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

14 NOV 1980

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Audit of the Permethrin (FMC 33297) 2-Year Mouse Toxicity/Oncogenicity Study (Mouse II), conducted at Bio/dynamics, Project No. 76-1695 (FMC Project Identification ACT 115.35)

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THRU: William Burnam, Acting Chief
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THRU: for Peter E. McGrath, Director
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INTRODUCTION

The intent of this FDA-EPA joint audit was to investigate the conduct of the Permethrin Mouse II study at Bio/dynamics. Because of the split up of the responsibilities of various aspects of this study beyond Bio/dynamics, it was necessary to expand the scope of this audit to include these facilities as well. Thus, this audit required visits to three FMC facilities, Princeton, Philadelphia and Richmond, California. In addition the FMC study monitor was interviewed in Cleveland, Ohio and Dr. Rapp, the pathologist who read the slides for Mouse II, was also interviewed in Princeton, New Jersey. Since three separate FDA field offices were involved in visits to these facilities and conduct of interviews, there will be four separate FDA audit reports submitted to the Agency.

Ih the report which follows, basic issues investigated at these facilities shall be integrated. No attempt will be made here to encompass all of the details of the FDA audit reports, since such an effort would be redundant.

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Our "bottom line" conclusions relative to the audit of the Mouse II study were listed in our previous memo of October 16, 1980. (See Attachment A) It was concluded that the chronic toxicity portion of this experiment was not useful while the oncogenic portion of the study was valid and demonstrated a potentially greater oncogenicity than previously reported, but slides of lung, liver, and masses were requested from the sponsor to complete our assessment.

One point should be reinforced prior to considering the following report. That is, although the scope of our audit was confined to Mouse II, it was necessary to consider some aspects of previously conducted Permethrin studies at Bio/dynamics including the rat feeding study, No. 74R-1022, FMC Project No. NCT 549.32 and the Mouse I study, No. 74-1100, FMC Project No. 605.35. In both of these studies diet fortification levels as well as control contamination were investigated in Richmond, California. In addition some daily observation and food consumption data from Mouse I were considered in our audit of Mouse II. This audit, however, did not include a comprehensive examination of the conduct of these other studies.

The audit of "Mouse II" was performed as a joint effort with FDA. Four members of the audit team were EPA scientists and two members were FDA investigators. Each EPA scientist prepared a report on the parameters he investigated during the audit. These are attached:

Dr. Louis Kasza, Pathology aspects including histopatholgy examination of selected liver and lung slides & correlation to gross findings (See Attachment B)

Dr. John Doherty, Comparison of gross findings to the edited version and to histopathology findings (See Attachment C)

Gary Burin, food consumption, compound intake, hematology, and environmental conditions (See Attachment D)

Laurence Chitlik, audit coordinator

I must also note that FMC was given about 10 days advance notice of our audit of this permethrin study. Potentially, this offered FMC extensive time to "prepare" for our visit. Although the advance notice, was due, in part, to some "last minute changes" in the EPA audit team, the whole idea of advance notice to the study sponsors is counterproductive to the intent of a "for-cause" investigation. Furthermore, such a procedure is <u>not</u> routinely utilized by the FDA for a "for-cause" audit. This EPA procedure should be changed.

In addition, it soon became apparent to the audit team that FMC had copies of internal Toxicology Branch memos. How many they had was not determined, but they produced copies of several including one of Dr. Doherty. It was explained at one point that they routinely get them from Registration Division. I also examined a critical analysis of one of our reviews performed by Dr. Rapp under a contract with FMC. The release of these internal EPA memos was unwise and potentially harmful. The documents' release prior to the Agency's final determination constitutes a violation of OPP standard operating procedures. In addition, if a sponsor had copies of a critical memo such as that of Dr. Gross, the potential would be there to make a fact finding audit very difficult. Such a possibility must be avoided in the future.

Audit Preparation Prior to the One-site Investigation

In preparation for the audit of this two-year oral toxicity/oncogenicity study of FMC 33297 (Permethrin), available reviews and memos were carefully examined. These included reviews by Drs. Engler and Panitch of the previous Bio/Dynamics rat and mouse studies (3/30/79), Dr. J. Doherty's review of the FMC letter of 1/18/80 and the review of the Permethrin study to be audited, Mouse II, as well as the rather comprehensive memo of Dr. Adrian Gross dated 5/30/80. The 8/16/80 memo from William Burnam was also noted. (Attachment S)) This memo listed many of the identical concerns noted in the 5/30/80 memo of Dr. Gross and recommended that an audit be carried out as soon as possible.

Our audit was therefore designed to investigate the specific issues raised, as well as carry out an over-all GLP type audit related to the conduct of this study. The issues were best spelled out in Dr. Gross' memorandum which comprehensively discussed previous reviewer concerns as well as his own concerns about the Mouse II study and other Permethrin studies conducted at Bio/dynamics. Normally, the reviewers memo, in this case Dr. Doherty's, would have been our primary focus. However, since Dr. Gross had compiled a very thorough assessment of the issues, from this and other studies, his memo was considered paramount in our laboratory audit, although Dr. Doherty's was also carefully considered. In addition, a meeting was held with Dr. Gross prior to the audit so that the audit team could obtain any other useful information Dr. Gross could offer prior to this investigation. It had been understood that Dr. Gross had spent extensive time in additional evaluation of the Mouse II study and we wished to utilize all available information so that our investigation addressed all of the Agency concerns.

Summary of Findings

Although the following summary is provided, a thorough examination of the complete report will result in a much more accurate understanding of the scope of the audit findings.

1. Although there were in-life observations which were of serious concern, Bio/dynamics did not have a pathologist present during the terminal sacrifice. The gross pathology of the "abdominal distention" and ano-genital (A-G) staining was therefore not defined in this study. Dr. Rapp stated that he was at the terminal sacrifice for only part of 1 day.

2. FMC obtained individual gross findings from Bio/dynamics on January 5, 1979. Attachment L indicates that FMC tabulated this data and found "increased liver findings" in the test groups, but failed to report these adverse findings to the Agency for at least nine months.

3. Abdominal distention was considered by Bio/dynamics to be compound related. This is clearly stated in the interim reports which were not submitted to the Agency. At the time of the 1 year report, these findings should have been considered as potentially adverse data and results should have been submitted to the Agency.

4. After the abdominal distention developed in Mouse II, it later developed in other Bio/dynamics mouse studies which followed. At least seven other mouse studies at Bio/dynamics were found to have the same finding. The likely source of the problem was the animal supplier. It is recommended that the scope of the problem be investigated by FDA; other laboratories and studies affected should be determined.

5. The processing of tissues and reading of slides was halted by FMC in early February, 1979 and did not resume until early April, 1979. Although histopathology examination was originally targeted for completion by Dr. Rapp for June, 1979, it was not completed until late November. His report was not finalized until February 9, 1980. Dr. Rapp was told by FMC not to hold open the original time slot for reading slides in this study and hence the anticipated schedule was not met.

6. Although no significant deviations were noted in Dr. Rapp's editorial changes of gross findings, the procedure is not justified.

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7. The tabulations of histopathology data were not prepared by Dr. Rapp; the data were tabulated by FMC personnel and forwarded to Dr. Rapp. He stated he carefully re-checked lung and liver tabulations and spot checked others, retyped that data on his stationery and then signed the report. Such a procedure is not justified and potentially dangerous. FMC personnel were not trained to properly perform this task. Some errors are visible in the pathology report. Tabulated individual animal findings have been requested from FMC.

8. A large number of tissues were not histopathologically examined. These tissues were not to be taken and examined as per the protocol until its revision effective July 17, 1978. This is less than five months before termination of the in-life phase of the study.

9. A second pathologist, Dr. John Ischmael of ICI examined selected liver and lung slides. No report by Dr. Ischmael was submitted to the Agency. We were informed in the November 6, 1980 meeting that no formal report was written. I requested in this meeting that he submit a statement to this effect and a summary of his findings which he reported to ICI management.

10. The autolysis rate is unacceptably high. Of the 202 mice found dead or sacrificed moribund up to 6/15/78, 155 showed partial autolysis and 8 were totally autolyzed. Eighty-one percent of the animals up to this date had at least some autolyzed tissues. From 6/16/78 to termination, 211 additional mice died or were sacrificed moribund. Of these 140 showed partial autolysis and 2 were totally autolyzed. That is, 67% of these animals had at least partially autolyzed tissues. It is therefore apparent that many moribund animals were not sacrificed.

    In addition, 4 mice of the terminal sacrifice also showed autolyzed tissues suggesting poor preservation techniques were utilized.

11. A separate report relating to in-life observations of abdominal distention and A-G staining in Mouse II was not submitted to the Agency.

12. A report on the effect of caging relative to the tumor incidence in Mouse II was not submitted to the Agency. This report was an attempt, without apparent success, to rationalize away the positive tumor findings in Mouse II. This report was commissioned just prior to the January 18, 1980 letter to the Agency and demonstrates by its existence that FMC found Mouse II to be a positive study.

13. An evaluation of bone marrow smears was not completed by FMC. Although a report by Dr. Bloom was written, he requested peripheral blood smears and bone marrow slides to complete his assessment. These slides were not provided by FMC, although the existence of these slides was verified. This incomplete report was not submitted to the Agency.

14. Control diet was contaminated with Permethrin during nearly all analyzed intervals in Mouse II.

15. Control diet was also contaminated in Mouse I and the rat chronic feeding study conducted at Bio/dynamics. Other samples for other weeks should be analyzed by FMC and results submitted to the Agency.

16. Diet analyses for Mouse II indicate that for nearly half of the time, fortification levels were more than 10% below targeted levels.

17. Diet fortification problems may exist in Mouse I and the rat study, but conclusions on this must await analysis of other diet samples by FMC and the final FDA report. Preliminary data received indicates some weekly intervals were underfortified by 20-25%. Also, preliminary data indicates diet levels were fed to the wrong test groups at least during week 24 in Mouse I.

18. Dr. Kasza's examination of selected liver slides revealed an additional tumor in females of Groups II and III and three additional tumors and two proliferative changes re-diagnosed as tumors in Group IV. Liver slides were chosen for his examination on the basis of gross findings and random selection. Slides have been requested for a complete review.

19. Histopathologic examination of randomly selected lung tissues also revealed some additional tumors and therefore slides have been requested for a complete review. (See Attachment B)

20. Diet preparation records are incomplete. (See Attachment D)

21. Enviromental conditions were not properly monitored. Temperature varied from 64 to 80°F and humidity was not controlled.

The following report classifies investigated issues and deficiencies into two basic groups. These groups are (A) previously defined issues and (B) issues and deficiencies determined during the conduct of this audit.

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A. Investigation of Issues identified in the Mouse II study by Dr. Gross in his memo of 5/30/80 and by William Burnam in his memo of 8/13/80.

1. When and by whom were the gross necropsies of those animals sacrificed at the termination of the study conducted? When and by whom were the initial gross necropsy notes on individual animals written?

Necropsies of the terminal sacrifice took place on Dec. 5-11, 1978. Prosectors interviewed did not recall that there was a pathologist present. No gross pathology sheets were signed by any pathologist. We were informed that Dr. John Tornaben was the responsible pathologist for this study under the over-all supervision of Dr. Knezevich. Dr. Tornaben is no longer employed by Bio/dynamics. Dr. Knezevich explained that although he "was probably not there" during the terminal sacrifice, he had looked at slides prepared from five animals found dead early in the study. This examination of tissues from 5 animals was carried out, it was explained, in an effort to identify the reason for the "abdominal distention" and A-G staining observed in many animals in the Mouse II study.

In consideration of the fact that Dr. William Tierney, the study director, (a) had observed and was concerned about the in-life observations i.e.- abdominal distention and the A-G staining, and (b) had considered these findings in a number of interim reports to be compound related and, (c) knew that the associated pathology was not defined and (d) both he and clearly Bio/dynamics understood that a pathologist should have been present during a scheduled sacrifice, why wasn't one there? Bio/dynamics was informed in a number of prior audits of deficiencies in their necropsy procedures; there is no justification for this additional episode. The only explanation offered was that they (Bio/dynamics) didn't consider what was performed as a "regular" necropsy. That is, only the selected organs as stated in the procedure were removed and the carcass was then preserved with remaining tissues intact.

Dr. Tierney indicated that once this necropsy was completed their portion of the study was finished. The wet tissues and the gross pathology sheets were obtained by FMC on January 5, 1979, nearly a month after the terminal sacrifice. Bio/dynamics personnel did not tabulate the necropsy findings.

(2) When and by whom were the gross necropsy findings tabulated?

It is apparent that FMC personnel could have tabulated the gross pathology findings within a few days after receipt of the gross pathology sheets from Bio/dyanamics.

Nelson Wilson was the FMC study monitor. He was responsible for keeping FMC management informed and he tabulated all significant findings as the study progressed, i.e.- mortalities, abdominal distention etc. It was also postulated by other FMC personnel, that he had been responsible for the tabulation of the gross findings for the first 200 animals that had died or were sacrificed moribund during the study. This tabulation (although not signed) was located (Attachment Y), but it is not nearly as relevant as the final tabulation. All of the microfiched records of Nelson Wilson, Dr. Gerry Schoenig, and all "available" correspondence files related to this study were examined, but no such tabulation was ever found. Nelson Wilson left FMC in June of 1979 and he was not available for our questions in Princeton, N.J.

Nelson Wilson interview

On November 3, 1980, Nelson Wilson was interviewed at the FDA offices in Cleveland, Ohio.

(a) Nelson Wilson stated that he was employed by FMC for about two and a half years beginning in January of 1977 and ending in mid- June, 1979. He came into the firm just after the animal mix-up problem was discovered in Mouse I.

(b) He stated that he knew nothing about diet fortification problems related to Permethrin studies conducted at Bio/dynamics. He was responsible for monitoring a number of studies including the entire in life portion of Mouse II.

(c) He thought diet analyses were carried out in Mouse I and the rat study but did not remember any details.

(d) He did many tabulations, including those pertaining to abdominal distention in Mouse II. He was not aware of the abdominal distention in Mouse I.

(e) He stated that he usually tabulated gross pathology findings soon after receiving the individual gross pathology sheets from Bio/dynamics. He received 2 copies of the gross pathology sheets from Bio/dynamics, would keep one and give the other one to American Histolabs. He stated that he would have tabulated the gross pathology for Mouse II, but did not remember when this was done. (He picked up tissues from Bio/Dynamics on January 5, 1979.)

(f) He stated that he was at the January 26, 1979 meeting with ICI but had to leave early to catch a plane. He remembered that in a conversation with Dr. Fletcher near the elevator that he learned ICI was not aware that Bio/Dynamics had proceeded with the slide processing and examination. They were sharing the costs and did not want to continue having the tissues processed and slides read.

(g) He stated that it was possible gross pathology was discussed at the January 26 meeting, but more likely the gross pathology was known by management prior to the March 30, 1979 meeting with the Agency. He stated that he would have been the person tabulating this data, but he was not certain precisely when he did it. He would have reported the findings to at least McCarthy and Fletcher.
(See Attachment L)

(h) It was Dr. McCarthy who told him to have Dr. Rapp stop reading the slides.
(See Attachments H, I, J, K, L)

Meeting with ICI Representatives at EPA on November 6&7, 1980

On November 6th, after the ICI and FMC meeting with HED toxicologists, I spoke briefly with Dr. R.E. Ridsdale, G.A. Willis, and M.H. Litchfield about ICI participation in the meeting with FMC on January 26, 1979; Dr. Ridsdale was a participant in this previous meeting (See Attachment K). I also asked whether ICI had played any role is stopping processing of tissues and reading of slides for Mouse II, as noted in the interview of Nelson Wilson on November 3, 1980.

These ICI representatives did not recall any such meeting. I was also informed that they had told FMC, upon learning of the existence of the Mouse II study (some 15 months after it had been in progress) that they thought it was unnecessary. They suggested to FMC at that time that gross findings from Mouse II should be compared to the Mouse I study and on the basis of this comparison, the Agency should be informed that it would not be necessary to complete histopathology in Mouse II.

On the morning of November 7, 1980, Dr. Robert Ridsdale of ICI Americas called and asked for a meeting at 11:00 a.m. of that day to more clearly state the ICI position on the Mouse II study. Attending this meeting were Dr. J.T. Braunholtz, Mr. G.A. Willis, and Dr. Ridsdale of ICI and myself and Bertram Litt of HED. William Burnam, was informed of this meeting. The following points were discussed:

a. The ICI representatives again stated that they were not aware of this January 26, 1979 meeting with FMC. I produced the minutes of this meeting and informed them that Dr. Ridsdale was a participant and I read a few lines from the memo. Dr. Ridsdale stated that he vaguely remembered such a meeting but did not recall anything else.

b. I again asked about any part ICI may have had in halting the completion of Mouse II and informed them that the reason stated in the minutes was cost.

They informed me that no costs were shared by ICI; Mouse II was completely the financial responsibility of FMC, as the ICI studies were solely paid for by ICI. They had suggested to FMC that the study should be stopped after a comparison of Mouse I and Mouse II gross pathology (and tissues were imbedded) because they felt that another study was simply a waste of scarce resources.

c. They stated that the first gross and histopathology results for Mouse II were received by ICI in January of 1980.

d. They stated that Dr. Engler was informally told in some meeting(s) in mid 1978, that they (ICI) were not conducting any additional oncogenicity testing. Dr. Ridsdale and Mr. Willis stated that Dr. Engler had actually asked the question.

Essentially, he (Dr. Engler) stated that he was aware that there may be as many as 3 additional ongoing Permethrin studies and he asked if ICI was conducting any additional chronic studies on Permethrin. ICI responded "No". They concluded that Dr. Engler was therefore aware of the Mouse II study at that time. Dr. Ridsdale agreed to supply his minutes of the related meeting(s), but thought that since the primary focus of the meeting(s) was tolerances, that the subject would not be included in the minutes. He is going to supply an additional statement to this effect.

e. Dr. Ridsdale made the point that he had spoken to Dr. Chan on January 2, 1979, and was told that he had reviewed the re-submissions and was satisfied that the regulatory requirement for these Permethrin studies was satisfied. This was the basis for ICI telling FMC that the Mouse II study was a waste of resources. At the same time, however, it was made clear to Dr. Ridsdale that Dr. Chan's recommendations had not been signed off by the Toxicology Branch Chief and were therefore not necessarily the Agency position.

3. What was the nature of the "editorial changes" in the records of the gross observations? Was Dr. Rapp present at the time of these observations?

The editorial changes did not involve deletion of grossly reported lesions. In addition to the "editorial" changes performed by Dr. Rapp, which were primarily only an improvement of terminology, there were some additions of lesions not described by prosectors at Bio/dynamics. These additions, were likely the result of technicians trimming the tissues at American Histolabs, in Rockville, Maryland.

Although no significant deviations were noted between the Bio/dynamics American Histolabs additions, the mechanics of this system are not justified and such procedures should not continue in future FMC studies. Too many hands were involved and the data trail is very difficult to follow.

Although Dr. Rapp stated during our interview that he was present for one day during the terminal sacrifice of Mouse II, this was not recalled by any of the prosectors interviewed by the FDA investigator. In fact, the prosectors did not recall any pathologist being present and no gross pathology sheets were signed by any pathologist. The procedure of modifying gross observations by Dr. Rapp at some later date, when he did not note the original findings, is not justified. It is obvious that prosectors should be properly trained before they do necropsies rather than attempting to correct their work sometime after the fact.

The additional gross findings noted by American Histolabs technicians are not at all defined. That is, the only way to determine that these changes were actually made is to compare the original handwritten gross pathology sheets to the final typed version. We are unable to ascertain without an additional visit to American Histolabs whether personnel were properly trained and supervised, but overall their additions were considered of minor significance. If such a procedure is utilized, it also must be properly documented, and it certainly was not in this FMC study.

4. The numbers of mice mentioned in Dr. Rapp's report is at variance with the number stated on the study.

Our investigation revealed that once Dr. Rapp had read the slides, the individual histopathology findings were sent back to FMC. The data were then put into their computer and eventually tabulated by FMC personnel, Drs. Don Nye and Roger Case, through the use of a program FMC was developing. We were informed that there were many "bugs" in the process which caused numerous re-runs and as one FMC toxicologist stated, "It was a nightmare". He also stated that:

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(a) Dr. Rapp's descriptions were not always consistent and therefore it was very difficult to properly categorize the data utilizing their pathology dictionary. On some occasions it was necessary to contact Dr. Rapp in order to alter terminology and obtain consistent diagnoses.

(b) The FMC toxicologists explained that they did not have the training in pathology to handle the task with any degree of confidence and that although several other pathology reports were computerized (ie- carbofuran) by them, they no longer computerize pathology data.

It was later learned in our interview with Dr. Rapp that he took the FMC computerized pathology data, re-checked the total lesions for lung and liver, "spot checked" some other findings, had the report typed on his own stationery and signed it.

This is a very significant irregularity for several reasons:

(a) The submitted report makes no mention of the fact that tabulation etc. was performed by FMC personnel. (This must be interpreted as intentionally misleading since the report was re-typed on Rapp's stationery).

(b) Although we did not note any major errors in the submitted tables, it would be an extremely difficult job to be able to determine any, since only summary tables without individual animal numbers were submitted. Special attention was devoted to liver and lung lesions but in order to completely validate the submitted tables, each and every individual pathology sheet would need to be examined and tabulated. Some errors were noted in the numbers of animals per group, for example, in Group II males and females and Group III females 76 animals per sex per group were reported versus the procedure which states 75 were actually used. In addition, 26 Bronchioalveolar adenomas were counted in Group IV females versus 28 reported in the table on page 17. It is for these reasons that tables of individual animal findings have been requested from FMC.

(c) The whole concept of a sponsor getting so deeply involved into the manipulation of a pathologist's findings is not justifiable. Dr. Rapp accepted a very significant responsibility for a correlation of data he did not perform.

5. Dr. Gross stated on page 7 of his 5/30/80 memo that in the March 30 meeting with FMC, the specific request from the registrants was for permission to abort the then ongoing study (Mouse II). Dr. Gross also states on page 8, that as early as December, 1978 it was known, at least at Bio/dynamics, that the proportion of female animals with grossly visible tissue masses in the liver at the mid and high level of exposure were highly significantly increased.

The audit team made an exhaustive effort to investigate these issues.

(a) It was determined that no correlation of gross findings was performed after the terminal necropsy at Bio/dynamics. A number of Bio/dynamics personnel were interviewed on this point including prosectors, the study director Dr. Tierney, Dr. Knezevich and others. Also, during our interview with Dr. Rapp we again raised the question as to whether he was aware of any tabulation of gross pathology findings, at any time during his involvement. He stated that he did not know of any tabulation.

(b) Nelson Wilson acted as the FMC study monitor. He left the firm in mid June, 1979 and was not available for an interview when the audit team visited FMC facilities. While at FMC in Princeton, a number of correspondence files related to Mouse II were examined. Mike Bruckheimer, the FDA investigator also obtained and examined the microfiched files of Nelson Wilson. The FDA investigator examined all of the microfiched records looking for information on the permethrin studies. Between the hard copy files and the michofiched records, it was evident that Wilson kept very close tabs on the study. He tabulated many findings including mortality, abdominal distention, and gross pathology for the 202 spontaneous deaths and moribund animals, etc. It was clear that his findings were also routinely forwarded to FMC management. What we were unable to locate was the gross pathology tabulation for the terminal sacrifice. FMC personnel stated that they did not know of its existence. FMC management was also questioned on this issue, including John J. Lauber, Manager of Product Registrations, John F. McCarthy, Director of Product Development and Registrations Research and Development, and Donald E. Bissing, Director of Research and Development, all of the Agricultural Chemical Group. No FMC personnel claimed to have known of a tabulation of gross lesions for the terminal sacrifice.

We did, however, uncover some limited documentation related to these issues. FMC decided at least as early as January 26, 1979 not to complete the Mouse II study. This is documented in the minutes of a meeting with ICI, dated 2/5/79. On page 4, the minutes stated:

> "The disposition of this study was explained. Slides will be prepared but not read as long as no differences in gross pathology are noted. EPA will be so notified. Costs will be the reason for not doing the histopathology." (See Attachment K)

Dr. Fletcher explained that after Dr. Chan essentially ruled out the oncogenic potential of Permethrin, that FMC felt there was no need for the additional Mouse II study. They were also aware, however, that Dr. Chan's recommendations were preliminary; the recommendations had not been signed off on at the Branch Chief level. Dr. Fletcher also stated that FMC was then going to prepare a letter to the Agency. Gross pathology data for the Mouse II study was going to be included in this document and FMC was going to request that it was not necessary to complete the study. This document was never sent to the Agency.

We were informed in our meeting with FMC on October 7, 1980 in Philadelphia (Donald Bissing, John Lauber, William Hymans and John McCarthy) that Dr. Fletcher was told to do the gross pathology tabulation on January 26, 1979. This report was also to include histopathology on the spontaneous death animals and gross pathology from the terminal sacrifice of December, 1978. They intended to have the data compared to Mouse I. Dr. McCarthy explained that he didn't receive the tabulation in February, and in March Dr. Gross raised the issue again. An examination of internal EPA documents revealed that Dr. Gross completed his first permethrin memo on March 19, 1979. In Dr. Gross' 5/30/79 memo, he states that he was asked to resolve an existing issue between the views of Dr. Panitch and Dr. Chan.

So in other words, it was apparent that a controversy related to the oncogenic potential of Permethrin existed and persisted within the Agency and that the issue had not been completely resolved by Dr. Chan, as FMC had stated. Furthermore, at least 7 weeks passed between the FMC decision to submit the gross pathology data to the Agency and the 3/19/79 memo of Dr. Gross, and over 2 months before the March 30th meeting with the Agency. At this meeting Dr. Gross states in his 5/30/80 memo, that he was first informed of the fact that Mouse II was "in progress". Thus, there was adequate time for FMC to have tabulated and supplied the Agency with this information.

Dr. Bissing stated in our October 7, 1980 meeting in Philadelphia that it was his decision to stop the Mouse II study. He also stated that sometime during the middle of March 1979, in a meeting with Herb Harrison, he learned of Dr. Gross' memo and the TOX concerns. He stated that the decision had therefore been made prior to the March 30 meeting to complete the Mouse II study. This is in sharp contrast to what is stated in Dr. Gross' 5/30/80 memo. Dr. Gross states that "the specific request by the registrants was for permission to abort the ongoing study."

In response to our questions as to why the gross pathology data was not submitted as originally planned, we were informed that since the decision had already been made to complete the study, that the tabulation and the accompanying letter were not necessary since no rationale was then needed to avoid completion of the study.

The Agency did not receive any formal notification of findings until January 18, 1980. This "notification" was also notably brief and incomplete. FMC management stated that they thought that Dr. Engler had been informed of the existence of the study many months prior to the March 30 meeting with the Agency. Dr. Engler was contacted after my return from the meeting with FMC management on October 7. He stated that he was aware of an additional permethrin study, but to his best recollection he thought the reference was to a Burroughs-Wellcome study. Recently, drafts of 2 Burroughs-Wellcome permethrin studies were received by the Agency. These studies were submitted by FMC.

One final note on this issue. Much of the cost for the in-life portion of the Mouse II study was absorbed by Bio/dynamics. With the exception of protocol additions (over the Mouse I protocol) the in-life portion of Mouse II was not a financial responsibility of FMC; costs were absorbed by Bio/dynamics. The only substantial costs would have been slide preparation and histopathology diagnoses by Dr. Rapp. The decision not to complete the study was made after Dr. Rapp had already completed an examination of tissues for the first group of 200 spontaneous deaths. This must be the case since he asked for re-cuts for these on 12/26/78. Therefore, although histopathology findings were not picked up by Nelson Wilson until 4/9/79, a substantial portion (about 1/3) of his examination of slides had already been completed.

Part of the reason for the delay in the completion of this study is also based upon the fact that on 2/9/79, Dr. Rapp was visited by FMC representative Nelson Wilson, and further readings were cancelled. Dr. Rapp had originally set aside a large block of time in his schedule to do Mouse II. He had originally scheduled the completion of histopathology for June, 1979. Dr. Rapp was not told to continue slide reading until 4/9/79, or approximately a week after the March 30 meeting with the Agency. Dr. Rapp finally completed his examination of slides in Mouse II and submitted this data to FMC on 11/20/79. He re-examined liver and lung sections from 12/31/79 to 1/8/80. His report was finalized February 9, 1980.

6. Large Numbers of Tissues not Examined

On page 14 of Dr. Gross' report, item 12, he makes reference to the fact that a large number of tissues including cecum and jejunum were not examined.

The explanation for this is a protocol change. A letter from Nelson Wilson requested that a number of additional tissues including gall bladder, epididymus, prostate, parathyroid, seminal vesicles, parathyroid, cecum, jejunum, esophagus, trachea, thymus, cervix, cervical lymph node, spinal cord (thoracic), sciatic nerve and skeletal muscle, were added to the list of tissues to be examined for all animals. In addition, middle ear, and nasal cavity with paranasal sinuses were added for histopathological examination of 10 per sex per level. (NOTE: The histopathology report notes that far more than 10/sex/level were examined for these several tissues).

Adrenals, lungs and spleen were added to the list of tissues to be weighed. Other changes included placing adrenals in a cassette to avoid loss.

It was learned from Bio/dynamics that although the protocol change was requested by Nelson Wilson of FMC on June 13, 1978, it was not placed into effect until July 17, 1978. This change is confirmed in an August 14, 1978 letter from Nelson Wilson to Dr. William Tierney, the study director. (See Attachments E, F and G)

The submitted report procedures do not by any means state the tissues to be examined. Only on page 4 of the pathologist's report is this clearly stated. At that, Dr. Rapp states the protocol change was June 24, 1978, which our audit was unable to substantiate. Dr. Rapp states that if tissues were available from animals prior to this date, these were also processed. It is apparent from the report that few if any of these tissues, were actually processed since tissues were not saved at necropsy for many of the spontaneous deaths prior to July 17, 1978.

7. Examination of some liver & lung slides was performed by a second pathologist

In early January, 1980, Dr. John Ischmael of ICI examined selected liver and lung slides and agreed that "liver and lung findings were of concern". Although no report was available to us, we were told that Dr. Ischmael spent about a day and a half examining slides. (See Attachment W) Dr. Kasza noted that it was apparent that selected slides had been pulled out of sequence and placed in separate boxes for examination. ICI was requested to furnish a copy of Dr. Ischmael's report in a meeting on November 6, 1980. We were informed that no formal report was written. It was then requested that he submit a statement as to his participation and conclusions to management.

8. Autolysis Rate

Over two thirds of the 600 animals on study died prior to the terminal sacrifice. Far too many animals were not sacrificed moribund and hence additional tissues were lost.

(See Attachment B, Dr. Kasza's report and tabulations performed during the audit by FDA investigators, Attachment X.) The number of autolyzed and lost tissues are listed on individual pathology sheets and can be tabulated for any organ. This information was available to the Agency reviewer and was submitted by FMC.

B. Issues and deficiencies identified during the conduct of the Mouse II audit.

1. Addendum Reports withheld by FMC

In addition to the GLP type audit (performed by Dr. T. Ellison) submitted with the Mouse II report, FMC had two other evaluations performed by Dr. Ellison on this study which were not submitted to the Agency.

a. "Analysis of the Physical Observation Data Obtained During the In-Life Phase of the Twenty-four Month Oral Carcinogenicity Study of FMC 33297 (Permethrin) in Mice" 1/29/80.

This report was made available to us by FMC and should be attached to the FDA audit report when it is submitted to the Agency. It is worthwhile noting that this report is a detailed evaluation of the "abdominal distention" and A-G staining that was discussed in a number of interim reports submitted by Bio/dynamics to FMC; none of these interim reports were submitted to the Agency. As early as the 1 year interim report, dated December 12, 1977, a description of what was thought of at the time as a very unusual finding was noted in the treated groups.

b. An Analysis of the Effect of Caging on the Incidence of Tumors and Mortality for the Twenty-four Month Oral Carcinogenicity Study of FMC 33297 (Permethrin) in Mice (Bio/dynamics study 76-1695, FMC Study #ACT115.35)

This evaluation was initiated by FMC on 1/7/80 although the report itself was completed 2/15/80. The positive oncogenicity findings in the Permethrin study were obviously recognized and this report was an effort to negate the findings due to other causes i.e.- virus. No association was demonstrated between caging, tumors and mortality and therefore FMC decided not to submit it. On the other hand, such a report would tend to strengthen the study findings and it should have been submitted to the Agency.

2. Bone Marrow Smears

In addition to the two addendum reports, the audit also determined that a separate report on bone marrow smears had not been submitted to the Agency. This report was released by FMC to the audit team and should be attached to the FDA audit report. The report made no specific conclusions since the pathologist, Dr. Bloom, had not received all of the necessary materials he requested to formalize his conclusions. He desired peripheral blood smears and bone marrow slides in order to perform a proper assessment.

It was also determined that some of the bone marrow smears had been damaged during shipment from Bio/dynamics to FMC and were supposedly not readable. No reference to this data or these problems were included in the FMC submissions to the Agency. Our audit did not reveal any effort to supply Dr. Bloom the requested slides. Such data is potentially very significant to the proper interpretation of this study and it should have been completed and submitted to the Agency.

3. Abdominal Distention & A-G Staining in Mouse II

Although the final report indicated a higher incidence of yellow staining of the ano-genital area in treated males than controls, as well as a "slightly greater incidence" of abdominal distention observed in treated males and females than it was in corresponding controls, the associated pathology for these observations was not determined. This issue was left totally unresolved in the submitted report. Furthermore, FMC chose not to submit an addendum report on these findings prepared by Dr. Ellison. (Item #B-1)

These findings were discussed with Dr. Rapp and Dr. Knezevich. Dr. Rapp essentially concluded that without corresponding histopathology, and gross pathology, that the observation of distended abdomen was not "real". Dr. Knezevich stated that he never saw an animal with distended abdomen in this study but that in another study he had seen it and considered the observation due to severe amyloidosis. He also indicated that he had examined tissues of 5 spontaneous death animals from Mouse II and had not determined any histopathology that could be associated with the abdominal distention and A-G staining. (See Attachment H)

During the exit meeting at Bio/Dynamics, Dr. Hogan stated that these same observations had been noted in at least seven other mouse studies. The laboratory agreed to take voluntary action to finally characterize these findings. The sponsors of the affected studies are to be informed of the problem, the laboratory is going to survey the severity of the finding in these studies and submit the information to FDA investigators. Bio/Dynamics stated that other testing laboratories had recently reported the same finding to them and their animal supplier turned out to be the same. (They have since changed supplier) These findings are therefore likely related to the animal supplier, Charles River Breeding Laboratories of Wilmington, Massachusetts, rather than being compound related. A follow-up course of action is advisable, to establish which other laboratories and studies may have been affected. These observations, abdominal distention and A-G staining, are also likely associated with reduced longevity.

4. Diet Analysis

(a) Dr. Ellison's report (FMC GLP audit report) indicated samples of diets were prepared during weeks, 1, 7, 10, 14, 30, 41, 42, 43, 44, 45, 56, 69, 81, 95 and 105. Actually many other samples were also taken (likely weekly), but these were not analyzed by FMC. Dr. Ellison, also noted several apparent discrepancies which were documented during our audit. The targeted

500 ppm sample (Group III males) from week 10 (2/4/77) actually had only 47.7 ppm, the targeted 20 ppm sample of week 43 (Group II males & females) actually had only 3.1 ppm and the targeted 20 ppm sample of week 44 actually had 139 ppm.

These diet fortification errors were reported, but our audit revealed that many additional samples were also taken and not analyzed. Since only a limited number of samples were analyzed, it is not possible to ascertain the scope of diet preparation errors, especially since actual analyses were not at regular intervals.

(b) Another problem which surfaced during examination of diet analyses records relates to the contamination of control diet and test level fortification.

These records were obtained from Dr. Don Nye of FMC. The results of our findings for the dose groups are included in the attached residue table. The analyses indicate that nearly half of the time, levels were more than 10% below targeted levels. Actual compound intake should therefore be considered when a risk assessment is performed.

Examination of available records indicated that there were almost no reported analyses of control diet, although samples were taken. The only 2 analyses of control diet samples were from week 42, demonstrating a level of 0.73 ppm and from week 44, with a level of 6.4 ppm. A footnote indicated for the week 42 control sample, "This residue level ususally found in diet samples due to contamination of blenders used in extraction of sample". At that time, there was no explanation for the 6.4 ppm level in the control diet for week 44, 9/30/77. Dr. Don Nye was then requested to supply all other control analyses. He stated that these data had never been forwarded from Richmond, California; he requested the data from there. The following control analyses were eventually provided:

Date of Sample	Residue in PPM
12/8/76	3.3
*1/6/77	Other diet levels analyzed
Week 7	None detected
2/4/77	1.28
3/4/77	1.98
6/24/77	0.38
*9/6/77	Other diet levels analyzed
9/9/77	0.24

(Chart continued)

Date of Sample	Residue in PPM
9/16/77	0.73
9/23/77	None detected (broken bag)
9/30/77	6.4 (broken bag)
10/7/77	0.11
12/23/77	None detected
3/24/78	0.18
6/16/78	0.08
9/22/78	0.28
12/1/78	0.23

*It is doubtful that control diets were not analyzed at these times, since test diets were analyzed at these same intervals.

The "potentially" contaminated control diet was apparently a surprise to FMC management. It was suggested to them, and they agreed, that a careful re-analysis should be performed if samples had been retained. It would therefore, not be prudent to make any conclusions about the contamination of control diet until this re-analysis is completed. (See Attachments M&N)

(c) Approximately six weeks into the Mouse II study, the diet preparation methods were changed. The vehicle was changed from corn oil to acetone.

Not only was the change made in the Mouse II experiment, but Mouse I and the rat chronic feeding study as well. This change was made very early in the Mouse II study and if there was a diet fortification problem with permethrin in this study, the first 6 weeks would not have a significant bearing. On the other hand, depending upon the scope of the problem, which I have asked FDA to continue to investigate at the FMC analytical facilities in Richmond, California, the test levels in the studies nearing completion, i.e.- the previous mouse study and the chronic rat study, may have been significantly affected and dose levels may actually have been lower than reported. I have examined the audit report of the permethrin rat study of 4/9/79 and can find no indication that FDA or EPA investigators looked into diet preparation records and/or analysis records at the time their audit was conducted. The revised protocol in the rat study examined in this earlier audit clearly stated that effective 1/4/77,

RESIDUE TABLE

FORTIFICATION LEVELS

Date	20 PPM		500 PPM		2000 PPM		2500 PPM		5000 PPM	
	Total Residue	Percent	Total Residue (100 ppm for 12-8-76 1-6-77)	Percent	Total Residue	Percent	Total Residue	Percent	Total Residue	Percen
12-08-76	-	-	73.8	73.8	-	-	2114	84.6	4244	84.9
1-06-77	-	-	90.5	90.5	-	-	2208	88.3	4656	93.1
2-04-77	22.1	110.5	47.7*	9.5	1907	95.4	2404	96.2	4892	97.8
3-04-77	23.9	119.5	477	95.4	2037	101.9	2518	100.7	4930	98.6
6-24-77	17.3	86.5	441	88.2	1788	89.4	2110	84.4	4534	90.7
9-09-77	19.7	98.5	439	87.8	1709	85.5	2269	90.8	4530	90.6
9-06-77	19.7	98.5	436	87.2	1760	88	2229	89.2	4448	89
9-23-77	3.1*	15.5	438	87.6	1716	85.8	2086	83.4	4495	89.9
9-30-77	139*	695	428	85.6	1556	77.8	2195	87.8	4398	88
10-07-77	17.7	88.5	423	84.6	1751	87.6	2275	91	4480	89.6
12-23-77	19.2	96	445	89	1756	87.8	2391	95.6	4659	93.2
3-24-78	20.5	102.5	432	86.4	1830	91.5	2460	98.4	4673	93.5
9-22-78	19.1	95.5	486	97.2	1654	82.7	2293	91.7	4644	92.9
6-16-78	19.2	96	465	93	1821	91.1	2279	91.2	4854	97.1
Terminal	18.6	93	473	94.6	2119	106	2369	94.8	5011	100.2

*Values greatly different than intended fortification levels.

NOTE: Percentage cis varied from 39-43, percentage trans from 57-61.

diet would be prepared using the acetone preparation method. The study was terminated only 4 1/2 months later.

The reason for diet preparation change in all three studies is at least partly known. On a memo dated 9/24/76 from G.J. Fujie, an FMC Chemist, is a handwritten note from Jerry Schoenig to a "Jack" indicating a potentially serious problem.

Dr. Schoenig wrote, "All samples are low. This is the reason we looked at technical material. I evaluated method of diet prep while I was down there last week. I think problem lies there. We are trying several new ways to prepare diet and then we will analyze." (Attachment P)

On 10/22/76, or over a month before Mouse II was initiated, is another memo from Dr. Schoenig also related to this problem. (Attachment Q) He stated, "As a next step in trying to resolve the problem relating to apparent deficiency of FMC 33297 in the animal diets, I have asked Dr. J. Killeen of Bio/dynamics to prepare diets in several different ways." Dr. Shoenig also states, "This is becoming a very critical issue and needs prompt resolution." Five different diet preparation methods were tried and analyses for groups II and IV, 20 and 500 ppm levels are attached to the 11/16/76 memo of G.H. Fujie.

It is therefore apparent that prior to the Mouse II study there was a problem with fortification of permethrin in diets to proper levels. This is the likely reason for the change in diet preparation from corn oil as the vehicle for the first 6 weeks, to acetone starting on January 17, 1977 in the Mouse II study. We also note that there is only one available analysis for the Mouse II study prior to this change. The dose levels reported to the Agency for previous studies may not be representative of what the animals actually received. All diet analyses records should be obtained for these earlier Permethrin studies.

(d) Food Consumption Comparison to Mouse I

During the audit of Mouse II, food consumption data from Mouse I was compared. It was blatantly apparent that mice of the same strain as the previous study (Mouse I) and of the same body weight often consumed nearly twice the amount of diet in Mouse II. Gary Burin verified the correctness of food consumption and compound intake in Mouse II. (See Attachment D)

Dr. Hogan, the study Director of Mouse I and Dr. Tierney (Mouse II) were asked for explanation as to this rather dramatic difference. They had apparently not noticed this previously and were unable to rationalize the difference. To date, I have not received any explanation.

5. Preliminary Findings of the FDA Investigation of the Diet Samples Analyzed at the FMC Facility in Richmond, California, (Received from FDA, 11/7/80)

   a. Permethrin Rat Study No. 74-1022

   Only seven weekly intervals of samples were analyzed by FMC in this study. Original findings for control diet revealed that at five of the seven intervals, control diet was contaminated with Permethrin. Reported values were 1.0, 2.2, (5.0, 9.6), 0.8, and (0.7 and 0.9) ppm. The 5.0 ppm level was reported for male control diet on 1/13/77 and the 9.6 ppm level was reported for female control diet on the same date. FMC's recent repeat analyses of this interval found residues of permethrin estimated at less than 0.2 ppm, but an interference peak was noted which inhibited quantitation by peak area integration and residues were therefore estimated by peak height measurement. Repeated analyses of only the 11/25/76 interval, originally 2.2 ppm, showed 0.33 ppm permethrin. No sample was available for re-analysis of the 1.0 ppm finding of 8/5/76.

   It is recommended that available stored diet samples be analyzed to determine whether other control diet contamination occurred. RCB should examine the data obtained by the FDA investigators, when the report is completed. Control diet contamination in this study may potentially render it not useful for assessment of chronic toxicity.

   Original analyses of test groups diets at these 7 intervals, with the exception of week 58, were generally within reasonable limits. Re-analyses of the 8/8/75 (week 7) and 1/8/76 (week 29) intervals demonstrated significant variation and reduction in levels, likely beyond that due to storage stability of Permethrin. Values ranged from a 7% reduction (expected) to an much as an 18% reduction from originally reported values.

   Re-analysis of other diet intervals is recommended since fortification levels determined in the re-analysis are generally 25% below originally targeted levels; this difference is not explained by storage stability alone. (See Attachment T)

(b) Mouse I, No. 74-1100

Nine weekly intervals of diet samples were analyzed by FMC in this study. Contamination of control diet was originally found in two of the nine weekly intervals; week 75, 11/22/76 at 2.0 ppm and week 104, 6/13/77 at 2.7 ppm. Recent re-analysis confirmed the previous contamination findings (the limit of detection was 0.1 ppm for each isomer). The re-analysis of the week 75 sample showed 0.32 ppm and the week 104 sample was found to have 9.3 ppm. In addition, at week 24, 0.35 ppm was detected. Three other intervals also showed levels of permethrin but these were below the reported limits of detection and an interference peak was present in these samples.

Also of concern in Mouse I was a mix-up of diet at week 24, 12/1/75, where Group II, the 20 ppm level actually received 387 ppm (Re-analysis confirmed 412 ppm) and Group IV, the 500 ppm level actually received 21.3 ppm (Re-analysis confirmed 14 ppm).

In addition, on 11/22/76 and 6/13/77 groups III and IV were confused, ie- group III was reported as the 500 ppm group and group IV as the 4000 ppm group. This error was discussed with Jim Wyman, the FDA investigator and he determined that the error (where groups were reversed) likely took place during the recording of the analysis at Richmond and is not a diet error at Bio/dynamics. The error will be documented in the FDA report.

Diet fortification levels were low (20-25%) during some of the intervals. (See Attachment U)

Also of note is that for a two week period (weeks 20 to 22) the 500 ppm received 5000 ppm. This is stated in the FMC report.

(c) Mouse II, No. 76-1695

Re-analysis of control diets confirmed a consistent contamination of control diet. Only the high contamination levels of 4.2 ppm at week 42 and 9.7 ppm at week 44 (re-analysis values) can be explained due to broken sample bags during shipment. These values should not be considered as actual diet comtamination levels. The broken bags may also explain the 139 ppm level in the Group II diet 20 ppm level at week 44, 9/30/77. (See Attachment V)

ATTACHMENT A

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Status of Permethrin Audit (Mouse II), analyses, and Section 18 request on Soybeans

FROM: Laurence Chitlik, Section Head#5
Toxicology Branch, HED (TS-769) 10/16/80

TO: Douglas Campt, Director
Registration Division (TS-767)

THRU: Christine Chaisson, Acting Chief
Toxicology Branch, HED (TS-769) Christine L. Chaisson 10/16/80

THRU: Peter E. McGrath, Director
Hazard Evaluation Division (TS-769)

Although preparation of the audit report for the Permethrin oncogenicity study conducted at Bio/Dynamics is still in progress, we have been able to reach a "botton line" at least in terms of the usefulness of the data. Our audit determined that;

(1) The study is not useful for assessment of chronic toxicity for a variety of reasons to be specified in our report, but including "potential" contamination of control diet and;

(2) The oncogenic findings in the study are confirmed and strengthened; Some additional tumors were identified by Dr. Kasza in his examination of liver slides based upon gross lesions and a random examination of other liver and lung slides.

Since the tumor incidence was altered due to our random examination of 5 additional liver sections per sex per group it has been decided in a meeting with Dr. Kasza, Ed Budd, Bertram Litt, Chris Chaisson and myself, that examination of all slides of lung, liver and masses will be required before a risk assessment can be performed.

In order to complete this task the registrant should be contacted and requests should be made for the following:

(1) All slides for all groups

A. Identified as No. 3, lung
B. Identified as No. 6, liver
C. Identified as No. 17, (masses)

(2) Individual animal incidence tables of microscopic findings.

The animal numbers should be listed in the heading, with organs and graded lesions for each animal identified. Data should be arranged by sex and test group with approximately 20-25 animals listed per page.

In reference to the Section 18 request by the State of Georgia for the use of Permethrin on Soybeans, as explained above we are unable at this time to supply you with a reliable risk assessment, based upon our recent audit findings, and the action is therefore being returned to you. Of special concern to us, beyond the relatively small human dietary consumption of soybeans, is the necessity of avoidance of secondary residues in milk and meat. Furthermore, soybean milk may constitute 100% of the diet for many infants. Unfortunately, these risks cannot be reliably quantitated at this time.

ATTACHMENT B

First Draft Copy

Oct., 4 , 1980

TO: Larry Chitlik
Head of Auditing Team

FROM: Louis Kasza, D.V.M., Ph.D
Pathologist

OBJECTIVE: Results of Pathologic Investigation FMC. Permethrin at E. Millestone, New Jersey, 9/24 - 26/80 (EPA Reg. Nos. 279-3013, Caswell No. 652BB-Ambush 2E, EPA No. 279-10182 RI, and Pounce 3.2 EC, EPA No. 279-GMRU) 8F2044 and 8F2034; Registrants: - FMC and ICI.

SUMMARY (Attached)

INTRODUCTION:

Groups of 75 mice/sex were fed Permethrin at dose levels in males of 0, 20, 500, and 2,000 ppm and in females at 0, 20, 2,500, and 5,000 ppm. Necropsy examinations were conducted on animals which died or were killed as moribund and on all animals at the terminal sacrifices. Histopathologic examination was performed with each of the 600 animals.

The results related to Hepatoma (H), Hepatocellular carcinoma (H.C.), Bronchioalveolar adenoma (B.A.), and Bronchiogenic carcinoma (B.C.) are summarized in the company's pathologic report as shown.

Mouse II

Sex	Male				Female			
Group	I	II	III	IV	I	II	III	IV
Dose Level (ppm)	0	20	500	2000	0	20	2500	5000
Liver								
Hepatoma	16	21	18	17	3	2	15	17
Hepatocellular carcinoma	4	6	13	5	0	2	3	0
Total Proliferative Nodules	20	27	31	22	3	4	18	17
Lung								
Bronchioalveolar adenoma	18	19	20	17	12	14	28	28
Bronchiogenic carcinoma	1	0	2	1	2	0	2	2

Based on this tabulation, the incidence of liver and lung tumors in female animals indicate oncogenicity related to this test compound (Doherty report). The company provided the sections from the livers (marked by 6), the lungs (marked by 3), and sections from masses including the liver and lung masses which were observed grossly (marked 17). These slides were separated for each animal and placed in separate boxes.

The objective of this study was to confirm the company's histopathology findings and correlate the results of the gross pathologic descriptions.

MATERIALS AND METHODS:

In all female groups, we selected all animals which had grossly observed pathologic changes in their livers. We examined the livers and lungs of all these mice, and we compared our histopathologic diagnoses to the company's diagnoses. On slide 3, there were at least two lung sections. On slide 6, in addition to spleen and kidneys, there were at least 2 liver sections. On slide 17, there were at least two sections either from livers or lungs if the animals had grossly detected masses. Altogether, a minimum of 6 sections was studied from each selected animal. In the female control group, 15 animals had grossly detected liver lesions. In the female low dose groups, 21 animals had grossly described liver lesions. In the middle dose group, 39 animals had grossly observed liver lesions. In the high dose female group, 34 animals had grossly detectable changes. In addition to the female groups which had macroscopic changes in the livers, 5 animals in each group (male and female) were randomly selected (Mr. Litt made the selection) for our histopathologic study. Also, from two animals all tissues which were listed in the report were checked microscopically to confirm

that the listed tissues were present for histopathologic observations. Several animals were selected to see if bone and bone marrow were present as indicated in the report. At least 650 sections were investigated in this study during our three-day visit in E. Millestone at the premises of FMC. The slides were identified and given to me for histopathologic observation by Dr. Doherty who previously reviewed the company's report.

Both in the liver and in the lungs, the principal lesions which we compared were the proliferative changes. They are:

Liver:
- Hepatocellular hypertrophy
- Hepatocellular hyperplasia, focal
- Nodular hyperplasia
- Hepatoma
- Hepatocellular carcinoma

According to the company pathologist, the last two lesions represent tumors. Hepatoma is considered as benign and the hepatocellular carcinoma as malignant tumors. For comparable results, we accepted this terminology although we were not entirely in agreement with it. Many pathologists in mice identify benign liver tumor as a hepatocellular adenoma (and not hepatoma) and the malignant liver tumor as hepatocellular carcinoma in mice.

Lungs:
- Bronchioalveolar adenoma
- Bronchiogenic carcinoma

Both lesions represent tumors. Adenoma is benign; carcinoma is malignant.

The facilities for microscopic investigation were sufficient. The quality of the slides was adequate to recognize tumors in spite of the fact that several of the slides showed some degree of autolysis and artifacts.

Liver sections which were not found:	633 637 640
Lung sections which were not found:	633 637 640 655 808 854

The slides were studied microscopically and the diagnoses made and written down. Then the diagnoses were compared to the company's diagnoses. The livers and lungs of approximately 18% of the animals were investigated.

RESULTS:

Approximately equal numbers of animals showed gross pathologic changes in the livers in control (15) and low dose groups (21). The middle dose group (39) and the high dose groups (34) showed gross lesions in similar distribution. It should be noted that in the high dose group at the terminal sacrifice only 22 animals, in middle 23 in low 33 and in control group 22 animals survived.

Histopathologic Findings:

In the following table, we summarize our histopathologic findings in female mice and compare them to the company pathologist's diagnoses using identical number of tissues and the same tissue selection.

MOUSE II (Liver)

Sex	FEMALE							
Group	I		II		III		IV	
Dose Level (ppm)	0		20		2,500		5,000	
No. of Animals	15	15	21	21	39	36	34	34
Diagnoses	Company	EPA	Co.	EPA	Co.	EPA	Co.	EPA
Liver								
Total Tumors (Hepatoma and Hepatocellular Carcinoma)	3	3	4	5	18	19	17	22

Except in the high dose group, there is no basic difference in total number of tumors between the company report and the EPA pathologist's findings. There was some difference in diagnoses related to hepatoma and hepatocellular carcinoma. The EPA pathologist diagnosed a few more carcinomas than the company pathologist. The results of our findings in the lungs are summarized in the following table:

MOUSE II (Lungs)

Sex	FEMALE							
Group	I		II		III		IV	
Dose Level	0		20		2,500		5,000	
No. of Animals	15	15	21	21	38	35	34	31
Diagnoses	Co.	EPA	Co.	EPA	Co.	EPA	Co.	EPA
Lungs								
Total Tumors (Bronchioalveolar Adenoma and Bronchiogenic Carcinoma)	6	5	3	4	16	17	15	17

There was a slight difference in the number of tumors which were diagnosed by the Company pathologist and the EPA pathologist. Regarding the incidence of

of lung tumors, this report has little value. The number of tumors is far less than the company pathologist reported in his summary incidence table. The reason is that we investigated only a limited number of lungs and the animals were selected based on gross lesions in the livers. These lung tissues should be considered rather as random selections where the numbers in high and middle doses were close to double the control and low dose groups.

However, we confirmed all company pathologist's tumor diagnoses, and, in addition, we found a few more lung tumors which were not diagnosed by the company pathologist.

In the randomly selected female mice, 5/group, differences in liver diagnoses were not found between the company pathologist and the EPA pathologist. In the lungs, one additional bronchioalveolar adenoma was found in the middle dose group (No. 666) by the EPA pathologist.

In the randomly selected male mice, 5/group, one additional hepatoma (321) was dianosed in the low dose group, and the company's diagnosis of two nodular hyperplasias in the high dose group was changed to hepatoma (715, 719). Differences in diagnoses related to lung tumors were not found in these groups.

We checked two animals (Nos. 105, 155) in order to see that all listed organs and tissues in the report were present on slides. The listed tissues in the report were identical to those we found on slides.

Also, at the request of the team leader, I spot-checked a few animals to see that the bones and bone marrows were prepared for histopathologic observation. The bones and bone marrows of all (150, 151, 153, 159) selected animals were prepared for microscopic examination.

Additional information: The objective of our investigation was restricted to tumors in liversand lungs. We also saw several spleens and kidneys sections which were on the same slides as livers. It occurred to me that amyloidosis and possible lymphosarcomaswere more frequently present than I anticipated. Without tabulation and critical evaluation of these lesions, no conclusion can be made, but I advised the project officer that he should make an effort to evaluate these lesions from the report with special attention to the severity of the lesions, the distribution of the lesions and the first occurrence of these lesions in different groups. The high incidence of pneumonia and marked nephritis in all groups reflect the poor general condition of the animals in this experiment.

On the pathologic data sheets there were proper descriptions of gross and microscopic changes. The organs and tissues which had no remarkable changes were listed. Adequate correlation was made between gross and microscopic findings. In a separate portion of the report, summary incidence tables were prepared However, there were no individual incidence tables prepared. This is a rather significant shortcoming in this report since without these tables, the justification of summary incidence tables is difficult, if not impossible. These tables should be requested and should contain the animal numbers in the heading, as well as organs and graded lesions per animal. Approximately 20 - 25 animals should be listed on one page.

We have some reservations related to the scientific design of this experiment. The low dose (20 ppm) group was extremely low in comparison to middle (500, 2, 500 ppm) and high dose (2, 000, 5, 000 ppm) groups. The reason for this unusual dosing should be explained by the company.

On each pathology sheet at the bottom in 1, 2, and 3, points, those organs were listed which were not suitable for histopathologic observation or they were lost. The numbers of these tissues were particularly high with the animals which died spontaneously. The unusually high (46-66%) spontaneous death indicates unsatisfactory observation of animal colonies and allowing the moribund animals to die. These animals, because of the rapidly developing autolysis, have limited value for histopathology. It can be considered that approximately 50% of the animals fall in this category. This and other mentioned factors raise the question of reliability in the investigation of the effects of Permethrin in this experiment.

We discussed several problems of this experiment related to pathology with Dr. Rapp, the contract pathologist. We clarified the terminology of neoplastic changes. Also, I made remarks with regard to the moderate number of differences in diagnoses. He checked a few of them (804, 818, 811, 816), and did not dispute the correctness of our diagnoses. We both agreed that they all represent proliferative changes, and he diagnosed them correctly, but I put some of these lesions in the category of hepatoma and hepatocellular carcinoma (both tumors), instead of modular hyperplasia (which is not considered to be a tumor) or hepatoma as Dr. Rapp did.

The differences ultimately do not change the general concept, such as tumor incidence which was considerably higher in middle and high dose groups than in control and low dose groups. The EPA pathologist's findings not only confirmed the oncogenicity of Permethrin, but strengthened it by the diagnosing of additional tumors, especially in the high dose groups.

CONCLUSION:

The EPA sent a group of people to audit the FMC Permethrin experiment at East Millestone, New Jersey. My duty was to investigate the pathologic aspects of this experiment. The immediate objective of my exercise was to justify the histopathologic findings of the company pathologist in the mouse II experiment. From the pathology report, approximately 109 animals were selected primarily on the basis of liver lesions observed grossly. I found good correlation between gross and histopathologic changes. The figures related to tumors in the livers were justified with the modification that a few more proliferative changes were diagnosed as tumor than were done by the company pathologist. This finding, however, did not alter the basic interpretation that tumors occurred in considerably higher number in middle and high dose groups than in control and low dose groups. This concept was strengthened by the additional few tumors which we diagnosed in the selected animals.

The experiment had some major shortcomings, e. g., the mortality rate was very high (in high dose male group only 12 animals were left out of 75 at the termination of experiment). Spontaneous death occurred in approximately 50% of the animals. This is an unusually high number. These animals have

limited value for histopathology because of rapidly developing autolysis. A large proportion of animals had pneumonia, chronic nephritis, amyloidosis which reflected the generally poor condition of animals in this experiment. All these shortcomings make the reliability of this experiment questionable. Because of some differences in diagnoses between the company pathologist and the EPA pathologist, a full scale histopathologic review of this experiment is advisable, unless enough data are available to make a final decision.

cc; W. Burnam
J. Doherty
G. Burin

SUMMARY (Pathologic Report, Permethrin audit) Louis Kasza,DVM., Ph.D.

In this report, we would like to emphasize that the incidence of liver and lung lesions is based on reviewing a limited number of tissues. It should not be considered as a complete review of the liver and lung sections. The primary objective was to justify the company pathologist's report. However, almost all livers which had grossly detected enlargements, masses, or nodules were included in our microscopic study. Almost 100% of the tumors which were diagnosed by the company pathologist occurred in livers which showed grossly detected enlargements, masses or nodules.

Groups of 75 mice/sex were fed Permethrin at dose levels in males of 0, 20, 500, and 2,000 ppm, and in females at 0, 20, 2,500, and 5,000 ppm respectively. Proliferative changes (hepatoma and hepatocellular carcinoma) were found in female mice by the company pathologist in 3 (0), 4 (20ppm), 18 (2,500 ppm), and 17 (5,000 ppm) incidence. We confirmed the correctness of these findings. In addition, we diagnosed a few nodular changes as hepatoma, and some hepatoma diagnoses were changed to hepatocellular carcinoma. The tumors in the investigated livers according to our findings are summarized as 3 (0 ppm), 5 (20 ppm), 19 (2,500 ppm), and 22 (5,000 ppm). Our findings, in agreement with the company pathologist's findings, confirm and strengthen the facts that the liver tumors are present in considerably higher incidence in high and middle dose groups than in control and low dose groups (See table page 5).

Our investigation of lung tumors, however, has little value regarding the incidence of tumors because the selection of animals took place based on liver gross lesions. Approximately double the number of animals were reviewed in middle and high dose groups than in low dose and control groups. Based on

the aformentioned, the selection of lungs represents a random selection. However, we confirmed all of the company pathologist's tumor diagnoses; and, in addition, we found a few lung tumors which were not diagnosed by the company pathologist, although these changes do not seem to alter the company's interpretation related to the tumor incidence in lungs (See Table, page 5).

In the randomly selected male group, we diagnosed two hepatomas in the high dose group which were diagnosed by the company pathologist as nodular changes. The format of the pathologist's report was satisfactory, although the tabulation of individual lesions should be provided. Some data (e.g., the high rate of spontaneous death, 46 - 66%) indicate that the colonies were not properly controlled. These make the reliability of this experiment questionable.

In spite of the fact that in principle there is agreement between the company pathologist and the EPA pathologist related to the establishing of oncogenicity in the liver, the differences which were presented may indicate that a full scale histopathological review of this experiment is warranted. unless enough data are available to make a final decision.

ATTACHMENT C

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DATE

SUBJECT *Audit of Permethrin Mouse Oncogenesis Study Conducted by Bio/Dynamics (Study No. 76-1695) and Visit to FMC Corporation.*

FROM *John Doherty*
Toxicology Branch/HED (TS-769)

TO *Larry Chitlik*
Toxicology Branch/HED (TS-769)

Background

The assignment to review "Mouse II" was given to me in April 1980. The approach I used in reviewing "Mouse II" was to consider the data in this study only ("Mouse II"). Using these data I lined up a basis for demonstrating that oncogenic effects in mice fed permethrin were noted in lungs and livers of the female mice and possibly also in male mice livers. Once the position that oncogenic effects in lungs and livers was established, the data in "Mouse II" were examined for oncogenic effects in other tissues. Examination of the data revealed that no other obvious dose dependent oncogenic effects in other tissues were evident at least to the extent that as firm an argument could be made for oncogenic effects as was made from the data with the lung and liver. It is important to note that at the time that "Mouse II" was reviewed there was in fact a lot of "pressure" to speed this review along. A considerable portion of the time available for the review of "Mouse II" was spent refuting the registrants claim that the oncogenic effects in lung and liver were of random occurrence. An extensive item by item comparison of the findings in "Mouse II" and the other oncogenic effects that were claimed by Dr. Panitch in her reviews with the results of "Mouse II" was not made.

The following is an account of my activities related to the audit of "Mouse II" Bio/Dynamics study, No. 76-1695 (24-month oral toxicity/oncogenicity study of FMC 33299 in mice) and visit to FMC offices. The audit/office visit took place during the week of September 22-26, 1980.

1. *For three days prior to and on Monday afternoon during the audit, the original hand written gross necropsy reports were checked out against the edited versions which had previously been submitted to EPA with the original study final report. A systematic checkout of the lungs and livers for all females for all test groups and the controls was completed. For males, a similar systematic checkout was made but time did not permit comparison of the control group lung, other lung and liver comparisons were made.*

EPA Form 1320-6 (Rev. 3-76)

It was concluded that the edited version represented a reasonably adequate transcription of the original gross necropsy report in as far as lung and liver tissues were examined such as:

A summary table comparing the female liver data from the original gross necropsy with the related edited gross necropsy, microscopic and histopathological findings is appended.

The few instances where discrepancies were noted that indicated a lack of transcription involved the original gross necropsy report indicating that the liver was "pale". This was not always transcribed (see mice 230 and 263 for examples). These livers, however, were not associated with particular histopathological findings.

Other examples of possible transcriptional differences noted included:

Mouse No. 237	*Original Gross Necropsy "several diffuse grey-green..." The rest was not intelligible.*	*Edited Version "surface mottled"*
Mouse No. 610	*Gross Necropsy*	*Edited Version*
	Liver mass 1.5 X 0.8 firm, tan my dk brown vascularized, on incision - solid excess dk red material (mass is c...#4)	*Liver mass 1.5 X 0.8 cm, right lobe, firm, dark red.*
Mouse No. 800		
	Mass - lobe visceral sulface mass 1.7 X 2.2 cm tan white dk red mottled, filled with dk red fluid. similar masses on all lobes.	*Masses, mottled filled with dark fluid*

2. *The final typed histology report showed many examples of additional gross necropsy findings that were not on the original gross necropsy reports. These findings were added by the persons responsible for trimming and preparing the tissues for histology.*

3. *The gross necropsy findings for the lungs and liver of both the males and females were compared with microscopic and/or histopathological findings and instances where the gross necropsy report indicated the presence of tissue masses that did not also show related histopathology were noted. (These tissues are indicated by a ▷ in the appended table.) This list was presented to Dr. Louis Kasza, pathologist, so that these tissues would be reexamined.*

The problems related to whether or not the gross necropsy reports correlated well with the histopathology report was examined more carefully by Dr. Kasza and is summarized in a separate memorandum

4. *During both the audit and while examining the slides, it was noted that amyloidosis and lymphosarcoma may occur at a higher rate than the pathologist first suspected (see Dr. Kaszas memo dated 10/4/80, p. 7). Thus some of the data were reexamined for the occurrence of these lesions.*

The control, mid dose and high dose females groups were reexamined for occurrence of amyloidosis (the low dose group was not). There were 21, 18, and 13 animals reported as having amyloidosis in the control, mide and high dose groups respectively, showing, if anything, an inverse relationship between feeding permethrin and occurrence of amyloidosis. The intensity (minimal, mild, moderate or marked) appeared to be uniform among the three groups examined. Also, it was noted that amyloidosis occurred far more frequently in mice that died spontaneously or were sacrificed moribund.

The data from all groups was reexamined for occurrence of lymphosarcoma. Firstly, the total occurrence of lymphosarcomas was tabulated from Table I of Dr. Rapps pathology report. It was noted that the high dose male group had 76 incidences of lymphosarcomas vs only 57 in the control. The total incidence of lymphosarcomas in females was essentially the same for all doses. The male data for the control and high dose group were reexamined for time of occurrence and total animals affected. A total of eight mice were affected with 6.88 incidences per animal for the control group and a total of 11 mice were affected with 6.82 incidences per animal in the high dose group. This is not considered to be dose related by this reviewer and there was no evidence that the onset of lymphosarcoma occurred earlier in the treated group.

ATTACHMENT D

MEMORANDUM

SUBJECT: Biodynamics Laboratory Audit concerning FMC 33297 (Permethrin), Study No. 76-1695 (Mouse II)

FROM: Gary J. Burin, Toxicologist
Toxicology Branch, HED (TS-769)

TO: Laurence Chitlik, Section Head#5
Toxicology Branch, HED (TS-769)

The following is a summary, per your request, of my findings concerning food consumption, body weight, diet preparation and other parameters examined during the course of last week's laboratory audit.

1) Food consumption and body weight data.

No major discrepancies were revealed in selected comparisons of raw data to food consumption calculations (means and standard deviations). The following test groups were examined; Week 77 Males Groups 1-4, Week 81 Males Groups 1-4, Week 47 Males Groups 1-2. The groups examined were chosen on the basis of data appearing unusual compared with previous and subsequent data.

In addition, recalculations of values appearing in the final report concerning food consumption in mg/kg/day for two of the above groups was performed;

Week 77 Males Group 2

$N = 56$
$\bar{x} = 18.946$
$\sigma = 1.92$

mean F.C. for 3 days = 16.054 (200-183.946)

$$g/kg/day = \frac{g/3\ days}{current\ body\ weight(kg)} \div 3\ days$$

$$= \frac{16.0549}{.0413} \div 3 = 129.6$$

(compared with 129.9 in final report)

Week 77 Males Group 3

$N = 55$
$\bar{X} = 183.78$
$\sigma = 1.94$

mean F.C. for 3 days = 16.22 (200-183.78)

$$= \frac{16.22}{.0417} \div 3$$

$$= 129.66$$

(compared with 129.9 in the final report)

In addition, the following mean body weights were recalculated from raw data;

Week 47 Males Group 1

$\bar{X} = 39.4$
$N = 70$
(compared with 39.4 in final report)

Week 47 Males Group 2

$\bar{X} = 40$
$N = 65$
(compared with 40 in final report)

Reviewing the body weight and food consumption raw data revealed various technician errors in weight measurements, most of which were properly indicated and initialed. Isolated cases of unusual data did exist, however, such as that of Animal No. 538, which, during week 10 food consumption measurement, was coded as "excessive spillage" and not used for calculation purposes. However, examination of the raw data in that instance indicated only 5 grams of food consumed in 3 days, certainly not justifying a description of excessive spillage.

In addition, a number of unusual food consumption and body weight values were found. These include drop in body weight averaging 1.15 g in all females between the weights recorded in week 56 and those recorded in week 61. Between week 61 and 65, an average gain in body weight of 1.08 was found in all females. This rather drastic fluctuation in body weight over an 8 week period suggests either an error in measurement or an unusual stress on the animals during this period.

Another ususual finding was the food consumption values of all male and female test groups at week 77. Female food consumption values decreased an average of 36.03 g/kg/day and male food consumption decreased an average of 22.08 g/kg/day compared to the previous measurement. In all test groups, the subsequent

food consumption measurements indicated an increase comparable to the decrease of the previous comparison with males regaining an average of 18 grams and females an average of 19.95 grams over the next two weighings (weeks 81-85).

2. Diet Preparation

Complete diet preparation records were found only for the period 6/23/78 to 12/1/78 (weeks 78-102). Previous to this period, various degrees of documentation of diet preparation records exist -

12/3/76 - 1/27/78 (weeks 1-59)

Diet preparation log combines records for a number of different studies including "Mouse II". Records do not indicate quantity of diet prepared but do indicate date of preparation, technician responsible for preparation and groups for which diet was prepared. Fresh diet appears to have been prepared weekly with at least two exceptions - the period 3/4/77 to 3/18/77 (weeks 64-66) and the period 1/13/78 to 1/23/78 (week 57-58). Fresh control diet was not prepared from 12/2/77 to 12/23/77 (weeks 52-54), 10/21/77 to 11/3/77 (weeks 47-48) or 9/2/77 to 9/16/77 (weeks 41-42).

1/27/78 - 6/23/78 (weeks 60-77)

No diet preparation records exist. Records of dispensing of material from the pharmacy do exist.

6/23/78 - 12/1/78 (weeks 78-102)

Complete diet preparation records exist.

3. Hematology

A comparison of the raw data regarding differential leukocyte measurement (the only hematology parameter examined) to the final report found no discrepancies.

4. Environmental Conditions

The "Unusual Environmental Condition" forms (attached) indicate wide fluctuations in temperature during the period for which these reports exist (5/22/78 to 11/16/78). Temperature varied from 64° to 80° with many days either above or below the acceptable range. A discussion of attempts to better control environmental conditions can be found in the attached "Unusual Environmental Condition" forms. Humidity was not monitored during the couse of the study.

5. Water Supply

The water supply used was an automatic type system connected to the Elizabethtown, New Jersey muncipal water supply. Water traveled through a conduit of copper piping to plastic lines leading to individual caging where animals were allowed to drink ad libitum. Technicians were reported to have bled water lines daily during the course of the study. According to Joseph Townsend, Director of Quality Assurance for Biodynamics, monitoring data showed the quality of the Elizabethtown water supply to vary greatly (based on historical monitoring) though no records were available for the study period.

cc: L. Kasza
W. Burnam
J. Doherty

ATTACHMENT D

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

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Addendum to Biodynamics Laboratory Audit Memo of 10/14/80
(Permethrin "Mouse II")

FROM: Gary J. Burin, Toxicologist
Toxicology Branch, HED (TS-769)

TO: Laurence Chitlik, Section Head#5
Toxicology Branch, HED (TS-769)

Per your request, the following addendum to my initial memo is an estimation of compound intake followed by a comparison to "targeted" intake.

Test Compound Intake

Based on actual residue determinations for selected weeks and reported food consumption it is possible to estimate test compound intake on a time weighted average basis. The following residue data was furnished by Michael A. Trepani of the FDA in a telephone conversation of October 19. Although this data was the only data available at the time, other residue analyses were performed and can be incorporated as they become available. Data is as follows (in ppm);

Groups	II	III	IV
Week 50 - Males	17.3	441.	1788
Week 42 - Males	3.1	438.	1716.
Week 56 - Males	19.2	445.	1756.
Week 95 - Males	19.1	486.	1654.
Week 105 - Males	18.6	473.	2119.
Week 30 - Females	17.3	2110.	4534.
Week 42 - Females	3.1	2086.	4495.
Week 56 - Females	19.2	2191.	4695.
Week 95 - Females	19.1	2293.	4644.
Week 105 - Females	18.6	2369.	5011.

Food consumption data for the above weeks are as follows (in g/kg/day);

Groups	II	III	IV
Week 30 - Males	131.8	123.7	123.2
Week 42 - Males	155.6	160.8	153.4
Week 56 - Males	166.3	174.3	174.6
Week 93 - Males	173.1	163.8	171.2
Week 102 - Males	157.4	158.7	133.8
Week 30 - Females	162.1	167.2	161.4
Week 42 - Females	178.0	171.2	182.2
Week 56 - Females	192.7	198.3	204.9
Week 93 - Females	187.6	184.8	182.5
Week 102 - Females	193.1	188.0	180.1

Using the following formula it is now possible to calculate time weighted average daily consumption;

Average Daily Compound Intake (mg/kg/day) =

$$\frac{\Sigma tRF}{T}$$

where

t = number of days during period for which residue level is applicable
R = residue level (%)
F = food consumption during the period for which residue level is applicable (mg/kg/day)
T = total number of days on test

Time weighted average daily compound intake for various test groups are as follow (in mg/kg/day);

Group	II	III	IV
Males	3.96	89.98	273.09
Females	4.10	398.42	829.68

Among the assumptions in making the above estimates were;

1. For calculation purposes, residue levels at week 93 were assumed to be the same as those measured at week 95 and residue levels at week 102 were assumed to be the same as those measured at week 105.

2. Data is corrected for the change in dosing which occurred at week 8 (February 4, 1977). It is assumed that prior to this change, residue levels were as intended i.e. 100, 2500 and 5000 ppm for both males and females.

"Target" Intake

The above compound intake estimates compare to the following "targeted" compound intakes (which are based on a conversion of 1 ppm in the diet to .150 mg/kg/day);

		Actual/ Targeted (%)
Group II Males & Females	20 ppm = 3 mg/kg/day	132, 137
Group III Males	500 ppm = 75 mg/kg/day	120
Group IV Males	2000 ppm = 300 mg/kg/day	91
Group III Females	2500 ppm = 375 mg/kg/day	106
Group IV Females	5000 ppm = 750 mg/kg/day	111

Thus test groups are estimated to have received from 91% to 137% of the targeted amount of test compound with all but one group (high dose males) receiving somewhat more than intended.

cc: W. Burnam
J. Doherty
L. Kasza

TS-769:TOX/HED:th:LCHITLIK:10-10-80

PROTOCOL CHANGE RECORD

Project No.

Original Protocol

Person:

Date:

Written by: _____ _____

Authorized by: _____ _____

Date submitted/received (circle one): _____

In-house protocol issued: _____

Revisions: _____

Number:	Date	Pages	Reason for Change	Authorized by:	Date:	Documentation Type	Documentation Date
Initial*							
1	3/8/77	pp 1, 2, 3, 4	Nursing initiated; dosage changes; change in clin grp; change in histopath (add'l tissues)	Nelson Wilson	1/14/77 1/26/77 letter		
2	5/2/77	p 3	saving of clin sample and recd drug analysis at term	Nelson Wilson	4/6/77 letter	MHRC letter	4/6/77
3		pp 1, 3	documentation of date terminal lab studies Clarif. tissue lists				
4	6/22/77	p 3	Clarification of Clin lab studies - at term.				
~~5~~		~~p 3~~	~~Indication that clin samples are to be pooled wkly~~	~~Nelson Wilson~~	~~10/27/77~~	~~letter~~	
	/ /		not receiving change of project monitor eff 12/27/77				
5	7/14/77	Pgs 1-7	Sponsor additions & clarifications	Nelson Wilson & Wilson	letter memo	6/13/03 6/27/2	
	/ /		Copies - final case	Nelson Wilson	/ /	/ /	

ATTACHMENT

ATTACHMENT F

bcc-GPSchoenig
CF-G138
ACT 115.35 file

August 14, 1978

Dr. William Tierney
Bio/Dynamics
Mettler Rd.
P. O. Box 43
East Millstone, NJ 08873

Dear Bill:

This letter will confirm the changes in protocol discussed in our telephone conversation concerning our repeat chronic mouse study, ACT 115.35 (Bio's 76-1695). Enclosed with this letter are copies of revised pages from the FMC protocol for this study. The changes have been incorporated into these pages and are explained below:

1. Add adrenals, lungs, and spleen to the list of organs to be weighed at terminal necropsy. The complete list now includes brain, spleen, liver, (gall bladder should remain attached but be drained prior to weighing), kidneys, adrenals, gonads, heart, and lungs.

2. The sacrifice procedure has been changed such that the prosector will remove the stomach and intestinal tract intact with the mesenteric lymph nodes and pancreas left attached. The stomach and cecum will be incised, flushed, and examined internally. The brain, spleen, liver, kidneys, adrenals, gonads, heart, lungs, and thymus will be removed from all animals. The epididymis or ovaries will be placed in a cassette (# 1), the adrenals in another cassette (# 2), and the thymus in a third cassette (# 3). These cassettes should be of such design as to prevent any tissue from washing into or out of the cassette.

2. (cont'd)

All other organs will remain in situ and the entire carcass will be fixed. An incision will be made to expose the trachea, thyroids, salivary glands, cervical lymph nodes, etc., for examination and better fixation. Similarly, two cross cuts in the thoracic spinal column will be made to facilitate fixation of the spinal cord. Any masses or lesions associated with any organ or tissue other than those which will have been removed will also remain in situ. This procedure will be followed unless it is adjudged that such a procedure will result in an inability to locate a particular mass or lesion, in which case, it should be removed, placed in a numbered cassette (one lesion per cassette), and clearly identified on the pathology sheet.

That should cover the changes which we have made, Bill. If you or Alex should have any questions about them, give me a call. See you soon.

Sincerely,

Nelson H. Wilson
Toxicologist
Research & Development Department

jag

cc-J. Cheetham, Bio/Dynamics

The relationship between palpable masses removed will be recorded, e.g., palpable abdominal mass determined to be large nodule on liver. The orifices of the mouth, outer ear, anus and vagina, the cranial cavity, the nasal cavity and paranasal sinuses and the thoracic and abdominal cavities and their associated tissues and organs as well as the cervial tissues and organs will be examined. In addition, the cut surfaces of the brain, spinal cord, lung, liver, heart, spleen, and kidneys will be examined.

The prosector will remove the stomach and intestinal tract intact with the mesenteric lymph nodes and pancreas left attached. The stomach and cecum will be incised, flushed, and examined internally. The brain, spleen, liver, kidneys, adrenals, gonads, heart, lungs, and thymus will be removed from all animals. The epididymis or ovaries will be placed in a cassette (# 1), the adrenals in another cassette (# 2), and the thymus in a third cassette (# 3). These cassettes should be of such design as to prevent any tissue from washing into or out of the cassette.

All other organs will remain in situ and the entire carcass will be fixed. An incision will be made to expose the trachea, thyroids, salivary glands, cervical lymph nodes, etc., for examination and better fixation. Similarly, two cross cuts in the thoracic spinal column will be made to facilitate fixation of the spinal cord. Any masses or lesions associated with any organ or tissue other than those which will have been removed will also remain in situ. This procedure will be followed unless it is adjudged that such a procedure will result in an inability to locate a particular mass or lesion, in which case, it should be removed, placed in a numbered cassette (one lesion per cassette), and clearly identified on the pathology sheet.

Eyes and testes will be fixed in Bouin's fixative. The remainder of the tissues will be fixed in 10% neutral buffered formalin and the tissue volume will not exceed 10% of the fixative volume in the container.

Interval: TERMINAL: Complete set of tissues from all survivors.

SPONTANEOUS DEATHS AND SACRIFICED AS MORIBUND: Complete set of tissues from all animals.

Tissues to be Examined: a/

b/ Brain (3 levels)	X	b/Adrenals	X	Mammary gland	X	b/Testes	X
Pituitary	X	Esophagus	X	Fat		Sem. Vesicles	X
Eyes and contiguous Harderian gland	X	Stomach	X	Spinal cord (cervical, thoracic)	X	Epididymis	X
Salivary gland	X	Duodenum	X	b/Spleen	X	Prostate	X
Tongue		Ileum	X	Pancreas	X	Skin	X
Thyroid/parathyroid	X	Jejunum	X	b/Liver (2 lobes)	X	Femur	
Lymph Nodes:		Cecum	X	Gall bladder	X	Skel. muscle	X
Meseneteric	X	Colon	X	b/Kidneys	X	Sciatic nerve (right & left)	X
Cervical	X	Rectum		Ureters		Other:	X
Thymus	X	Uterus	X	Urinary bladder	X	1. Middle ear and nasal cavity with paranasal sinuses	
b/ Heart	X	b/Ovaries	X			2. Bone marrow smear.	
Lungs w/mainstem bronchi	X	Oviducts				3. Any tissues with lesions.	
Trachea	X	Cervix	X			4. Gross masses	
Sternum	X	Vagina					
		Vulva					

a/ Tissue not taken at necropsy shall be noted on the necropsy sheet by the prosector along with an explanation why they were not taken (could not find, lost, autolytic, etc.).

b/ Preserved and examined (all survivors at termination)

ATTACHMENT C

ATTACHMENT H

To Case, RS

Date June 12, 1979

cc Fletcher, MJ

From Wilson, NH

Subject Slides of Tissues from "Mouse II", ACT 115.35

During the in-life portion of this study, a physical observation of "distended and/or hardened abdomen" was noted. In trying to determine the cause of this observation, selected tissues from 5 animals which had exhibited this finding prior to their deaths were examined histopathologically. This examination was conducted at Bio/dynamics, and the slides, wet tissues, and blocks are still held there. The results were inconclusive concerning the etiology of the "distended abdomens". We should have Bio/dynamics document the fact that this tissue processing and evaluation was done. They should also document the results of this examination.

Tissues from these same 5 animals were sent to American Histolabs for processing and then to Bill Rapp for evaluation. The results of his examination indicated some of the tissues to be missing. The actuality is that they did exist and were on slides at Bio/dynamics. I suggest that we write an explanation of what occurred and include it and both sets of data for each animal (i.e., Bio/dynamics' and Rapp's) in the study file. The animals and tissues involved are tabulated below. Rapp now has these slides and will revise his analysis for these animals.

GROUP & SEX	ANIMAL NUMBER	TISSUES PROCESSED AT BIO/DYNAMICS
II Male	361	Liver, spleen, mesenteric lymph nodes (Bio/dynamics' path. no. 77-1809)
III Male	524	Liver, spleen, mesenteric lymph nodes, mass (Bio/dynamics' path. no. 77-1810)
III Female	643	Liver, spleen, mesenteric lymph nodes (Bio/dynamics' path. no. 77-1811)
IV Male	752	All tissues (Bio/dynamics' path. no. 77-1812)
	754	All tissues (Bio/dynamics' path. no. 77-1813)

NHW:mjm

EXHIBIT #
FMC Corp
Sept 24, 1980 et al
Inv. Bruckheimer & EPA

54

ATTACHMENT I

To Files - NCT 549.32, 605.35

Date June 12, 1979

cc Case, RS
Fletcher, MJ

From N. H. Wilson

Subject Labelling of Slides from Chronic Rat and Mouse Testing with FMC 33297, NCT 549.32 and NCT 605.35

I was informed by Bill Rapp that during his examination of tissues from the mouse study, he noted in several cases that the designation on the slides which should indicate whether the animal died on study or at a scheduled sacrifice was incorrect. He informed me that the animal numbers were in all cases accurately presented and that we should, therefore, rely on these for slide identification rather than on the type of death designation.

Dr. Rapp also suggested that we document the fact that the assignment of the tissues to specific slides was not always consistent. Therefore, if a tissue for an animal is not found where expected in some future search, it should be sought on other slides for that animal.

Dr. Billups, who examined the tissues from the rat study, did not indicate to me that such problems existed for the rat study. However, since tissues from both studies were processed and slides labelled by the same histology lab, I am assuming that the problems do exist for both studies.

NHW:mjm N. H. Wilson

70-3

EXHIBIT #
FMC Corp
Sept 24, 1980 et al
Inv. Bruckheimer & EPA

ATTACHMENT J

To M. J. Fletcher

Date June 11, 1979

cc Case, RS

From N. H. Wilson

Subject Procedures to Assure Accountability from In-Life to Gross Necropsy to Microscopic Examination in Ongoing Toxicology Studies

Several steps are being taken at each stage of the ongoing studies which require histopathologic examination to assure a good accountability for tissue masses and lesions. The procedures which we are following are described below.

In-Life Physical Observations:

All physical observations, including palpable masses, are recorded weekly during the studies. If no abnormalities are found for an animal during a given week that fact is also recorded. Each observation is numbered and is sought in subsequent weeks until it disappears. During the week that a particular observation is no longer noted, a recording of "not evident" is made.

Gross Necropsy Observations:

On the necropsy sheet for each animal is a section where the last set of in-life physical observations for that animal is recorded. At the time of necropsy each of these physical observations is commented on by the pathologist in the "gross necropsy findings" section of the necropsy sheet. If a particular abnormality is not able to be located by the pathologist, a recording of "not evident" is made. If a lesion is not uniquely identifiable after removal from the carcass, it is placed in a labelled cassette or porous bag before being placed in fixative and reference to that label is made on the necropsy sheet. As other tissues are removed from the animal they are placed on a grid which has a space for each tissue required by protocol. Following removal of all organs, a checklist is filled out as each organ is placed in fixative.

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Histopathologic Evaluation:

At Ameri an Histolabs (AHL), a 3-carbon tissue dispersal and inventory sheet for each animal is filled out as each tissue is placed on its respective slide. In addition to the listing of tissues specifically required by protocol, there is space provided on the sheet to document additional slide preparations of gross lesions. Once all slides have been prepared, AHL removes one of the copies of the sheet and forwards the other two, along with the slides, to the pathologist. During his examination, the pathologist indicates on these copies the tissues which are present, those which are missing (this usually should correspond to those noted as missing by AHL), and those where an inappropriate or insufficient section of the tissue precluded examination. He then keeps a copy for his files and returns the last copy of the sheet to AHL where a final search through the wet tissue and blocks is made for any missing or inappropriately sectioned organs. If some of these tissues are found or resectioned, the new slides are forwarded to the pathologist who indicates issuance of new slides for a tissue on his copy of the sheet.

The procedures described seem to have been operating effectively to date. They should insure a far better correlation between in-life, gross necropsy, and histopathologic observations than we have experienced in past studies.

NHW:mjm

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ATTACHMENT K

Route List Below February 5, 1979

Hymans WE

POUNCE® INSECTICIDE - MEETING WITH ICI ON JANUARY 26, 1979

ROUTE LIST
Bissing DE
Fletcher MJ
Graham JR
James AR
Lauber JJ
McCarthy JF
Robinson RA
Wilson NH
Wolfe JH

On January 26 representatives from FMC and ICI met to discuss items concerning the conditional registration of Permethrin. Present from ICI were Dick Herrett, Bob Ridsdale and Bob Hawk. Representing FMC were Don Bissing, Art James, Jack Lauber, Bob Robinson, Joe Wolfe and Bill Hymans. For each topic discussed I have summarized the present status and outlined the course of action approved by the participants. In cases where responsibilities or schedules were not specified at the meeting, I have superimposed my own judgment. Please respond if you foresee problems carrying out this program.

I. SCHEDULE

Bill Hymans in conjunction with Bob Ridsdale will set up a meeting with EPA Fish and Wildlife personnel for early in the week of March 5 to discuss all protocols. Those having responsibility for protocol development should have their information to the Registration Department for review by February 26 or sooner. In areas where EPA has promised to respond (see following) Bill and Bob will be responsible for obtaining this information.

II. AQUATIC STUDIES

A. MacroInvertebrate Acute Study
1. Lead responsibility - Bob Hawk (ICI)

2. Status - EPA has approved using Mayfly nymph as the test organism. ABC Laboratories will be contracted to carry out the study.
3. Course of action - Bob Hawk will secure a protocol from ABC Labs for EPA approval and ask ABC for duplicate reports on completion of this study, one to each company. Nelson Wilson will be the FMC contact.

B. Daphnia Life Cycle Study
1. Lead responsibility - Bob Hawk (ICI)
2. Status - ABC will be contracted to carry out the study. EPA will respond on their preference of protocols, semi-static or flow through. Both have already been discussed with ABC.
3. Course of action - When EPA decision is made, Bob Hawk will secure a protocol from ABC for EPA approval. Duplicate reports will be requested. Nelson Wilson will be FMC contact.

C. Food Chain Accumulation Study
1. Lead responsibility - Bob Robinson (FMC)
2. Status - Two protocols are under consideration, Schoetger-Johnson (SJ) and modified-Metcalf. Bob Robinson estimated that SJ would take five to six months to complete. Double that if a benchmark chemical were run for comparison. Estimate for a Metcalf was four months including a benchmark. EPA has seen one example of SJ and is aware of the difficulties in using that protocol. They will provide us with all information they have on the subject including the chemical and registrant involved in the study they have seen. In this area, EPA draws guidance from ASTM for protocol development. Bob Robinson and Bob Hawk reported on conversations each have had with industry and government experts on this subject and the consensus was that the modified Metcalf is preferable to SJ because it is ecologically more relevant and because a data base exists from which one can better interpret results. For either study, label position must be addressed. EPA has said they will respond on this question.

No information was available about the model ecosystem study carried out by ICI-UK. Hawk estimated that the report would be completed in four to six weeks.

3. Course of action - Bob Robinson in conjuction with Bob Hawk will prepare a protcool for a modified Metcalf study and develop a case for its use. This case will also refute the need for SJ. It was agreed that any source of information useful to this task should be tapped short of direct conversation with Schoetger-Johnson. Bob Robinson will also consider candidate laboratories for carrying out the study once an acceptable protocol is in hand.

   The merit of using the ICI-UK study to satisfy all or part of this requirement will be judged when the study is available.

D. Field Study - Aerial Application
   1. Lead responsibility - Bob Hawk (ICI)
   2. Status - Irrespective of site location, it was agreed that Union Carbide should be contracted tc carry out the study especially the parts concerned with aquatic organisms. FMC and ICI will carry out themselves or monitor closely the Agricultural aspects of the study. EPA will respond with their ideas concerning parameters such as pond size and depth, distant from crop to water, etc. The first site for consideration will be ICI-Goldsboro.
   3. Course of action - Bob Hawk will determine the feasibility of using the Goldsboro site. If unsatisfactory, Union Carbide will be brought in on the site selection process and close contact will be kept with Art James in this respect. The protocol will be developed using that of the Texas study as a base. For the EPA meeting in early March, as complete a description of the site as is possible should be included.

E. Monitoring
   1. Lead responsibility - Bill Hymans (FMC)
   2. Status - EPA has only said they want monitoring in high use areas. While it was agreed that five locations would be used, six (three each, Ambush and Pounce) seems more logical.

Three will be running water, three static. Sampling will be in duplicate every two weeks for one season's duration. Analysis, to be done by FMC, will be for residue in water only. If not satisfactory to EPA, the first fallback in addition to water will be sediment, second fallback fish. EPA is considering monitoring as a condition of emergency exemptions for lettuce in California and Arizona. The impact of this on our program must await issuance of the exemptions.

3. Course of action - Bill Hymans will develop the protocol.

F. Willis Paper

It was agreed that the Willis Paper will not be submitted to EPA at this time. Its greatest present value seems to be in assisting protocol development.

III. DATA COMPENSATION - OFFER TO PAY

It was agreed that both companies would submit identical documents. Appendix A certifying reliance only on data used (not cite-all) will be employed. Joe Wolfe will contact his counterpart at ICI, Kent Riegle and jointly draft offer to pay letters for exchange.

IV. FMC 24 MONTH MOUSE STUDY

The disposition of this study was explained. Slides will be prepared but not read as long as no differences in gross pathology are noted. EPA will be so notified. Costs will be the reason for not doing the histopathology.

r711B42
rs24

ATTACHMENT L

FMC Agricultural Chemical Division
Middleport

Interoffice

To J. F. McCarthy
Phil (2315)

From N. H. Wilson

Subject TOXICOLOGY MONTHLY PROGRESS REPORT
APRIL, 1979

Date May 4, 1979

cc DTAiello, Phil(2317)
PHEastburg, Phil(2313)
MJFletcher, Phil(2717)
JRGraham
ARJames, Phil(2312)
HKLatourette, Phil(2602)
RLGates
Tox file
CF-Toxicology

Attached is the April Monthly Progress Report.

NHWilson

jag
attach.

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1. Study: 2-year dietary toxicity/oncogenicity study in mice.

Results: The 24 month terminal sacrifice of this backup study has been completed. Dose levels of 0, 20, 500, and 2000 ppm in males and 0, 20, 2500, and 5000 ppm in females were evaluated.

In-life findings included a mortality effect noted in the male mice of the 2000 ppm group and an increased incidence of a physical observation termed distended abdomen noted in the 500 and 2000 ppm males and all female treated groups. Tabulation of gross necropsy observations for all animals indicates an increase incidence of liver findings in all treated groups.

Histopathologic evaluation of tissues from 200 animals which died or were sacrificed as moribund by 18 months of study has been conducted. A statistically significant increase in the incidence of hepatocellular hypertrophy was noted in males and females of the high dose group. An increased incidence of amyloidosis and/or subepithelial mononuclear leukocyte infiltrate was noted in many organs from animals primarily of the high dose group. In addition, an increased incidence of endometrial hyperplasia was noted in females of the mid and high dose levels. The incidence of tumors of specific site (eg., hepatomas or bronchioalveolar adenomas) as well as that of tumors of diffuse or nonspecific sites (eg., lymphosarcomas or undifferentiated sarcomas) was examined. There was no indication in these 200 animals of any carcinogenic effect of FMC 33297. Histopathologic examination of the tissues of the remaining 400 animals is ongoing.

The final report is due December, 1979.

2. Study: Acute oral, dermal, and inhalation toxicity studies and a skin irritation test with FMC 33297 3.2 EC with 25% oil.

Results: These studies have been reported. The acute oral LD_{50}'s and 95% confidence intervals for males, females, and males and females combined were determined to be 1.45 g/kg (1.11-1.90 g/kg), 1.33 g/kg (1.03-1.71 g/kg), and 1.35 g/kg (1.12-1.63 g/kg), respectively. The acute dermal LD_{50} was determined to be >2.0 g/kg. The material was found to be mildly irritating to the skin. The four-hour aerosol inhalation LC_{50} was determined to be >6.67 mg/l.

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ATTACHMENT M

FMC Agricultural Chemical Group
Richmond

Interoffice

FMC Philadelphia
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To J. F. McCarthy

Date October 3, 1980

From G. H. Fujie

cc DEBissing-JJLauber
WEHymans-WMQuillman
MJFletcher-RSCase
DNye-JHWolfe-JRCraham
OHFullmer

Subject PERMETHRIN DIET ANALYSIS - BIO/DYNAMICS MOUSE AND RAT STUDIES

Per your request, this is a status report on the Bio/Dynamics Mouse and Rat Diet Sample Re-analysis Program.

We have completed solvent blank analysis and have obtained blank samples with no permethrin background (<0.01 ppm each isomer). We will continue to analyze blank samples with each set of diet samples analyzed to check on laboratory contamination.

Mouse II (76-1695) untreated diet samples have all been extracted. Initial analysis at the 0.1 ppm sensitivity level have been unsuccessful due to inadequate sample cleanup. We are currently sorting this problem out.

To check storage stability, we plan to analyze diets from the first two intervals of each study. Per your instructions we will analyze samples from all fortification levels.

In regard to Mouse I (74-1100) and Rat (74-1022) diet samples, we will need to repeat all check analyses as in our original work we did not quantitate at the 0.1 ppm (each isomer) level. The procedure used was to scan only with the required sensitivity to do Group II (20 ppm) analyses.

No projected completion dates can be made until we have a workable analytical method. We will utilize overtime to complete this project as soon as possible.

/lw

C4

ATTACHMENT IV
pert 4/29
ma

FMC Agricultural Chemical Group
Philadelphia

Interoffice

To JR Graham

Date September 29, 1980

From JF McCarthy

cc DE Bissing
JJ Lauber
WE Hymans
WM Quillman
MJ Fletcher
RS Case
D Nye
JH Wolfe
GH Fujie

Subject PERMETHRIN - DIET ANALYSIS - MOUSE AND RAT STUDIES PERFORMED AT BIO/DYNAMICS

Confirming our telephone conversation of September 29, 1980, the Richmond Laboratory should proceed at once to re-analyze the following retained diet samples received from Bio/dynamics:

1. All Mouse II control diet samples
2. All Mouse I and 2-year rat control diet samples if there is any indication in the laboratory records that positive responses for permethrin were observed.
3. A sufficient number of treated diet samples from these studies to determine if any deterioration of permethrin occurred during storage.

The following sequence should be followed:

1. Solvent blanks. A sufficient number should be run through the entire procedure to establish unequivocally the absence of any amount of background.
2. All the control diet samples. Start with Mouse II first; then proceed, if necessary as noted above, with Mouse I control samples and finish with the 2-year rat control samples.
3. The treated diet samples necessary to establish permethrin storage stability. Samples from all the studies at all the dose levels should be looked at to establish conclusively the stability question. Please inform me on which samples you plan to analyze to answer the stability question. I may want to add some.

65

Please keep me informed totally on the progress of this project and any problems or deviations from the above plan that may be necessary. I want a phone report on progress on October 2 and a written status report on Monday, Oct. 6. Also, on Oct. 2, let me know when a final report will be issued. I would like to have this by the end of next week, if possible. The information is needed as quickly as humanly possible, but quality and exactness should not be sacrificed for the sake of speed.

One final point - I want three separate final reports - one each for the three studies.

JFM

P.S. Don Nye would like the laboratory notebook pages containing analytical information on control diets from Mouse I and the 2-year rat study. Please see that these are sent as soon as possible.

JFM:ns

ATTACHMENT O

FMC Philadelphia

Pc [redacted] 4/11/79

Interoffice

To Route List

Date April 9, 1979

From Fletcher MJ MJF

cc Bissing DE
Lauber JJ ✓
McCarthy JF
Reed SK

Subject Meeting with EPA Regarding FMC 33297 Toxicology Issues

As a result of several telephone calls between EPA and FMC personnel concerning the Pounce toxicology package, a meeting was held at the EPA offices at 821 Crystal Mall #2, Washington, D.C. on Friday, March 30, 1979 at 1:00 p.m. Those present included:

EPA - Dr. Adrian Gross, Dr. Sin Lam Chan
ICI - M. Litchfield, J. Ischmael, R. Ridsdale, R. Herrett, G. Willis
FMC - J.F. McCarthy, J.J. Lauber, W.E. Hymans, M.J. Fletcher

JFM explained why both ICI and FMC representatives were present, introduced each one and then asked Dr. Gross to itemize by study the concerns which led to the reversal of the agency's previous position regarding the acceptability of our toxicology submission.

Dr. Gross described his entrance into the subject, which was by way of Dr. Panitch's internal reviews and questions contained in her letter of April 13, 1979 (what none of us knew at that time was that Gross had not read FMC's supplemental submission of September, 1978 in response to Panitch's letter). Gross pointed out that his two general areas of concern were the adequacy of the studies and the responses observed in the studies. He described his visits to Bio/dynamics as a member of the FDA inspection teams. He mentioned that Bio/dynamics demonstrated deficiencies in pathology, especially in the area of follow-up of external tissue masses ante-mortem, and poor gross to microscopic accountability.

Specific areas in the rat study commented upon by Dr. Gross included:

- Pre- and post-mortem cataract accountability.
- The increase in control lung lesions after step sectioning with no concomitant increase in treated group lung lesions.

FMC Philadelphia

APR 10 1979

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He questioned whether more tissues were evaluated in the control group than in the treated group.

- the general accountability problem of external tissue masses ante-mortem and poor gross through microscopic accountability.

- The thyroid tumor incidence.

Specific areas in the mouse study included:

- Time-to-tumor of lymphosarcomas, especially of the two lower dosages.

  He suggested a reanalysis of the data and asked whether the historical incidence in female mice was available.

- Undifferentiated sarcoma.

As soon as it became obvious that Gross did not use our supplemental submission for his evaluation, I mentioned that we had indeed addressed most of these concerns (originally Panitch's) in this document. We then gave him a copy. It was pointed out how the accountability situation was addressed; our arguments concerning total lymphosarcomas; our analysis of undifferentiated sarcomas (Gross then performed a Fishers exact test and concurred that a non-significant result is obtained).

During the conversations, Dr. Gross stated the following: "We are not going to call this a carcinogen" and "We are not going to class this as a carcinogen."

As soon as Gross realized that his recent evaluation of our data did not include all the available information (our supplemental report), he quickly agreed to review the rat study data again for acceptability. He is aware of "Mouse II" and wants us to finish it up and submit it to the agency. He stated that before the meeting he was going to recommend both a rat and a mouse study. But he has changed his current thinking and will only require a mouse study (the completion of "Mouse II"). At the end of the meeting he stated that Dr. Chan (who after review of our supplemental submissions previously found all of Panitch's concerns answered to his satisfaction) would with Gross's assistance study and reevaluate our supplemental submission and inform us of their appraisal of the rat study in approximately four weeks. At this time, they will not ask us to submit a new rat study. "Mouse II" must be completed on schedule and submitted to the agency.

Dr. Gross impressed me as being a competent, reasonable, and even-handed scientist. However, I judge we have been treated unfairly in this case due to confusion at the agency caused by Gross' ascension to a new position as Chief of the Section and incomplete preparation prior to evaluation of our total submission.

r526B5
LJB72

FMC Agricultural Chemical Richmond

ATTACHMENT P JR 6

Interoffice

To G. P. Shoenig

Date September 24, 1976

cc JRGraham
JFMcCarthy
OHFullmer

From G. J. Fujie

Subject DIET SAMPLES - FMC 33297 FORTIFYING STANDARD

We received and analyzed two samples of FMC 33297 technical material from Bio/Dynamics. A portion of each technical material was weighed out, diluted with hexane and compared with our FMC 33297 standard using glc. Both technical materials were determined to be 97% pure. Both materials had cis-trans ratios of approximately 40:60.

mc

Jerry/
Do you plan to have diet samples fortified and analyzed 0, 3, 7 days?
Jack

Jack - This has already been done
while there was no decrease as a func
of time, all samples were low. This is
the reason we looked at technical mat
I evaluated method of diet prep while I
was down there last week. I think proble
lies there. We are trying several new ways t
prepare diet and then we will analyze. J-

FMC Agricultural Chemical Division
Middleport New York

ATTACHMENT Q

Interoffice

To O. H. Fullmer
Richmond

Date October 22, 1976

cc JRGraham

From G. P. Schoenig

Subject MORE DIET SAMPLES CONTAINING FMC 33297

As a next step in trying to solve the problem relating to the apparent deficiency of FMC 33297 in the animal diets, I have asked Dr. J. Killeen of Bio/Dynamics Laboratories to prepare the diets in several different ways. He will be sending you frozen samples of these diets for analysis during the week of 10/25/76. This is becoming a very critical issue and needs prompt resolution. Anything you folks can do to process these samples at your earliest convenience would certainly be appreciated.

Jag

70-3

FILE COPY (ATTACHMENTS)

August 13, 1980

Lab Audit of the "Twenty-Four Month Oral Carcinogenicity of FMB 33297 in Mice" (Permethrin) at Bio/dynamice Inc. Project No. 76/1695

William Burnam, Acting Chief
Toxicology Branch, HED (TS-769)

Dr. Diana Reisa
Special Pesticide Review Division (TS-791)

I recommend that a laboratory audit be carried out as soon as possible on the permethrin mouse study at Bio/dynamics (Mouse II) to clarify major issues which severely compromise our branch's ability to estimate the carconogenic risk of this pesticide.

Our chief concerns include, but are not limited to the following:

1. When and by whom were the gross necropries of those animals sacrificed at the termination of the study conducted? When and by whom were the initial gross necropry notes on individual animals written?

2. Were these prosectors under the supervision of a pathologist while performing these task?

3. What was the nature of the "editorial changes" in the records of the gross observations? Was Dr. Rapp present at the time of these observations?

4. When and to whom were the fixed tissues transmitted after sacrifice? When and who selected and trimmed the fixed tissue to be sent for slide preportion?

5. The numbers of mice mentioned in Dr. Rapp's report is at variance with the number started on the study.

6. There are many problems regarding the flow of information from Bio/dynamics to various subcontractors which can only be answed by a audit.

Although, I realize that Dr. John Doherty of Toxicology Branch reviewed the original submission on Mouse II, I believe that Dr. Adrian Gross should be in charge of the audit. The problems associated with all aspects of the audit are of such magnitude that only someone with Dr. Gross's background in pathology, statistics and laboratory procedures could adequately undertake such a task.

cc: Dr. Peter McGrath
Mr. Douglas Campt
Dr. Adrian Gross

OPP:HED:TOX: B.BURNAM:gjd 7/28/80 X77395 TS-769 Rm. 820 CM 2

FMC Agricultural Chemical Division
Richmond

ATTACHMENT R

Interoffice

To G. P. Shoenig

Date November 16, 1976

cc JRGraham
JFMcCarthy
OHFullmer

From G. H. Fujie

Subject FMC 33297 DIET SAMPLES

Analyses have been completed on diet samples from Groups II and IV of the three and twenty-four month oral toxicity/carcinogenity study of FMC 33297 on rats (project No. 74-1022). Diet samples were received in triplicate (five methods of preparation), therefore only a single analysis was done for each sample. All residue ppm values tabulated below were corrected for method recovery (82-100%).

ams

attach

ATTACHMENT 2 R

GROUP II DIET SAMPLES (20 ppm)

Method		RESIDUE ppm cis	trans	total	C/T ratio (%)
I		8.0	11.5	19.5	
		8.3	11.5	19.8	
		8.0	10.7	18.7	
	Ave.	8.1±0.1	11.6±0.1	19.3±0.4	42/58
II		7.2	10.1	17.3	
		8.3	12.0	20.3	
		6.9	9.6	16.5	
	Ave.	7.5±0.6	10.6±1.0	18.0±1.6	42/58
III		7.5	10.1	17.6	
		8.0	11.2	19.2	
		7.2	10.1	17.3	
	Ave.	7.6±0.3	10.5±0.5	18.0±0.8	42/58
IV		8.5	12.5	21.0	
		8.5	12.3	20.8	
		8.8	12.3	21.1	
	Ave.	8.6±0.1	12.4±0.1	21.0±0.1	41/59
V		7.2	10.1	17.3	
		8.3	12.0	20.3	
		8.3	11.7	20.0	
	Ave.	7.9±0.5	11.3±0.8	19.2±1.3	41/59

GROUP IV DIET SAMPLES (500 ppm)

I		205	319	514	
		216	286	502	
		191	300	491	
	Ave.	204±10	302±14	502±9.4	41/61
II		184	267	451	
		265	414	679	
		228	357	585	
	Ave.	225±33	346±61	572±94	39/61
III		195	305	500	
		177	281	458	
		195	305	500	
	Ave.	189±8.5	297±11	486±20	39/61
IV		172	271	443	
		181	286	467	
		177	271	448	
	Ave.	177±3.7	276±7.1	453±10	39/61
V		181	281	462	
		200	319	519	
		205	314	519	
	Ave.	195±10	305±17	500±27	39/61

ATTACHMENT T

RAT

74-1022

Sampling Date	Week	Shipping Date	Receiving Date	Analysis Date	Finalize Data Date	Memo Date
8/5/75	7	8/13/75[a/]		10/9/75 10/10/75	10/10/75	10/13/75
1/8/76	29	1/8/76		1/20/76	1/20/76	1/21/76
8/5/76	58	8/5/76		8/23/76	8/23/76	9/2/76
11/25/76	75	11/29/76		12/17/76 12/20/76	12/20/76	12/22/76
1/13/77	82	1/25/77		1/27/77	1/27/77	2/1/77
12/2/76	76	12/5/77		12/15/77	12/15/77	12/15/77
6/16/77	104	12/5/77		12/15/77	12/15/77	12/15/77

[a/] Date received in Middleport

Rat
74-1022

Memo Date	Sampling Date	WK	animal Group	Dose	Original PPM	Repeat PPM	% change
10-13-75	8-8-75	7	I	0	ND<1.3	<0.2	
			II	20	18.7	15.8	-16%
			III	100	94.5	87.1	-8%
			IV	500	554	515	-7%
Extra Group? →			V	2500	2503	2061	-18%
1-21-76	1-8-76	29	I	0	ND<3	<0.2	
			II	20	17.1	14.8	-13
			III	100	87.8	79.8	-9
			IV	500	477	395	-17
9-2-76	8-5-76	58	I	0	1.0	No sample available	
			II	20	15		
			III	100	78		
			IV	500	408		
"	?	Day 0	IV	500	473	Storage Stability Samples	
		Day 3	IV	500	382		
		Day 7	IV	500	406		
11-16-76 — Mixing study Samples							
12-22-76	11-25-76	75	I	0	(2.2)	0.33	
			II	20	19.3		
			III	100	102.7		
			IV	500	435		

Rat
74-1022

Memo Date	Sampling Date	Wk	animal Group	Dose	Original PPM	Repeat PPM	% change
2-1-77	1-13-77	82	I ♂	0	(5.0)	<0.2	Int Tech PK.
			I ♀	0	(9.6)	<0.2	
			II ♂	20	19.6		
			II ♀	20	20.9		
			III ♂	100	98.5		
			III ♀	100	99.6		
			IV ♂	500	467		
			IV ♀	500	487		
12-15-77	12-2-77	76	I	0	(0.8)	<0.2	(Int-Secc PK
			II	20	19.2		
			III	100	97		
			IV	500	492		
	6-16-77	104	I ♂	0	(0.7)	<0.2	
			I ♀	0	(0.9)	0.04	
			II	20	20.6		
			III	100	99		
			IV	500	494		

ATTACHMENT U

MOUSE I

74-1100

Sampling Date	Week	Shipping Date	Receiving Date	Analysis Date	Finalize Data Date	Memo Date
8/7/75	7	8/13/79[a/]		10/9/75 10/10/75	10/10/75	10/13/75
12/1/75	24	12/16/76[b/]		1/16/76	1/21/76	1/21/76
1/8/76	30	1/8/76		1/20/76	1/20/76	1/21/76
6/7/76	51	6/8/76		6/11/76 6/14/76	6/14/76	6/15/76
8/9/76	60	8/10/76		8/23/76	8/23/76	9/2/76
11/22/76	75	11/29/76		12/17/76 12/20/76	12/20/76	12/22/76
1/3/77	81	1/3/77		1/6/77	1/6/77	1/7/77
1/17/77	83	1/25/77		1/27/77	1/27/77	2/1/77
6/13/77	104	12/5/77		12/15/77	12/15/77	12/15/77

[a/] Date received in Middleport

[b/] Date shipped from Middleport to Richmond

Memo Date	Sampling Date	wk	Animal Group	Dose	Original ppm	Repeat ppm	% chan.
10-13-75	8-7-75	7	I	0	- No sample		
			II	?	" "	—	
			III	100 ppm	97.3	—	
			IV	?	- No sample		
1-21-76	12-1-75	24	I	0	ND<3	0.35	
	Note on memo states "mislabeled?"		II	20	387	412	+6
			III	4000	3148	3081	-2
			IV	500	21.3	14.0	-3
"	1-8-76	30	I	0	ND<3	0.09	
			II	20	21.5	17.9	-1
			III	4000	4167	3978	-5
			IV	500	551	581 (Interference	+5
6-15-76	6-7-76	51	I	0	ND<3ppm	0.06	
			II	20	26.2		
			III	4000	3118		
			IV	500	408		
9-2-76	8-9-76	60	I	0	ND<5	(Interference ppm 0.06	
			II	20	9.7		
			III	4000	3097		
			IV	500	404		

Memo Date	Sampling Date	wk	Animal Group	Dose	Original PPM	Repeat PPM	
9-2-76	?	Day 0	IV	500	377	—	Storage stability sampl
		Day 3	IV	500	422		
		Day 7	IV	500	442		
12-22-76	11-22-76	75	I	0	(2.0)	0.32	
			II	20	20.3		
	Flip/Flop		III	500	439		
			IV	4000	3235		
1-7-77	1-3-77	81	I	0	NO < 1.2		No sa avail.
			II	20	19.9 ± 0.7		
			III	4000	4145 ± 57		
			IV	500	501 ± 5		
2-1-77	1-13-77	83	I	0	NO < 40 ppm		0.4
			II	20	19.5		
			III	4000	3661		
			IV	500	482		
12-15-77	6-13-77	104	I	0	(2.7)		9.3
			II	20	17.7		
	Flip Flop		III	500	454		
			IV	4000	3256		

Attachment V

MOUSE II

76-1695

Sampling Date	Wk.	Shipping Date	Receiving Date	Analysis Date	Finalize Data Date	Memo Date
12/8/76	1	12/9/76		12/17/76 12/20/76	12/20/76	12/22/76
~~1~~1/14/77	7	1/18/~~76~~77		1/27/77	1/27/77	2/1/77
2/4/77	10	3/17/77		3/28/77 3/29/77	3/29/77	3/30/77
3/4/77	3 mo	3/17/77		3/28/77 3/29/77	3/29/77	3/30/77
6/24/77	30	1/17/78		2/17/78 2/21/78	2/22/78	2/24/78
9/23/77	43	1/17/78		2/17/78 2/21/78	2/22/78	2/24/78
12/23/77	56	1/17/78		2/17/78 2/21/78	2/22/78	2/24/78
9/16/77	42	3/7/78		3/13/78 3/14/78	3/14/78	3/15/78
9/30/77	44	3/7/78		3/13/78 3/14/78	3/14/78	3/15/78
9/9/77	41	3/27/78		4/6/78	4/7/78	4/7/78
10/7/77	45	3/27/78		4/6/78	4/7/78	4/7/78
3/24/78	69	3/27/78		4/6/78	4/7/78	4/7/78
6/16/78	81	6/26/78		8/21/78	8/21/78	8/22/78
9/22/78	95	9/25/78		10/3/78	10/4/78	10/5/78
12/1/78	105	12/4/78		12/13/78 12/21/78	12/14/78 12/21/78	12/21/78

Mouse II
76-1695

Memo Date	Sampling Date	WK	animal Group	Dose	original PPM	Repeat PPM	
12-22-76	12-8-76	1	I	0	(3.3)	2.0	-
			II	100	73.8	65.2	-1
			III	2500	2114	2047	-3
			IV	5000	4244	4599	+8
2-1-77	1-14-77	7	I	0	ND <12ppm		
			II	100	90.5		
			III	2500	2208		
			IV	5000	4656		
3-30-77	2-4-77	10	I	0	(1.3)	0.45	-
Dose change ⟶			II	20	22.1	22.1	0
			III ♂	500	47.7	48.1	+1
			III ♀	2500	2404	2159	-10
			IV ♂	2000	1907	1679	-12
			IV ♀	5000	4892	4489	-8
3-30-77	3-4-77	3mo.	I	0	(2.0)	2.1	
			II	20	23.9	22.9	-4
			III ♂	500	477		
			III ♀	2500	2518		
			IV ♂	2000	2037		
			IV ♀	5000	4930		

Memo Date	Sampling Date	Wk	Animal Group	Dose	Original PPM	Repeat PPM	% change
2-24-78	6-24-77	30	I	0	(0.39)	0.72	
			II	20	17.3		
			III ♂	500	441		
			III ♀	2500	2110		
			IV ♂	2000	1788		
			IV ♀	5000	4534		
"	9-23-77	43	I	0	ND <0.1	0.52	
			II	20	[redacted] 3.1		
			III ♂	500	438		
			III ♀	2500	2086		
			IV ♂	2000	1716		
			IV ♀	5000	4495		
"	12-23-77	56	I	0	ND <0.1		0.14
			II	20	19.2		
			III ♂	500	445		
			III ♀	2500	2391		
			IV ♂	2000	1756		
			IV ♀	5000	4659		

Memo Date	Sampling Date	WK	Animal Group	Dose	Original PPM	Repeat PPM	% change.
3-15-78	9-16-77	42	I	0	(0.73)	4.2	
			II	20	19.7		
	Broken Bags		III ♂	500	436		
			III ♀	2500	2229		
			IV ♂	2000	1760		
			IV ♀	5000	4448		
"	9-30-77	44	I	0	(6.4)	9.7	
			II	20	139		
	Broken Bags		III ♂	500	428		
			III ♀	2500	2195 ~~2229~~		
			IV ♂	~~5000~~ 2000	1556 ~~4398~~		
			IV ♀	5000	4398		
4-7-78	9-9-77	41	I	0	(0.24)	No sample available	
			II	20	19.7		
			III ♂	500	439		
			III ♀	2500	2269		
			IV ♂	2000	1709		
			IV ♀	5000	4530		

Mouse II

Memo Date	Sampling Date	wk	Animal Group	Dose	Original ppm	Repeat ppm	% chan
4-7-78	10-7-77	45	I	0	(0.11)	0.70	
			II	20	17.7		
			III ♂	500	423		
			III ♀	2500	2275		
			IV ♂	2000	1751		
			IV ♀	5000	4480		
11	3-24-78	69	I	0	(0.18)	0.07	
			II	20	20.5		
			III ♂	500	432		
			III ♀	2500	2460		
			IV ♂	2000	1830		
			IV ♀	5000	4673		
8-22-78	6-16-78	81	I	0	(0.08)	0.13	(in Tenbene PK pres
			II	20	19.2		
			III ♂	500	465		
			III ♀	2500	2279		
			IV ♂	2000	1821		
			IV ♀	5000	4854		

Memo Date	Sampling Date	Wk	Animal Group	Dose	original PPM	Repeat PPM	% change
10-5-78	9-22-78	95	I	0	(0.28)	0.16	(In Test PK)
			II	20	19.1		
			III ♂	500	486		
			III ♀	2500	~~1654~~ 2293		
			IV ♂	2000	1654		
			IV ♀	5000	4644		
12-21-78	12-1-78	105	I	0	(0.23)	0.09	
			II	20	18.6		
			III ♂	500	473		
			III ♀	2500	2369		
			IV ♂	2000	2119		
			IV ♀	5000	5011		

ATTACHMENT W

File

January [illegible]

Wolfe, JF

Fletcher, MJ

FMC 33297, Permethrin, Mouse II
Chronic Study - Histopathological Observations

On Friday, December 28, 1979, the first print-out of the pathology summary tables of Mouse II was obtained from the Princeton R&D computer. I screened the data from the print-out, although the numbers had not been verified, to determine whether there were any obvious areas of interest. Knowing that some questions had been raised regarding lung findings (alveologenic adenoma) in FMC Rat Study #1 and that some questions had been raised regarding a small increase in lung adenoma and liver findings (hepatocyte vacuolation, liver hypertrophy) in ICI mouse study, I directed my attention to liver and lung findings in Mouse II. The print-out revealed the data in Table I for liver (hepatoma, hepatocellular carcinoma) and lung (bronchioalveolar adenoma).

I notified Dr. J. F. McCarthy, ACG R&D, on that date (December 28, 1979) that some unusual findings had been observed on the print-out. These findings were different than those observed in Mouse I (Table II).

I then phoned Dr. W. A. Rapp, Ph.D., DVM, the board certified pathologist who evaluated the tissue slides. I met with Dr. Rapp on Monday, December 31, 1979 to show him the data, discuss the findings and to attempt to understand their significance. He stated that he wished additional time to re-examine the lung and liver slides. The slides of those animals which were terminally sacrificed were given to Dr. Rapp on Monday, December 31, 1979. The remaining slides of those animals which had died spontaneously or were sacrificed as moribund were retrieved from American HistoLabs on Wednesday, January 2, 1980. These slides were transferred to Dr. Rapp on Friday, January 4, 1980. Dr. Rapp then completed his re-examination of all the slides after Friday, January 4, 1980. He verified all the data as presented in Table I except for increasing the number of Group IV female bronchioalveolar adenoma from 27 to 28. At this time I notified Joseph Wolfe, FMC Counsel, of the differences of liver and lung findings between Mouse I and Mouse II. ICI Americas was then informed of these unusual findings. A meeting with ICI was called for Thursday January 10, 1980. On Wednesday, January 9, 1980, Dr. M. Litchfield, Dr. J. Ishmael and Mr. G. Willis from ICI U.K. arrived in the U.S. to review the pathology data and some aspects of the in-life portion of the study. They also met with Dr. Rapp at the Princeton R&D Center at that time. Dr. Ishmael, an ICI pathologist, also reviewed the slides and confirmed Dr. Rapp's diagnosis (especially those for hepatocellular carcinoma).

At the FMC-ICI meeting on Thursday, January 10, 1980, I presented the unusual liver and lung findings from Mouse II and the group decided that this information should be submitted to the EPA no later than January 14, 1980.

On Friday, January 11, 1980, FMC and ICI representatives met to prepare a letter which was to be submitted to the Agency describing the findings of Mouse II.

Although the significance of these data are not well understood at this time, the following points with relation to these findings are worthy of note:

1) Proliferative liver lesions in the mouse are not unusual. They are spontaneous events. However, they are apparently related to compound administration in Mouse II.

2) Bronchioalveolar adenoma are not unusual findings in the CD-1 mouse used in this study. However, there is apparently a relationship to compound administration in the two top doses of Group III and Group IV of the females on study.

MJF:mjm

TABLE 1

MOUSE II UNUSUAL PATHOLOGICAL OBSERVATIONS

GROUP	I		II		III		IV	
SEX	M	F	M	F	M	F	M	F
DOSE	0	0	20	20	500	2500	2000	5000
OBSERVATION								
HEPATOMA	16	3	21	2	18	15	17	17
HEPATOCELLULAR CARCINOMA	4	0	6	2	13	3	5	0
BRONCHIOALVEOLAR ADENOMA	18	12	19	14	20	28	17	28
BRONCHIOGENIC CARCINOMA	1	2	0	0	2	2	1	2

TABLE II

MOUSE II vs. MOUSE I

COMPARISON OF PATHOLOGICAL OBSERVATIONS

GROUP	I		II		III		IV	
SEX	M	F	M	F	M	F	M	F
MOUSE I DOSE	0	0	20	20	500	500	4000	4000
MOUSE II DOSE	0	0	20	20	500	2500	2000	5000
OBSERVATION								
I HEPATOMA	3	2	1	1	9	4	4	1
II HEPATOMA	16	3	21	2	18	15	17	17
I HEPATOCELLULAR CARCINOMA	4	0	0	0	5	0	7	0
II HEPATOCELLULAR CARCINOMA	4	0	6	2	13	3	5	0
I BRONCHIOAVEOLAR ADENOMA	6	11	7	8	4	7	5	9
II BRONCHIOAVEOLAR ADENOMA	18	12	19	14	20	28	17	28
I BRONCHIOGENIC CARCINOMA	0	0	0	0	0	0	0	0
II BRONCHIOGENIC CARCINOMA	1	2	0	0	2	2	1	2

Attachment X

mice sacrificed at terminal necropsy (12/5/78 to 12/11/78). This pick-up was made by Mr. Wilson on 1/4/79 and consisted of 400 animals. Dr. Tierney explained that although the total seen above is slightly above the 600 mice figure, the extra jars may have constituted a few cases where 2 containers were used for one animal.

AUTOLYSIS DATA

Dr. Kasza had reported that during his review, during this data audit inspection, he found an unusually high (46%-66%) spontaneous death rate which would indicate unsatisfactory observation of animals allowing the moribund animals to die. He further points out that because of rapidly developing autolysis, the animals have limited value for histopathology. He figured that approximately 50% of the animals fall into this category.

Investigators Trapani and Bruckheimer reviewed the pathology sheets prepared by Dr. Rapp and hand tabulated the relationship of autolysis within specified necropsy time frames. The time frames chosen were:

1. Terminal sacrifice (12/5-11/78) - 187 mice.

2. Onset of study through 6/15/78 (first pick-up cate of tissues by FMC) - 202 mice.

3. Period between 6/15/78 and the day prior to scheduled terminal necropsy (6/16 thru 12/4/78) - 211 mice.

TOTAL = 600 mice

1. Terminal Sacrifice:

There were 187 mice necropsied between 12/5-12/11/78. A total of 4 mice showed autolysis (Rapp report) in one or more tissues (#305; 427; 439; and 720). This represents a figure of 2%.

2. Onset of study through 6/15/78:

We calculated that 202 mice died in this period based upon our review of Appendix B of the Bio/dynamics study report.

Based on Dr. Rapp's report, we tabulated 155 showing partial autolysis (at least one soft tissue) and 8 showing total autolysis resulting in 163 or 81% of the 202.

3. 6/16/78 to the day prior to scheduled necropsy:

We calculated that 211 mice were in this category using Appendix B. Dr. Rapp's report was used to tabulate the autolysis rate: 140 showing partial; 2 showing total and 69 showing none. The total autolysis for the 142 animals represents 67% of the 211 in this category.

ATTACHMENT 4

From Nelson Nelson Reader
Fib during April, 1979

DEQ
9-29-80

FIRST 200 ANIMALS WHICH DIED SPONTANEOUS
OR WERE SACRIFICED AS MORIBUND - ACT 115.35

Group	I		II		III		IV	
Tissue & Finding / Sex	M	F	M	F	M	F	M	F
Adrenals (no. of tissues examined)	17	16	16	15	11	24	37	21
Amyloidosis	0	3	1	1	2	6	8	4
Congestion, post-mortem	0	1	0	1	0	1	0	4
Vacuolar change, cortex	0	1	0	2	0	0	0	0
Lymphosarcoma	0	1	0	0	0	1	2	1
Thymus (no. of tissues examined)	0	3	1	0	1	1	0	1
Lymphosarcoma	0	3	1	0	1	1	0	1
Thyroid (no. of tissues examined)	15	13	13	10	11	19	28	22
Amyloidosis	0	0	0	0	0	6	6	4
Mononuclear luekocyte infiltrate	0	0	0	0	0	0	1	0
Lymphosarcoma	0	1	0	0	0	0	0	0
Parathyroid (no. of tissues examined)	8	2	7	2	4	7	9	7
Amyloidosis	0	0	0	0	0	1	3	1
Testes (no. of tissues examined)	19	-	16	-	14	-	34	-
Amyloidosis	0		0		1		0	
Degeneration	0		0		1		0	
Brain (no. of tissues examined)	24	21	17	15	16	26	38	22
Perivascular mononuclear leukocyte infiltrate	0	0	0	0	0	2	0	0
Lymphosarcoma	0	0	0	0	1	0	0	0
Trachea (no. of tissues examined)	19	17	14	11	14	24	34	21
Lymphosarcoma	0	0	0	0	1	0	0	1
Skin (no. of tissues examined)	21	17	16	13	14	23	40	20
Chronic dermatitis	0	0	0	0	0	0	0	1
Subacute dermatitis	0	0	0	0	0	1	2	0
Lymphosarcoma	0	0	0	0	2	0	1	1

FIRST 200 ANIMALS WHICH DIED SPONTANEOUS OR WERE SACRIFICED AS MORIBUND - ACT 115.35

Tissue & Finding	Group	I		II		III		IV	
	Sex	M	F	M	F	M	F	M	F
Spinal Cord									
Lymphosarcoma		0	0	0	0	1	0	0	0
Gall Bladder (no. of tissues examined)		1	1	1	2	1	1	2	3
Edema		0	0	0	0	0	0	0	1
Esophagus (no. of tissues examined)		22	19	18	14	16	24	38	23
Lymphosarcoma		0	0	0	0	0	0	0	1
Cervix (no. of tissues examined)		-	10	-	5	-	4	-	7
Amyloidosis			0		0		0		1
Lymphosarcoma			0		0		0		1
Sciatic Nerve (no. of tissues examined)		24	20	18	16	11	23	36	20
Lymphosarcoma		0	0	0	0	0	0	0	1
Ileum (no. of tissues examined)		7	5	3	3	9	12	14	12
Amyloidosis		0	1	1	1	2	3	3	2
Subacute enteritis		0	0	0	0	0	0	0	1
Submucosal lymphoid hyperplasia		1	0	0	0	0	3	4	1
Lymphosarcoma		0	0	0	0	1	0	0	0
Colon (no. of tissues examined)		8	9	7	5	9	14	16	11
Amyloidosis		0	0	0	0	0	0	3	1
Chronic serositis		0	0	0	0	0	0	1	0
Submucosal lymphoid hyperplasia		2	4	5	0	3	5	6	2
Lymphosarcoma		0	0	0	0	1	0	0	0
Jejunum (no. of tissues examined)		0	1	0	0	0	0	0	0
Amyloidosis		0	1	0	0	0	0	0	0

FIRST 200 ANIMALS WHICH DIED SPONTANEOUS
OR WERE SACRIFICED AS MORIBUND - ACT 115.35

Tissue & Finding	Group	I		II		III		IV	
	Sex	M	F	M	F	M	F	M	F
Mammary Gland (no. of tissues examined)		20	17	16	12	23	23	34	20
Cystic		0	0	0	0	1	0	0	0
Galactocele		0	0	0	0	0	0	1	0
Lymphosarcoma		1	1	0	1	0	1	0	2
Urinary Bladder (no. of tissues examined)		16	11	9	9	6	16	21	15
Chronic cystitis		0	0	0	0	1	0	0	0
Subepithelial mononuclear leukocyte infiltrate		0	0	0	1	0	1	5	4
Lymphosarcoma		2	1	0	0	0	1	1	0
Duodenum (no. of tissues examined)		7	5	5	4	9	11	19	11
Amyloidosis		0	0	2	0	2	3	4	3
Chronic serositis		0	0	0	0	0	0	1	0
Subacute enteritis		0	0	0	0	0	0	0	1
Submucosal lymphoid hyperplasia		0	1	0	0	0	0	2	0
Fibrosarcoma		0	1	0	0	0	0	0	0
Lymphosarcoma		0	0	0	0	2	0	0	0
Ovaries (no. of tissues examined)		-	23	-	19	0	24	0	22
Amyloidosis			6		1		6		4
Congestion			0		0		1		0
Congestion, post-mortem			1		0		0		0
Follicular cyst			1		2		1		1
Hematocyst			1		1		0		0
Perinovarian cyst			0		1		0		1
Fibrosarcoma			1		0		0		0
Lymphosarcoma			0		1		1		1
Uterus (no. of tissues examined)		-	23	-	18	-	24	-	20
Amyloidosis			0		0		1		1
Endometrial hyperplasia			8		6		14		13
Hemorrhage			0		1		1		0
Hydrometra			0		1		0		0
Leiomyoma			0		0		1		0
Lymphosarcoma			0		0		1		2
Sarcoma, undiff.			0		0		1		0

FIRST 200 ANIMALS WHICH DIED SPONTANEOUS OR WERE SACRIFICED AS MORIBUND - ACT 115.35

GROUP	I		II		III		IV	
Tissue & Finding — SEX	M	F	M	F	M	F	M	F
Salivary Gland (no. of tissues examined)	20	18	16	17	15	24	39	22
Amyloidosis	0	0	0	0	0	2	2	4
Mononuclear leukocyte infiltrate	3	5	0	4	3	9	8	2
Lymphosarcoma	2	2	1	0	1	2	4	4
Stomach (no. of tissues examined)	22	10	12	10	8	18	26	17
Acute Gastritis	0	0	0	0	0	1	0	0
Abcess	1	0	0	0	0	0	0	0
Amyloidosis	0	0	0	0	0	4	2	2
Chronic serositis	0	0	0	0	0	0	1	0
Congestion, post-mortem	1	0	0	0	0	0	0	0
Mononuclear leukocyte infiltrate	1	0	0	0	0	0	0	0
Submucosal lymphoid hyperplasia	1	0	0	1	0	0	1	0
Fibrosarcoma	0	1	0	0	0	0	0	0
Lymphosarcoma	0	0	0	1	1	2	0	1
Pancreas (no. of tissues examined)	22	17	14	11	15	23	35	23
Amyloidosis	0	0	0	0	1	1	0	3
Chronic pancreatitis	0	0	1	0	0	0	1	0
Mononuclear luekocyte infiltrate	2	1	0	1	0	1	0	0
Subacute pancreatitis	0	0	0	0	0	0	1	0
Fibrosarcoma	0	1	0	0	0	0	0	0
Lymphosarcoma	3	0	2	1	2	3	4	1
Reticulum cell sarcoma	0	0	0	0	1	0	0	0
Bone Marrow (no. of tissues examined)	23	19	19	15	13	25	40	23
Lymphosarcoma	5	3	1	1	2	2	7	6
Prostate (no. of tissues examined)	21	-	16	-	13	-	24	-
Acute suppurative prostatitis	0		0		0		2	
Mononuclear luekocyte infiltrate	1		0		0		0	
Lymphosarcoma	2		1		0		0	

FIRST 200 ANIMALS WHICH DIED SPONTANEOUS OR WERE SACRIFICED AS MORIBUND - ACT 115.35

Tissue & Finding	Group	I		II		III		IV	
	Sex	M	F	M	F	M	F	M	F
Heart (no. of tissues examined)		26	22	21	18	16	26	42	23
Amyloidosis		0	0	0	0	2	2	6	3
Chronic epicarditis		1	1	0	0	0	0	0	0
Chronic pericarditis		0	0	0	0	0	1	0	0
Interstitial fibrosis		0	0	0	0	1	0	1	0
Mineralization		0	0	0	0	0	0	0	1
Mononuclear leukocyte infiltrate		1	2	0	1	1	0	1	0
Myofiber degeneration		1	0	0	0	1	0	1	1
Thrombus		0	0	0	0	0	1	3	1
Adenocarcinoma, origin unknown		0	0	0	0	0	0	0	1
Fibrosarcoma		0	0	0	1	0	0	0	0
Lymphosarcoma		0	1	0	0	2	2	2	4
Spleen (no. of tissues examined)		25	21	18	6	14	25	40	23
Amyloidosis		1	4	1	0	0	3	3	3
Chronic inflammation		0	0	0	0	0	0	1	0
Congestion, post-mortem		0	1	0	0	0	0	0	0
Hematocyst		0	1	0	0	0	0	0	0
Lymphoid depletion		1	0	0	0	0	0	0	0
Lymphoid hyperplasia		0	3	1	3	1	3	1	1
Pigmentation (hemosiderosis)		5	5	4	3	4	5	9	9
Hemangiosarcoma		0	1	0	0	0	1	1	0
Lymphosarcoma		6	4	3	4	3	5	8	5
Reticulum cell sarcoma		0	0	0	0	1	0	0	0
Kidneys (no. of tissues examined)		25	22	17	16	13	26	37	23
Amyloidosis		1	4	3	1	2	6	7	4
Chronic interstitial nephritis		4	4	1	1	1	6	1	3
Congestion, post-mortem		0	2	1	1	0	1	0	0
Hyalinized tubular casts		1	1	1	0	0	1	1	0
Hydronephrosis		0	0	1	0	1	0	0	1
Mononuclear leukocyte infiltrate		12	9	7	8	6	11	20	8
Pyelitis, non-suppurative		1	1	0	1	1	1	1	0
Subacute interstitial nephritis		1	0	0	1	0	0	0	0
Tubular dilatation		0	1	1	0	1	2	2	0
Cysts, cortex		0	0	0	0	0	0	2	0
Lymphosarcoma		6	5	2	2	3	5	6	2

FIRST 200 ANIMALS WHICH DIED SPONTANEOUS
OR WERE SACRIFICED AS MORIBUND - ACT 115.35

Tissue & Finding	Group	I		II		III		IV	
	Sex	M	F	M	F	M	F	M	F
Liver (no. of tissues examined)		25	22	17	15	15	24	39	23
Amyloidosis		1	4	2	0	2	4	5	4
Coagulation necrosis		1	2	0	1	1	1	1	0
Chronic inflammation		0	0	0	0	0	0	1	0
Congestion, post-mortem		10	5	2	3	3	9	12	4
Hepatocellular degeneration		0	0	0	0	0	0	1	0
Hepatocellular hypertrophy		6	1	4	1	3	10	21	6
Hepatocellular vacuolization centrolobular		1	0	0	0	0	0	0	0
Mononuclear leukocyte infiltrate		6	6	1	3	1	6	8	3
Nodular hyperplasia		2	0	0	0	0	0	1	1
Fibrosarcoma		0	1	0	0	0	0	0	0
Pigmentation (hemosiderosis)		1	0	0	0	0	0	0	0
Hemangiosarcoma		0	0	0	0	0	1	1	0
Hepatocellular carcinoma		1	0	1	0	1	0	2	0
Hepatoma		3	0	2	0	2	1	7	1
Lymphosarcoma		6	4	2	2	3	2	7	4
Reticulum cell sarcoma		0	0	0	0	1	0	0	0
Sarcoma, undiff.		0	0	0	1	0	0	0	0
Lung (no. of tissues examined)		25	21	19	18	15	26	34	23
Acute suppurative pneumonia		1	1	0	0	0	0	0	0
Amyloidosis		0	0	0	0	2	1	3	1
Chronic pneumonia		0	3	0	2	2	3	5	3
Congestion, post-mortem		10	8	12	4	4	8	19	13
Interstitial leukocyte infiltrate		0	1	0	0	0	0	0	0
Interstitial pneumonitis		10	5	8	6	7	9	20	7
Adenocarcinoma, origin unknown		0	0	0	0	0	0	0	1
Bronchioalveolar adenoma		3	3	2	3	2	3	4	2
Carcinoma, metastatic		1	0	0	0	0	0	0	0
Fibrosarcoma		0	0	0	1	0	0	0	0
Hemangiosarcoma		0	1	0	0	0	0	0	0
Hepatocellular carcinoma		0	0	1	0	1	0	0	0
Lymphosarcoma		3	4	1	2	3	6	6	4
Reticulum cell sarcoma		0	0	0	0	1	0	0	0

FIRST 200 ANIMALS WHICH DIED SPONTANEOUS
OR WERE SACRIFICED AS MORIBUND - ACT 115.35

Tissue & Finding	Group	I		II		III		IV	
	Sex	M	F	M	F	M	F	M	F
[illegible] Node									
		0	0	1	0	0	3	2	2
[illegible]penitis		0	0	0	0	0	0	1	0
Congestion, post-mortem		0	1	0	1	0	1	1	2
Lymphoid hyperplasia		0	0	1	0	0	0	0	0
Reticuloendothelial cell hyperplasia		1	1	1	0	0	1	0	0
Fibrosarcoma		0	1	0	0	0	0	0	0
Lymphosarcoma		5	5	2	3	2	4	9	5
Reticulum cell sarcoma		0	0	0	0	1	0	0	0
Sub-Q or Skin Mass									
Abcess		0	1	0	0	0	0	0	0
Epidermal cyst		0	0	1	0	0	0	0	0
Hemangiosarcoma		0	1	0	0	0	0	0	0
Internal mass									
Adenocarcinoma, origin unknown		0	0	0	0	0	0	0	1
Fibrosarcoma		0	0	0	1	0	0	0	0
Hematocyst		0	0	0	1	0	0	0	0
Hemangiosarcoma		0	0	0	0	0	1	0	0
Lymphosarcoma		1	1	0	1	1	1	0	1
Sarcoma, undiff.		0	0	0	2	0	1	1	0

ACT 115.35 - HISTOPATH ON FIRST 200 SD'S

LYMPHOSARCOMA DATA

Group	I		II		III		IV	
SEX	M	F	M	F	M	F	M	F
# of animals which died on or after 5/9/77*	25	20	18	17	14	22	40	21
# of above animals with most or all tissues not examined	1 (#158)	0	2 (#304, 345)	1 (#466)	3 (#552, 555, 559)	0	2 (#716, 764)	1 (#841)
# of above animals evaluted	24	20	16	16	11	22	38	20
# of lymphosarcoma	6	6	3	6	4	6	9	6
Incidence	6/24	6/20	3/16	6/16	4/11	6/22	9/38	6/20
	25	30	19	38	36	27	24	30

* First lymphosarcoma found in animals which died on 5/9/77 (Gr I -, #247)