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

AUG - 8 1986

005336

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: FMC 54800 (BIFENTHRIN)

TO: Mr. George LaRocca, PM 15
Registration Division (TS-767C)

FROM: Byron T. Backus, Toxicologist Byron T. Backus 7/28/86
Toxicology Branch (TS-769C)

THROUGH: Marcia van Gemert, Ph.D.
Section Head, Review Section III M. van Gemert 8.5.86
Toxicology Branch (TS-769C)

and

Theodore M. Farber, Ph.D.
Branch Chief
Toxicology Branch (TS-769C) 8/8/86

EPA Reg. No. 279-3055
EPA I.D. Nos. 5G3235, 5G3238, 5G3289, 6G3313, 6G3315, 6G3314,

Project Nos. 1397, 1391, 1392, 1393, 1394, 1395, 1396

Tox. Chem. 463F

Action Requested:

The Registration Division has requested a toxicology review of a 2-year rat chronic feeding/oncogenicity study, a rat reproduction/2-generation study, and a mouse oncogenicity study. The mouse oncogenicity study constitutes 6(a)(2) data as it indicates that FMC 54800 is oncogenic in this mammalian species.

Conclusions and Recommendations:

1. The mouse oncogenicity study has been classified as core minimum data. FMC 54800 was dietarily administered at 0, 50, 200, 500 and 600 ppm for 87 weeks to males and 92 weeks to females. Conclusions are the following:

   a. The test material has been demonstrated to be oncogenic. Incidences of leiomyosarcomas of the urinary bladder in male mice were 2/48, 6/50, 8/50, 7/50 and 14/49 at 0, 50, 200, 500 and 600 ppm respectively. While incidences of this tumor were elevated in all male groups exposed to FMC 54800 (and the P value associated with positive trend was 0.00053) only the incidence at 600 ppm was significantly (with $p < 0.01$) different from that of controls.

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b. Incidences of combined bronchioalveolar adenocarcinomas and adenomas in the lungs were 14/50, 26/50, 23/50, 19/50 and 23/48 for females at 0, 50, 200, 500 and 600 ppm respectively. Incidences were significantly different ($p < 0.05$) at 50, 200 and 600 ppm (and elevated, but not significantly so, at 500 ppm) relative to the controls. Time-to-tumor tests for positive trend and heterogeneity were not significant ($p = 0.108$ and 0.131 respectively). However, in a number of mouse studies conducted with Permethrin (structurally similar to this compound) there were similar significantly increased incidences of these tumors in females (see the attachment to this memorandum consisting of an excerpt from Permethrin - Assessment of Chronic and Oncogenic Effects - A Summmary relating to the FMC mouse II study conducted at Bio/Dynamics Inc. under project no. 76-1695). It is tentatively concluded that the increased incidences (38-52%) of pulmonary tumors in females receiving Bifenthrin (as compared to 28% in controls) were compound-related. The lack of a strong correlation between incidences and dietary levels of Bifenthrin in this recently conducted study may indicate a relatively low degree of slope in tumor incidences vs. dietary exposure in the range from 50 to 600 ppm.

It is noted that information (copies from the open literature) received 5-30-86 indicates high incidences of spontaneous pulmonary tumors in female Swiss-Webster mice. These data have not yet been fully evaluated.

c. The incidence of lymphoblastic leukemia was significantly increased (22/49) in highest-dose females relative to their control group (12/50). However, since combined incidence of lymphoid tumors in the 600 ppm females (23/49 or 47%) was not statistically different from that of controls (19/50 or 38%) it is concluded that occurrence of lymphoblastic leukemia in females was not related to exposure to Bifenthrin. The reported incidences for the intermediate dose groups must be regarded cautiously since in many group II, III and IV mice (particularly those sacrificed at termination) mesenteric and mediastinal lymph nodes, as well as the thymus, were not examined unless they were enlarged or otherwise obviously abnormal. It is noteworthy that in some cases (such as group IV females #2706, 2712 and 2726) diagnosis of lymphoblastic leukemia/lymphosarcoma was made from infiltrates into kidneys and lung or uterus as lymph nodes were not microscopically examined.

d. In some mouse studies conducted with Permethrin, there were indications of possible oncogenicity with respect to the liver. It is tentatively concluded that the dose-related trend of increased incidence (with $P = 0.025$) of combined adenocarcinomas and adenomas of the liver of male mice (2/49, 2/50, 4/50, 4/50 and 7/49 at

0, 50, 200, 500 and 600 ppm respectively) is directly related to exposure to Bifenthrin, even in the absence of apparent preneoplastic changes.

e. An oncogenic NOEL was not established. In the case of leiomyosarcomas of the urinary bladder in male mice, group II (lowest dose or 50 ppm) mice showed an elevated incidence of this tumor type relative to their controls. While this elevated incidence at 50 ppm was not statistically significant relative to that of controls, it was part of a well-defined dose-related trend. Group II females showed a statistically significant elevated incidence of bronchioloalveolar adenomas and adenocarcinomas relative to their controls.

2. The 2-year rat feeding/oncogenicity study has been classified as core minimum data. FMC 54800 was adminstered in the diet at 0, 12, 50, 100 and 200 ppm. Conclusions are as follows:

a. While there was no conclusive evidence of dose-related oncogenicity, incidences of pancreatic islet cell adenoma (combined sexes) were 1/97, 0/48, 0/40, 0/47 and 4/99 for 0, 12, 50, 100 and 200 ppm respectively. Incidences of fibrosarcoma in male rats were 0/50, 1/50, 0/50, 0/50 and 3/50 for 0, 12, 50, 100 and 200 ppm respectively. The registrant should supply historical control data as to the incidences of these tumor types in rats of this strain at this testing facility.

b. The 200 ppm dietary level can be accepted as being reasonably close to an MTD, based on a preliminary 28-day study in which deaths accompanied by tremors occurred at 300 ppm in 6/10 males by day 12 and 1/10 females by day 20.

c. At 100 ppm 1/50 females showed tremors for 3 days. At 200 ppm all males and females showed tremors at some time (usually starting during the first month of the study, with the incidence decreasing during the middle part of the study, and then increasing later). The incidence of tremors at 100 ppm in this study (1/50 females and 0/50 males) was lower than that previously observed in a 90-day feeding study (FMC study no. A83-818, dated Jan. 31, 1984). The difference might be related to the higher amount (10%) of trans isomer in the 90-day study, versus the 2% in this study.

d. Females at 200 ppm showed a statistically significantly lower (8-10%) body weight than their respective controls from week 13 through 96.

e. Three of 28 group 5 (200 ppm) females (and none of 40 control females) had retinal atrophy, a distribution pattern with $p = 0.065$. Although not statistically significant

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this must be considered, given the reporting of the study (with comparatively little data from females of the 12, 50 and 100 ppm groups) as a possible effect.

f. Males at 100 ppm showed a statistically significantly higher mean body weight from week 2 to week 6 relative to their controls, and during the first 80+ weeks of the study this group consistently had elevated body weights relative to their controls (although differences were not usually statistically significant). In the previously reviewed 90-day rat feeding study (FMC no. A83-818, dated Jan. 31, 1984; review #004501) males and also females at 100 ppm had greater (but not statistically significantly different) mean weight gains at termination than their respective controls. The higher mean weight of males at 100 ppm during the first 80+ weeks of this 2-year study must then be considered as a possible (not necessarily adverse) effect.

g. While there was a statistically significant decrease in the "red blood cell levels" in 200 ppm males at 24 months this was a somewhat equivocal finding as the effect was not observed in females, and it seems that male controls had a relatively high count on that date relative to their previous readings.

h. While not statistically significant, 200 ppm males had increases in mean liver (10.9%) and kidney (28%) weights at 24 months with respect to control values. Males and females at 200 ppm, as well as males at 100 ppm, showed elevated (although not to a statistically significant level) liver and kidney organ-to-body weight ratios relative to controls, and these should be considered as possible effects.

3. The rat reproduction/2-generation study has been classified as core minimum data. FMC 54800 was added to the diet at 0, 30, 60 and 100 ppm. The NOEL is 30 ppm, while the LEL is 60 ppm (weight depression in dams during lactation and some gestation periods), with these effects more pronounced (and statistically significant) at 100 ppm. At 100 ppm there were tremors in dams during and up to 2 weeks after lactation. The NOEL for fetal toxicity was 100 ppm (HDT), but in a preliminary study there was considerable fetal mortality at 200 ppm.

4. Before the Toxicology Branch can consider temporary and/or permanent tolerances for Bifenthrin, a peer review of the findings and a risk assessment have to be conducted, taking into consideration additional information from the registrant (such as the rat historical control data specified above).

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Attachment:

Pages 4-10 from the Toxicology Branch report titled Permethrin - Assessment of Chronic and Oncogenic Effects - A Summary, dated September 3, 1982.

Data Evaluation Reports (attached):

1. Geiger, L. E., Barbera, J. & Ballester, E. Oncogenicity study of FMC 54800: lifetime feeding study in albino mice. Study no. A83-974, conducted at the FMC Toxicology Laboratory. Report issued February 3, 1986; received at EPA 3-13-86; in Acc. 261948, 261949, 261950, 261951, 261952, 261953, 261954 and 261955.

2. McCarty, J. D., Barbera, J., Ballester, E. J. & Geiger, L. E. Oncogenicity study of FMC 54800: 2 year (734 day) feeding study in albino rats. Study no. A83-952, conducted at the FMC Toxicology Laboratory. Report issued January 31, 1986; received at EPA 3-13-86; in Acc. 261940, 261941, 261942, 261944, 261945, 261946 and 261947.

3. DeProspo, J. R., Barbera, J., Ballester, E. J. & Geiger, L. E. Multi-generation reproduction study with FMC 54800 technical in rats. Study no. A83-977, conducted at the FMC Toxicology Laboratory. Report issued January 31, 1986; received at EPA 3-13-86; in Acc. 261933, 261934, 261935, 261937, 261938 and 261939.

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Permethrin

Assessment of Chronic and Oncogenic Effects

A Summary

September 3, 1982

O.E. Paynter, Chief

E.R. Budd, Section Head

B.D. Litt, Biostatistician

Toxicology Branch
Hazard Evaluation Division
Office of Pesticide Programs
Environmental Protection Agency

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since lung adenomas were also present in the FMC Mouse II and B-W Mouse studies, Toxicology Branch is cautious about disregarding the effect. There were no notable patterns of other (including rare, unusual, or malignant) neoplasms which occurred in treated or in the control animals.

As is the case in the majority of oncogenic studies, the time from initiation of exposure to tumor development can not be precisely determined in this study. However, the majority of the lung neoplasms were found at death or sacrifice of the animals late in the 98-week study interval. The mean time, in weeks, from initiation of the study to tumor discovery for control and each treated group is: males - 84.8, 91.5, 92.6, and 85.3; females - 81.3, 81.2, 86.7, and 87.3. This data does not suggest shortening of the latency period.

FMC Mouse II Study - Bio/Dynamics Inc.
Project No. 76-1695
October 9, 1979

Doses of 0 (control), 20, 500, and 2000 ppm of Permethrin were administered in the diet to groups of 75 male Charles River CD-1 strain mice and doses of 0 (control), 20, 2500 and 5000 ppm to groups of 75 female mice for 104 weeks. Initial concerns regarding abdominal distention and amyloidosis in animals of this study have been dismissed as having no significant influence on the interpretation of results. Relevant non-oncogenic effects observed during the study were increased mortality in males at 2000 ppm, increased liver weights in females at 2500 and 5000 ppm, and increased lung weights in females at 5000 ppm. Histopathologically, "focal areas of alveolar cell proliferation" (increased numbers of lung cells) was observed with dose-related incidence in Permethrin treated females . The incidence of this lesion is presented for both sexes in Table III.

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Table III

FMC Mouse II Study

Mice Exhibiting Multifocal Alveolar Cell Proliferation

Males		Dosage Group	Females	
No.	%		No.	%
1/75	1.3	I	3/75	4.0
7/75	9.3	II	5/76	6.6
5/74	6.8	III	11/75	14.7
1/75	1.3	IV	13/75	17.3

Multifocal hepatocytomegaly (increased liver cell volume) was observed with increased frequency in both sexes at the high dose levels and to a lesser extent in the other treated groups. Necrosis of the liver did not follow a dose-related pattern.

Table IV

FMC Mouse II Study

Livers Exhibiting Multifocal Hepatocytomegaly or Necrosis at Time of Sacrifice (S) or Death (D)

Males*						Dosage Group	Females*					
Cytomeg.			Necro.				Cytomeg.			Necro.		
S	D	Tot.	S	D	Tot.		S	D	Tot.	S	D	Tot.
0	1	1/75	0	13	13/75	I	0	0	0/74	2	6	8/74
2	4	6/75	2	6	8/75	II	1	2	3/76	3	8	11/76
3	4	7/75	2	5	7/75	III	3	3	6/76	1	6	7/76
3	11	14/75	1	10	11/75	IV	3	6	9/75	2	6	8/75

* Denominators are tissues examined.

The mechanistic explanation of the hepatocytomegaly in this study is not obvious. It might be related to the enzyme induction observed or strongly suggested in all three mouse studies as discussed on p. 19 of this Summary, or it might be a precursor of necrosis (as suggested by the data in Table IV) and therefore a manifestation of Permethrin toxicity.

With regard to oncogenic effects, the initial pathology report (submitted with the study and dated February 7, 1980) indicated an increased incidence of bronchioalveolar adenomas in female mice. A second reading of the same lung slides (performed under contract to EPA; report dated February 23, 1981) also reported an increased incidence of alveolar cell neoplasms in female mice. Data from the second report is presented in Table V.

Table V

FMC Mouse II Study

Total Mice with Bronchioalveolar Neoplasms
(Adenoma and/or Carcinoma)

Males		Dosage Group		Females	
No.	%		ppm for Q	No.	%
23/75	30.7	I	0	15/75	20.0
20/75	26.7	II	20	24/76	31.6
28/74	37.8	III	2500	35/75	46.7
21/75	28.0	IV	5000	44/75	58.7

The second pathology report also presented separate incidences for pulmonary adenomas and carcinomas. These are presented in Table VI.

Table VI

FMC Mouse II Study

Mice with Bronchioalveolar Adenoma (BA) or Carcinoma (BC)

Males				Dosage Group	Females			
BA		BC			BA		BC	
No.	%	No.	%		No.	%	No.	%
16/75	21.3	7/75	9.3	I	10/75	13.3	6/75	8.0
17/75	22.7	5/75	6.7	II	18/76	23.7	7/76	9.2
20/74	27.0	13/74	17.7	III	26/75	34.7	11/75	14.7
17/75	22.7	4/75	5.3	IV	37/75	49.3	15/75	20.0

N.B. [Caution must be exercised in comparing numerators in Tables V and VI. Because some mice bore both BA and BC the numerators in these Tables are not addable.]

The incidence of pulmonary neoplasms in male mice, Tables V and VI, does not appear to be related to treatment with Permethrin. The numbers of male mice with alveolar cell adenomas, with alveolar cell carcinomas, or with adenomas and/or carcinomas demonstrate no dose-dependence or suggestion that Permethrin treated males had a higher incidence of neoplasms that did the male controls. The slightly higher incidences observed in Group III are considered by TB to be within the limits of normal biological variation. (See Table 2, p. 25 for incidence of pulmonary tumor variability in CD-1 male mice, the mouse used in this study).

The denominators, presented below, for female mice are slightly modified from those given in the EPL Pathology Report, 2/23/81, and given in Table VI. Those mice for which the individual pathology sheets stated that autolysis had occurred, and no further diagnosis was made, were deleted.

The number of female mice with alveolar cell adenomas (10/74, 18/72, 26/74 and 37/75), with alveolar cell carcinomas (6/74, 7/72, 11/74 and 15/75) and with adenomas and/or carcinomas (15/74, 24/72, 35/74 and 44/75) demonstrate a significant dose-response. Statistical analyses of the incidence data for lung neoplasms in female mice revealed the following:

(1) Alveolar cell carcinomas (alone), when analyzed by Peto's Prevalence Method, demonstrated a significant positive time-adjusted dose-response trend with a one sided p value of 0.006. Pair-wise comparisons using chi-square, with Yates correction, gave a one sided p value of 0.15 and 0.032 for control vs 2500 ppm and control vs 5000 ppm respectively.

(2) Alveolar cell neoplasms (adenomas and/or carcinomas), when analyzed by Peto's Prevelence Method, demonstrated a significant positive trend with a one sided p value of 8.49×10^{-8}. Pair-wise comparisons using chi-square, with Yates correction, also gave significant values of 0.0005 and < 0.00001 for control vs 2500 ppm and control vs 5000 ppm respectively.

As stated previously in this document, most oncogenicity studies do not allow evaluation of "time to tumor" with a high degree of certainty. This is true in this study. However, Table VII presents an analysis, by Peto's Prevalence Method, of the female lung tumor/dose-response trends using the incidences given above.

Table VII

FMC Mouse II Study

Lung Tumor/Dose-Response Trends* for Female Mice

Study Interval (Months)	Alveolar Cell Carcinomas (alone) (p value)	Alveolar Cell Neoplasms - (Adenomas and/or Carcinomas) (p value)
1-12	0	0.59
13-18	0.3	0.33
19-21	0.4	0.003
22-24	0.002	0.006
1-24**	0.01	8.48×10^{-4}
24***	0.14	3.45×10^{-6}
Total Study****	0.006	8.49×10^{-8}

* Peto's Prevalence Method (one sided significant test)
** for entire in-life phase of study
*** for animals at terminal sacrifice only
**** for all animals in entire study

For alveolar cell carcinomas (alone), there was a significant dose-related increase in tumors during the final 3 months of the study which largely accounted for the significant increases also observed for the entire life phase of the study (months 1-24) and for the total study (all animals). For alveolar cell neoplasms (adenoma and/or carcinoma), there was a significant dose-related increase in tumors during the final 6 months of the study and significant increases for the entire life phase of the study, for the terminal sacrifice animals and for the

total animals in the study. Historical control data for the specific mouse used in this study (CD-1) indicate that these tumor types regularly appear late in the lifespan, at a mean of 22.3 and 22.5 months of age for males and females respectively (1). In view of this, the total data base does not suggest a decrease in latency.

The above data is interpreted by TB as clearly indicating that administration of dosage levels of 2500 ppm and 5000 ppm of Permethrin to female mice in this study resulted in a significant dose-related increased incidence of alveolar cell neoplasms (adenoma and/or carcinoma). For alveolar cell carcinomas (alone), the data is somewhat less convincing at the dosage level of 2500 ppm, but there is nevertheless clear evidence of a significant dose-related increase in alveolar cell carcinomas, particularly at 5000 ppm. Permethrin apparently enhanced the normally expected spontaneous lung tumor incidences in the females, only, in this study.

The incidences of male and female mice with liver neoplasms, provided by the second pathology report, are presented in Table VIII.

Table VIII

FMC Mouse II Study

Total Mice with Liver Neoplasms

Males		Dosage Group	Females	
No.	%		No.	%
22/73	30.1	I	6/74	8.1
29/73	39.7	II	7/76	9.2
34/70	48.6	III	25/76	32.9
25/69	36.2	IV	30/75	40.0

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Reviewed by: Byron T. Backus, Toxicologist Byron T. Backus 7/28/86
Section III, Tox. Branch (TS-769C)

Secondary Reviewer: Marcia van Gemert, Ph.D., Section Head
Section III, Tox. Branch (TS-769C) M. van Gemert 8.5.86

DATA EVALUATION REPORT

STUDY TYPE: Oncogenicity - mouse TOX. CHEM. NO.: 463F

ACCESSION NUMBERS: 261948, 261949, 261950, 261951, 261952, 261953, 261954, 261955 MRID NO.:

TEST MATERIAL: FMC 54800 technical

SYNONYMS: Bifenthrin, Brigade, Capture, Talstar

STUDY NUMBER(S): A83-974

SPONSOR: FMC Corporation

TESTING FACILITY: FMC Toxicology Laboratory
76 Fourth St.
Somerville, NJ 08876

TITLE OF REPORT: Oncogenicity study of FMC 54800: lifetime feeding study in albino mice.

AUTHOR(S): Geiger, L. E., Barbera, J. and Ballester, E. J.

REPORT ISSUED: February 3, 1986

STUDY CLASSIFICATION: Core Minimum Data (as an oncogenicity study)

CONCLUSIONS:

1. The test material has been demonstrated to be oncogenic. Incidences of leiomyosarcomas of the urinary bladder in male mice were 2/48, 6/50, 8/50, 7/50 and 14/49 at 0, 50, 200, 500 and 600 ppm respectively. While incidences of this tumor were elevated in all male groups exposed to FMC 54800 (and the P value associated with positive trend was 0.00053) only the incidence at 600 ppm was significantly ($p < 0.01$) different from that of controls.

2. Incidences of combined bronchiolar-alveolar adenocarcinomas and adenomas in the lungs were 14/50, 26/50, 23/50, 19/50 and 23/48 for females at 0, 50, 200, 500 and 600 ppm respectively. Inci-

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dences were significantly different ($p < 0.05$) at 50, 200 and 600 ppm (and elevated, but not significantly different, at 500 ppm) relative to controls. Time-to-tumor tests for positive trend and heterogeneity were not significant ($p = 0.108$ and 0.131 respectively). However, in a number of mouse studies conducted with Permethrin (structurally similar to FMC 54800) there were significantly increased incidences of these tumors in females. It is tentatively concluded that the increased incidences (38-52%) of pulmonary tumors in females receiving FMC 54800 (as compared to 28% in controls) were compound-related. The lack of a strong correlation between incidences and dietary levels of FMC 54800 in this recently conducted study may indicate a relatively low degree of slope in tumor incidences vs. dietary exposure in the range from 50 to 600 ppm.

It is noted that information (copies from the open literature) received 5-30-86 indicates high incidences of spontaneous pulmonary tumors in female Swiss-Webster mice. These data have not yet been fully evaluated.

3. The incidence of lymphoblastic leukemia was significantly increased (22/49) in highest dose (600 ppm) females relative to their control group (12/50). However, since combined incidence of lymphoid tumors in the 600 ppm females (23/49 or 47%) was not statistically different from that of controls (19/50 or 38%) it is concluded that occurrence of lymphoblastic leukemia in females was not related to exposure to FMC 54800. The reported incidences for the intermediate dose groups must be regarded cautiously since in many group II, III and IV mice (particularly those sacrificed at termination) mesenteric and mediastinal lymph nodes, as well as the thymus, were not examined unless they were enlarged or otherwise obviously abnormal. It is noteworthy that in some cases (such as group IV females #2706, 2712 and 2726) diagnosis of lymphoblastic leukemia/lymphosarcoma was made from infiltrates into kidneys and lung or uterus as lymph nodes were not microscopically examined.

4. In some mouse studies conducted with Permethrin, there were indications of possible liver oncogenicity. It is tentatively concluded that the dose-related trend (with $P = 0.025$) of increased incidence of combined adenocarcinomas and adenomas in the liver of male mice (2/49, 2/50, 4/50, 4/50 and 7/49 at 0, 50, 200, 500 and 600 ppm respectively) is directly related to exposure to FMC 54800, even in the absence of apparent preneoplastic changes.

5. An oncogenic NOEL was not established. In the case of leiomyosarcomas of the urinary bladder in male mice, group II (lowest dose or 50 ppm) mice showed an elevated incidence in this tumor type relative to their controls. While the elevated incidence at 50 ppm was not statistically signifi-

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cant, it was part of a well-defined dose-related trend. Group II females showed a statistically significantly elevated incidence of bronchiolar alveolar adenomas and adenocarcinomas relative to their controls.

6. The highest dose tested (600 ppm) is acceptable as an MTD, based on the high incidence of tremors, as well as occasional jerks, twitches and clonic convulsions and the deaths of 4 animals (2 males, 2 females) during the early part of the study.

7. Non-oncogenic effects were statistically significantly lower mean body weights in males of the 500 and 600 ppm groups relative to their controls during the first 20 weeks of the study, as well as occasionally significantly lower mean body weights in females at 500 and 600 ppm relative to their controls during the first 6 weeks of the study. Associated with these lower mean body weights were lower mean food consumption values in 500 and 600 ppm males and females during the first few weeks of the study; food consumption values were statistically significantly different from those of controls only during the first week.

Classification: (oncogenicity study) core minimum

Classification: (long-term feeding) core supplementary (no urinalyses or blood chemistry work. NOEL for tremors: 200 ppm; LEL for tremors: 500 ppm.

A. MATERIALS:

1. Test compound: FMC 54800 technical, described as FMC notebook no. E2392-105, purity 88.35%, with an isomer ratio of [redacted]

2. Test animals: Species: mouse. Strain: Swiss-Webster, Tac(SW)fBR. Age: 43 days at initiation of study-(30 days old when received).
Source: Taconic Farms, 33 Hover Ave., Germantown, NY
Weight: (males) 24.7-29.5 g at initiation of study
(females) 18.8-24.1 g at initiation of study

B. STUDY DESIGN:

1. Dose levels were selected (refer to vol. 1, p. 6) on the basis of results from two 28-day dietary studies. In the first, no effects were noted at 0, 50, 100, 200 or 300 ppm. In the second, the concentrations were 0, 500, 600, 750 and 1000 ppm. At 1000 ppm all female mice died by day 12 and 7/10 males by day 7. At 750 ppm 5/10 females died by day 6 but there were no mortalities among males.

2. Animal assignment - 50 males and 50 females were assigned by a computer-randomization program to each dosage group. Equality of initial group body weights was tested and confirmed using analysis of variance. The following is a listing of the test groups:

Test Group	Dose in diet (ppm)	Main Study weeks male	Main Study weeks female	Interim Sac. months male	Interim Sac. months female
1. Control	0	87	92	None	
2. Low (LDT)	50	87	92	None	
3.	200	87	92	None	
4.	500	87	92	None	
5. Highest (HDT)	600	87	92	None	

Mice were individually housed in stainless steel cages with wire bottoms.

In addition to the 250 mice/sex directly participating in the study, there were 12 mice/sex which were in the same room receiving the control diet. Every 3 months 3-6 of these mice were sent to Microbiological Associates (Bethesda, MD) for microbial and viral screening.

3. Diet preparation: The appropriate amount of test material was heated to 70-80° C until liquified. For each batch of diet prepared the appropriate amount of liquefied FMC 54800 was added to acetone and dissolved. Each solution was added to approximately 500 g of Purina Laboratory Chow #5002 and hand mixed until dry. Premixes were then added to sufficient Laboratory Chow to achieve concentrations of 50, 200, 500 or 600 ppm. Control diets were prepared in a similar fashion, with incorporation of the appropriate amount of acetone.

Control and treated feeds were sealed in plastic bags and closed containers and stored at room temperature until fed to the mice.

There is a report (FMC Study A82-776, GLP E314-76E in appendix F of volume 8) titled: "Homogeneity and Stability Study of FMC 54800 in Rodent Chow," in which technical FMC 54800 was mixed (to a concentration of 50 ppm) with Purina Chow #5002 and, according to the analytical results, the test material was homogeneous and stable in the dietary sample for a period of one year. The technical FMC 54800 assayed for stability was approximately [redacted] isomer (the material fed to the mice was [redacted]

4. Animals received food (Purina Rodent Chow #5002, mixed with the appropriate amount of FMC 54800) and water ad libitum.

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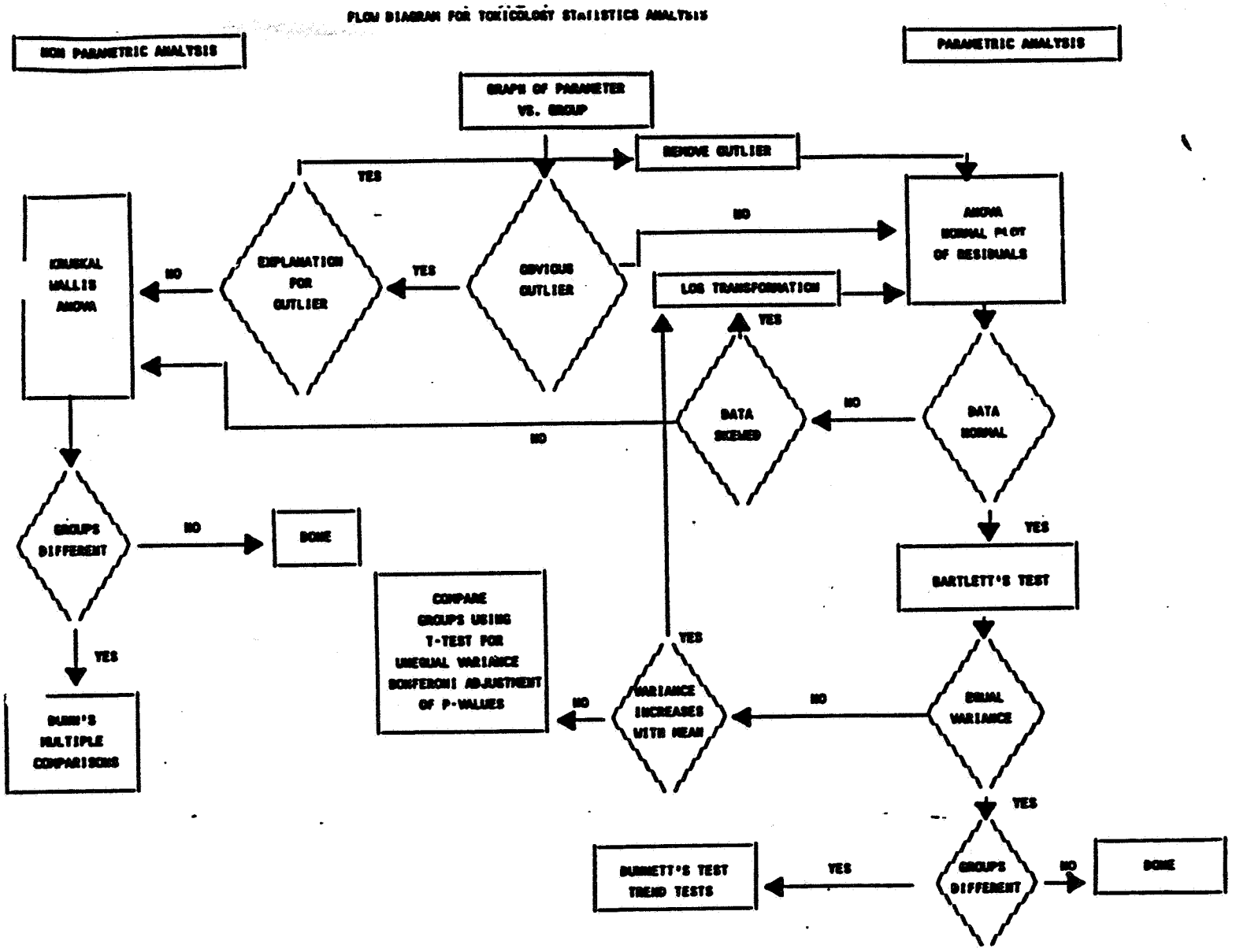
5. Statistics - The following procedures were utilized in analyzing the data:



6. Quality Assurance: There is a copy of a quality assurance statement signed by L. E. Geiger, Ph.D., D.A.B.T. in vol. 1, page 2, along with a listing of when on-site inspections were conducted (p. 3).

C. METHODS AND RESULTS:

1. Observations: Mice were examined twice a day for mortality and once daily for appearance, behavior, signs of toxicity and moribundity.

Results

Toxicity: The following clinical observations showed an increased incidence in group IV (500 ppm) and group V (600 ppm) males and females (from vol. I, p. 57-58 and 59-60):

Number of observations (number of animals):

Males:	Group 1	Group 2	Group 3	Group 4	Group 5
Clonic convulsions	0	0	0	1(1)	6(4)
Jerks and twitching	0	0	0	18(7)	126(20)
Tremors	0	2(1)	6(2)	1077(50)	1875(50)

Females	Group 1	Group 2	Group 3	Group 4	Group 5
Clonic convulsions	0	0	0	21(3)	21(7)
Jerks and twitching	0	0	0	111(23)	225(29)
Tremors	0	0	3(2)	1846(50)	2088(50)

Almost all of the tremors are reported (vol. 1, p. 18) as occurring during the first 3 months of the study. A check of the individual animal data for group 4 and group 5 mice (males: vol. 2, p. A180-A224; females: vol. 2, p. A284-A344) indicates that most of the mice with tremors had them only during the first 60 days of the study.

The tremors occurring in one group 2 male (C2363) occurred relatively late in the study (days 343 and 344), and the animal subsequently died with fluid in the lungs on day 371. However, the tremors observed in two group 3 males (C2376 and C2377) were on days 37, 38 and 39. This was during the period when tremors were quite frequent in group 4 and 5 mice, so these tremors are considered related to exposure to FMC 54800.

Mortality: There were no statistically significant dose-related differences between groups with respect to survival, although two 600 ppm males, two 600 ppm females and one 500 ppm female died with compound-induced symptoms (refer to vol. 1, p. 15). From vol.

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1, p. 35 and 38:

SURVIVORS AT TERMINATION/INITIAL NUMBER OF ANIMALS

	Group 1 0 ppm	Group 2 50 ppm	Group 3 200 ppm	Group 4 500 ppm	Group 5 600 ppm
Males	14/50	19/50	24/50	13/50	19/50
Females	18/50	13/50	15/50	21/50	18/50

2. Body weight: Individual body weights were taken weekly the first 13 weeks of the study and monthly thereafter. Final body weights were obtained just prior to necropsy.

Results:

Mean body weights of group 5 (600 ppm) males were depressed relative to controls from week 1 through 78, and were significantly "different" in the period from week 2 through 20. Mean body weights of group 4 (500 ppm) males were generally lower relative to controls from week 1 to study termination, and were significantly "different" at weeks 5, 8, 9, 12 and 13. Mean body weights for group 2 (50 ppm) and group 3 (200 ppm) males were generally lower than controls through about week 32, but the only significantly "different" value was for group 2 males at week 5.

Among the females, mean body weights for all groups receiving the test material were consistently depressed relative to controls from week 1 through termination, but the only statistically significant "differences" occurred at weeks 1, 5 and 6 for group 4 (500 ppm) females and at weeks 1, 2 and 5 for group 5 (600 ppm) females.

3. Food consumption and compound intake: Food consumption was measured weekly the first 13 weeks of the study and at monthly intervals thereafter.

Results:

Food consumption: Mean food consumption was generally lower in group 4 and 5 males during the first five weeks relative to their controls, but it was significantly "different" in these groups only during the first week. At week 20 group 5 males actually had a higher mean food consumption than their controls which was significantly ($p \leq 0.05$) different from the control value, but this was an isolated occurrence. A significantly ($p \leq 0.01$) "different" (and lower) mean food consumption level in group 3 males at 48 weeks was also an isolated occurrence.

Mean food consumption for group 4 and 5 females was lower than controls during the first two weeks of the study, but was significantly (at $p < 0.01$) "different" only for the first week. From week 3 through 44 group 4 females consistently had higher mean food values than their controls, although there was never any significant "difference." Group 2 (50 ppm) females consistently had higher mean food consumption levels than controls from week 1 through 61, and differences were statistically significant ($p < 0.05$) at weeks 4, 9 and 10.

Food efficiency - not calculated.

Compound intake - mean compound consumption in terms of mg/kg/week is given in volume 1, table 7 (males) and table 8 (females). For females the mean values for both group 4 (500 ppm) and group 5 (600 ppm) ppm) were above 1000 mg/kg/week in the period from week 3 to 13. Group 4 (500 ppm) females actually had a higher mean consumption of the test compound for week 3 on a body weight basis than did group 5 females (600 ppm), with 1180.0 mg/kg/week (500 ppm) and 1153.9 mg/kg/week (600 ppm). For males, the highest values on a body weight basis occurred at weeks 2 (939.4 mg/kg/week) and 7 (939.4 mg/kg/week) in group 5 (600 ppm) animals.

4. Opthalmalogical examinations: not performed.

5. Bleeding (via a tail clip) of 10 mice/sex/group was conducted at 12 and 18 months, and also at terminal sacrifice. The CHECKED (X) parameter was examined.

a. Hematology -

	Hematocrit (HCT)		Total plasma protein (TP)
	Hemoglobin (HGB)	X	Leukocyte differential count
	Leukocyte count (WBC)		Mean corpuscular HGB (MCH)
	Erythrocyte count (RBC)		Mean corpuscular HGB conc. (MCHC)
	Platelet count		Mean corpuscular volume (MCV)

Results: From vol. 1, p. 17: "there were no biologically significant differences between the high dose and control groups in either males or females... differential blood counts on the intermediate dose groups were not conducted because there were no biologically significant differences between the high dose and control groups." Individual data (vol. 2, p. A101-A112) show no significant (either statistically or biologically) differences.

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b. Clinical Chemistry - not performed.

6. Urinalysis - not performed.

7. Sacrifice and Pathology -

All mice that died as well as those sacrificed (ether anesthetization and exsanguination) at termination (87 weeks for males, 92 weeks for females) were subjected to a full gross necropsy. Representative sections of the following CHECKED (X) tissues were preserved in buffered neutral formalin.

X	Digestive system	X	Cardiovasc./Hemat.	X	Neurologic
	Tongue	X	Aorta	X	Brain (3 sections)
X	Salivary glands	X	Heart		Periph. nerve
X	Esophagus	X	Bone Marrow	X	Sciatic nerve
X	Stomach		Lymph nodes	X	Spinal cord (3 levels)
X	Duodenum	X	mediastinal	X	Pituitary
X	Jejunum	X	mesenteric	X	Eyes
X	Ileum	X	Spleen		Glandular
X	Cecum	X	Thymus	X	Adrenals
X	Colon		Urogenital		Lacrimal gland
X	Rectum	X	Kidneys	X	Mammary gland (females)
X	Liver (2 sections)	X	Urinary bladder	X	Parathyroids
X	Gall bladder	X	Testes	X	Thyroid
X	Pancreas	X	Epididymides		Other
	Respiratory	X	Prostate	X	Bone (sternum with bone marrow)
X	Trachea	X	Seminal vesicle		
X	Lung	X	Ovaries	X	Skeletal muscle
		X	Uterus	X	Skin
		X	Vagina	X	All gross lesions
				X	and masses

The wet weight of the brain (with brain stem), both kidneys, liver and both gonads were taken from 10 animals/sex/group at the final sacrifice.

Results -

a. Organ weights at termination: individual organ weights (from 10 mice/sex/group) are reported in vol. 2, p. A345-A354. Group means are in vol. 1, p. 61 (males) and p. 62 (females).

The only significant differences with respect to control values occurred with mean absolute kidney weights in group 2 and group 5 males, but there was no evident dose-related trend, and there was no indication of an effect in females:

Mean kidney weights (gm) at termination	Group 1 0 ppm	Group 2 50 ppm	Group 3 200 ppm	Group 4 500 ppm	Group 5 600 ppm
Males	0.946	0.761**	0.848	0.877	0.799*
Females	0.612	0.645	0.633	0.648	0.647

*Reported as significantly different from control, $p \leq 0.05$
**Reported as significantly different from control, $p \leq 0.01$

No significant differences existed with respect to mean percent organ/body weight ratios (vol. 1, p. 63-64) or mean organ to brain weight ratios (vol. 1, p. 65-66).

b. Gross pathology - nothing remarkable found at this level except for bladder masses in some 600 ppm animals (refer to neoplastic microscopic pathology, below).

c. Microscopic pathology.

1) Non-neoplastic:

There were slight increases in incidences of glandular hyperplasis of the stomach (not significant by Fisher's exact test) and retinal atrophy (statistically significant by Fisher's exact test) in males and females of the highest (600 ppm) dose group. Highest-dose males also showed an increased incidence of cortical atrophy of the adrenal gland and bilateral germinal epithelial degeneration of the testes (vol. 1, p. 20); the latter condition was also significantly elevated in males of groups 2 and 3. From vol. 1, p. 24:

Finding	Group 1 0 ppm	Group 2 50 ppm	Group 3 200 ppm	Group 4 500 ppm	Group 5 600 ppm
Stomach-glandular hyperplasia				-	
males	6/49 (12%)	8/50 (16%)	7/50 (14%)	9/50 (18%)	8/48 (17%)
females	5/48 (10%)	6/50 (12%)	5/49 (10%)	5/50 (10%)	9/48 (19%)
Eye - retinal atrophy					
males	14/48(29%)	12/29(41%)	8/25(32%)	11/36(31%)	24/49(49%)*
females	14/49(29%)	12/37(32%)	11/35(31%)	8/29(28%)	23/49(47%)*
Testes - bilateral germinal epithelial degeneration	4/49 (8%)	8/32(25%)*	8/26(31%)*	8/38 (21%)	12/49(24%)*

*Statistically significant at $p \leq 0.05$ as judged by Fisher's exact test.

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2) Neoplastic:

The following neoplastic findings occurred with statistically significantly increased incidences in highest-dose (600 ppm) males or females and/or showed dose-related trends:

i) Leiomyosarcomas of the urinary bladder: these occurred only in males surviving 250 or more days. The following is a comparison of overall incidences and incidences for males which survived 250 or more days:

Finding	Group 1 0 ppm	Group 2 50 ppm	Group 3 200 ppm	Group 4 500 ppm	Group 5 600 ppm
Urinary bladder - males - Leiomyosarcomas(overall)	2/48 (4%)	6/50 (12%)	8/50 (16%)	7/50 (14%)	14/49 (29%)**
Urinary bladder leiomyosarcomas in males surviving 250+ days	2/48 (4%)	6/48 (13%)	8/49 (16%)	7/46 (15%)	14/45 (31%)

**Statistically significant at $p < 0.01$ by Fisher's exact test (see vol. 1, p. 23 & 25).

Note: no statistical evaluation was done on the incidence of this tumor type among males surviving 250+ days.

Most urinary bladder leiomyosarcomas were detected microscopically. However, in at least 4 males these tumors were macroscopically evident. Three (#2476, #2477, #2506) of these males were in the 600 ppm group, while one (#2463) was in the 500 ppm group. Also, male 2520 (at 600 ppm) is reported (vol. 6, p. C1143) as having abdominal distention related in some way to the urinary bladder (for which the only finding was a leiomyosarcoma). The following are individual findings (from vol. 6) relating to the leiomyosarcomas of the urinary bladder in those mice in which these tumors were macroscopically evident:

Animal Number	Group	Days	Urinary bladder finding
2463	4 - 500 ppm	605	1 mm whitish focus
2476	5 - 600 ppm	516	Mass (2-1/2 cm. diameter attached to urinary bladder
2477	5 - 600 ppm	598	Urinary bladder distended and filled with yellow fluid, dimension 3 cm in diameter; inside the bladder a soft white mass was present.
2506	5 - 600 ppm	460	Mass on dorsal surface of urinary bladder; nodular, apprx. 8 mm. diameter.)

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In some males with leiomyosarcoma of the urinary bladder, that organ was distended. Possibly associated with this, there was a higher (but not statistically significant) incidence of abdominal distention in males of the 600 ppm group. From vol. 1, p. 57:

Finding	Group 1 0 ppm	Group 2 50 ppm	Group 3 200 ppm	Group 4 500 ppm	Group 5 600 ppm
Abdominal distention - number of males	17	22	15	13	23

Although leiomyosarcomas of the bladder were observed in females, there was no dose-related correlation; from vol. 4, p. C268:

Finding	Group 1 0 ppm	Group 2 50 ppm	Group 3 200 ppm	Group 4 500 ppm	Group 5 600 ppm
Urinary bladder females:Leiomyosarcomas	1/50 (2%)	2/50 (4%)	4/50 (8%)	1/50 (2%)	0/49 (0%)

ii) Bronchioalveolar adenocarcinomas and adenomas in females: the combined incidences of these tumors were elevated in all groups exposed to FMC 54800, although no dose-related trend appeared to be otherwise present:

Finding	Group 1 0 ppm	Group 2 50 ppm	Group 3 200 ppm	Group 4 500 ppm	Group 5 600 ppm
Lung - females: combined bronchioalveolar adenomas and adenocarcinomas	14/50 (28%)	26/50* (52%)	23/50* (46%)	19/50 (38%)	23/48* (48%)

For the increased incidences of bronchioalveolar adenocarcinomas and adenomas in the females, it is noted (vol. 1, p. 21) that: "...time-to-tumor tests for positive trend and heterogeneity indicated that there was no significant trend and that the incidence rates are not significantly different between groups."

iii) Lymphoblastic lymphosarcoma/leukemia in females: The incidence of this tumor type was 12/50 (24%) in controls and 22/49 (45%) in highest-dose (600 ppm) females, and comparison of the two groups showed the distribution was significant at p = 0.024 by Fisher's exact test. However, when incidences for composite lymphosarcoma (7/50 in controls, 1/49 in highest-dose females) are added in, these incidences become 19/50 and 23/49, and are no longer significantly different.

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From vol. 1, p. 24 and vol. 4, p. C25:

Finding-females	Group 1 0 ppm	Group 2 50 ppm	Group 3 200 ppm	Group 4 500 ppm	Group 5 600 ppm
Lymphoblastic lymphosarcoma/ leukemia	12/50 (24%)	14/50 (28%)	17/50 (34%)	10/50 (20%)	22/49* (45%)*
Composite lymphosarcoma	7/50 (14%)	4/50 (8%)	3/50 (6%)	6/50 (12%)	1/49 (2%)
Combined incidence	19/50 (38%)	18/50 (36%)	20/50 (40%)	16/50 (32%)	23/49 (47%)

*Statistically significant at p = 0.024 by Fisher's exact test.

Incidences given for the "intermediate" groups must be regarded cautiously. For many group 2, 3 and 4 mice (particularly those sacrificed at termination) mesenteric and mediastinal lymph nodes, as well as the thymus, were not examined, except if enlarged or obviously abnormal. In some case (such as group 4 females #2706, 2712 and 2726) diagnosis of lymphoblastic leukemia/lymphosarcoma was made from infiltrates in kidneys and lung or uterus as lymph nodes were not microscopically examined in these animals.

In vol. 1, p. 22 it is stated (with respect to the elevated incidence of lymphoblastic leukemia):

"Time-to-tumor tests revealed no significant trends for either the mortality or onset functions while the prevalence function was significant. As noted by the study pathologist, combining all lymphoid tumors in female mice results in an incidence pattern of 38%, 38%, 40%, 32% and 47% for groups I through V respectively. None of the treatment groups are significantly different that the control group as judged by pairwise comparisons with the control using Fisher's exact test on the combined incidence data."

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iv) There was a dose-related increased incidence in combined hepatocellular adenomas and adenocarcinomas in males; the following is a listing of the individual males with these tumors and cumulative incidences (from vol. 4, p. C260-C261):

Incidences of combined hepatocellular adenomas and adenocarcinomas in males

Finding males)	Group 1 0 ppm		Group 2 50 ppm		Group 3 200 ppm		Group 4 500 ppm		Group 5 600 ppm	
	A-14	B-35	A-19	B-31	A-24	B-26	A-13	B-37	A-19	B-30
Hepatocellular adenomas	2318	2285		2352 2362	2410 2418	2411	2424	2456	2479 2500	2494 2507 2520
Hepatocellular adenocarcinomas					2375			2431 2438		2504 2514
Cumulative incidence	1/14	1/35	0/19	2/31	3/24	1/26	1/13	3/37	2/19	5/30
	2/49		2/50		4/50		4/50		7/49	

A = animals surviving to termination; B = animals sacrificed moribund or dying during study; value following = number of animals in each subgroup.

Most of the males with liver tumors had been on the study for 500 or more days (although #2507 had been on the study for only 391 days); recalculating incidences in terms of animals surviving 300+ days:

Finding	Group 1 0 ppm	Group 2 50 ppm	Group 3 200 ppm	Group 4 500 ppm	Group 5 600 ppm
Hepatocellular adenomas and adenocarcinomas in males surviving 250+ days	2/48(4%)	2/47(4%)	4/47(9%)	4/44(9%)	7/45(16%)

The increased incidence of these tumors in group V males is not statistically significant relative to their controls; however, the positive trend associated with the dose-related increased incidence is reported as significant at p = 0.022 (volume 1, p. 23) using the method of Kodell et al. (1983).

It is stated (vol. 1, p. 21) that: "Hepatocellular tumor incidence rate was elevated in high dose males (4%, 4%, 8%, 8%, 14%, control thru high dose males, respectively), however, no predisposing hepatic changes were present in the livers of treated animals. The cause of these hepatocellular lesions could not directly, from a biological standpoint, be considered to have been directly associated with treatment."

There is no indication from mean liver weights at termination of a compound-related effect involving this parameter in either males (vol. 1, p. 65) or females (vol. 1, p. 66).

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D. DISCUSSION

There is no doubt that the test material causes leiomyosarcomas in the bladders of male mice. As part of a positive dose-related trend, the incidence of this tumor type was elevated even at the lowest dose level (50 ppm) of FMC 54800 tested (6/50 mice, as compared to 2/48 controls) although it must be noted that there is no statistical difference between these incidence values.

While most leiomyosarcomas of the urinary bladder were detected microscopically, there were at least 4 males in which these tumors were evident at gross necropsy. Three were in the 600 ppm group, while the other was in the 500 ppm group. This correlation of the largest tumors of this type with highest dietary doses of the test material is further evidence of a cause-and-effect relationship.

In the pathology summary of the submitted report it is stated (vol. 1, p. 20) that:

> "Microscopic evaluation of the tissues revealed an apparent treatment associated increase in leiomyosarcomas of the urinary bladder wall of male mice...The tumor incidence was increased in males, especially at the high dose level. The percent involvement was 4, 12, 16, 14, and 29, control thru high dose, respectively. The tumors were slow growing, did not metastasize, and were not responsible for the death of any of the affected mice."

This reviewer does not consider this statement to be entirely accurate. The probable cause of death of mouse #2476, dead on day 516, is reported (vol. 6, p. C1093) as neoplasia. The only tumor in this animal was a 2-1/2 cm diameter leiomyosarcoma attached to the urinary bladder. The probable cause of death (on day 460) for animal #2506 is also reported (vol. 6, p. 1125) as neoplasia. The only tumor reported for this animal was a nodular leiomyosarcoma about 8 mm in diameter on the dorsal surface of the urinary bladder.

and that, as part of this trend, incidence of this tumor type in lowest dose (50 ppm) males is elevated (although not to a statistically significant level) with respect to controls, particularly in those animals surviving to termination. Incidences of this tumor (with no calculations as to statistical significance) in males were the following (from information in vol. 4, p. C263):

	Group 1 0 ppm	Group 2 50 ppm	Group 3 200 ppm	Group 4 500 ppm	Group 5 600 ppm
Males surviving to termination	0/14 (0%)	4/19(21%)	5/24(21%)	3/13(23%)	5/19(26%)
Males dying before termination	2/35 (6%)	2/31 (6%)	3/26(12%)	4/37(11%)	9/30(30%)

All female groups exposed to FMC 54800 had elevated incidences of combined bronchioloalveolar adenocarcinomas and adenomas relative to controls, and these were statistically significant for 50, 200 and 600 (but not 500) ppm animals. Incidences of these tumors did not appear to correlate with dietary level of FMC 54800:

Incidences of combined bronchioalveolar adenocarcinomas and adenomas in females

	Group 1 0 ppm	Group 2 50 ppm	Group 3 200 ppm	Group 4 500 ppm	Group 5 600 ppm
All females	14/50 (28%)	26/50(52%)	23/50(46%)	19/50(38%)	23/48(48%)
Females surviving to termination	7/18 (39%)	8/13(62%)	8/15(53%)	11/21(52%)	7/17(41%)

It is stated (vol. 1, p. 21) that while there are significantly elevated combined incidences of brochiolar-alveolar adenocarcinomas and adenomas in group II, III and V female mice, "time-to-tumor tests for positive trend and heterogeneity indicated that there was no significant trend and that the incidence rates are not significantly different between groups."

However, it has been concluded that dietary exposure to Permethrin, a structurally similar pyrethroid, is associated with an increased incidence of lung adenocarcinomas and adenomas in female mice (refer to the EPA document titled Permethrin - Assessment of Chronic and Oncogenic Effects - A Summary, dated September 3, 1982 - see attachment at the end of this DER). In a 104-week study conducted for FMC by

Bio/Dynamics Inc. (Project No. 70-1695, dated October 9, 1979) there was an increased incidence of bronchioalveolar neoplasms in female mice correlating with dosage level of Permethrin:

Incidences of Bronchioalveolar neoplasms in female mice dietarily exposed to Permethrin

Group I 0 ppm	Group II 20 ppm	Group III 2500 ppm	Group IV 5000 ppm
15/75(20.0%)	24/76(31.6%)	35/75(46.7%)	44/75(58.7%)

Note that the slope of the dose-response is gradual between 20 and 5000 ppm Permethrin. In the FMC 54800 study there was only a 12X factor between lowest (50 ppm) and highest (600 ppm) dose levels. The lack of a pronounced dose-trend in the data for FMC 54800 may reflect a gradual (or low degree) of slope response, similar to that for Permethrin. Incidences for bronchioalveolar tumors in females exposed to FMC 54800 (38 to 52%) are similar to those (31.6 and 46.7%) observed at 20 and 2500 ppm in the broader dosage range Permethrin study.

Tentatively, the combined bronchioalveolar adenoma and adenocarcinoma incidences of 38-52% in female mice exposed to FMC 54800 seem high in comparison with most values from the open literature (refer to material received 5-30-86 from the registrant), so that at this time the elevated incidences of these tumors must be regarded as an effect of exposure to 54800, and must be considered in any risk assessment of this chemical.

Statistically, the combined incidence (47%) of lymphoid tumors in females at 600 ppm was not significantly different from that of controls (38%). Combining incidences of leukemias (except myelogenous) and all lymphomas in mouse studies is acceptable (p. 92, Hazard Evaluation Division Standard Evaluation Procedure for Oncogenicity Potential, Paynter, 1984). Reported incidences for the intermediate female groups (38%, 40% and 32%) must be regarded as possibly low, because the mediastinal and mesenteric lymph nodes of many of these animals (particularly those sacrificed at termination) were not examined.

Although the incidence of lymphoblastic leukemias/sarcomas in 600 ppm females surviving to termination was elevated with respect to controls, the incidences for those animals dying prior to termination were similar (11/32 in 600 ppm females, 10/32 in controls). This correlates with the statement in the report that: "Time-to-tumor tests revealed no significant trends for either the mortality or onset functions while the prevalence function was significant."

The conclusion then is that the increased incidence of lymphoblastic leukemias/sarcomas was not caused by exposure to FMC 54800.

Although somewhat equivocal considering the lack of preneoplastic lesions in the liver, the statistically significant dose-related trend of increased incidence of combined hepatocellular adenomas and adenocarcinomas in males must be considered as an effect. There were some possible indications, although nothing conclusive, of a similar effect in at least one mouse oncogenicity study with Permethrin.

Besides tremors, non-oncogenic effects in 500 and 600 ppm mice included statistically significantly lower mean body weights in males relative to controls during the first 20 weeks of the study and occasionally significantly lower mean body weights in females of these groups during the first 6 weeks of the study. Probably associated with these lower mean weights were lower mean food consumption values in both sexes at 500 and 600 ppm during the first few weeks of the study, although mean food consumption values were significantly different from controls only during the first week.

No non-oncogenic effects were observed in 50 and 200 ppm animals.

The 600 ppm dosage level can be accepted as an MTD, based on the data from a preliminary study in which a considerable number of mortalities occurred at 750 and 1000 ppm, as well as the occurrence of a few deaths at 600 ppm in this study.

Overall, the study is acceptable in demonstrating the oncogenicity of the test material.

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Section III, Tox. Branch (TS-769C) M. van Gemert 8.5.86

DATA EVALUATION REPORT (II)

STUDY TYPE: Oncogenicity - rat

TOX. CHEM. NO.: 463F

ACCESSION NUMBERS: 261940, 261941, 261942, 261944, 261945, 261946, 261947

MRID NO.:

TEST MATERIAL: FMC 54800 technical

SYNONYMS: Bifenthrin, Brigade, Capture, Talstar

STUDY NUMBER(S): A83-952

SPONSOR: FMC Corporation

TESTING FACILITY: FMC Toxicology Laboratory
76 Fourth St.
Somerville, NJ 08876

TITLE OF REPORT: Oncogenicity study of FMC 54800: 2 year (734 day) feeding study in albino rats.

AUTHOR(S): McCarty, J. D., Barbera, J., Ballester, E. J. and Geiger, L. E.

REPORT ISSUED: January 31, 1986

STUDY CLASSIFICATION: Core Minimum Data (oncogenicity and chronic feeding).

CONCLUSIONS:

1. While there was no conclusive evidence of dose-related oncogenicity, incidences of pancreatic islet cell adenoma (combined sexes) were 1/97, 0/48, 0/40, 0/47 and 4/99 for 0, 12, 50, 100 and 200 ppm respectively. Incidences of fibrosarcoma in male rats were 0/50, 1/50, 0/50, 0/50 and 3/50 for 0, 12, 50, 100 and 200 ppm respectively. The registrant should supply historical control data as to the incidences of these tumor types in rats of this strain at this testing facility.

2. The 200 ppm dietary level can be accepted as being reasonably

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close to an MTD, based on a preliminary 28-day study in which deaths accompanied by tremors occurred at 300 ppm in 6/10 males by day 12 and 1/10 females by day 20.

3. At 100 ppm 1 (out of 50) female showed tremors for 3 days. At 200 ppm all males and females showed tremors at some time (usually during the first month of the study, with the incidence decreasing for the middle part of the study, and then increasing later). The incidence of tremors at 100 ppm in this study (1/50 females and 0/50 males) was lower that that previously observed in a 90-day study (FMC study no. A83-818, dated Jan. 31, 1984; review #004501). The difference might be related to the higher amount [redacted] of trans isomer in the 90-day study, versus [redacted] in this study.

4. Females at 200 ppm showed a a statistically significantly lower (8-10%) body weight than their respective controls from weeks 13 through 96.

5. Three of 28 group 5 (200 ppm) females (and none of 40 control females) had retinal atrophy, a distribution pattern which approaches statistical significance at $p \leq 0.05$. This must be considered, given the reporting of the study (with comparatively little data from females of 12, 50 and 100 ppm) as a possible effect.

6. Males at 100 ppm showed a statistically significantly higher mean body weight from week 2 to week 6 relative to controls, and during the first 80+ weeks of the study this group consistently had elevated mean body weights relative to their controls (although differences were not usually statistically significant). In a previously reviewed 90-day rat feeding study (FMC study no. A83-818, dated Jan. 31, 1984; review #004501) males (also females) at 100 ppm had a greater (although not statistically significant) mean weight gain at termination than controls. The higher mean weight of males receiving 100 ppm of test material must then be considered as a possible (but not necessarily adverse) effect.

7. While there was a statistically significant decrease in the "red blood cell levels" in 200 ppm males at 24 months, this was a somewhat equivocal finding as the effect was not observed in females, and it looks as if male controls had a relatively high count on that date relative to some of their previous readings.

8. While not statistically significant, 200 ppm males had increases in mean liver (10.9%) and kidney (28%) weights at 24 months with respect to control values. Males and females at 200 ppm, as well as males at 100 ppm, showed higher (but not statistically significantly so) liver and kidney organ-to-body weight ratios relative to controls, and these should be considered as possible effects.

Classification (oncogenicity & long-term feeding): core minimum data. NOEL: 50 ppm; LEL (tremors) 100 ppm; possible (not statistically significant) higher kidney and liver organ-to-body weight ratios in males at 100 and 200 ppm, as well as females at 200 ppm. At 200 ppm females had an increased incidence of retinal atrophy.

A. MATERIALS:

1. Test compound: FMC 54800 technical, FMC notebook no. E2392-105, 88.35% purity with an isomer [redacted] The amount added to the diet was adjusted to account for the purity so that the diets contained 12, 50, 100 or 200 ppm in terms of the active ingredient.

2. Test animals: Sprague-Dawley rats, 39 days old at the initiation of the study, obtained from Taconic Farms, 33 Hover Avenue, Germantown, NY 12526. These rats had been acclimated for a period of 14 days before the initiation of the study.

B. STUDY DESIGN:

1. Dose levels: These were selected (refer to vol. 1, p. 6) on the basis of results of a 28-day range-finding study in which all rats at 400 ppm died by day 15, and 6/10 males and 1/10 females at 300 ppm died by day 20.

2. Animal assignment: 50 males and 50 females were assigned by a computer randomization program to each dosage group. Equality of initial group body weights was tested and confirmed using analysis of variance. The following is a listing of the test groups:

Test Group	Dose in diet (ppm)	Main Study days male	Main Study days female	Interim Sac. months male	Interim Sac. months female
1. Control	0	734	734	None	
2. Low (LDT)	12	734	734	None	
3.	50	734	734	None	
4.	100	734	734	None	
5. Highest (HDT)	200	734	734	None	

Rats were individually housed in stainless steel cages with wire bottoms.

In addition to the 250 rats of each sex directly participating in the study, there were 24 rats which were in the same two rooms receiving the control diet. Every 3 months

3 rats from each room were sent to Microbiological Associates (Bethesda, MD) for general health, microbial and viral screening. (Note: this would be 6 rats shipped out each quarter, or a total of 48 rats - instead of 24 - for the entire 2-year study period).

3. Diet preparation: The appropriate amount of test material was heated to 70-80° C until liquified. For each batch of diet prepared the appropriate amount of liquefied FMC 54800 was added to acetone and dissolved. Each solution was added to approximately 500 g of Purina Laboratory Chow #5002 and hand mixed until dry. Premixes were then added to sufficient Laboratory Chow to achieve concentrations of 12, 50, 100 or 200 ppm. Control diets were prepared in a similar fashion, with incorporation of the appropriate amount of acetone.

Control and treated feeds were sealed in plastic bags and closed containers and stored at room temperature until fed to the rats.

There is a report (FMC Study A82-776, GLP E314-76E in appendix F of volume 7) titled: "Homogeneity and Stability Study of FMC 54800 in Rodent Chow," in which technical FMC 54800 was mixed (to a concentration of 50 ppm) with Purina Chow #5002 and, according to the analytical results, the test material was homogeneous and stable in the dietary sample for a period of one year. The technical FMC 54800 assayed for stability was approximately [redacted] cis isomer and [redacted] trans isomer (the material fed to the mice had a ratio of [redacted]

Animals received food and water ad libitum. It is noted (page F56, appendix F, volume 7) that the following deviations occurred:

i. According to the records, during week 27 some females in the 100 ppm group may have received the nominal 50 ppm diet (or an incorrect diet number was recorded).

ii. "During week 22 Group V animals received diet number 110 for four days. This was a nominal 200 ppm diet that analyzed at 110.3 ppm." On a few other occasions (p. F56, appendix F, vol. 7) some of the analyzed diets were outside the acceptable ± 10% range for 50, 100 and 200 ppm, and ± 20% for 12 ppm.

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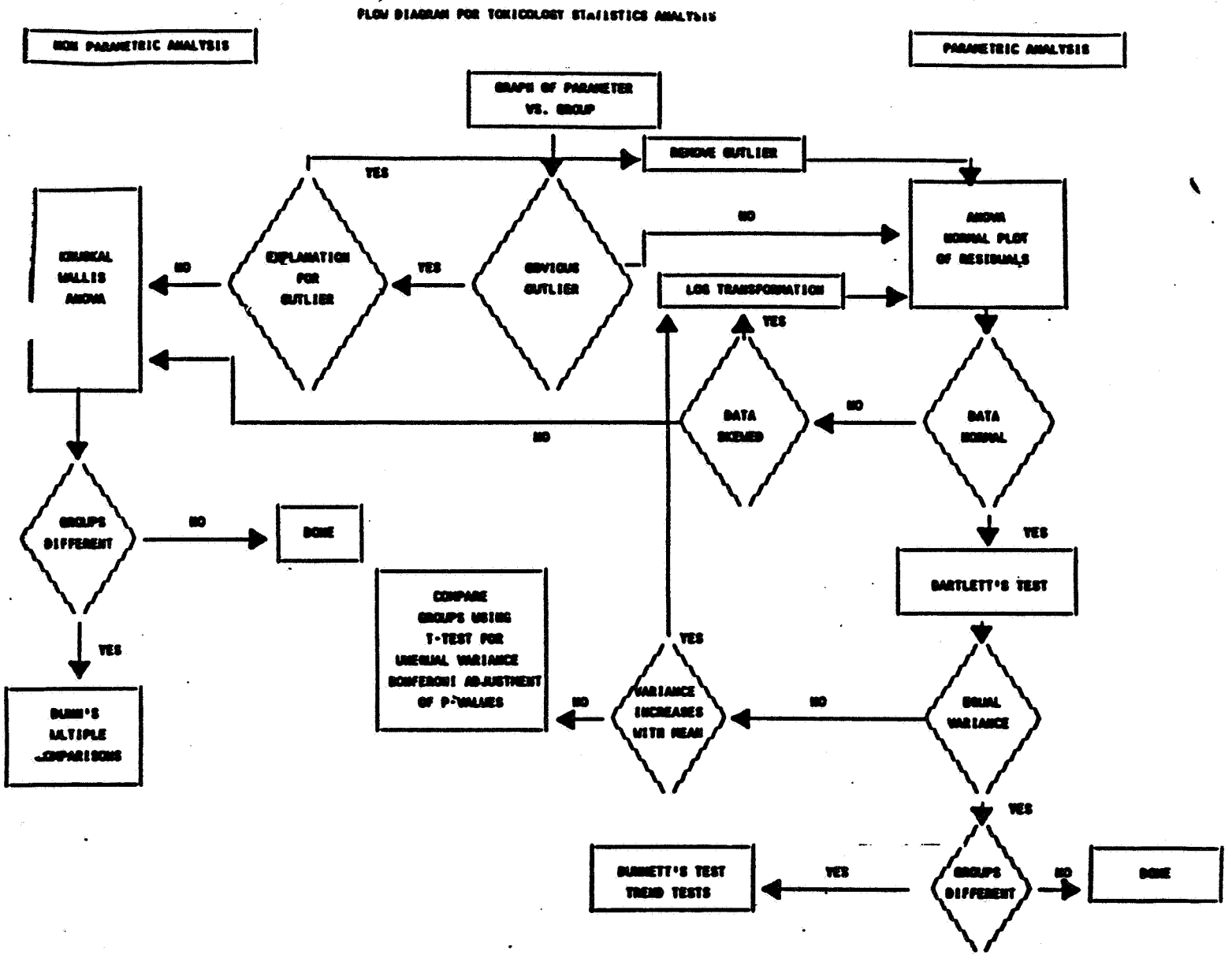
4. Statistics - The following procedures were utilized in analyzing the data:



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5. Quality Assurance: There is a copy of a statement of compliance signed by J. D. McCarty, M.S. in vol. 1, page 2, along with a quality assurance statement reporting when on-site inspections were conducted (p. 3-4) signed by W. D. Barta.

C. METHODS AND RESULTS:

1. Observations: Rats were examined twice a day for mortality and once daily for appearance, behavior, signs of toxicity and moribundity. A detailed examination, including palpation for masses, was performed once a week.

Results

Toxicity: The following clinical observations showed an increased incidence in group V (200 ppm) males and females (from vol. I, p. 63 & 64):

Number of observations (number of animals):

Males:	Group 1 0 ppm	Group 2 12 ppm	Group 3 50 ppm	Group 4 100 ppm	Group 5 200 ppm
Abrasion	0	7(1)	39(1)	0	1250(11)
Alopecia	0	0	12(1)	0	1670(10)
Tail lacerated	0	0	0	0	158(4)
Tremors	0	5(1)	0	0	3893(50)

Females	Group 1 0 ppm	Group 2 12 ppm	Group 3 50 ppm	Group 4 100 ppm	Group 5 200 ppm
Abrasion	10(1)	8(1)	976(1)	37(2)	1343(10)
Alopecia	0	47(1)	53(2)	192(1)	2813(15)
Tail lacerated	21(2)	2(1)	42(1)	0	875(6)
Tremors	0	8(1)	4(1)	3(1)	9616(50)

Tremors are reported (vol. 1, p. 20) as occurring in all group 5 males during days 4 through 28, and in all group 5 females during days 4 through 30 and again from days 36 through 38. Tremors were not observed in group 5 males from days 133 through 307, but then reappeared sporadically. The frequency of tremors in group 5 females decreased in the period from weeks 21-84, but then increased in the period from weeks 89-104. Tremors were observed in one group 4 female (A7624 - see vol. 3, p. A546) on study days 3, 4 and 5, at a time when most group 5 females were showing this symptom.

The tremors occurring in one group 2 male (A7228 - see vol. 3, p. A346) were present relatively late in the study (days 554-558). Those in the group 2 female (A7507 - see vol. 3, p. A480) were on days 628-635 and in the

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group 3 female (A7590 - refer to vol. 3, p. A531) were on days 553, 622, 623 and 632.

Mortality: There were no significant dose-related differences between groups with respect to survival. From vol. 1, p. 31:

SURVIVORS AT TERMINATION/INITIAL NUMBER OF ANIMALS

	Group 1 0 ppm	Group 2 12 ppm	Group 3 50 ppm	Group 4 100 ppm	Group 5 200 ppm
Males	24/50*	23/50*	23/50	17/50	23/50
Females	29/50	28/50	35/50	34/50	28/50

*According to information in vol. 2, p. A1-A2 22/50 group 1 males and (p. A3-A4) 22/50 group 2 males survived to termination.

2. Body weight: Individual body weights were taken weekly the first 13 weeks of the study and monthly thereafter. Final body weights were obtained just prior to necropsy.

Results: The following are mean weights at representative intervals during the study; from vol. 1, p. 35-38:

Mean group weights (grams) - males

Week	Group 1 0 ppm	Group 2 12 ppm	Group 3 50 ppm	Group 4 100 ppm	Group 5 200 ppm
1	189.5	192.5	191.4	194.3	185.9
2	237.2	240.3	237.5	245.6**	232.3
13	434.8	440.8	430.0	446.8	421.7
27	495.6	507.7	493.6	514.0	476.8
54	567.7	575.1	577.3	589.1	544.9
79	589.4	592.4	605.1	608.2	576.8
FINAL	510.9	525.5	523.9	531.5	528.5

**Significantly different from control, $p < 0.01$ as determined by ANOVA and Dunnett's Test.

Mean group weights (grams) - females

Week	Group 1 0 ppm	Group 2 12 ppm	Group 3 50 ppm	Group 4 100 ppm	Group 5 200 ppm
1	149.1	151.8	150.2	150.3	148.1
2	173.2	176.3	173.3	174.8	170.7
13	265.0	268.4	261.7	262.2	255.0*
27	294.2	298.9	290.3	290.9	277.8**
54	344.6	347.6	337.8	336.7	319.2**
79	377.6	376.0	366.3	370.9	343.4**
FINAL	378.4	397.8	361.6	360.9	335.0*

*Significantly different from control, $p < 0.05$ as determined by ANOVA and Dunnett's Test.
**Significantly different from control, $p < 0.01$ as determined by ANOVA and Dunnett's Test.

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Mean body weights of group 5 (200 ppm) males were depressed relative to controls from week 1 through 92 but were never significantly "different" during this period. Mean body weights of group 4 (100 ppm) males were generally higher relative to controls from week 1 through 88, and were significantly "different" from controls at weeks 2-6, 8 and 18. For females, mean body weights for group 5 (200 ppm) rats were generally lower than controls, and, with the exception of week 100, were significantly "different" from controls from week 13 through termination. Body weights for group IV (100 ppm) and group III (50 ppm) females also tended to be slightly depressed relative to controls during this same period, but the differences were never statistically significant.

3. Food consumption and compound intake: Individual food consumption was measured weekly for the first 13 weeks of the study and at monthly intervals thereafter.

Results: From the mean food consumption values given in volume 1, p. 39-40 (males) and p. 41-42 (females), mean food consumption in group 5 males tended to be somewhat less (but usually not significantly "different") than their controls; group 4 males tended to have greater mean food consumption than controls and, through week 54, differences were sometimes significant (this correlates with what was observed with mean body weights). During weeks 1-23 mean food consumption in group 5 females tended to be slightly lower (but was usually not significantly different) than corresponding control values. After week 27 mean food consumption in group 5 females tended to be slightly higher than controls. Group 4 females tended to have slightly higher (but usually not significantly different) food consumption than controls.

Food efficiency - not calculated.

Compound intake: Mean compound consumption in terms of mg/kg/week is reported in volume 1, p. 43-44 (males) and p. 45-46 (females). For group 5 males, mean compound consumption was above 100 mg/kg/week through week 5; for group 5 females mean compound consumption was $\geq$ 100 100 mg/kg/week through week 9. This may be at least a partial explanation for the higher incidence of tremors in group 5 females.

4. Ophthalmological examinations: Eyes of all rats were examined before the initiation of the study; any rat with an eye abnormality was eliminated. Eyes of surviving rats were examined at termination.

Results: There was no indication of any dose-related ophthalmological effect (refer to vol. 3, p. A743-A745).

5. Bleeding: Blood was obtained at 6, 12 and 18 months and at termination (24 months) via puncture of the orbital sinus plexis from 10 rats/sex/dosage level which had been fasted overnight. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)		Total plasma protein (TP)
X	Hemoglobin (HGB)	X	Leukocyte differential count
X	Leukocyte count (WBC)	X	Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)	X	Mean corpuscular HGB conc. (MCHC)
X	Platelet count	X	Mean corpuscular volume (MCV)

Results: From vol. 1, p. 19: "the only significant alterations...were an increased mean corpuscular hemoglobin concentration in Group IV females at the 12-month bleeding, (and) an increase in RBC count in the Group III females at the 18-month bleeding. These findings are...without biological significance and (are) unrelated to the consumption of FMC 54800 as they did not occur in the Group V animals or at any other bleeding intervals. A statistically significant decrease in Group V male red blood cell levels at 24-months is dose-related and is judged to be the result of consumption of FMC 54800." However, the control mean at 24 months was somewhat higher than the previous measurements for this group. From vol. 1, p. 47-50:

RBC Counts (x 10^{12} cells/liter) in male mice (S.D's in parenthesis):

Month	Controls	Group 2	Group 3	Group 4	Group 5
6	8.21(0.567)	8.11(0.371)	8.35(0.501)	8.51(0.326)	8.16 (0.926)
12	7.80(0.477)	7.80(0.380)	7.71(0.473)	7.92(0.542)	7.75 (0.347)
18	7.83(0.594)	7.67(1.087)	7.39(1.234)	8.00(0.585)	7.96 (0.513)
24	8.44(0.628)	8.15(0.557)	7.93(0.453)	7.79(1.144)	7.45*(0.686)

*Significantly different from control at $p < 0.01$ as judged by Kruskall-Wallis followed by Dunn's Test for Multiple Rank Comparisons.

Two group 5 males (A7376, A7386) had relatively low values for RBC counts at 24 months; without values from these two rats the mean RBC count was 7.74 x 10^{12} (with a S.D. of 0.31) cells/liter for that group on that date.

Despite the fact that the mean RBC count for group 5 males at 24 is statistically "different" from the control value, this reviewer considers this as a somewhat equivocal finding, partly because it occurs at only one time (24 months), partly because the control value is relatively high at 24 months, and also because there is no indication

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of any similar effect in females. From vol. 1, p. 51-54:

RBC Counts (x 10^{12} cells/liter) in female mice (S.D's in parenthesis):

Month	Controls	Group 2	Group 3	Group 4	Group 5
6	7.83(0.390)	8.06(0.424)	7.83(0.507)	7.70(0.253)	7.40(0.546)
12	7.31(0.177)	7.61(0.485)	7.41(0.265)	7.05(0.557)	7.27(0.247)
18	7.37(0.386)	7.74(0.355)	7.76*(.195)	7.52(0.355)	7.03(0.933)
24	7.36(0.917)	7.39(0.982)	7.60(0.704)	7.64(0.982)	7.51(0.686)

*Significantly different from control at $p < 0.05$ as judged by Kruskall-Wallis followed by Dunn's Test for Multiple Rank Comparisons.

b. Clinical Chemistry

X		X	
	Electrolytes		Other
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorus	X	Total Cholesterol
X	Potassium	X	Globulin ("calculated")
X	Sodium	X	Glucose
	Enzymes	X	Total Bilirubin
	Alkaline phosphatase	X	Total Serum Protein
	Cholinesterase		Triglycerides
X	Creatinine phosphokinase		
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)		
X	Serum aspartate aminotransferase (also SGOT)		

Results: From vol. 1, p. 19: significant reductions in mean sodium in group 4 and 5 males (1.5 and 1.3% respectively) at 6 months were not biologically significant as sodium levels in males at other times and in females were not affected. At 12 months group 4 females had reduced creatinine phosphokinase activity and group 2 females had increased glucose levels, but significant changes in these parameters did not occur in group 5 females.

6. Urinalysis: Urine was collected overnight at 6, 12, 18 and 24 months from 10 nonfasted rats sex/dosage group housed individually in stainless steel metabolism cages. The CHECKED (X) parameters were examined.

X		X	
X	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific gravity		Bilirubin
	pH	X	Occult Blood
X	Sediment (microscopic)		Nitrate
X	Protein		Urobilinogen

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Results: Individual data from males are in vol. 2, p. A237-A276, and for females in vol. 2, p. A277-A316. Examination of the data indicates the statement in vol. 1, p. 20 that: "Ingestion of FMC 54800 did not alter any of the urinalysis parameters at 6, 12, 18 or 24 months." is correct.

7. Sacrifice and Pathology: All rats that died and were sacrificed at termination were subjected to gross pathological examination and the representative sections of the CHECKED (X) tissues were preserved in buffered neutral formalin for histological examination. Double-checked (XX) organs were also weighed from 10 animals/sex per group at the final sacrifice.

X	Digestive system	X	Cardiovasc./Hemat.	X	Neurologic
	Tongue	X	Aorta	XX	Brain
X	Salivary glands	X	Heart	X	Sciatic nerve
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes	X	Eyes
X	Duodenum	X	Spleen		Glandular
X	Jejunum	X	Thymus	X	Pituitary
X	Ileum		Urogenital	X	Adrenals
X	Cecum	XX	Kidneys		Lacrimal gland
X	Colon	X	Urinary bladder	X	Mammary gland
X	Rectum	XX	Testes	X	Parathyroids
XX	Liver	X	Epididymides	X	Thyroid
	Gall bladder	X	Prostate		Other
X	Pancreas	X	Seminal vesicle	X	Bone
	Respiratory	XX	Ovaries	X	Skeletal muscle
X	Trachea	X	Uterus	X	Skin
X	Lungs	X	Vagina	X	All gross lesions and masses

The second sciatic nerve was preserved in formalin at necropsy from 10 animals sex/group. These nerve specimens were dissected from muscle and teased to separate the axon bundles. Each specimen was stained using 1) hematoxylin and eosin, 2) luxol fast blue and 3) a modified Bielschowsky's (silver stain) for myelin.

Microscopic examination was performed on all preserved tissues from all animals in group 1 (control) and group 5 (highest dose), as well as all animals which died or were sacrificed (moribund) in the course of the study.

Lungs, liver, kidneys, sciatic nerve and spinal cord were examined from all animals in groups 2, 3 and 4.

All gross lesions and "target tissues" were examined from all animals, as were the special stains of the sciatic nerves from 10 animals sex/dose group.

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Results:

a. Organ weights:

Mean liver and kidney weights in group 5 males were elevated (but were not significantly different than) over those of their controls. From vol. 1, p. 65:

Terminal mean organ weights (in grams) of 10 males/group:

Organ	Group 1 0 ppm	Group 2 12 ppm	Group 3 50 ppm	Group 4 100 ppm	Group 5 200 ppm
Liver	15.717†	17.424	15.877	17.182	17.442
Kidneys	4.271	5.284	4.451	4.984	5.477

†Liver weight for A7182M revised from 11.817 to 18.201 grams after reweighing (refer to vol. 4, p. B15) and the higher weight was used in calculations (refer to vol. 3, p. A634).

For females, however, mean liver and kidney weights in group 5 (200 ppm) rats were somewhat lower than those of controls; from vol. 1, p. 66:

Terminal mean organ weights (in grams) of 10 females/group:

Organ	Group 1 0 ppm	Group 2 12 ppm	Group 3 50 ppm	Group 4 100 ppm	Group 5 200 ppm
Liver	11.520	11.941	11.527	10.700	10.957
Kidneys	2.952	2.890	3.117	2.793	2.764

However, group 5 males and females both show somewhat (but not statistically significant) elevated liver and kidney-to-body-weight ratios; from vol. 1, p. 67 and 68:

Organ-to-body-weight ratios (%) for males (10 rats/group)

Organ	Group 1 0 ppm	Group 2 12 ppm	Group 3 50 ppm	Group 4 100 ppm	Group 5 200 ppm
Liver*	8.07	10.10	8.70	9.73	10.32
Kidneys	2.927	3.422	3.094	3.342	3.275

Organ-to-body-weight ratios (%) for females (10 rats/group)

Organ	Group 1 0 ppm	Group 2 12 ppm	Group 3 50 ppm	Group 4 100 ppm	Group 5 200 ppm
Liver*	8.46	8.00	8.37	7.89	8.72
Kidneys	3.255	3.176	3.094	3.000	3.369

*Liver-to-body-weight ratios reported in vol. 1, p. 67-68 are one-tenth the values given here, apparently due to a consistent miscalculation.

There were no indications of any significant or relevant differences between groups with respect to brain or gonadal weights.

One question that this reviewer had was whether

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Reviewed by: Byron T. Backus, Toxicologist Byron T. Backus 8/31/86
Section III, Tox. Branch (TS-769C)

Secondary Reviewer: Marcia van Gemert, Ph.D., Section Head
Section III, Tox. Branch (TS-769C) M van Gemert 8.5.86

DATA EVALUATION REPORT (III)

STUDY TYPE: 2-Generation Reproduction - Rat

TOX. CHEM. NO.: 463F

ACCESSION NUMBERS: 261933, 261934, 261935, 261937, 261938, 261939 MRID NO.:

TEST MATERIAL: FMC 54800 technical

SYNONYMS: Bifenthrin, Brigade, Capture, Talstar

STUDY NUMBER(S): A83-977

SPONSOR: FMC Corporation

TESTING FACILITY: FMC Toxicology Laboratory
76 Fourth St.
Somerville, NJ 08876

TITLE OF REPORT: Multi-Generation Reproduction Study with FMC 54800 Technical in Rats

AUTHOR(S): DeProspo, J. R., Barbera, J., Ballester, E. J. and Geiger, L. E.

REPORT ISSUED: January 31, 1986

STUDY CLASSIFICATION: Core Minimum Data

CONCLUSIONS:

1. Dietary exposure to 100 ppm FMC 54800 (Bifenthrin) causes tremors in female rats during lactation and in the 2-week period following lactation.

2. Since group 3 females frequently showed noticeably (even though not statistically significant) lower mean weights than controls at times when group 4 females had significant weight depressions, it is concluded this was an effect of exposure to FMC 54800 at 60 ppm. An additional consideration is that there is no indication that group 4 females showing the highest incidence of tremors tended to have a greater weight loss than other rats in this

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group.

3. There was no conclusive indication of any effect on group 2 (30 ppm) females.

4. Reproductive parameters were not affected at the highest dose level (100 ppm), nor was there any evidence of fetal toxicity. In a preliminary study considerable pup mortality had occurred by day 14 at a maternal dietary exposure level of 200 ppm FMC 54800.

5. It is stated (vol. 1, p. 5) that: "The...(NOEL) for toxicity was determined to be 30 ppm...while the lowest observable effect level (LOEL) was determined to be 60 ppm... based on the decreased F_1 absolute ovarian weights." It is noteworthy that F_1 females at 30 ppm had a lower (but not significantly different) mean ovarian weight than their controls, and there was a dose-related trend in this parameter among all F_1 groups. However, when ovary-to-body-weight ratios for F_1 females are examined, none of the FMC 54800 exposed groups are statistically different from controls, and the mean ovary-to-body-weight ratios for all 4 groups range from 0.036 (100 ppm) to 0.040 (controls).

6. There was no evidence of any histological effect, either at the macro- or microscopic level.

7. This reviewer concludes that the study is acceptable, and that a LOEL of 60 ppm can be set based on dose-related lower weights in P_1 and F_1 females during the first and second lactation periods, as well as for the second gestation, and significantly decreased mean absolute ovarian weights in F_1 females at 60 and 100 ppm. The NOEL is 30 ppm.

A. MATERIALS:

1. Test compound: FMC 54800 technical, FMC notebook no. E2392-105, 88.35% purity with an isomer of [redacted] The amount added to the diet was adjusted to account for the purity so that the diets contained 0, 30, 60 or 100 ppm in terms of the active ingredient.

2. Test animals: TAC(SD)fBR rats, 8 weeks old at the initiation of the study, obtained from Taconic Farms, 33 Hover Avenue, Germantown, NY 12526. These rats were acclimated for a period of 7 days before the start of the study.

B. STUDY DESIGN:

1. Dose levels: These were selected (refer to vol. 1, p. 6) on the basis of a range-finding study in which groups of 5 presumed-pregnant rats were exposed to dietary concentrations of 0, 12, 50, 100 or 200 ppm FMC 54800 from gestation day 0 through lactation day 21. Lactation phase mean body weight gains were depressed by 28.7%, 51.5% and 114% in the 50, 100 and 200 ppm group dams respectively, while all pups from 2 of the 4 litters at 200 ppm died within 14 days of birth. Overall pup mean body weight gain for the remaining 200 ppm litters was depressed by 36% on lactation day 21.

2. Animal assignment: Animals selected for the P_1 generation were individually weighed and randomly assigned to each of the treatment groups by a computer randomization program. Homogeneity of initial body weights was confirmed by analysis of variance. The following is a listing of the test groups:

Test Group	Dose in diet (ppm)	Number of animals/group male	female
1. Control	0	25	25
2. Low (LDT)	30	25	25
3.	60	25	25
4. High (HDT)	100	25	25

Rats were individually housed in stainless steel cages with wire bottoms, except for females from the end of gestation and throughout lactation, when plastic nesting box cages with nesting material were provided.

The following gives the study schedule (from vol. 1, p. 9):

Study initiation	April 3, 1984
Mating for F_1a Litter	May 29-June 18, 1984
F_1a Terminal Sacrifice	July 14-30, 1984
Mating for F_1b Litter	August 6-27, 1984
P_1 Terminal Sacrifice	September 20-October 8, 1984
Inititation F_1 Generation	October 9, 1984
Sacrifice excess F_1b rats	October 11-12, 1984
Mating for F_2a Litter	December 26, 1984 - January 15, 1985
F_2a Terminal Sacrifice	February 9-26, 1985
Mating for F_2b Litter	March 6-26, 1985
F_1 Terminal Sacrifice	May 8-9, 1985
F_2b Terminal Sacrifice	May 9-10, 1985

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Breeding began for P_1 animals after 8 weeks of exposure, and for F_1 animals after 11 weeks. Females without evidence of mating within 7 days were paired with a different male. If mating was not evident after the second pairing, the female was considered barren.

The first day upon which pups were observed was considered day 0 for that litter. The number of pups born alive or stillborn, and the numbers cannibalized or found dead were recorded. Live pups were sexed and individually weighed; any gross anomalies were recorded. If possible, lungs of dead pups were excised and floated on water to differentiate stillborns from those which had died shortly after birth.

Litters in excess of 8 pups were reduced to 8 on lactation day 4 using a randomization table. If possible, 4 males and 4 females were kept. However, if litters contained less than 4 of either sex on day 4, enough of the other sex were kept to make the total 8.

Pups were allowed to nurse for 21 days prior to weaning. In addition to being weighed at birth, pups were individually weighed on lactation days 4, 7, 14 and 21.

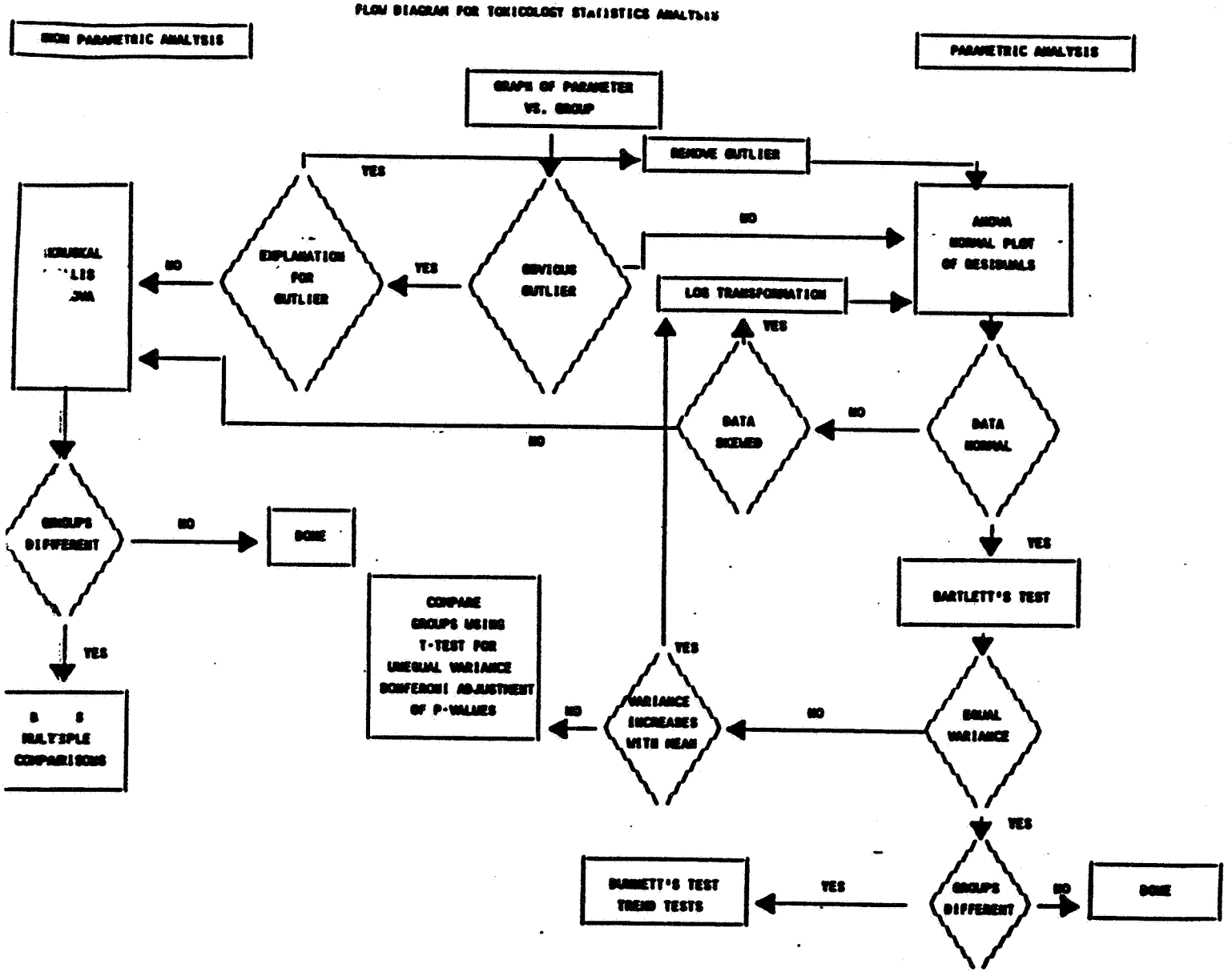
Rats were selected for the F_1 generation from the F_1b litters using a random numbers table, with one male and one female, if possible, being selected from each litter for each group. An additional 10 males and 10 females were randomly selected from each group of the the F_1b litters for necropsy; similarly, 10 male and 10 female weanlings from each F_2b group litter were necropsied, along with any pups with abnormal appearance or behavior.

3. Diet preparation: The test material was heated to 70-80° C until liquified. For each batch of diet prepared the appropriate amount of liquefied FMC 54800 was added to acetone and dissolved. Each solution was added to approximately 500 g of Purina Laboratory Chow #5002 and hand mixed until dry. Premixes were then added to sufficient Laboratory Chow to achieve concentrations of 30, 60 or 100 ppm. Control diets were prepared in a similar manner, with addition of a similar amount of acetone.

   Control and treated feeds were sealed in plastic bags within closed containers and stored at room temperature until fed to the rats.

4. Animals received food (Purina Rodent Chow #5002, mixed with the appropriate amount of FMC 54800) and water ad libitum.

5. Statistics - The following procedures were utilized in analyzing the data:



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6. Quality Assurance: There is a copy of a statement of compliance signed by J. R. DeProspo, M.S., in vol. 1, page 2, along with a quality assurance statement reporting when on-site inspections were conducted (p. 3) signed by W. D. Barta.

C. METHODS AND RESULTS:

1. Observations: Rats were examined daily for appearance, behavior, signs of overt toxicity and moribundity, and twice a day for mortality.

All P_1 rats were individually weighed once a week for the first 8 weeks, as were all F_1 rats for the first 11 weeks. All dams were weighed on gestation days 0, 6, 15, 20 and on lactation days 0, 7, 14 and 21.

Weekly individual food consumption was measured for all P_1 rats during the first 8 weeks and for all F_1 rats for the first 11 weeks, but was suspended during mating. Male food consumption measurements were made after completion of the matings, while dam food consumptions were measured during gestation periods but not while lactation was occurring.

Results - P_1 Generation

Toxicity: There was a high incidence of tremors (affecting females only) in group 4 rats of the P_1 generation. The occurrence of clonic convulsions in one female of this group may also have been related to exposure to FMC 54800 (see vol. 1, p. 47):

Number of observations (number of animals involved):

Females:	Group 1 0 ppm	Group 2 30 ppm	Group 3 60 ppm	Group 4 100 ppm
Clonic convulsions	0	0	0	1(1)
Tremors	0	0	0	410(23)

A check of individual clinical observations (vol. 2, p. A68-A73) shows that tremors occurred only in the period of 9-35 days following birth of the first litter, and 9 to 22 days after birth of the second litter (but rats were sacrificed at day 22 or 23 after birth of the second litter, so if sacrifice had not intervened there may have been further occurrences of tremors).

Number of observations (number of animals involved):

Symptom	Group 4 - days 9-35 after 1st delivery	Group 4 - days 9-22 after 1st delivery	Group 4 - days 9-22 after 2nd delivery
Tremors	304 (23)	197 (22)	106 (15)

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Mortality: There was no significant difference in mortality between groups, with group survival ranging from 96-100% by sex for the P_1 generation. From vol. 1, p. 32 & 33:

Survival to termination - P_1 rats:

	Group 1 0 ppm	Group 2 30 ppm	Group 3 60 ppm	Group 4 100 ppm
Males	24/25 (96%)	24/25 (96%)	24/25 (96%)	24/25 (96%)
Females	25/25(100%)	24/25 (96%)	24/25 (96%)	25/25(100%)

Body weights: Males at 60 and 100 ppm tended to have slightly higher mean body weights (although differences were not statistically significant) than their corresponding controls. Mean group weights at representative intervals are given below (from vol. 1, p. 34):

Mean group weights (grams) - males

Week	Group 1 0 ppm	Group 2 30 ppm	Group 3 60 ppm	Group 4 100 ppm
INITIAL	284.0	284.3	284.1	284.3
1	309.5	316.1	315.2	316.1
2	336.7	338.2	343.0	344.2
13	473.9	471.0	490.2	482.9
17	494.6	495.4	506.2	509.1
FINAL	520.9	528.6	540.7	537.0

There were no statistically significant differences between mean body weights of exposed females and their controls through week 8. At week 17, however (after gestation and lactation) mean body weights for groups 2, 3 and 4 were somewhat lower than controls, and in the case of group 4 females the difference was statistically significant. From vol. 1, p. 35:

Mean group weights (grams) - females

Week	Group 1 0 ppm	Group 2 30 ppm	Group 3 60 ppm	Group 4 100 ppm
INITIAL	189.8	189.8	190.0	189.7
1	198.1	198.2	200.6	197.6
2	214.6	212.9	215.0	213.6
17	315.3	312.9	307.4	303.8*
FINAL	341.2	335.1	334.5	332.3

*Significantly different from the control, $p < 0.05$ as determined by Chi Square and Fisher's Exact Test.

There were no statistically significant differences in mean maternal weights during the first gestation period; however, mean body weights for all 3 exposed groups tended to be lower than their controls through lactation. From vol. 1, p. 36:

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Mean group weights (grams) - females

Lactation Day	Group 1 0 ppm	Group 2 30 ppm	Group 3 60 ppm	Group 4 100 ppm
0	311.0	309.5	299.1	302.1
7	335.3	329.4	324.0	316.2†
14	347.0	341.2	336.3	328.0**
21	327.1	323.4	317.6	316.4

†Significantly different from control, $p < 0.05$ as judged by Kruuskal-Wallis followed by Dunn's Test for Multiple Rank Comparisons.

**Significantly different from the control, $p < 0.01$ as determined by Chi Square and Fisher's Exact Test.

In Group 4 females there was no evident correlation between the incidences of tremors and weight gain or loss during the lactation period following birth of the F_1a litter. Female 2319 gained 33 grams from lactation day 0 to day 21, but tremors were recorded from this rat 15 times from day 9 through 23; female 2327 gained 10 grams in the same period but no tremors were reported for this animal.

In the second gestation, group 3 and 4 females tended to have mean body weight gains that were somewhat less than those of their controls, although differences were not statistically significant. From vol. 1, p. 37:

Mean group weights (grams) - P_1 females
Second gestation period

Gestation Day	Group 1 0 ppm	Group 2 30 ppm	Group 3 60 ppm	Group 4 100 ppm
0	309.3	312.0	307.6	303.5
6	333.6	334.0	329.0	323.6
15	368.5	365.3	360.9	355.7
20	441.8	442.5	428.2	420.5

During the second lactation period, group 3 and 4 females tended to have lower mean body weight than their controls. From vol. 1, p. 37:

Mean group weights (grams) - P_1 females
Second lactation period

Lactation Day	Group 1 0 ppm	Group 2 30 ppm	Group 3 60 ppm	Group 4 100 ppm
0	356.0	347.6	347.3	342.9
7	373.9	368.1	365.2	359.1
14	378.4	369.5	367.2	357.6++
21	358.2	355.3	347.4	349.1

++Significantly different from the control, $p < 0.01$ as determined by ANOVA and Dunnett's Test.

Examination of individual weight data (vol. 2, p. A28) and comparison with individual clinical observations (vol. 2, p. A68-A73) for group IV P_1 females gives no

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indication of any relationship between individual weight gain and/or loss and the incidence (or lack of) tremors in that individual during the lactation period following birth of the F_1b litters.

Food consumption: Group 3 (60 ppm) males tended to have a higher mean food consumption than any of the other groups, but this was statistically significant with respect to controls only for week 12. There were no statistically significant differences between group 4 males and their controls during any of the time intervals in which food consumption was measured.

There were no significant differences between female groups with respect to mean food consumption during any of the intervals in which this parameter was measured.

Results - F_1 Generation

Toxicity: Tremors were noted in females (but not males) of the F_1 generation. From vol. 1, p. 87:

Number of observations (number of animals involved):

Females:	Group 1 0 ppm	Group 2 30 ppm	Group 3 60 ppm	Group 4 100 ppm
Tremors	0	0	0	269(22)

As with the P_1 rats, tremors occurred only in group 4 females only in the period 3-35 days after birth of litters. There was a higher incidence following the first delivery than the second (the same number of dams were involved, but some which did not have tremors during the first lactation period had tremors during the second).

Number of observations (number of animals involved):

Symptom	Group 4 - days 3-35 after 1st delivery	Group 4 - days 3-33 after 2nd delivery
Tremors	172 (16)	97 (16)

Mortality: Group survivals ranged from 96-100% by sex for the F_1 generation, with no significant differences between groups. From vol. 1, p. 72 & 73:

Survival to termination - F_1 rats:

	Group 1 0 ppm	Group 2 30 ppm	Group 3 60 ppm	Group 4 100 ppm
Males	24/25 (96%)	25/25(100%)	25/25(100%)	24/25 (96%)
Females	25/25(100%)	24/25 (96%)	24/25 (96%)	24/25 (96%)

Body weights: Exposed males of all groups tended to have slightly lower mean body weights (differences were not

statistically significant) than their controls. Mean group weights at representative intervals are given below (from vol. 1, p. 74):

Mean group weights (grams) - F_1 males

Week	Group 1 0 ppm	Group 2 30 ppm	Group 3 60 ppm	Group 4 100 ppm
1	219.0	220.2	221.3	211.2
7	435.6	420.4	428.4	421.6
13	514.0	486.2	491.5	492.2
20	557.3	528.2	536.2	530.8
26	581.6	554.5	559.5	551.2
FINAL	601.4	576.7	582.8	579.2

There were no statistically significant differences between mean body weights of exposed F_1 females and their controls through week 11, or at termination (week 31?).

Mean group weights (grams) - F_1 females

Week	Group 1 0 ppm	Group 2 30 ppm	Group 3 60 ppm	Group 4 100 ppm
1	161.2	165.6	166.2	164.4
7	249.8	251.8	249.6	249.8
11	276.7	275.7	272.9	274.6
FINAL	348.6	343.7	345.3	339.2

There were no statistically significant differences in mean maternal weights during the first gestation and first lactation periods. From vol. 1, p. 76:

Mean group weights (grams) - F_1 females

Day	Group 1 0 ppm	Group 2 30 ppm	Group 3 60 ppm	Group 4 100 ppm
First gestation period				
0	274.8	276.1	273.2	276.0
6	293.5	293.0	292.1	294.6
15	326.9	324.7	325.4	323.7
20	393.8	392.3	389.3	388.2
Day	First lactation period			
0	308.1	306.5	307.5	306.6
7	325.7	325.6	325.3	318.2
14	338.0	338.7	338.0	331.5
21	317.4	328.8	325.7	315.3

In the second gestation and lactation periods, groups of exposed females tended to have mean body weights somewhat less than those of controls, although differences were not statistically significant. From vol. 1, p. 77:

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Mean group weights (grams) - F_1 females

Second gestation period

Day	Group 1 0 ppm	Group 2 30 ppm	Group 3 60 ppm	Group 4 100 ppm
0	320.3	311.6	309.3	317.6
6	335.0	330.2	325.2	330.1
15	368.6	360.0	356.5	359.7
20	441.1	433.1	422.0	425.6

Second lactation period

Day				
0	352.6	351.4	349.9	348.4
7	362.6	360.0	358.4	358.4
14	368.8	364.7	363.5	358.8
21	359.0	349.8	351.6	346.2

Food consumption: Group 4 males tended to have lower mean consumption than controls from week 2 through 29, but this was statistically significant only at week 10. There were no significant differences in food consumption between any of the F_1 exposed female groups and their controls for any time interval for which food consumption was measured.

2. Reproductive performance:

Parameters measured:

$$\text{Mating Index} = \frac{\text{Number of matings observed} \times 100}{\text{Number of mating opportunities required*}}$$

*Defined as the number of estrus cycles

$$\text{Fecundity Index} = \frac{\text{Number of delivered pregnancies} \times 100}{\text{Number of copulations†}}$$

†Confirmed by sperm in vaginal smears or assumed by observation of sperm plugs.

$$\text{Male Fertility Index} = \frac{\text{Number males impregnating one or more females} \times 100}{\text{Number of males mated}}$$

$$\text{Female Fertility Index} = \frac{\text{Number delivered pregnancies} \times 100}{\text{Number females mated with males}}$$

$$\text{Gestation Index} = \frac{\text{Number of females delivering litter} \times 100}{\text{Number of females considered pregnant}}$$

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Results:

The P_1 and F_1 generations showed no indications of any dose-related trends with respect to any of the reproductive parameters (mating, male fertility, female fertility, and gestation indices) examined.

3. Delivery Data:

In the P_1 generation F_1a and F_1b litters there were no significant differences between dose groups or evidence for dose-related trends with respect to total number of pups delivered, pups/litter, live birth index, number of stillborn pups, or number of pups cannibalized immediately after birth.

In the F_2a litter of the F_1 generation it is noted in the text (vol. 1, p. 22) that: " A statistically lower live birth index (live pups/pups born) and a statistically higher incidence of stillborn pups were observed in the 30 and 60 ppm groups for the F_2a litter. These findings were attributed to the lack of heat experienced on January 20 p.m. -January 21 a.m., 1985. When the delivery data for litters born during, or shortly after the heat failure are excluded from the overall litter data, there are no apparent treatment-related differences...these findings were not observed in any of the F_1a, F_1b or F_2b litters...The number of pups cannibalized during the F_2a litter was statistically higher in the 100 ppm group. This isolated finding was not considered to be related to treatment since it was not observed in any other treatment group in any other litter (i.e. F_1a, F_1b or F_2b). There were no other statistical differences."

F_1 Generation F_2a litter - from table 65, vol. 1, p. 96:

Group (ppm)	Total of Pups Delivered	Pups/Litter ± S.D.	Live birth index	No. of pups stillborn	No. of pups cannabilizedt
0	278	11.6 ± 1.9 (N = 24)	276/278 (99.3%)	2/278 (0.7%)	0/276 (0.0%)
30	274	11.9 ± 2.7 (N = 23)	263/274 (96.0%)*	11/274 (4.0%)*	3/263 (1.1%)
60	273	11.9 ± 2.5 (N = 23)	262/273 (96.0%)*	11/273 (4.0%)*	3/262 (1.1%)
100	260	10.4 ± 3.4 (N = 25)	252/260 (96.9%)	8/260 (3.1%)	6/252 (2.4%)

†Pups presumed to have been born alive
*Significantly different from the control, $p < 0.05$ as determined by Chi square and Fisher's Exact Test.

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In the F_2b litter of the F_1 generation there were no significant differences between groups with respect to delivery parameters.

4. Progeny Survival:

There were no consistent significant dose-related differences between groups with respect to progency survival for either the first or second litters of the P_1 and/or F_1 generations.

5. Progeny Weights:

In the P_1 generation F_1a litter mean weights for male and female pups from exposed litters were somewhat (but not significantly) lower than controls. From tables 29 and 30, vol. 1, p. 60-61:

Males (mean wts in grams with S.D. in parenthesis)

Lactation Day	Group 1 0 ppm	Group 2 30 ppm	Group 3 60 ppm	Group 4 100 ppm
0	7.3 (0.55)	7.1 (0.54)	7.1 (0.73)	7.3 (0.47)
7	20.5 (2.52)	19.3 (2.60)	19.4 (2.58)	19.3 (1.87)
21	66.6 (6.25)	63.0 (7.68)	63.3 (5.49)	63.9 (5.23)

Females (mean wts in grams with S.D. in parenthesis)

Lactation Day	Group 1 0 ppm	Group 2 30 ppm	Group 3 60 ppm	Group 4 100 ppm
0	6.9 (0.59)	6.8 (0.50)	6.6 (0.79)	6.9 (0.44)
7	19.6 (2.40)	18.6 (2.49)	18.7 (2.51)	18.6 (1.75)
21	62.6 (6.49)	60.2 (5.50)	60.3 (5.57)	61.1 (4.98)

However, in subsequent litters, there were no indications of any dose-related differences in mean weights between control pups and those exposed to the test material.

6. Litter Clinical Observations:

Examination of the summary observations in tables 33-34 (vol. 1, p. 64-65) and tables 73-74 (vol. 1, p. 104-105) indicates there were no consistent or significant differences between dose groups at any one time with respect to such parameters as cannibalization of individual pups, missing pups (presumed cannibalized), individual pups found dead, or any other observations.

7. Litter Anomalies:

In table 34 (vol. 1, p. 65) it is stated that "missing tail" occurred in one group 3 F_1b litter. According to table 22, p. A208 (vol. 2) this involved 2 pups of the litter of dam AA2305. A missing tail is also reported for one pup on day 0 in an F_2b group 4 litter (table 74, vol. 1, p. 105; also table 46, vol. 3, p. A458). There

is no other indication of any possible malformations or anomalies. (In vol. 1, p. 12 it is stated that: "any gross anomalies were recorded." This reviewer has not been able to find any indication - other than the missing tails - that anomalies occurred. However, it would be nice to have a statement somewhere in the report that they did not occur).

8. Mean Progeny Organ Weights:

No organ weights are reported from rats of either the F_1a litter of the P_1 generation or the F_2a litter of the P_2 generation.

For the 10 rats/sex/group representing the F_1b litter, group 4 males had slightly higher mean absolute organ weights for adrenals, heart and testes over the controls, but differences were not statistically significant. Group 4 females showed significantly higher mean absolute weights for adrenals, heart and ovaries over their controls. Group 4 males and females had higher (but not statistically significant) mean absolute liver and kidney weights than their controls; and a dose-related increase appears to be present in both males and females with respect to kidney weights. From tables 35 & 36, vol. 1, p. 66-67:

Males (absolute organ wts in grams with S.D. in parenthesis)

Organ	Group 1 0 ppm	Group 2 30 ppm	Group 3 60 ppm	Group 4 100 ppm
Adrenals	0.032(0.0059)	0.036(0.0080)	0.034(0.0086)	0.036(0.0057)
Heart	0.728(0.1407)	0.785(0.1069)	0.771(0.1187)	0.780(0.0581)
Kidneys	1.560(0.3091)	1.673(0.2046)	1.687(0.2951)	1.704(0.1700)
Liver	7.915(2.0653)	8.836(1.6016)	8.422(1.6067)	9.020(1.3800)
Testes	1.787(0.6252)	2.070(0.4704)	2.005(0.6047)	2.174(0.2588)

Females (absolute organ wts in grams with S.D. in parenthesis)

Organ	Group 1 0 ppm	Group 2 30 ppm	Group 3 60 ppm	Group 4 100 ppm
Adrenals	0.032(0.0060)	0.037(0.0102)	0.035(0.0063)	0.042*(0.0088)
Heart	0.592(0.1008)	0.659(0.0968)	0.641(0.0753)	0.679*(0.0540)
Kidneys	1.250(0.2171)	1.320(0.1964)	1.339(0.1904)	1.411 (0.1617)
Liver	6.310(1.3070)	7.027(1.4543)	6.586(1.1265)	7.316 (0.7634)
Ovaries	0.047(0.0140)	0.068(0.0329)	0.061(0.0144)	0.073¶(0.0176)

*Significantly different from control at $p < 0.05$ by ANOVA and Dunnett's Test.
¶Significantly different from control at $p < 0.01$ by Kruskal-Wallis followed by Dunn's Test for Multiple Rank Comparisons.

However, there were no statistically significant differences between groups with respect to organ to body weight ratios as expressed in percent.

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Group 4 females did have a significantly elevated ($p < 0.05$ by Kruskal-Wallis followed by Dunn's Test for Multiple Rank Comparisons) ovary-to-brain weight ratio with respect to their controls, and groups 2 and 3 were elevated (not significantly) with respect to controls for this parameter. From table 40, vol. 1, p. 71:

F1b females - organ/brain weight ratio (percent) with S.D. in parenthesis

Organ	Group 1 0 ppm	Group 2 30 ppm	Group 3 60 ppm	Group 4 100 ppm
Ovaries	2.679(0.7405)	3.805(1.8019)	3.476(0.7200)	4.057†(0.9404)

†Significantly different from control at $p < 0.05$ by Kruskal-Wallis followed by Dunn's Test for Multiple Rank Comparisons.

For the 10 rats/sex/group representing the F_2b litter there were no statistically significant dose-related differences (or even any possible indications of dose-related trends) in mean absolute organ weights, mean organ-to-body or mean organ-to-brain weight ratios (refer to tables 75 through 80, vol. 1, p. 106-111).

9. Adult Organ Weights:

P_1 Generation:

In the P_1 generation there was a statistically significant elevation in mean absolute weight (as well as organ-to-body weight ratio) for the brain in group 4 females, and this appeared to be part of a dose-related trend. From table 20, vol. 1, p. 51:

Females (absolute organ wts in grams with S.D. in parenthesis)

Organ	Group 1 0 ppm	Group 2 30 ppm	Group 3 60 ppm	Group 4 100 ppm
Brain	2.029(0.0910)	2.078(0.1084)	2.084(0.0806)	2.122¶(0.1103)

¶Significantly different from control at $p < 0.01$ by ANOVA and Dunnett's Test.

In the males of this generation there was no evidence of any elevation in either the mean absolute brain weight or brain-to-body weight ratio (refer to vol. 1, tables 19 and 21, pages 50 and 52).

No other statistically significant differences or possible dose-related trends were evident for adrenal, heart, kidney, liver, testes or ovarian weights or organ-to-body weight ratios.

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F_1 Generation:

Males at all dosage levels showed a reduced mean heart weight, but this was statistically significant only for group 2. Also, there was a slight (not statistically significant) elevation in mean absolute liver weight in group 4 males. From table 59, vol. 1, p. 90:

Males (absolute organ wts in grams with S.D. in parenthesis)

Organ	Group 1 0 ppm	Group 2 30 ppm	Group 3 60 ppm	Group 4 100 ppm
Heart	1.754(0.1598)	1.653*(0.1102)	1.700(0.1691)	1.702(0.1696)
Liver	19.76(2.8118)	19.57 (2.3767)	19.82(2.7237)	20.567(2.9666)

*Significantly different from control $p < 0.05$ as determined by ANOVA and Dunnett's Test.

However, there were no significant differences in mean organ-to-body weight ratios between any exposed male group and the controls.

Among females, mean absolute ovary weights were depressed significantly in groups 3 and 4, with what appears to be a dose-related trend including group 2; from table 60, vol. 1, p. 91:

Females (absolute organ wts in grams with S.D. in parenthesis)

Organ	Group 1 0 ppm	Group 2 30 ppm	Group 3 60 ppm	Group 4 100 ppm
Ovaries	0.138(0.0184)	0.130(0.0157)	0.126*(0.0156)	0.122¶(0.0236)

*Significantly different from control at $p < 0.05$ by ANOVA and Dunnett's Test.
¶Significantly different from control at $p < 0.01$ by ANOVA and Dunnett's Test.

On p. 5, vol. 1, it is stated: "The...(NOEL) for toxicity was determined to be 30 ppm...while the lowest observable effect level (LOEL) was determined to be 60 ppm...based on the decreased F_1 absolute ovarian weights."

However, there were no significant differences between any of the exposed groups and controls with respect to ovary-to-body weight ratios.

10. Adult Organ Histology:

P_1 Generation:

No dose-related changes were found in any of the organs examined from 25 males and 25 females of the control and high-dose (group 4) groups. One group 4 male had unilateral testicular aplasia. Two mammary gland neoplasms were observed, one (a mammary fibroadenoma) in a control female and one (a mammary adenocarcinoma) in a group 4 female.

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F_1 Generation:

No dose-related changes were found in any of the organs examined from 25 males and 25 females of the control and high-dose (group 4) groups. One group 4 female died with generalized septicemia, while one group 4 male sacrificed in a moribund condition had lymphocytic leukemia. Three mammary neoplasms were observed, one (a mammary fibroadenoma) in a group 4 male, and mammary adenocarcinomas in a control male and group 4 female. A squamous cell papilloma was present on the nose of one of the group 4 males.

D. DISCUSSION:

The principle finding of this study is that dietary exposure to 100 ppm FMC 54800 causes tremors in female rats during lactation and in the period (approximately 2 weeks) following lactation.

For the P_1 generation females at week 17 mean body weights of exposed rats were somewhat lower than controls, and in the case of group 4 (100 ppm) females differences were statistically significant. From vol. 1, p. 35:

Mean group weights (grams) - P_1 females

Week	Group 1 0 ppm	Group 2 30 ppm	Group 3 60 ppm	Group 4 100 ppm
INITIAL	189.8	189.8	190.0	189.7
17	315.3	312.9	307.4	303.8*

*Reported as significant at $p < 0.05$.

Group 3 and 4 P_1 females in their second gestation had mean body weight gains somewhat less than those of their controls, with what appears to be a dose-related trend even though none of the differences (even for 100 ppm females) were statistically significant with respect to control values. From vol. 1, p. 37:

Mean group weights (grams) - P_1 females
Second gestation period

Gestation Day	Group 1 0 ppm	Group 2 30 ppm	Group 3 60 ppm	Group 4 100 ppm
0	309.3	312.0	307.6	303.5
6	333.6	334.0	329.0	323.6
15	368.5	365.3	360.9	355.7
20	441.8	442.5	428.2	420.5

Somewhat similar (but not always statistically significant with respect to control values) findings of lowered mean body weights for group 3 and 4 females occurred in the second lactation period:

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Mean group weights (grams) - P_1 females
Second lactation period

Lactation Day	Group 1 0 ppm	Group 2 30 ppm	Group 3 60 ppm	Group 4 100 ppm
0	356.0	347.6	347.3	342.9
7	373.9	368.1	365.2	359.1
14	378.4	369.5	367.2	357.6++
21	358.2	355.3	347.4	349.1

++Significantly different from the control, $p < 0.01$ as determined by ANOVA and Dunnett's Test.

Somewhat similar body weight depressions occurred in the F_1 generation.

Since group 3 females frequently showed noticeable (even though not statistically significant) lower mean weights than controls at times when group 4 females had significant weight depressions, it is concluded this was an effect of exposure to FMC 54800 at 60 ppm. An additional consideration is that there is no indication that group 4 females showing most tremors tended to have the greatest weight loss of animals in this group.

There was no conclusive indication of any effect on group 2 (30 ppm) females.

Reproductive parameters were not affected at the highest dose level (100 ppm), nor was there any evidence of fetal toxicity. In a preliminary study considerable pup mortality had occurred by day 14 at a maternal dietary exposure level of 200 ppm FMC 54800.

While it is stated (vol. 1, p. 5) that: "The...(NOEL) for toxicity was determined to be 30 ppm...while the lowest observable effect level (LOEL) was determined to be 60 ppm ...based on the decreased F_1 absolute ovarian weights." this is equivocal as it was only observed in F_1 adult females (and not in F_1b or F_2b weanlings). Also, when ovary-to-body weight ratios for F_1 females are examined, none of the FMC 54800 exposed groups are statistically different from controls for this parameter.

There was no evidence of any histological effect, either at the macro- or microscopic level.

This reviewer concludes that the study is acceptable, and that a LOEL of 60 ppm can be set based on dose-related lower weights in P_1 and F_1 females during the first and second lactation periods, as well as for the second gestation.

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the 10 rats/sex/dosage group whose organ weights were taken were representative of each of their respective groups. The 10 rats/sex/group surviving to termination with the lowest numerical designations were the ones used as "representatives." Comparing mean terminal weights of groups (all survivors) with those of the 10 rats from that group gives the following:

	Mean terminal body weight (gm) (group)	Mean terminal body weight (gm) 10 rats/sex/group
Male controls	510.9	537.8
Group 5 (200 ppm) males	528.5	534.8
Female controls	378.4	357.1
Group 5 (200 ppm) females	335.0	323.3

b. Gross pathology:

The statement is made (vol. 1, p. 22) that "no treatment related findings were observed at necropsy," and this appears to be essentially correct. However, a few minor findings (such as necrotic tail tips, swollen hind feet) may have been associated with tail lacerations and abrasions, previously noted as having been observed more frequently in group 5 rats than in the other groups in this study.

c. Microscopic pathology:

1) Non-neoplastic: The following findings occurred more frequently in group 5 rats than in controls or the intermediate dose groups:

Incidences (all animals; as reported vol. 5, p. C24-C37):

Organ and finding.	Group 1 0 ppm M	F	Group 2 12 ppm M	F	Group 3 50 ppm M	F	Group 4 100 ppm M	F	Group 5 200 ppm M	F
Pituitary congestion	0/32	0/44	0/24	0/25	0/27	0/22	0/29	0/27	0/37	3/36
Stomach: nonglandular gastritis	1/40	0/49	0/27	0/20	1/25	0/14	0/29	0/16	3/48	2/49
Eye: retinal degeneration	0/31	0/42	0/19	0/7	0/21	0/4	0/21	0/6	0/40	3/28

The text discussion (vol.5, P.C9) does not specifically address any of these increased (but presumably not to a statistically significant level) incidences.

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It is stated (vol. 1, p. 22, and again in vol. 5, p. C9) that: "The type and/or incidence of all non-neoplastic morphologic changes were considered unremarkable. The tissues from rats of this study were noteworthy, in that, with few exceptions, there was a minimal incidence and severity of the degenerative and inflammatory lesions common to aged rats."

According to the text (vol. 5, p. C9) "In the brain a curious lesion of loose granulation tissue occurred as a focal lesion in one Group 5 rat. The granulation tissue replaced a relatively large area of the brain tissue, appeared as a repair/replacement process, was grossly observed, and was otherwise unremarkable." (Presumably this is female #7642, described in vol. 6, p. C947 as having "Brain: #Granulation tissue/focal, marked).

There were no biologically significant differences between dosage groups with respect to findings in the sciatic nerve histopathology carried out on 10 animals/sex/group (refer to vol. 5, p. C17-C18.

2) Neoplastic: The most common tumors and their incidences were as follows (vol. 5, p. C40-C52):

Organ and tumor type	Group 1 0 ppm		Group 2 12 ppm		Group 3 50 ppm		Group 4 100 ppm		Group 5 200 ppm	
	M	F	M	F	M	F	M	F	M	F
Pituitary adenoma	8/32	27/44	8/24	21/25	8/27	20/22	14/29	23/27	9/37	19/36
Adrenal cortical adenoma	9/47	17/49	5/33	8/34	8/33	12/32	5/32	11/33	15/50	13/48
Adrenal medullary neoplasm benign	4/47	1/49	4/33	1/34	2/33	0/32	6/32	0/33	5/50	0/48
medullary neoplasm malignant	3/47	1/49	3/33	0/34	5/33	1/32	1/32	0/33	4/50	0/48

According to the text (vol. 1, p. 22): "The type and incidence of neoplasms in rats of this study were without any definition of a relationship to the test material or dose level of the dietary exposure."

However, the following occurred more frequently in group 5 rats (of one or both sexes) than in their corresponding controls or the intermediate dose groups:

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Incidences (all animals; as reported vol. 5, p. C47 and C52):

Organ and tumor	Group 1 0 ppm		Group 2 12 ppm		Group 3 50 ppm		Group 4 100 ppm		Group 5 200 ppm	
	M	F	M	F	M	F	M	F	M	F
Pancreas: islet cell adenoma	1/47	0/50	0/25	0/23	0/27	0/13	0/31	0/16	3/50	1/49
Fibrosarcoma	0/50	0/50	1/50	1/50	0/50	1/50	0/50	1/50	3/50	1/50

Additionally, one group 5 male (7369; refer to vol. 6, p. C698) had leiomyosarcoma of the salivary gland and lung (?-tumor had possibly originated in salivary gland and metastasized to the lung). One group 2-male (7266) had a leiomyosarcoma of the urinary bladder, but this finding was not present in any other rat. One group 4 female (7630) had a malignant ependymoma of the brain; one group 5 male (7399) had a benign ependymoma of the brain.

For the pancreatic islet cell adenomas the following is a listing of the animals with this finding:

Rat #	Sex	Dose Group	Days on study at death or sacrifice	Remarks
7182	M	1 (0 ppm)	734	
7398	M	5 (200 ppm)	734	
7402	M	5 (200 ppm)	681	8 mm mass
7415	M	5 (200 ppm)	735	
7662	F	5 (200 ppm)	706	

For the fibrosarcomas, the following is a listing of the rats with this finding (among males with this tumor type 3/4 were group 5 animals):

Rat #	Sex	Dose Group	Days on study at death or sacrifice	Remarks
7516	F	2 (12 ppm)	365	vagina+uterus
7567	F	3 (50 ppm)	712	widespread
7601	F	4 (100 ppm)	436	mass on heart
7660	F	5 (200 ppm)	454	vagina+uterus
7247	M	2 (12 ppm)	734	mass on head
7392	M	5 (200 ppm)	488	forelimb mass
7407	M	5 (200 ppm)	384	abdominal mass
7409	M	5 (200 ppm)	635	skin mass

D. DISCUSSION:

The 200 ppm highest dose level can be accepted as being reasonably close to an MTD, based on a preliminary 28-day study in which deaths accompanied by tremors occurred at a 300 ppm dietary level in 6/10 males by day 12 and in 1/10 females by day 20.

While there was no conclusive evidence of dose-related oncogenicity, there were presumably statistically non-significant increased incidences of pancreatic islet cell adenomas in 200 ppm rats of both sexes, and of fibrosarcoma in male rats at 200 ppm. The registrant should supply some historical control data as to the incidences of these tumor types in rats of this strain at this testing facility.

Terminal organ weights were obtained from only 10 rats per sex/dietary group at termination. According to the Subdivision F Guidelines (p. 122) for an oncogenicity study "At least the liver, kidneys, brain, and testes should be weighed wet as soon as possible after dissection to avoid drying. For these organs, at least ten rodents per sex/group should be weighed."

Since organ weights were not obtained from all rats at termination, this results in additional uncertainty as to the interpretation of some of the findings. 200 ppm males had increased mean liver (10.9%) and kidney (28%) weights relative to their controls, although the mean terminal weight of the ten 200 ppm males from which organ weights were obtained was slightly less than that of the corresponding controls (534.8 to 537.8 grams). Males and females at 200 ppm, as well as males at 100 ppm, showed higher (but not at a statistically significant level) liver and kidney organ-to-body weight ratios. Considering the uncertainty, these must be tentatively considered as possible compound-related effects.

Males at 100 ppm showed a statistically significantly elevated mean body weight relative to controls from week 2 through week 6, and during the first 80+ weeks of the study this group consistently had elevated (but not always to a statistically significant level) mean body weights compared to controls. In a previously reviewed 90-day rat feeding study (FMC no. A83-818, dated Jan. 31, 1984) males (also females) at 100 ppm showed a greater mean weight gain than their controls at termination. The higher mean weight of males receiving 100 ppm of test material in this study must then be considered as a possible (but not necessarily adverse) effect.

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Three of 28 group 5 (200 ppm) females (and none of 40 controls) had retinal atrophy, a distribution which approaches statistical significance. This finding must be considered as a possible dose-related effect.

While there was a statistically significant decrease in red blood cell counts in 200 ppm males at 24 months, this was a somewhat equivocal finding as the effect was not observed in females, and it appears as if male controls had a relatively high mean count on that date relative to some of the previous (6, 12 and 18 month) values.

Tremors that were associated with ingestion of the test material occurred in all rats at 200 ppm, but in only 1/50 females and 0/50 males at 100 ppm. The incidence of tremors observed in this study was lower than that of the 100 ppm group in the previously reviewed 90-day feeding study. The difference might be related to the higher amount (10%) of trans isomer in the 90-day study, as compared to the 2% in this study.

Despite the neurological symptoms, there was no evidence of any pathological changes involving the sciatic nerve in slides prepared from 10 rats/sex/group following termination.

The NOEL then is 50 ppm. The LEL (tremors in 1/50 females, possible increases in organ-to-body weight ratios for the liver and kidneys in 100 ppm males) is 100 ppm, with possible increases in organ-to-body weight ratios for kidneys and liver in 200 ppm males and females and tremors occurring at some time in all rats of this dosage level. An increased incidence in retinal atrophy in 200 ppm females must also be considered as a possible effect.

The study can be accepted as core minimum data for both chronic feeding and oncogenicity.