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

OFFICE OF  
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: June 24, 1981

SUBJECT: Review of Additional Information Pertaining to the Permethrin  
Chronic/Oncogenicity Rat Study Submitted by FMC Corporation  
CASWELL#652BB Acc. Nos.#097421, 097419, 097418, 099964

FROM: Gary J. Burin, Toxicologist  
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TO: Chris Chaisson, Acting Branch Chief  
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Background Information:

On June 15, 1977, the FMC Corporation submitted a combined chronic/oncogenicity study of Permethrin conducted with Long-Evans rats to the EPA. This study was originally reviewed by Martha Panitch on March 30, 1978 (see Section I of Discussion, below, for a summary of that review). Dr. Panitch noted an increase in alveologenic tumors in treated animals compared to controls and stated that "An oncogenic effect appears to be present, but of low potency". However, she classified the study as "Supplementary Data" based on her finding that an unacceptable number of tissue masses were not accounted for by gross or histologic examination.

Subsequent additional pathologic evaluations were performed and submitted by FMC Corporation:

1. Additional tissues were examined from masses and gross lesions that were not originally examined histologically. Slides were read by Dr. Billups of Environmental Pathology Services (Acc. No. 097421).
2. Lung tissue slides were re-examined by Dr. William Busey of Experimental Pathology Laboratories (Acc. No. 097419).
3. Wet tissues of the lung were step sectioned and slides of the step-sectioned lungs were evaluated by Dr. Billups (Acc. No. 097418).

Thus, three separate examinations of the lung occurred - Drs. Busey and Billups each examined the same set of tissues and submitted separate reports and Dr. Billups examined step-sectioned lungs and submitted an additional (third) report.

In addition, FMC Corporation independently compiled and submitted the following:

4. Gross-Histological correlation of findings based on the updated histological report from Dr. Billups (Acc. No. 099964).

On January 5, 1979, S.L. Chan included the findings of the aforementioned materials in his review of Permethrin petitions 8F2024 and 8F2044. He concluded that the study should now be upgraded to "Core-Minimum" status and that, based on the findings of step-sectioned lungs, that "an oncogenic potential for Permethrin is ruled out in the rat". His review of the step-sectioning was brief, consisting only of the following paragraph:

"Since step-sectioning of the lung represents a much more thorough approach, results from this procedure should be viewed as the answer to this issue. It is seen that there is no significant increase in alveogenic tumors between the control and treated male rats. However, it is a little surprising that many more tumors were uncovered by this procedure."

Finally, per the suggestion of Chris Chaisson, Acting Branch Chief, this reviewer has re-examined the additional materials submitted by FMC Corporation in response to the concerns initially raised by Dr. Panitch. The following is a summary of the findings of this re-examination.

### Conclusions

This reviewer has examined the additional submissions (cited above) and has concluded the following:

1. The incidence of alveogenic lung tumors is significantly elevated in males treated with Permethrin compared to untreated males ( $p = .016$ ).<sup>1</sup> No tumor type other than alveogenic lung tumors is significantly elevated in either sex, although trends were noted for pheochromocytomas in males and alveogenic lung tumors in females (See Section II. of Discussion).
2. The procedure used in the step-sectioning of lung involves a serious bias which precludes its use in the assessment of the incidence of alveogenic lung tumors. This bias probably leads to an underestimation of the number of tumors found in the step-sectioned lungs of Permethrin treated animals (See Section III. of Discussion).

<sup>1</sup>Fisher's exact test based on animals surviving to final sacrifice. All alveogenic lung tumors in males were found at final sacrifice.

3. The confirmation of tissue masses found at the final in-life palpation through gross necropsy and histologic observation, although neither complete nor thorough, is minimally satisfactory for the purposes of this study. The follow-up of gross lesions through histologic examination can be considered to be satisfactory. This study should thus be considered "Core Minimum" (See Section IV. of Discussion).

## Discussion

- I. Study Summary (Abstracted from the M. Panitch review of March 30, 1978): Dose levels of 0, 20, 100 and 500 ppm of Permethrin were administered, in the diet, to 60 male and 60 female rats per dose level. Corn oil was used as a vehicle for the first 19 months and acetone was used for the remainder of the study. Ten male and 8 female Long-Evans 100 ppm animals were sacrificed after 1 year. Behavior was monitored daily and animals were palpated weekly.

Food consumption was measured weekly for 14 weeks, biweekly until week 26 and monthly to the end of the study. Ophthalmologic examination occurred at 3, 6, 12, 18 and 24 months.

Hematology, blood chemistry and urinalysis was performed after 6 months and at termination.

Gross and histopathology examinations were performed on all animals.

Periodic diet analyses confirmed target dose levels with means of 18.5, 93.3 and 483.7 ppm found in samples of the 20, 100 and 500 ppm diets.

The only behavioral effect noted was tremors in two high dose females. Mortality patterns, body weights, food consumption, urinalysis, ophthalmology and hematology did not appear to be effected by treatment.

Total blood protein, albumin and A/G ratio were reduced, to varying degrees, in 100 or 500 ppm females. The 12th month measurement of alkaline phosphatase indicated a decrease among all female treatment groups although the high dose females were not significantly effected. Terminal bone marrow smears found significantly elevated segmented neutrophil counts in 20 and 500 ppm females but not in females of the 100 ppm dose group. Absolute liver weights were significantly increased in 100 and 500 ppm males and 500 ppm females. Ovarian weight was significantly elevated among 500 ppm females. No compound related gross or histopathology was noted, although a number of tumor types were noted in treated but not control animals (including alveologenic adenomas and carcinomas). The review concluded that "an oncogenic effect appears to be present, but appears to be of low potency."

It also was concluded that the study was Supplementary Data due to the lack of "reports of histology for each gross lesion." A systemic No Effect Level of 20 ppm was recommended based on the increased liver weights found in 100 and 500 ppm males.

## II. Review of Additional Histopathology Reports

Subsequent to the M. Panitch review, an additional histopathology evaluation (Acc. No. 097421) was submitted by the registrant, who noted that this second report superceded the first and that it contained "information on additional tissues which had been processed histopathologically and corrections of some transpositional and typographical errors."

A summary of the revised tumor incidence, based on this second pathology report, has been complied by this reviewer and is present below.

Tables I-IV are the revised incidences of the most common tumors in this study. Tables V and VI are the revised overall incidences of tumors found in males and females. Table VII presents the incidence of lung tumors diagnosed by Dr. William Busey of Experimental Pathology Laboratories (Acc. No. 097419). It is noted that Table II suggests a trend in the occurrence of alveologenic tumors in females although the comparison of treated and untreated animals did not find the difference to be statistically significant. Chromophobe adenomas are slightly elevated in treated females, but not to an extent that is considered statistically or biologically significant. Endometrial polyps, were somewhat elevated among treated females. It is also noted that adrenal pheochromocytomas are elevated in treated males though the comparison with untreated males is not statistically significant (Table IV).

Among treated males, alveologenic tumors are significantly increased based on the diagnoses of both Drs. Billups and Busey (Tables III, IV, VI),  $p = .041$  and  $p = .016$ , respectively. All alveologenic tumors in males, and all but two of those found in females, were discovered at terminal sacrifice, suggesting that this type of tumor is late appearing. As Dr. Busey notes in his report, this type of tumor is infrequently encountered in the rat. Benirschke et al<sup>2</sup> has noted the rarity of lung tumors in rats and cites several extensive studies that have reported a very low incidence, giving further support to the hypothesis that the increase of this type of tumor found in this study is biologically as well as statistically significant.

<sup>2</sup>Benirschke, K. Garner, F.M., Jones, T.C. Pathology of Laboratory Animals, Vol. II, Springer-Verlag, New York, 1978, pp. 1081-1082.

TABLE I

Proliferative Lesions among  
Female Terminal Sacrifices

	<u>Control</u>	<u>20 PPM</u>	<u>100 PPM</u>	<u>500 PPM</u>
Adrenal Phenochromocytoma (including invasive)	1/36	1/35	2/37	0/38
Alveologenic Adenomas and Carcinomas	1/36	0/36	1/37	1/39
Islet cell adenomas of the Pancreas	3/36	0/36	2/37	1/39
Endometrial Polyp	1/34	4/36	5/36	3/38
Chromophobe Adenoma of the Pituitary	17/33	22/36	27/36	22/39
Fibroadenoma of the mammary	14/36	8/36	9/34	9/38

TABLE II

Proliferative Lesions among  
All Females

	<u>Control</u>	<u>20 PPM</u>	<u>100 PPM</u>	<u>500 PPM</u>
Adrenal Phenochromocytoma (including invasive)	2/58	2/54	2/49	0/56
Alveologenic Adenomas and Carcinomas	1/60	0/58	2/58	2/58
Islet cell adenomas of the Pancreas	3/56	1/53	2/52	1/54
Endometrial Polyp	2/57	5/58	6/56	4/55
Chromophobe Adenoma of the Pituitary	31/55	36/59	38/57	34/57
Fibroadenoma of the mammary	17/58	10/57	13/56	13/57

5

TABLE III

Proliferative Lesions among  
Male Terminal Sacrifices

	<u>Control</u>	<u>20 PPM</u>	<u>100 PPM</u>	<u>500 PPM</u>
Thyroid Adenoma	3/41	1/36	4/39	1/35
Adrenal Pheochromocytoma (including invasive)	1/42	3/37	2/39	4/37
Alveologenic Adenoma & Carcinoma	1/43	3/37	6/39	5/38
Islet cell adenoma of the Pancreas	2/43	6/37	3/39	4/37
Chromophobe Adenoma of the Pituitary	23/43	19/35	17/38	13/33
Skin Papilloma	0/41	2/36	4/37	1/36
Skin Fibroma, subcutaneous	5/41	1/36	2/37	0/36

TABLE IV

Proliferative Lesions among  
All Males

	<u>Control</u>	<u>20 PPM</u>	<u>100 PPM</u>	<u>500 PPM</u>
Thyroid Adenoma	3/53	1/45	6/44	2/48
Adrenal Pheochromocytoma (including invasive)	1/57	3/57	2/45	5/53
Alveologenic Adenoma & Carcinoma	1/59	3/57	6/57	5/56
Islet cell adenoma of the Pancreas	3/55	7/54	4/54	5/52
Chromophobe adenoma	27/56	25/53	21/52	21/50
Skin Papilloma of the Pituitary	1/56	2/56	4/55	1/56
Skin Fibroma, subcutaneous	7/56	1/56	2/55	0/56

Total Numbers of Animals with Neoplasm  
Number of Animals (Males)

TABLE V

	<u>Control</u>	<u>20 ppm</u>	<u>100 ppm</u>	<u>500 ppm</u>
a. with single neoplasm	26	20	23	22
b. with multiple neoplasms of the same histo. type	0	0	0	1
c. with multiple neoplasm of different histo. types	20	18	14	16
d. a+b+c	46	38	37	39

TABLE VI

Number of Animals (Females)

	<u>Control</u>	<u>20 ppm</u>	<u>100 ppm</u>	<u>500 ppm</u>
a. with single neoplasm	31	31	26	27
b. with multiple neoplasms of the same histo. type	0	1	0	3
c. with multiple neoplasm of different histo. types	18	16	21	17
d. a+b+c	49	48	47	47

TABLE VII

Alveologenic Tumors Found by Dr. Busey

	<u>Control</u>	<u>20 ppm</u>	<u>100 ppm</u>	<u>500 ppm</u>
Males	1 (60)* (45)**	3 (57) (37)	8 (57) (39)	6 (58) (39)
Females	1 (60)	1 (60)	1 (59)	2 (59)

\* (Total number examined)

\*\* (Total number of terminal sacrifice animals examined)

7



III. Review of Acc. No. 09748, Step-Sectioned Lungs of Rats from Biodynamics Study No. 74-R-1022 (NOTE: This review was written prior to the meeting of June 9, 1981 between registrants of Permethrin and the EPA. The review of the detailed step-sectioning procedures (requested by EPA at that meeting) will be dealt with in the form of an addendum to this memo after the requested material is submitted.)

In an attempt to provide a more thorough examination of the change of the lung suggested in the initial histopathology report of this study, additional sectioning of lung tissue was undertaken. This step-sectioning was performed by American HistoLabs, Inc. of Silver Spring, Maryland and slides were read by Environmental Pathology Services of Rockville Maryland. Tissues were supplied by the laboratory which performed the in-life and necropsy phases of this investigation, Bio/dynamics Inc. of East Millstone, New Jersey.

The following procedure was reported to have been followed in the step-sectioning investigation:

"All remaining tissue from lungs of male rats from a two-year oral feeding study of FMC Compound 33297 (Final Report submitted August 22, 1978 to FMC Corporation) was processed by American HistoLabs, Inc., Silver Spring, Maryland. Sections of lung were cut at a thickness of 6 micrometers from both paraffin blocks and wet tissues from right and left lungs. Sections were cut at 250 micrometer intervals until the tissue was depleted. Slides were prepared, and odd-numbered step-sections were stained with hematoxylin and eosin. These slides were examined only for proliferative lesions."

In cases where a proliferative lesion was suggested upon examination of the odd-numbered step-sections noted above, even-numbered step-sections were also stained and examined. The effect of this procedure was to allow a more thorough observation of cell and tissue morphology in up to 10 intervals of each lobe of examined lung with additional intervals available if a suggestion of a proliferative lesion warranted further investigation.

However, a serious bias was introduced into the quantification of observed tumors resulting from an unequal number of sections being examined from each of the four groups on test.

The control group clearly was examined extensively, other groups were examined to a much lesser degree. The control group averaged 24.63 sections examined per animal, the 20, 100 and 500 ppm averaged 18.25, 14.46 and 16.56 ~~slides~~ <sup>sections</sup>, respectively. Findings for each sections of lung tissue are reported in columns which apparently correspond to various lobes of the lung. An examination of areas of the lung examined further illustrates the bias - all but one control animal had more than two lobes of the lung examined, 25/57 of the 20 ppm group, 36/61 of the 100 ppm group and 32/59 of the 500 ppm group had only two lobes of the lung examined. No explanation was offered for this apparent skewing in the extent of examination of animals treated with FMC 33297 compared to control animals. However, assuming that the probability of discovering a tumor is related to the extent to which the tissue is examined, it is clear that proportionally

more tumors would be expected to be found in the control group compared to treated animals as a result of this bias in this step-sectioning procedure. If an attempt was made to correct for the bias and the results from step-sectioning were limited to the lobes designated as the right and left lobes of the lung (which were examined to a similar extent in treated and control groups) and the findings for lobes A, B&C are disregarded, the number of alveologenic tumors discovered by step-sectioning would be 1, 4, 9 and 8 for the control, 20, 100 and 500 ppm males, respectively.

In conclusion, It is recommended that a risk assessment of the carcinogenic potential of FMC 33297 not be based upon the findings of the step-sectioned lungs as those findings contain an inherent bias which renders them unacceptable. Previous histopathology examinations, by Dr. William Busey of Experimental Pathology Labs and Dr. Leonard Billups of Environmental Pathology Services, are likely to be a more accurate comparison of the prevalence of alveologenic tumors. It is recommended that either of these two histopathology reports serve as the basis for the oncogenic risk assessment of Permethrin in Long-Evans rats.

IV. Comparison of In-Life Tissue Masses, Gross Autopsy Findings and Histopathology Acc. No. 099964

In response to the EPA request for a detailed audit of in-life palpable masses observed prior to final sacrifice and corresponding gross and histological findings, FMC has compiled an animal-by-animal accounting of masses and gross and microscopic findings which might explain each observed mass. In addition, FMC has attempted to correlate all gross lesions with histological findings.

A review of the in-life mass/gross/histological comparison reveals that even if all tissue mass/gross observation associations proposed by FMC are accepted, only 72.1% of all palpable masses found during the last in-life examination were accounted for during gross observation, leaving 27.9% of all masses unaccounted for during gross necropsy and thus probably not followed up microscopically. It is clear that if a significant number of these were associated with malignant or benign tumors, the number of tumors diagnosed in this study may be seriously underestimated. However, further examination of the characteristics of the for masses that were not accounted for during gross necropsy indicates that this is probably not the case.

Five male and five female animals from each dose group were sacrificed and necropsied at the Mayo Clinic where special neurological studies were conducted on those animals. Of the 138 masses which, according to FMC, were not accounted for during gross necropsy, 44 were found in animals sacrificed at the Mayo Clinic. In addition, a spontaneous disappearance of a small number (5-10%) of masses might be expected.

Of the palpable masses that were unaccounted for during gross necropsy in animals necropsied at Biodynamics, the distribution among control and treated males was similar (13, 8, 9 and 7 in the 0, 20, 100 and 500 ppm groups, respectively). Among females a somewhat greater number of masses was found in treated groups compared to controls (11, 24, 23 and 12 in the 0, 20, 100 and 500 ppm groups respectively).

The second aspect of the FMC audit concerned the microscopic follow-up of tissue with gross lesions. An examination of this data found a large percentage (94.7%) of tissues with gross lesions that were, in fact, examined microscopically. Furthermore, the gross/microscopic relationships presented by FMC and those performed independently by this reviewer suggest that, although the correlation is not as exact as might be desired, the extent of a lack of correlation does not seriously compromise this study. The results of the correlation performed by this reviewer for 100 ppm females are presented in the final table, Table VII.

cc: A. Gross  
L. Kazsa  
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Animal #

Gross. Path

Histopath.

Table VIII, p. 1

Animal #	Gross. Path	Histopath.		
419	R. Thyroid Pituitary	Enlarged Mass	Adenocarcinoma, thyroid Chromophobe adenoma	+ +
421	R. Lung Pituitary	Mass Mass	Multiple abscess Chromophobe adenoma	+ +
423	Spleen		Lymphosarcoma	+
427	R. Base of Tail Pituitary Ventral FLL	Mass Mass Mass		- + +
428	Pituitary Tissues	Mass Black, soft	Chromophobe adenoma Autolysis	+ +
445	Pituitary	Mass	Chromophobe adenoma	+
447	L. Adrenal Pituitary	Dark brown, irregular shape Mass	Congestion Chromophobe adenoma	+ +
452	Pituitary	Mass	Chromophobe adenoma	+
453	Lung L. Axilla	Mass Mass	Alveologenic adenoma Fibroadenoma, mammary	+ +
455	Spleen Lung Liver	Mass L. Lobe consolidated, nodules Mass	Foreign body, bronchopneumonia Fibrosis, necrosis	- + +
460	A-G. area Pituitary	nodules Black & white	Squamous cell carcinoma Chromophobe adenoma	+ +
463	R. Kidney <del>Pituitary</del> Pituitary	Granules Mass	Glomerulonephrosis Chromophobe adenoma	+ +
464	Pituitary	Mass	Chromophobe adenoma thymic hyperplasia	+ +

Animal #	Gross Path.	Histopath.	
449	R. Ovary Yellow Nodule L. Inguinal Mass	Fibroadenoma of mammary	+
450	R. Axilla Mass	Fibroadenoma of mammary	+
	L. Inguinal Mass	"	+
	L. Lateral Abdomen Mass	"	+
	R & L Uterine Horn Nodules	Endometrial polyp and mesovarium abscess	±
451	R. Anterior ILL Mass	Fibroadenoma, mammary	+
	Pituitary Mass	Chromophobe adenoma	+
455	R. Inguinal Mass		-
	Pituitary Red, soft	Chromophobe adenoma	+
458	Pituitary Black		-
459	Pituitary Black, soft		-
	Nodule Red and white, smooth hard	Squamous skin papilloma	+
461	R. Horn of Uterus Mass	Keratin cyst	+
	Mass	Squamous cel. carcinoma	+
462	R. Axilla Mass		-
465	Mammary tissue Dark brown & firm		-
	Pituitary Black, ground & firm	Chromophobe adenoma	+
466	R. Inguinal Mass	Ductal dilatation (?)	+
	R. Axilla Mass	Fibroadenoma, mammary	+
	Pituitary Mass	Chromophobe adenoma	+
467	Pituitary Mass	Chromophobe adenoma	+
	Vagina Mass	Squamous papilloma	+
	L. Abdomen Mass	Multifocal hematocyst, abdominal cavity	+

410	R-Uterus	Mass	Pyometra	+
	Pituitary	Mass	Chromophobe Adenoma	+
411	L. Axilla	Mass	Ductal dilatation (4)	+
	Horn of Uterus	Focal Enlarg. & Growth	Squamous metaplasia, endometrial (2), cervicitis	+
	Adrenal L & R	Purple	Bilateral congestion & Pheochromocytoma	+
412	Pituitary	Mass	Hemosiderosis	-
413	L. Lateral thorax	Mass	Fibroadenoma of mammary	+
	R. Lateral thorax	Mass	Ductal dilatation (2)	+
	Stomach	Peduncle		-
	Pituitary	Mass	Chromophobe adenoma	+
414	Pituitary	Mass	Chromophobe adenoma	+
416	Perineal area	Numerous Yellow firm nodules	Nematodiasis	-
417	R. Adrenal	Mass	Hematocyst	+
	R. Lateral thorax	Mass	Fibroadenoma of mammary	+
	Pituitary	Mass	Chromophobe adenoma	+
418	L. Perineal	Mass	Epidermal inclusion cyst	+
	Uterus	Mass	Endometrial polyp	+
425	Kidney	Pitted, red with clear, cyst-like areas	Glomerulonephrosis	+
	R. Axilla	Mass	Fibroadenoma of mammary	+
	Pituitary	Mass	Chromophobe adenoma	+
430	Uterus	Mass	Endometritis (3), diffuse	+
	L. Perineal	Mass	Fibroadenoma of mammary	+
	L. Inguinal	Mass	Adenoma of mammary Papillary	+
	Pituitary	Mass	Chromophobe adenoma	+

Animal #	Gross Path.	Histopath.	
431	L. Vagina Mass	Ductal dilatation, gland <sup>cl. tonsy</sup>	+
	L. Kidney Cystlike structure	cuticle cyst	+
432	Pituitary Nodule	Chromophobe adenoma	+
434	R. Anterior Vagina Mass		-
	Uterus Nodule	Endometritis	+
	Pituitary Mass	Chromophobe adenoma	+
435	R. Uterus Mass	leiomyoma	+
	R. lateral Thorax Mass	Ductal dilatation, skin	+
	Mammary Nodular growths	Multifocal granulomas	+
439	L. Axilla Mass	Fibroadenoma of mammary	+
	Pituitary Mass	Chromophobe adenoma	+
	Base of tail Mass		-
441	Adrenal Enlarged foci		-
	Pituitary Mass	Chromophobe adenoma	+
443	L. Inguinal Mass	Ductal dilatation (4)	+
	R. Inguinal Mass	Papillary adenoma, mammary	+
	R. Perineal Mass		-
448	R. Inguinal Mass	Ductal dilatation, mammary	+
	L. Inguinal Mass	"	+
	R. Axilla Mass	"	+
	Mass	mastitis, chronic	+
	Mass	adenoma, papillary, gland <sup>mammary</sup>	+