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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

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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.

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 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)	Weeks 1-4	5	5-26	27-52	53-78	79-105[f]	Total
0[#]	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.

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

[f]Final 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 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)	Weeks 1-4	5	5-26	27-52	53-78	79-107[f]	Total
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.

[f]Final 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)

	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

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 $p < 0.01$.

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)	Weeks 1-26	27-52	53-68	69-79[f]	Total
0[#]	2/120	5/118	13/113	20/100	40/120 (33)[n]
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.

[n]Negative trend.

[f]Final 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)	Weeks 1-26	27-52	53-65	66-80[f]	Total
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.

[f]Final 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)

	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 - Charles River CD-1 Mouse Study

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 $p < 0.01$.

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