details can be found in section D of this document).

I. Studies in the rat

Rat Study #1: Administration of PBO in the diet to Fischer 344 rats resulted in statistically significant increases in liver adenomas and carcinomas in both sexes of the rat at doses which the CPRC determined to be excessively toxic to the rats. Dosing was considered to be excessive based on the presence of gastrointestinal hemorrhage at all dose levels.

Rat Study #2: Administration of PBO in the diet to Sprague Dawley rats was associated with a slight increase (statistically significant positive trend only) in thyroid follicular cell tumors in both sexes of the rat (combined adenomas/carcinomas in males and adenomas in females). The presence of thyroid hyperplasia was considered to be supportive of the neoplastic response. A very slight numerical increase in hepatocellular tumors was not considered to be related to treatment, but was attributed to normal variability. The dosing in this study was considered to be adequate.

Rat Study #3: Administration of PBO in the diet to Fischer 344 rats was associated with very slight numerical increases in thyroid follicular cell tumors in both sexes, which the CPRC did not consider to be treatment-related. Dosing in this study was also considered to be adequate.

Rat Study #4: Administration of PBO in the diet to Fisher 344 rats was associated with a numerical increase in neoplastic nodules in the livers of male rats, at a dose considered to be excessive, based on increased mortality and a high incidence of ulcers in the digestive tract. There were no apparent compound related increases of tumors in female rats, which also were considered to be dosed excessively, based on increased mortality and a high incidence of ulcers.

II. Studies in the mouse

Mouse Study #1: Administration of PBO in the diet to CD-1 mice was associated with statistically significant increases in hepatocellular adenomas, carcinomas and combined adenomas/carcinomas in males, and statistically significant increases in adenomas in female mice. There were also statistically signif-

icant positive trends for these tumors. In male rats adenomas were increased at all doses, but the increases were significant only at the mid and high doses, and the carcinomas only at the high dose. The incidence of these tumors exceeded that of the available historical controls. The CPRC considered the dosing in this study to be adequate, but noted liver hemorrhages at the high dose. Nevertheless, the tumor response occurred at lower doses not showing hemorrhages.

Mouse Study #2: Administration of PBO in the diet to CD-1 mice was associated with statistically significant increases in hepatocellular adenomas and carcinomas at both doses in male mice. In addition there was a statistically significant increase in hemangioendothelial sarcomas at the high dose in male mice. (No data were reported for female mice.) The CPRC considered the high dose to have been excessive and, since this was only a 12 month study, that the low dose (there were only 2 doses) would also have been excessive, had the study been run for 18 months. The CPRC did note the early appearance of these tumors and the early increased deaths possibly attributed to the liver tumors.

Mouse Study #3: Administration of PBO in the diet to B6C3F1 mice was associated with a numerical increase in liver hepatocellular tumors in female mice. In male mice there was also an increase in adenomas of the lacrimal gland (statistically significant positive trend, only), the significance of which was unclear to the CPRC. The dosing in this study was considered to be excessive.

The Group C classification for PBO was primarily based on the statistically significant increases in liver tumors in both sexes of the CD-1 mouse, by both pair-wise and trend analysis, at doses that were considered to be appropriate for carcinogenicity assessment (Mouse #1). Data from safrole and piperonyl sulfoxide, structurally related analogs, which are also associated with liver (and other) tumors in rodents, provided additional support.

The consensus of the CPRC was that it was inappropriate to apply a low-dose extrapolation methodology (Q*) to the animal data. This was based on only slight increases in thyroid follicular tumors in Rat #2, increases in liver tumors only at excessive doses in Rat #3 and #4, and in Mouse #2 and #3, and generally low concern for mutagenicity. Therefore, the CPRC recommended the use of the MOE and RfD methodologies to be applied for the estimation of human risk.

A. Individuals in Attendance at the meeting:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Stephanie Irene

William Burnam

Karl Baetcke

Marcia Van Gemert

Kerry Dearfield

Elizabeth Doyle

Marion Copley

Hugh Pettigrew

[redacted]

Yin Tak Woo

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

John Doherty[1]

Lori Brunsman

Lucas Brennecke[2]
(PAI/ORNL)

3. Other Attendees:

Bernice Fisher, Krystyna Locke, Stanley Gross (HED) and Amber Aranda (OGC)

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[1]Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

[2]Signature indicates concurrence with pathology report.

B. Material Reviewed

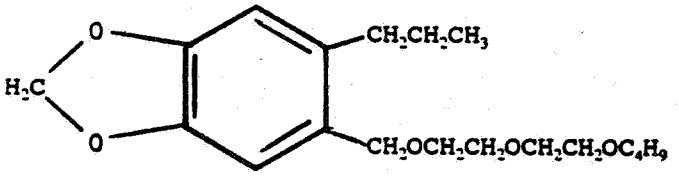
The material available for review consisted of DER's, one-liners, data from the literature and other data summaries prepared and/or supplied by Dr. John Doherty, and tables and statistical analysis by Lori Brunsman. The material reviewed is attached to the file copy of this report.

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 raw agricultural commodities (RACs).

Following the Data-Call-In Notice (January 1989), 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 in the order in which they were discussed at the Peer Review meeting. 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). Submitted as a prepublication monograph under MRID No.: 42839601 and 42920201. HED Document No.: 010658. Note: The DER was based on the prepublication monograph.

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 did not extract the data for an independent statistical assessment.

Table 1 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.

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).

Table 1. Liver tumors and Focal Hyperplasia in F344 rats (Rat Study No.: 1) 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%
Butler	(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
Original	(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.

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 in the stomach and cecum, lung and kidneys.

Table 2. Non-neoplastic pathology in Fischer 344 rats (Rat Study No.: 1) 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 significant $p < 0.05$.

-*Liver*. The incidence of "focal hyperplasia" in the liver was presented in Table 1.

-*"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 died with 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 are also considered excessive.

2. Rat Study #2. ***Rat Carcinogenicity Study***. Bio-Research Study No.: 81690, August 27, 1987, Accession No.: 40323701, HED Document No.: 006668.

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 combined adenomas/carcinomas (males) based on positive trends. Tables 3 (males) and 4 (females) illustrate the tumor incidence and present a statistical assessment of the thyroid data.

Historical control data for thyroid tumors from the testing laboratory were 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.

Table 3. Piperonyl Butoxide - Charles River Sprague-Dawley Crl-CDR Rat Study No.: 2. Male Thyroid Follicular Cell Tumor Rates[-] and Exact Trend Test and Fisher's Exact Test Results (p values)

Dose (mg/kg/day)				
	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[n]	0.375
Carcinomas (%)	1[b]/115 (1)	0/58 (0)	0/58 (0)	2/53 (4)
p =	0.090	0.665[n]	0.665[n]	0.234
Combined (%)	3/115 (3)	1/58 (2)	0/58 (0)	4/53 (8)
p =	**0.040**[•]	0.589[n]	0.291[n]	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.
[n]Negative change from control.
[a]First adenoma observed at week 79, dose 500 mg/kg/day.
[b]First 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 No.: 2. Female Thyroid Follicular Cell Tumor Rates[-] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (mg/kg/day)			
	0[#]	30	100	500
Adenomas[@] (%)	1/116 (1)	0/57 (0)	1[a]/58 (2)	3/58 (5)
p =	**0.029**[•]	0.671[n]	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.
[n]Negative change from control.
[a]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 $p < 0.01$.

Liver tumors were found in the dosed animals as indicated by Table 5.

Table 5. Pathological findings in the liver of Crl:CDBR rats (Rat Study No.: 2) 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	0	0	0	0	2	[None reported]				
total	1	1	0	0	3	0	0	0	1	1

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

c. Non-neoplastic Lesions. The liver weight in all dosed groups were determined to be increased (9% for males and 20% for females in the 30 mg/kg/day dose group). In the female group dosed with 100 mg/kg/day and above there was 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. Body weight gain decreases in the first 90 days in the high dose group only (<10%). 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. The dose levels were considered adequate.

3. Rat Study #3. *Rat Carcinogenicity Study.* NCI (Blue Book) Study No.: 120. Frederick Cancer Research Center, Published 1979. No DER has been prepared for this study.

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. The 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.

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 illustrates the neoplastic and non-neoplastic findings in the thyroid.

Table 6. Thyroid neoplastic and non-neoplastic pathology in Fischer 344 rats (Rat Study No.: 3) dosed with piperonyl butoxide.

Lesion	Males			Females		
	Control	5000	10000	Control	5000	10000
N	19	49	48	19	48	49
Tumors						
Follic. cell adenoma	0	0	2	0	0	0
Follic. cell carcinoma	0	1	1	0	0	1
Total	0	1	3			1
	1	5	4	4	4	2
C-cell adenoma	0	1	0	0	2	1
C-cell carcinoma	1	6	4	4	6	3
Total						
Non-neoplastic	8	10	12	-	-	-
C-cell hyperplasia 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 observed in the other studies with rats.

-*Lymphomas.* Three types of malignant lymphomas were noted 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"[3]	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 NCI study 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 NCI report assessed the lymphoma data as follows:

> "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."[4]

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

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 for body weight became more evident and a clear dose response was noted. The dose levels are considered adequate.

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[3]NOS = not otherwise specified.

[4]NCI study report, page 32.

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. 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. Tox Branch I notes, however, that there were "neoplastic nodules" with incidence rates of 2%, 2% and 6% in the liver of males for the control, 0.5% and 1% dose groups, respectively. Furthermore, the male high dose group had hepatocellular carcinomas at an incidence rate of 2%, but the control and low dose groups did not have this tumor.

Among the females, there were also "neoplastic nodules" with an incidence rate of 2% in the 0.5% dose group but none in the control or high dose group. No indications of compound related increases in thyroid tumors were presented. The incidences rates for c-cell adenoma were 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 incidences 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. 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 excessive.

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.

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. In male mice there were statistically significant increases in hepatocellular adenomas and combined adenoma/carcinoma at the mid and high doses, and in carcinomas at the high dose (all at $p < 0.01$). There were also statistically significant positive trends for adenomas, carcinomas and combined adenoma/carcinoma (all at $p< 0.01$). In female mice there was a statistically significant increase in hepatocellular adenomas at the high dose ($p< 0.01$) with a statistically significant positive trend ($p< 0.01$). There were no carcinomas reported in the females. Tables 7 (males) and 8 (females) illustrates the tumor incidences 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% for males and 0-0.7% for females based on 8 studies run for 18 months. The incidence for both male (all dose groups) and female adenomas (high dose group) and carcinomas for males (high dose group) were in excess of these historical control data.

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. The dose levels were considered adequate in both sexes.

Table 7. Piperonyl Butoxide - Mouse Study #1 (CD-1 strain).

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

	Dose (mg/kg/day)			
	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.
[a]First adenoma observed at week 61, dose 0 mg/kg/day.
[b]First carcinoma observed at week 69, dose 300 mg/kg/day.
[c]One animal in the 30 mg/kg/day dose group had both an adenoma and a carcinoma.
[d]Five 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 - Mouse Study #1 (CD-1 strain).

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

	Dose (mg/kg/day)			
	0[#]	30	100	300
Adenomas[@] (%)	4[a]/116 (3)	1/58 (2)	1/60 (2)	10/57 (18)
p =	**0.000****	0.459[n]	0.444[n]	**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.
[n]Negative change from control.
[a]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 $p < 0.01$.

6. Mouse Study #2. *Mouse Carcinogenicity Study.* O. Takahashi et. al. in Arch Toxicol 68:467-469 (1994). 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 for 12 months. These dose levels correspond to 0, 6000 or 12000 ppm or 0, 857 or 1714 mg/kg/day.

b. Discussion of Tumor Data. Hepatocellular adenoma and carcinoma were induced in a dose related manner with incidence rates of 1.9%, 24.5% and 75% for the combined tumor types in 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.

Table 9. Neoplastic and non-neoplastic lesion in male CD-1 mice dosed with PBO for 12 months (Mouse Study #2, Takahashi study, 1994).

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

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 (Table 9).

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 CPRC considered the high dose to have been excessive and, since this was only a 12 month study, that the low dose (there were only 2 doses) would also have been excessive, had the study been run for 18 months.

7. Mouse Study #3. ***Mouse Carcinogenicity Study.*** NCI (Blue Book) Study No.: 120. Published 1979[5]. No separate DER has been prepared for this study.

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 mice/sex 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, respectively, strongly indicating a dose response but statistical significance was not attained (according to the NCI report[6]).

Lacrimal gland. In males there was a statistically significant increase trend (Cochran-Armitage) in adenomas of the lacrimal gland as indicated by there being 0/20, 0/50 and 4/50

[5]Refer to footnote number 1 in the NCI report for a description of the test material.

[6]Page 109, Table F2, in the NCI study report.

(8%) in the control, low and high dose groups, respectively[7]. 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.

c. Non-neoplastic Lesions.

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

Thyroid. In females "cystic follicles" were slightly elevated in the dosed animal with there being incidences of 0, 1 (2%) and 5 (11%) for the control, low and high dose groups, respectively. In males, "follicular cysts" were present at incidences of 0, 1 (2%) and 2 (4%). There was also one finding 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. The NCI report[8] indicated a dose related effect on body weight. The initial dose levels were considered excessive and had to be decreased to prevent severe reactions.

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 ^{14}C 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.

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[7]Page 13B of the NCI study report.

[8]Page 34, Figure 3 of the NCI study report.

2. Mutagenicity PBO has been tested in several mutagenicity studies. Studies concluded to be acceptable have been submitted for bacterial and mammalian gene mutations, in vitro chromosomal aberrations and sister chromatid exchange and an unscheduled DNA synthesis assay. The acceptable studies satisfy the three categories of mutagenicity testing (pre-1991 guidelines) of gene mutations, structural chromosomal aberrations and other mechanism of genotoxic effects.

a) *Salmonella* assay - (MRID Nos.: 00143499 and 42004502, refer to HED Document No.: 003438, 007674 and 009789) did not indicate that piperonyl butoxide induced gene mutations in *Salmonella typhimurium* strains with and without metabolic activation.

b) In vitro gene mutations in chinese hamster ovary cells (MRID No.: 00147693, and 00146135 and HED Document No.: 004508). This study was negative for inducing gene mutations in cultured CHO cells at the hprt locus.

c) *In vitro* cytogenetics in Chinese hamster ovary (CHO) cells (refer to HED Document Nos: 003438, 007674 and 011005 and MRID No.: 43013801 and 00143499). These studies provide evidence that piperonyl butoxide does not induce chromosome aberrations in CHO cells *in vitro*.

d) *In vitro* sister chromatid exchange study (MRID No.: 00143499, HED Document Nos.: 003438 and 007674) did not indicate sister chromatid exchanges in CHO cells with and without metabolic activation.

e) In vitro unscheduled DNA synthesis (UDS) in primary rat hepatocytes (MRID No.: 42004503, HED Document No.: 009789) was negative for inducing UDS in primary rat hepatocytes.

f) As part of the National Toxicology Program testing of PBO, in addition to negative Salmonella, aberration and SCE results (part of evidence presented above), PBO induced an increase in gene mutations at the tk locus in mouse lymphoma cells without activation (activation conditions not tested).

Based on the total weight of evidence presented for PBO, there is no significant concern for the mutagenicity of PBO.

3. Developmental Toxicity

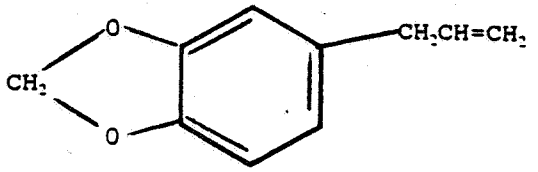
No effects were noted in a rat developmental toxicity study

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).

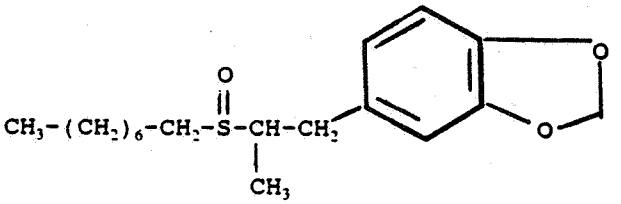
4. Structure-Activity and Functional Activity Correlations

Piperonyl butoxide is structurally related to:

a. 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 safrole is as follows:



b. Piperonyl sulfoxide. Piperonyl sulfoxide (CAS 120-62-7) is 1,2-(methylenedioxy)-4-(2-octylsulfinyl)propyl)benzene and is illustrated as follows:



Piperonyl sulfoxide is also an insecticide synergist that also affects the microsomal oxidase system. Piperonyl sulfoxide was concluded to be carcinogenic for male B6C3F1 strain mice by the NCI (DHEW Publication No.: (NIH) 79-1379). More specifically, the low (350 ppm) and high (700 ppm) piperonyl sulfoxide test dose groups were associated with higher incidence of hepatocellular carcinomas with there being 6/18 (33%), 31/50 (62%) and 46/50 (92%) for the control, low and high dose groups. Females in the high dose group also had the highest incidence of hepatocellular carcinomas (6/49 or 12%) vs only 6% in the control or low dose groups.

c. 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

have recently (February 22, 1995) been presented to the HED Carcinogenicity Peer Review Committee.

5. Acute, Subchronic, and Chronic Toxicity Studies

Acute toxicity. Piperonyl butoxide has acute oral LD_{50}s of 4.7 (4.39-5.03) mg/kg in males and 4.1 (3.53-4.86) mg/kg in females i.e. it is not regarded as a very acutely toxic substance. There were no mortalities to rabbits dosed dermally with 2 gm/kg and the LC_{50} for inhalation toxicity was established to be > 5.9 mg/l. Piperonyl butoxide is in 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 considered 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 for both sexes 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 2.4% dietary level 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 1.2% dose 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. Only minor (<10%) body weight decreases in the first 90 days in the high dose group only were evident. There was, however, a consistent decrease in body weight reaching 22% for males and 21% for females at termination. Thus, the dose levels were considered adequate.**

Males had a significant increased trend in thyroid follicular cell combined adenomas/carcinomas ($p < 0.05$); females had a significant increased trend in thyroid follicular cell adenomas ($p < 0.05$). 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. Only minor body weight decreases were noted. The study is further compromised because no individual animal data were provided and HED has not done independent statistical analyses. The study is an NCI Blue Book report and no independent statistical analysis was performed.**

Males in 5000 (one carcinoma) and 10000 (2 adenomas and one

carcinoma) ppm dose groups had thyroid follicular cell adenomas and/or carcinomas. The single incident of a thyroid adenoma in females was in the high dose group. Neither male or female controls had follicular cell tumors. In males the two dosed groups also had increased incidence of C-cell adenomas and/or carcinomas.

Females had increased incidence of "malignant lymphomas" of two types and both the trend ($p < 0.007$) and pair-wise comparisons ($p < 0.020$) 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. Body weight was only slightly affected in females, but this sex is still considered to have been tested at an adequate dose.**

In male mice there were statistically significant increases in hepatocellular adenomas and combined adenoma/carcinoma at the mid and high doses, and in carcinomas at the high dose (all at $p < 0.01$). There were also statistically significant positive trends for adenomas, carcinomas and combined adenoma/carcinoma (all at $p< 0.01$).

In female mice there was a statistically significant increase in hepatocellular adenomas at the high dose ($p< 0.01$) with a statistically significant positive trend ($p< 0.01$).

Mouse Study #2. **CD-1 strain mice (males only reported) 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 CPRC considered the high dose to have been excessive and, since this was only a 12 month study, that the low dose (there were only 2 doses) would also have been excessive, had the study been run for 18 months. 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/carcinoma. The males also had significantly increased hemangioendothelial sarcoma in the high dose group ($p < 0.05$) and an incident in the low dose group but no 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 the initial high dose was excessive. 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 and to piperonyl sulfoxide a chemical demonstrated to produce liver tumors in male mice. The structurally and functionally related chemical piperonyl sulfoxide also resulted in liver tumors. The functionally related chemical (MGK-264) resulted in marginal increases in thyroid tumors and liver tumors in males and female mice.

3. Based on several mutagenicity studies in which PBO was considered to be negative, there is presently no large concern for the mutagenicity of PBO.

4. Carcinogenicity in animals -- PBO

After a full evaluation of all of the data and supporting information regarding animal carcinogenicity, the Committee concludes that exposure to PBO resulted in an increased incidence of hepatocellular tumors in both sexes of the CD-1 mouse (adenomas, carcinomas, combined adenomas/carcinomas in males and adenomas in females). The incidence of these tumors exceeded that of historical controls. Data from safrole and piperonyl sulfoxide, structurally related analogs, which are also associated with liver (and other) tumors in rodents, provided additional support.

The relevance of the tumor data to an evaluation of PBO's potential for human carcinogenicity is discussed in Part G of this document.

G. Classification of Carcinogenic Potential:

The Peer Review Committee considered the criteria contained in the EPA's "Guidelines for Carcinogen Risk Assessment" [FR51: 33992-34003, 1986] for classifying the weight of evidence for carcinogenicity.

The Peer Review Committee agreed that PBO should be classified as a Group C - possible human carcinogen and that for the purpose of risk characterization the Margin of Exposure (MOE) and RfD approaches should be used for quantification of human risk.

The decision to classify PBO as a Group C carcinogen was based on evidence of increased incidence of hepatocellular tumors in both sexes of the CD-1 mouse (adenomas, carcinomas, combined adenomas/carcinomas in males and adenomas in females). The incidence of these tumors exceeded that of historical controls. Data from safrole and piperonyl sulfoxide, structurally related analogs, which are also associated with liver (and other) tumors in rodents, provided additional support.

The CPRC felt that it was inappropriate to apply a linear low-dose extrapolation methodology (Q*) to the animal data. Therefore, the CPRC recommended the use of the MOE and RfD methodologies to be applied for the estimation of human risk. The selection of a NOEL for calculating the MOE utilizes only those biological endpoints which are related to tumor development (refer to Table 10 below).

The weight of the evidence indicated that both thyroid and liver tumors were associated with increases in liver weight and/or the presence of hyperplastic changes. Table 10 was prepared to illustrate the NOEL and LEL for liver and thyroid tumors and non-neoplastic or hyperplasia changes. This table can be used for selecting the NOEL and LEL for MOE determinations.

Table 10. Summary of neoplastic and non-neoplastic liver and thyroid effects in rat, mouse and dog studies with piperonyl butoxide

Study	Carcinogenic Effects	Non-neoplastic Effects
Rat Study #1. Takahashi/1994 Fischer 344 strain, control, 0.6%, 1.2% and 2.4%	Liver tumors-both sexes at 547 mg/kg/day (original analysis) but only at 547 and 1052 mg/kg/day by Butler's analysis. No data on thyroid	NOEL for liver < 547 mg/kg/day absolute and relative liver weight of females increased at 0.6%. At 1052 mg/kg/day: focal hyperplasia (females only) and increased liver weigh in males; hemangiosarcoma-like lesion (males). At 1877 mg/kg/day increases in gamma GTP and hemangio-sarcoma -like lesion (females). No data on thyroid.
Rat Study #2 BioResearch/1987 Sprague-Dawley strain, control 30, 100 and 500 mg/kg/day.	Positive trends for thyroid tumors. Marginal increase in liver tumors: total of 3 in high dose (500 mg/kg/day) males vs one in each control group and one in the mid (100) and high (500 mg/kg/ day) dose female groups but none in the control.	NOEL and LEL for thyroid: 100 and 500 mg/kg/day: "pigment in follicles", and hyperplasia of follicles in both sexes. NOEL for Liver: < 30 mg/kg/day: All dosed groups had increased liver weight. At 100 mg/kg and above there was "focal mixed cells" and at 500 mg/kg/day there was "hypertrophy of hepatocytes".
Rat Study #3 NCI/1979 Fischer 344 strain, control, 5000 and 10000 ppm or 250 and 500 mg/kg/day.	Marginal increases in thyroid follicular cell tumors at 250 (1 incident and 500 (3 incidents) in males and 500 (1 incident) mg/kg/day in females. none in controls No evidence for liver tumors.	Thyroid: No evidence increased hyperplasia. Liver: None.
Mouse Study #1 Bushy Run/1993 CD-1 strain, control, 30, 100 and 300 mg/kg/day.	liver tumors at 30 and 100 mg/kg/day both sexes.	NOEL and LEL = 30 and 100 mg/kg/day. At 100 mg/kg/day: liver weight increase. At 300 mg/kg/day: liver hemorrhage (males) and hypertrophy (males and females).
Mouse Study #2 Takahashi/1994 CD-1 strain, control, 0.6% and 1.2% or 857 or 1714 mg/kg/day.	Liver tumors at 857 mg/kg/day and above. Liver tumors in males (females not reported) and hemangiosarcoma.	NOEL < 857 mg/kg/day. At 857 mg/kg/day: Hepatocellular hyperplasia and postnecrotic peliosis.
Mouse Study #3 NCI/1979 B6C3F1 strain, control, 148 and 298 (TMA) mg/kg/day.	Not considered positive by NCI but 5% (control), 4% (148) and 10% (298 mg/kg/day) incidence of hepatocellular carcinomas in females but not stat. signif.	NOEL and LEL = 148 and 298 mg/kg/day: At 298 mg/kg/day: Slight elevations in "nodular hyperplasia" in males.

Rat Study #1. Takahashi/1994 Fischer 344 strain, control, 0.6%, 1.2% and 2.4%	Liver tumors-both sexes at 547 mg/kg/day (original analysis) but only at 547 and 1052 mg/kg/day by Butler's analysis. No data on thyroid	NOEL for liver < 547 mg/kg/day absolute and relative liver weight of females increased at 0.6%. At 1052 mg/kg/day: focal hyperplasia (females only) and increased liver weigh in males; hemangiosarcoma-like lesion (males). At 1877 mg/kg/day increases in gamma GTP and hemangio-sarcoma -like lesion (females). No data on thyroid.
Dog Study IRDC/1993 Beagle dog, control, 2.9, 15.5 or 52.8 mg/kg/day.	No thyroid or liver tumors reported.	NOEL and LEL = 2.9 and 15.5 mg/kg/day: At 15.5 mg/kg/day: alkaline phosphatase and increased relative liver weight.

Based on this table, there is no NOEL for liver effects since at the lowest dose level from all of these studies there is increased liver weight in females (with a trend for increased liver weight in males). The increase in liver weight was 20%, was statistically significant, and liver weight relative to brain and body weight were also elevated (refer to HED Document No.: 006668). Thus, the MOE determined from this study may require an additional modification or uncertainly factor to account for the study not demonstrating a true NOEL.

The Reference Dose (RfD) for piperonyl butoxide has not been determined and therefore a comparison of NOEL's and LEL's for all study types could not be presented. Based on an overview of the "one liners" and FSTS it appears that the NOEL and LEL for developmental toxicity and other studies are greater than 30 mg/kg/day.

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

Study Identification	Organs of Concern for Neoplasia
Rat Study #1. 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	Liver tumors (adenomas and carcinomas) all dose levels. Dose levels considered excessive.
Rat Study #2. Bio-Research # 81690. August 27, 1987. Sprague-Dawley Crl-CDR strain, control, 30, 100 or 500 mg/kg/day for two years. GUIDELINES	Positive trend for thyroid follicular cell tumors. Dose levels considered adequate.
Rat Study #3. 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: Thyroid follicular tumors Lymphomas Doses considered adequate.
Rat Study #4. National Institute Hygienic Sciences, Tokyo, Japan in Fd. Chem. Toxic. 23:675-682 (1985). Fischer F344/DuCrj strain rats, controls, o.5% and 1.0% for 2 years.	Authors concluded study does not demonstrate carcinogenicity. Ileocaecal ulcers in both treated groups in both sexes. Doses considered excessive.
Mouse Study #1. Bushy Run Research Center, #91N0134, August 27, 1993. CD-1 strain mice, control 30, 100 or 300 mg/kg/day for 78 weeks. GUIDELINES.	Liver tumors in both sexes. Doses considered adequate in both sexes.
Mouse Study #2. 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).	Liver Tumors in both doses. Hemangioendothelial sarcoma in high and possible low dose group. Doses considered excessive.
Mouse Study #3. NCI Blue Book Study, 1979. B6C3F1 strain mice. Control,1036 and 2804 ppm for 112 weeks. SUPPLEMENTARY	Lacrimal gland tumors have increased trend in males. Dose levels excessive in initial phase of study.