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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: February 10, 1982

SUBJECT: Permethrin Oncogenicity Evaluation: FMC-Mouse II Study
Liver and Lung Pathology Findings in Females

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Lung and liver pathology for individual female animals in the FMC-Mouse II study summarized and analyzed in this report have been taken from the EPL report of Task Number 44b which is dated February 23, 1981. The dates of death for each animal were taken from the original FMC report of the subject study amended for 5 or 6 animals where the date of death was verified by telephone conversation of Litt and A. Gross (EPA) with FMC personnel.

The proportion of liver (A) and lung (B) carcinoma bearing female mice is summarized in Table 1 as are those for adenoma and/or carcinoma in Table 2. These summaries partition the deaths into those occurring during the first year, months 13-18, months 19-21 and months 22-24 and for the animals examined after the final, or planned, kill. The dose/response trend during each of these intervals; the cumulative time-adjusted dose/response for animals dying during the entire feeding period and separately for the complete set of data was computed using Peto's prevalence method. This is a test of the Null hypothesis that there is no difference in response rates among the 4 dose groups or that the response rate decreases as dose goes up against the alternative that the response rate increases as the dose of permethrin increases (Annex to Supplement 2 of the IARC Monographs, 1980). This procedure adjusts for time to death for events which are not definitely fatal.

If time is to be used as a factor for increasing the sensitivity of statistical analyses of dose-response relationships, it is necessary that the stage of tumor development at detection, or diagnosis, should be comparable for all animals in the study. As it is not (at this time) possible to detect cancer at the moment or even the day of initiation some other point in time such as the day that skin tumors are first seen, or the day when mammary tumors can first be palpated or the day of death must be used. But day of death from tumor or other natural causes during the study differs from death at planned kills, such as after end of study period, due to fact that planned kills forshorten (or statistically censor) the lifetime of a large percentage of study animals. Moreover even when animals die during the course of study, many animals die with tumors that did not cause their death. Thus the more fatal the tumor type and the shorter the interval between initiation and expected death due to tumor, the more closely will time to death approximate time to tumor. In carcinogenicity studies it is customary to refer to the total proportion of study animals examined who exhibit the tumor of interest as the two year incidence rate for that tumor. The following definitions of incidence and prevalence are quotations from MacMahon, Pugh and Ipsen's first (1960) edition of 'Epidemiologic Methods':

> "The incidence rate of a disease is defined as the number of cases of a disease that come into being during a specified period among a specified unit of population.
>
> Period prevalence consists of the point prevalence at the beginning of a specified period of time plus all cases that arise during the period. Period prevalence is a particularly complex measure, since both point prevalence and incidence are incorporated in it."

Using these definitions one will observe that incidence rates are computed using the number of animals (or persons) at risk of the event during a stated interval while prevalence rates are based only upon the number (of animals or persons) examined for the condition during the interval. Thus tumors diagnosed as the result of planned, interim or terminal, kills are appropriately analyzed by prevalence methods. Tumors occurring during study which did not or probably did not kill the tumor bearing animals should also be more appropriately analyzed by prevalence methods. Fatal tumors observed during the course of study may be analyzed by, the more conservative, life-table methods for incidence rates. Regardless of the tumor type the meshing of the proportion of tumor-bearing animals detected at planned kills and those detected following unplanned deaths (and/or morbid deaths) may be appropriately accomplished using prevalence procedures.

Tables 1 and 2 can also be used to evaluate survival among the females in the study: during the first year survival was higher among controls and low dose females than among the mid- and high-dose groups; the pattern is slightly modified during months 13-18 as the low dose group appears to be doing best. During the last 6 months there is an increased death rate in all groups but most markedly among control animals. At the end of study there is a borderline statistically significant difference between control and low dose animals, i.e. Yates Corrected-Chi Square is 2.9 for a two-tailed P = .087. However as control and high dose have identical 2 year survival rates it is sufficient to note that the null hypothesis of no difference in survival rates among all 4 groups cannot be rejected as $P \leq 0.20$ for the entire 2x4 contingency table.

The carcinogenicity findings displayed in Table 1 demonstrate no evidence of an increase in permethrin related liver carcinoma. There is however an increase in the dose/response trend for lung carcinomas during the last three months of study (P=0.002). This is the primary source of the during study dose/response effect of P=0.01 and for the total study significance of P=0.006. As convincing as the lung carcinoma findings may be the addition of the adenoma bearing animals to those with carcinoma further clarify the pattern of the oncogenicity demonstrated in the data and the relevant statistics in Table 2. The lung findings increase with permethrin dose and with time. And a high level of statistical significance noted during months 19-21 of the experiment is maintained during the last 3 months and increased by several orders of magnitude by the final kill. These consistent and reliable estimates of P<.001 during the study and P<0.0001 for the entire study are useful for making inferences about the oncogenic potential of Permethrin in mice.

Data from studies of permethrin metabolism have indicated that the liver is a target organ in that enzyme induction occurs and liver weights increase both absolutely and relative to body weight. These phenomena may alter the dose/response for any type of tumor but will most certainly effect the time and dose level at which liver tumors appear. The pattern of the liver adenoma and/or carcinoma findings in mouse II is less consistent then was the case for lung tumors. The dose/response relationship at the final kill ($P=3.98 \times 10^{-11}$) is diluted almost 50% for the entire study ($P=5.53 \times 10^{-10}$) due to the just signficant P=0.025 finding among animals dying during the experiment. The variable levels of effect seen during the second year of study (P=0.058 at 13-18 months, P=0.49 at 19-21 and P=0.027 at 22-24 months) may be a reflecction of other changes in liver function occurring among progressively less responsive animals. Whatever the reason, the lower degree of consistency in the liver oncogenicity responses during this study indicates that the lung data are a more reliable indicator of Permethrin related oncogenicity in mice than are liver data.

Life-table analysis of liver and lung adenoma and/or carcinoma dose/response rate have also similar results showing a highly statistically signfificant dose-response and also reduced latency for the lung tumors.

Pairwise comparisons to test for increased response in treated animals at a specific dose compared to controls by Fisher's Exact Test or by Chi Square corrected for continuity by Yates correction one shown in Table 3 along with the distribution of findings selected by the branch pathologist, L. Kasza. Highly statistically significant P values are shown for increased liver adenoma and/or carcinoma, hepatocytomegaly and pigmetation of the liver among both high and mid-dosed groups. As hepatocytomegaly may be a relevant indicator of serious liver damage, the Armitage-Cochran Test for Linear Trend has been computed and the linearity component has a P=.003: the departure from linearity is not significant (P>.50) indicating that hepatocytomegaly may be expected to increase incrementally as the dose of Permethrin increases. Similar findings are shown for pairwise comparisons of lung cancer, lung adenoma and/or carcinoma and for adenomatosis. Because adenomatosis is in many ways biologically similar to lung adenoma the groups of females with adenomatosis have been examined as a single group and separately for the sub-group which have only adenoma or less advanced lung disease and also separately for animals with adenomatosis and/or more advanced lung tumors. The only partion of adenomatosis (4) which contributes new information is the sub-group who are free of more advanced disease. Here there is a significant linear trend among mice dying during study and the total data set has a linear trend which is significant at the P=.0185 level of statistical significance, (by the Armitage-Cochran test.) The increase of the mid-dose response shows boderline-statistical significance for those with adenomatosis only (P .07) both among dosing study deaths and for the entire study. However in the high dose group the statistical significance is clearly P <.01 for this condition indicating that the risk of adenomatosis may be increased by Permethrin.

From the preceeding it is concluded that the data set of female mice with lung tissues usable for pathology examination who were diagnosed as having or not having aleveolar or bronchiolar adenoma and/or carcinoma should be used for extrapolating the risks to low doses, ie 10^{-3} to 10^{-8}. This has been done by the Mantel-Bryan procedure, the Multi-Stage Model (Crump's Global 79' Program) and Rai and Van Ryzin's Multi-Hit program. Both the multi-stage and multi-hit programs indicated the one-hit as the parametric model of choice. However, as this model had a statiscally significant lack of fit, P<.05 there seems to be little mathematical basis for using parametric models which are conventionally fitted. Also because of the possible effect of chemicals induction coupled with lack of mutagenic evidence (mutagenic tests have all been negative) only the Mantel-Bryan procedure for low-dose extrapolation

remains in the current aramamentarium. Results from the Multi-Stage Model and for the Mantel-Bryan Procedure are are shown in Table 4: Virtually safe doses for mice represent the lower 95% confidence bound on expected risks of carcinoma and/or adenoma. The risks are displayed as increasing orders of magnitude. These valuses can be reinterpreted as the upper 95% bound on the risk which might be expected at the dose levels displayed. In addition to virtually safe dose levels for mice, Table 4 displays virtually safe dose levels for humans. The human VSD are computed from the mouse VSD by use of the surface area correction used by CAG and discussed by Mantel & Sneiderman (J. Cancer Research, June 1975, pg. 1385). This procedure assumes that rodents are less sensitive than are humans as indicated by the one-third power of the animal to human weight when the dosage in mg/kg/day is used for life-time dosing study in the animal species of interest. This adjustment also assumes (as do most low dose risk extrapolation procedures) that there is no operative threshold associated with the dose-response relationship being studied. To the extent that these assumptions are subject to practical proof they could overstate the true risks to man. But because cancer is such a critical condition it is imporant to protect against false negatives while looking for safe techniques to increase the size of the estimates of virtually safe dose levels. This cannot be done simply by reducing the level of the confidence bounds used as examination of the relationships between confidence limits and standard deviations shows:

99%	confidence	limits	are	computed	using	3 SD's
95%	"	"	"	"	"	2 SD's
75%	"	"	"	"	"	1.4 SD's

Thus there is little advantage to using confidence bounds at levels lower than 95%. More knowledge explaining induction, promotion and development of particular neoplasms is needed so that more informative models for extrapolation can be developed. The use of such biological criteria may also be a more fruitful way for extrapolating from mouse to man.

TABLE I

Permethrin FMC - Mouse II Carcinogenicity - Dose Relationships

Dose Levels/Study Interval (Months): Number Positive/Number Examined							
	1-12	13-18	19-21	22-24	During Study	Final Kill	Total Study
	A) Liver Carcinoma						
Control							4/74
4.05	Data Insufficient for Interim Analysis						3/66
375							3/75
750							2/74
				No Statistically Significant Effects			

Dose Levels/Study Interval (Months): Number Positive/Number Examined							
	1-12	13-18	19-21	22-24	During Study	Final Kill	Total Study
	B) Lung Carcinoma						
Control	0/6	2/12	2/15	0/19	4/52	2/22	6/74
4.05	0/7	1/9	0/10	3/12	4/38	3/24	7/72
375	0/11	0/13	4/9	4/16	8/49	3/25	11/74
750	0/9	3/14	1/14	7/16	11/53	4/22	15/75
TOTAL Obs.	0/33	6/48	7/48	14/63	27/192	12/103	39/295
D (O-E)	0	326.5	220.7	2919.4	3466.5	1069.9	4536.4
Variance	0	728.8	806.3	1033.3	1499.7	984.3	1793.8
$Z = \frac{D\ (O-E)}{\sqrt{Variance}}$	0	0.45	0.27	2.825	2.3115	1.087	2.529
P =	0	~.3	~.4	~.002	.01	.14	.006

Z - Statistic shown is the one-tail test of Time-Adjusted Trend using Peto's Prevalence Method (Supplement 2 to IARC, 1980)

TABLE II

Permethrin FMC Mouse II - Oncogenicity Relationships

Dose Levels/Study Interval (Months): Number Positive/Number Examined							
	1-12	13-18	19-21	22-24	During Study	Final Kill	Total Study
	Liver Adenoma and/or Carcinoma (No. Obs./No with Tumor)						
Control	0/5	0/13	1/15	3/19	4/52	2/22	6/74
4.05	0/5	0/7	3/10	1/10	4/32	3/24	7/66
375	0/11	2/3	3/9	8/17	13/50	12/25	25/75
750	0/9	2/3	2/14	8/16	12/52	19/22	31/74
TOTAL Obs.	0/30	4/40	9/48	10/62	33/186	36/103	69/289
D (O-E)	0	1009.66	23.29	3065.97	4,098.92	9,424.17	13,523.1
Variance	0	640.98	1193.63	1590.31	2,089.18	1,449.42	2218.95
$Z = \frac{D\ (O-E)}{\sqrt{Variance}}$	0	1.575	0.0195	1.927	1.962	6.502	6.094
P =	0	(0.058)	(0.492)	(0.027)	0.025	3.98×10^{-11}	5.53×10^{-10}
	Lung Adenoma and/or Carcinoma (No. Obs./No with Tumor)						
Control	1/6	4/12	3/15	3/19	11/52	4/22	15/74
4.05	1/7	2/9	2/10	5/12	10/38	14/34	24/72
375	2/11	4/13	4/9	8/16	18/49	17/25	35/74
750	1/9	5/14	9/14	10/16	25/53	19/22	44/75
TOTAL Obs.	5/33	15/14	18/48	26/63	64/192	54/103	118/295
D (O-E)	-146.4	439.22	3039.41	3075.20	6407.34	7049.83	13,456.87
Variance	631	1021.7	1105.6	1225.5	2041.1	1567.6	2,573.6
$Z = \frac{D\ (O-E)}{\sqrt{Variance}}$	-0.232	0.43	2.749	2.509	3.139	4.497	5.23
P =	(0.59)	(0.33)	(0.003)	(0.006)	8.48×10^{-4}	3.45×10^{-6}	8.49×10^{-8}

Z = Statistic shown is the one-tail test of Time-Adjusted Trend using Peto's (Supplement 2 to IARC, 1980) Prevalence Method.

TABLE III. Permethrin Mouse II

Distribution and Pairwise Comparisons of Selected Liver and Lung Pathology Findings

Findings During Study Proportion TBA				P Values		Diagnostic Category	Findings During & After Study P Values		Findings During & After Study Proportion TBA			
750	375	4.05	0	0 vs 375	0 vs 750		0 vs 375	0 vs 750	0	4.05	375	750
						Liver						
12/52	13/50	4/32	4/52	0.013	0.026	Adenoma and/or carcinoma	<.0002	< .0001	6/74	7/66	25/75	31/7
				-----	-----	Hepatocytomegaly	< .015	0.0015	0/74	3/66	6/75	9/74
				-----	-----	Pigmentation	0.00015	0.0019	18/74	19/66	41/75	36/74
						Lung						
4/52	4/38	8/49	11/53	NS(.15)	.05	Carcinoma	NS(.15)	0.032	6/74	7/72	11/74	15/75
11/52	10/38	18/49	25/53	< .07B	<.005	Adenoma and/or carcinoma	0.0005	<.00001	15/74	24/72	35/74	44/75
0/52	3/38	5/49	9/53	0.024*	0.0015*	Multifocal Adenomatosis	0.025	<.00001	3/74	5/72	11/74	13/75
0/41	3/28	3/30	7/28	0.07B*	0.001*	MA Only	0.07B	<.006	3/59	4/48	6/38	8/31
0/11	0/10	2/19	2/25	NS(0.40)*	NS(0.50)*	MA + AD or CA	NS(.16)*	NS(.26)*	0/15	1/24	5/36	5/44

All P Values are 1 Tail tests *indicates Fishers Exact Test, all others Chi-Square with Yates Correction

•B indicates, Borderline significance 0.1 > P > .05
NS indicates, P value significance at P > 0.10 therefore events may be due to change
TBA refers to Tumor Bearing Animals

TABLE IV. Permethrin Mouse II

Risk Assessment Based on Lung Adenoma and/or Carcinoma

Relevant Data Dose:	0	4.05	375	750
#TBA/# Examined:	15/74	24/72	35/74	44/75

Multi-Stage Model

Virtually Safe Dose (Lower 95% CB)		Expected Cancer Rate or Attributable Level of Risk
Mice (mg/kg/d)	Humans*	
5.88×10^{-6}	4.66×10^{-7}	1×10^{-8}
5.88×10^{-5}	4.66×10^{-6}	1×10^{-7}
5.88×10^{-4}	4.66×10^{-5}	1×10^{-6}
5.88×10^{-3}	4.66×10^{-4}	1×10^{-5}
5.88×10^{-2}	4.66×10^{-3}	1×10^{-4}
5.88×10^{-1}	4.66×10^{-2}	1×10^{-3}

Equation Produced By Multihit Model is Equal to a One Hit Model:
Q(0) 0.332
Q(1) 0.001054
Q92) 0.0000

Goodness of Fit of Model to data $P < .05$

Mantel-Bryan Procedure

Vitrually Safe Dose (Lower 95% CB)	
Mice	Humans*
1.22×10^{-3}	9.68×10^{-5}
3.16×10^{-3}	2.51×10^{-4}
8.82×10^{-3}	7.00×10^{-4}
2.71×10^{-2}	2.15×10^{-3}
9.54×10^{-2}	7.57×10^{-3}
4.06×10^{-1}	3.22×10^{-2}

VSD for Humans based on the surface area correction of 12.6
e.g. $1.22 \times 10^{-3} \times 1/12.6 = 9.68 \times 10^{-5}$
$12.6 = (60{,}000 \text{ mg}/30 \text{ mg})^{1/3}$