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

February 12, 1999

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Cancer Assessment Review Committee Meeting on Malathion

FROM: Sanjivani Diwan
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Addressees

Attached for your review is a package on Malathion prepared by Brian Dementi.

A meeting to review the carcinogenicity classification of this chemical is scheduled for Wednesday February 24,1999 at 10:00 am in Room 813, CM2.

Addressees

K. Baetcke
L. Brennecke
L. Brunsman
W. Burnam
K. Dearfield
V. Dellarco
S. Diwan
V. Dobozy
R. Hill
Y. Ioannou
N. McCarroll
E. Rinde
J. Rowland
J. Stewart
C. Swentzel
L. Taylor
Y. Woo

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

FEB 12 1999

MEMORANDUM

FROM: William Burnam, 2/12/99

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Additional Malathion Information for Malathion Cancer Peer Review

For all those of you who asked "why is my Malathion package so small?", I submit the following information:

Attachment A: Dr. Dementi's overview for the 1997 Cancer Peer Review (without its attachments)

B: The 1990 Final Cancer Peer Review

C: DER of Pivotal Rat Cancer Study

D: DER of Pivotal Mouse Cancer Study

E: DER of Maloxon Rat Study

F: Qualitative Assessment of Malathion Rat Based on DER in attachment C

G: Qualitative Assessment of Malathion Mice Study Based on DER D

H: Brief Qualitative Assessment of Male Mouse Re-read of Liver Slides

I: Summary of September 24 and October 8, 1997 meeting

Prior to the meeting, please also review the non-cancer information in the DER's. Check Attachment I regarding our opinion of the adequacy of the dosing and relevancy of certain tumors.

I think that the two hours allotted may not be enough so I am checking on perhaps an additional Wednesday afternoon session or maybe Thursday the 25th.

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

(A)

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

**MEMORANDUM**

**SUBJECT:** Cancer Assessment Review Committee Meeting on **MALATHION**

**FORM:** Jess Rowland Jess Rowland 9/8/97
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

**TO:** Addressees

Attached for your review is a package on Malathion prepared by Dr. Brian Dementi. Please note that there are 12 Attachments. The total package is 708 pages.

A meeting to review the carcinogenicity classification of this chemical is scheduled for **Wednesday, September 24, 1997, at 10:00 am in Room 817, CM2**

Addressees

K. Baetcke
L. Brennecke
L. Brunsman
M Copley
K. Dearfield
V. Dellarco
V. Dobozy
R. Hill
P. Hurley
Y. Ioannou
N. McCarroll
H. Pettigrew
E. Rinde
J. Rowland
J. Stewart
L. Taylor
Y. Woo
B. Dementi
A. Protzel

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: MALATHION/Evaluation of Carcinogenic Potential by the Health Effects Division Carcinogenicity Peer Review Committee (Second Evaluation)

TO: Jesudoss Rowland, M.S. Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

FROM: Brian Dementi, Ph.D., DABT Brian Dementi 9/4/97
Toxicologist
Toxicology Branch I, Health Effects Division (7509C)

THRU: Alberto Protzel, Ph.D. 9/4/97
Senior Scientist
Toxicology Branch I, Health Effects Division (7509C)

Attached is an overview of the carcinogenic potential of malathion, including data on mutagenicity and metabolism. These data are the results of evaluation of studies submitted to the Agency by the Registrants of malathion. The material available for review consists of DERs, and other data summaries prepared and/or supplied by Dr. Brian Dementi, Ms. Nancy McCarroll and tables and statistical analysis by Lori Brunsman.

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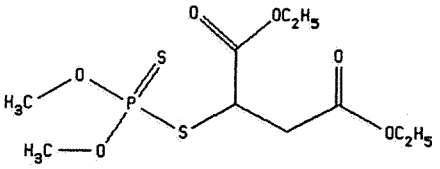
# Cancer Peer Review Document for Malathion

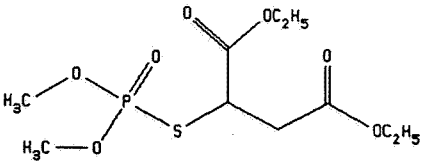
## C. Background Information:

Chemical Name: Malathion

Synonym: Mercaptosuccinic acid diethylester; S-ester with O, O-dimethyldithiophosphate



Malathion



Malaoxon

PC Code: 057701

CAS No. 121-75-5

Tolerance: Tolerances for malathion in 40 CFR 180.111 are listed as 8 ppm for a variety of food crops.

Historical: The Registration Standard for malathion of February 1988 identified a number of toxicology Guideline testing deficiencies. These required studies have been submitted and reviewed, and the Registration Eligibility Document (RED) is expected to be completed by early 1998.

In 1990, the malathion carcinogenicity data base was considered by the HED Cancer Peer Review Committee. At that time five National Cancer Institute (NCI) carcinogenicity studies plus a contract lab carcinogenicity study constituted the principal body of information on carcinogenicity under review by that committee. Specifically, the five NCI studies included studies of malathion in Osborne-Mendel rats, F344 rats and B6C3F1 mice, and of malaoxon (principal malathion metabolite) in F344 rats and B6C3F1 mice. The contract lab study was a 2-year malathion study in Sprague-Dawley rats performed by Food and Drug Laboratories, Waverly, New York.

After evaluating results of these six studies, which took into consideration the registrant's assessment of the studies plus an NTP reexamination of selected tissues in three of the NCI studies (malathion Osborne-Mendel and F344 rat studies and malaoxon F344 rat study), the Cancer Peer Review committee placed malathion in carcinogen category D, with the requirement that additional testing be completed. The



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Registration Standard had required a new malathion carcinogenicity study in B6C3F1 mice, a malaoxon chronic toxicity/carcinogenicity in F344 rats and a malathion chronic toxicity study in F344 rats. The cancer peer review endorsed these three new studies, but in addition required that the malathion study in F344 rats be upgraded from a chronic toxicity study to a combined chronic toxicity/ carcinogenicity study. Referable background document: **Attachment No. 1**: April 12, 1990 Cancer Peer Review for Malathion. This document contains several supporting documents including HED's July 27, 1989 draft "American Cyanamid Company Response to Malathion Registration Standard"; HED's reviews of the five NCI studies; Dr. Adrian Gross' April 24, 1984 assessment of three of the NCI studies; a 1985 journal publication by Huff et al containing a NTP reevaluation of three of the NCI studies; a June 14, 1984 letter of Ernest E. McConnell to John A. Moore; four reviews of the Food and Drug Laboratories chronic toxicity/carcinogenicity study of malathion in the Sprague-Dawley rat (It is noteworthy that this bioassay received no less than four reviews. The original HED review was rated Guideline with no chemical related carcinogenic findings. The second review performed by Dynamac (contractor) identified a number of chronic toxicity and questionable carcinogenicity findings and was rated Core Supplementary. The third review performed in HED declared the study invalid due to numerous claimed deficiencies. This particular review received no secondary or tertiary review, i.e., it was one person's opinion. A "Qualitative and Quantitative Risk Assessment of Combined Toxicity and Oncogenicity Study in Rats" was performed on this study by an HED statistician and constitutes part of this record. The study was also audited on March 11-13, 1986. As presented in the final report of the audit dated 7/31/87, a number of deficiencies were identified. In the fourth review which involved reexamination of histopathology slides by an independent pathologist, as required by the Cancer Peer Review Committee, no clear evidence of carcinogenicity was identified. However, this last review of the same slides reviewed earlier by other pathologists yielded remarkable differences in diagnoses. Of course, this last review was not in evidence at the last cancer peer review, but has been added to that Cancer Peer Review package, **Attachment No. 1**, for review by the current Cancer Peer Review Committee members.); selected pages from the 1988 Registration Standard for Malathion; plus other items. Also now being introduced into this attachment is a copy of a publication by M. D. Reuber (1985), Environmental Research, 37, 119-153, setting forth independent assessments of the five NCI studies in question performed in 1978-1980. This article appeared in the same volume of Environmental Research immediately preceeding the above mentioned publication by Huff, et al concerning the



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same subject. Rueber (1985) concluded that malathion was carcinogenic for both sexes in the NCI Osborne-Mendel and F344 rat studies and for male B6C3F1 mice. He also concluded that malaoxon was carcinogenic in both sexes in F344 rats and that the malaoxon study in B6C3F1 mice was unsatisfactory.

An effort will be made later, under the Weight of the Evidence section in this document, to summarize and analyze findings in the older documents in discussing the three recent cancer studies.



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D. **Evaluation of Carcinogenicity Evidence**

1. Evaluation of Carcinogenicity Study with Malathion in B6C3F1 Mice, 1994. (DER) **Attachment No. 2.**

Reference: RW Slauter: "18-Month (Oral (Dietary) Oncogenicity Study in Mice." Report Date 10/12/94. MRID 43407201. Study No. 668-001. Testing facility: International Research and Development Corporation (IRDC), Mattawan, MI.

a. Experimental Design: Technical malathion (96.4% a.i.) was administered in the diet to groups of 65 male and 65 female B6C3F1 BR strain mice at dose levels of 0 (control) 100, 800, 8000 or 16000 ppm (equivalent to 0, 17.4, 143, 1476 or 2978 mg/kg/day in males and to 0, 20.8, 167, 1707 or 3448 mg/kg/day in females). Ten mice/sex/group were sacrificed at 12 months and the remaining survivors were sacrificed at 18 months. The study was classified Core Guideline.

b. Discussion of Tumor Data: "A treatment-related increased incidence of hepatocellular tumors was observed in both male and female mice in this study at 8000 ppm and 16000 ppm. For male mice which died during the final 6 months of the study or were killed at the terminal sacrifice at 18 months, the percent incidences of hepatocellular adenomas were 1.9%, 7.3%, 3.6%, 21.8% and 94.1%; of hepatocellular carcinomas were 0.0%, 10.9%, 5.5%, 10.9% and 2.0%; and of combined hepatocellular adenomas/carcinomas were 1.9%, 18.2%, 9.1%, 32.7% and 96.1% for the 0 (control), 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively. For female mice which died during the final 6 months of the study or were killed at the terminal sacrifice at 18 months, the percent incidences of hepatocellular adenomas were 0.0%, 1.8%, 0.0%, 17.0% and 80.8%; of hepatocellular carcinomas were 1.8%, 0.0%, 3.7%, 1.9% and 3.8% and of combined hepatocellular adenomas/carcinomas were 1.8%, 1.8%, 3.7%, 18.9% and 84.6% for the 0 (control), 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively." (**Attachment No. 2**, p. 2)

There were no effects on survival rates at any dose for both sexes (Attachment 3). As summarized in Tables 1 and 2 of this document, notable increases in hepatocellular tumors were observed in the study in mice of both sexes.



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Table 1. Malathion - $B_6C_3F_1$ Mouse Study (From: Attachment 3, L. Brunsman HED Memo dated 5/8/97).

Male Liver Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

| | Dose (ppm) | | | | |
|---|---|---|---|---|---|
| | 0 | 100 | 800 | 8000 | 16000 |
| Adenomas (%) | 1/54 (2) | 6[a]/54 (11) | 2/55 (4) | 13/55 (24) | 49/51 (96) |
| p = | 0.000** | 0.056 | 0.507 | 0.001** | 0.000** |
| Carcinomas (%) | 0/54 (0) | 6/54 (11) | 3[b]/55 (5) | 6/55 (11) | 1/51 (2) |
| p = | 0.345 | 0.014* | 0.125 | 0.014* | 0.486 |
| Combined (%) | 1/54 (2) | 10[c]/54 (19) | 5/55 (9) | 18[d]/55 (33) | 49[d]/51 (96) |
| p = | 0.000** | 0.004** | 0.107 | 0.000** | 0.000** |

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 54. Also excludes week 53 interim sacrifice animals.

[a]First liver adenoma observed at week 53, dose 16000 ppm, in an interim sacrifice animal. Second liver adenoma observed at week 79, dose 100 ppm, in a terminal sacrifice animal.

[b]First liver carcinoma observed at week 65, dose 800 ppm.

[c]Two animals in the 100 ppm dose group had both an adenoma and a carcinoma.

[d]One animal in each of the 8000 and 16000 ppm dose groups had both an adenoma and a carcinoma.

Note: Interim sacrifice animals are not included in this analysis. One animal in the 16000 ppm dose group of the interim sacrifice group had a liver adenoma.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.



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Table 2. Malathion - $B_6C_3F_1$ Mouse Study. (From: Attachment 3, L. Brunsman HED Memo dated 5/8/97).

Female Liver Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

| | Dose (ppm) | | | | |
|---|---|---|---|---|---|
| | 0 | 100 | 800 | 8000 | 16000 |
| Adenomas (%) | 0/55 (0) | 1/53 (2) | 0/53 (0) | 9/52 (17) | 42[a]/51 (82) |
| p = | 0.000** | 0.491 | 1.000 | 0.001** | 0.000** |
| Carcinomas (%) | 1[b]/55 (2) | 0/53 (0) | 2/53 (4) | 1/52 (2) | 2/51 (4) |
| p = | 0.183 | 0.509 | 0.486 | 0.738 | 0.471 |
| Combined (%) | 1/55 (2) | 1/53 (2) | 2/53 (4) | 10/52 (19) | 43[c]/51 (84) |
| p = | 0.000** | 0.743 | 0.486 | 0.003** | 0.000** |

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 54. Also excludes week 53 interim sacrifice animals.

[a]First liver adenoma observed at week 78, dose 16000 ppm.

[b]First liver carcinoma observed at week 79, dose 0 ppm.

[c]One animal in the 16000 ppm dose group had both an adenoma and a carcinoma.

Note: Interim sacrifice and accidental death animals are not included in this analysis. One animal in the 16000 ppm dose group which was killed accidentally had a liver adenoma.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.



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Perspectives on the findings are presented in the DER (**Attachment No. 2**) and the January 13, 1997 letter of Brian Dementi to Daniel Barolo. (**Attachment No. 4**)

The results show statistically significant hepatocellular tumorigenic responses in mice of both sexes at the 8000 and 16000 ppm dose levels. It should be noted that this study was required in the 1988 Registration Standard for malathion to address an "equivocal" tumorigenic response in male mice only, at the 16000 ppm dose level in a 1978 National Cancer Institute Study. The 1980 HED review of that study is included. (**Attachment No. 1**) The registration standard required that the new study employ the same dose levels as those of the NCI study, namely, 8000 and 16000 ppm. Additional dose levels of 100 and 800 ppm were introduced by the registrant. In this new study, the statistically significant increase in hepatocellular tumors at the 100 ppm level in males is of particular concern (See Table 1). It is also noteworthy that in contrast to the 1978 study, where there was no compound-related tumorigenic response in females, a positive response was observed in the new study in females at both the 8000 and 16000 ppm dose levels. Hence, now the finding in B6C3F1 mice involves both sexes. Furthermore, in the new study, tumor incidences in males were much higher (98%) at the high dose level, and the incidence in females is quite high (86%) as well.

The DER for the study expresses uncertainty as to the interpretation of the statistically significant finding in males at the lowest dose level and appears to defer interpretation until the HED statistical evaluation is performed. As stated previously, statistical treatment of liver adenomas and carcinomas has been completed, (**Attachment No. 3**), and shows a pairwise statistically significant incidence of hepatocellular carcinomas in males at 100 ppm.

Dr. Dementi's 1/13/97 letter to Mr. Barolo expresses the following concerns with regard to the findings in male mice: statistically significant hepatocellular tumorigenic responses were observed at 100, 8000 and 16000 ppm. The five fold combined carcinoma/adenoma incidence relative to the control at 800 ppm dose level (where $p = 0.107$) though not statistically significant at the $p \leq 0.05$ criterion of significance,

cannot be dismissed as a positive finding, nor can it be used to dismiss the finding at 100 ppm as a real effect of the test material especially given the clear evidence at the higher doses that the liver is a target for the tumorigenicity of malathion. The finding of three and possibly four examples of liver tumor multiplicity among the ten male mice in the 100 ppm groups with liver tumors, serves as an added reason to conclude the finding is a compound related effect. Specifically, among the ten low dose group males affected, there were two mice with both an adenoma and a carcinoma of differing lobes of the liver, one with carcinoma in each of two lobes of the liver and one with a very large carcinoma spanning two lobes of the liver, which according to an expert pathologist could be two carcinomas that arose independently in the respective lobes and subsequently fused, or is an example of an advanced single carcinoma. Only further histopathological assessment would answer the question. By contrast, in the control group there was but one male mouse with one adenoma of the liver, i.e., no multiplicity. Individual animal (MRID 43407201) pathology sheets for the four low dose group male mice in question are appended. (**Attachment No. 5**)

The claim in the study report that essentially all liver tumors at 16000 ppm were adenomas begs confirmation by an independent pathology working group. See the 1/13/97 letter of Brian Dementi to Daniel Barolo (**Attachment No. 4**) in addition to the letters of Drs. Joseph Haseman and Robert Maronpot of NTP. (**Attachment No. 6**) Furthermore even if essentially all liver tumors in the 16000 ppm group were benign tumors it cannot be interpreted to mean that the effect at that dose level is limited to a benign tumor response, given the complexity of pathologic diagnosis and the propensity of mouse liver adenomas to transform to carcinomas post week 80 of the in-life phase of B6C3F1 mouse studies. This study was restricted to 78 weeks.

The NCI Study, in which the high dose male liver tumor incidence (17/55) consisted of 11/55 carcinomas and 6/55 adenomas, was of longer duration than the IRDC study. The NCI study was 95 weeks in-life (80 weeks dosing), while the IRDC Study was 78 weeks. Hence, more time for transformation of adenomas to carcinomas was available in the former study. Also, by more contemporary NTP standards, all "neoplastic nodules" may not be identified as adenomas, i.e. in contemporary assessments total adenomas will likely be less than



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total neoplastic nodules, as certain of the latter lesions will drop out as not being tumors.

c. Comparison with Historical Control Data: Historical control data for liver tumors in male B6C3F1 mice for studies conducted at IRDC are reproduced here from the MRID Submission (p. 1404) (**Attachment No. 7**). See pages 25-26 of the 1995 DER (**Attachment No. 2**) for the Reviewer's comments on the historical control data. According to the DER with respect to adenomas, "It should be noted that the percent incidences in the control (1.9%), 100 ppm (7.3%) (more correctly 11%, per HED's statistical analysis) and 800 ppm (3.6%) groups were far below the percent incidence range reported for the male historical control animals for 18 month studies (14.3-21.7%). For hepatocellular carcinomas during the same period, percent incidences were 0.0%, 10.9%, 5.5%, 10.9% and 2.0% for the control, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups, respectively. Although increased at all doses compared to the concurrent control group, the response was not dose related. The percent incidence at 100 ppm (10.9%) and at 8000 ppm (10.9%), however, did exceed the upper historical control percent incidence for 18-month studies (0.0-6.4%)." (pp. 25-26) Examination of this historical data from IRDC discloses that there were five studies comprising the 18-month studies. The dates on these studies were 1985/87, 85/87, 88/89, 88/90 and 89/90. While the historical range for liver carcinomas among male mice was indeed 0.0-6.4%, in three of the five studies, the incidence was zero, in one the incidence was 2.2% (one mouse involved) and in one study the incidence was 6.4% (three mice involved). In the five studies combined, there were 4 carcinomas in a total of 205 control mice, or a mean incidence of 2.0% in the five studies. Yet, in the IRDC malathion study, carcinomas were identified in all four dose groups, where the 100, 800 and 8000 ppm groups well exceeded this mean historical control value. The finding of but one carcinoma in the 16000 ppm group is a curiosity, and suggests the need for reexamination of the histopathology slides. However, the anomalous response may have its explanation in possibly differing metabolic affects across the wide dose range in this study. The IRDC historical data base (5 studies, 205 male mice total) must be viewed as small and therefore of questionable reliance. However in considering this matter in the design of the study, the registrant concluded that the data base was adequate. NTP does not have an historical data base for 78-week mouse



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studies, as their studies are 2-year studies. Dr. Joseph Haseman in his letter to Dr. Dementi (**Attachment No. 6**), in an effort to estimate historical liver tumor incidences for 78-week studies, concluded it to be approximately 26%(25/95). However, his approach to deriving the number is qualified, the number of animals is small (95) and no distinction is made between adenomas and carcinomas.

The fact remains that in the IRDC study incidences of combined tumors in males and females are remarkable in the 8000 and 16000 ppm groups. In males the incidence of adenomas and carcinomas at the 100 ppm level is remarkably increased relative to the contemporaneous control group, the most important and most relevant control group. In male mice, liver tumor incidence as viewed across all dose groups is an obvious finding. The apparent inversion in incidence between the 100 ppm and 800 ppm groups, while not understood, may have its explanation in mechanisms of tumor inducting, i.e., given the wide dose range in this study, 100-16000 ppm, the potential for varied metabolic or mechanistic effects across doses is considerable, and such may well explain the varied tumorigenic findings as valid.

The Office of Sciences and Technology Policy (OSTP) (1985) (Fed. Reg. vol. 50, no. 50 "Chemical Carcinogens: A Review of the Science and Its Associated Principles" pp. 10372-10442), a principal authoritative source on chemical carcinogenesis, in discussing the use of historical control data says that: "Historical control data can be valuable when used appropriately, especially when the differences in incidence rates between treated and concurrent negative controls are <u>small</u> (emphasis added) and can be shown to be within the anticipated historical incidences." (p. 10418). In this particular study with malathion, the differences between concurrent control and the 100 ppm group liver tumor incidences among male mice is not small, in fact is over ten fold and solidly statistically significant. Furthermore, though adenomas at 100 ppm be inside the historical control range, carcinoma incidence is well outside the highest and mean incidences for carcinomas. Furthermore, the liver is clearly a target organ in this study.

The DER claims "The study report also indicated that 35 male mice at the 16000 ppm had multiple adenomas present whereas multiple adenomas were not present in any other group of male mice." (p. 25) This statement

conveys the fact that "multiplicity", a weighing factor in carcinogenesis evaluations, was evident only at the highest dose level. As stated earlier, an independent examination of individual animal histopathologic data sheets for the control and 100 ppm dose groups in the MRID while not disclosing any multiplicity in terms of multiple adenomas alone, does show three and possibly four examples of tumor multiplicity in the 100 ppm group. So in terms of multiplicity as a weighing factor in carcinogenesis assessment, 30% (possibly 40%) of mice with liver tumors in the 100 ppm dose group exhibited multiplicity of the type described, which enhances the concern with respect to the 100 ppm male dose group.

There were no other compound related tumorigenic findings in this mouse bioassay.

d. Non-neoplastic Lesions: According to the DER, there were gross histopathologic findings of the liver . "Increased numbers of liver masses, compared to control groups, were observed in the 100 ppm, 800 ppm, 8000 ppm and 16000 ppm male groups and the 16000 ppm female group at the terminal sacrifice." (p. 16) The incidences were 0, 8, 4, 5 and 18 for male and 1, 0, 3, 2 and 10 for females for the 0, 100, 800, 8000 and 16000 ppm groups, respectively. "Also at the terminal sacrifice, increased numbers of liver nodules were observed in the 8000 ppm and 16000 ppm male and females groups." (p. 16) The incidences were 5, 2, 3, 10 and 19 for males and 1, 2, 0, 9 and 29 for females for the 0, 100, 800, 8000 and 16000 ppm groups, respectively. The gross findings of liver masses across all dose groups in the case of males tends to support a hepatotoxic effect of malathion at all doses for which there is no NOEL, and to that degree supports the tumorigenic findings.

The DER also claims in the case of male mice a very small increase in enlarged hepatic lymph nodes in malathion treated groups at 800 ppm, 8000 ppm and 16000 ppm." (p. 16) In the case of females, an increased incidence of focus/foci (tan/yellow) was observed in the livers of the 16000 ppm group.

"Treatment-related increases in liver weight were observed in male mice at 8000 ppm and 16000 ppm and in female mice at 16000 ppm at 12 and 18 months." (p. 18)



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A number of findings in this study show that the liver of mice of both sexes is affected by malathion. This organ is a target of this test material. We must presume this reflects an effect on the part of the liver to detoxify malathion. The male gender appears more remarkably affected than females, even though doses at each dose level in terms of mg/kg/day was greater in females. The 1978 NCI study exhibited liver tumorigenic responses in males only. Taken together the data support the male mouse to be more susceptible, which in turn tends to support the statistically significant finding in the male low dose group of the IRDC study as real.

e. Adequacy of dosing for Assessment of Carcinogenic Potential:

This study was a requirement in the 1988 Registration Standard. Furthermore, the Standard required that the study be conducted in the same strain of mouse (B6C3F1) and incorporate the same dosage levels (8000 and 16000 ppm) as employed in the 1978 NCI study in order to resolve so called equivocal increases in hepatocellular tumors in males. The NCI report for the 1978 study identified no significant effects on mortality in either sex. There were dosing related clinical signs in both sexes, and deficits in body weight gain in males of about 14% and 36% at low and mid doses, respectively, and of about 20% at both dose levels in females. The HED review found the study acceptable (core minimum). In the current (1994) study, "Mortality rates, clinical signs of toxicity and hematological parameters were not affected by treatment with malathion at any dose." (**Attachment 2**, pp. 2-3) There were decreased absolute body weights at 8000 and 16000 ppm in both sexes, ranging 14.3-20.0% in males and 9.7-16.1% in females throughout the entire duration of the study. The NOEL for plasma and erythrocyte cholinesterases was 100 ppm, and that for brain cholinesterase inhibition was 8000 ppm for both sexes. The study is rated core Guideline and concluded in the DER to satisfy the Guideline requirement for a carcinogenicity study in mice.

In summary, dosing is considered adequate **except** in not identifying a NOEL for hepatocellular tumorigenicity in male mice.

2. Evaluation of Combined Chronic Toxicity/Carcinogenicity Study With Malathion in F344 Rats, 1996 (DER) (**Attachment No. 8**)

Reference: Daly, W.I.: "A 24-Month Oral Toxicity/Oncogenicity Study of Malathion in the Rat via Dietary Administration", February 27, 1996. MRID Number: 43942901, Lab. Study No.: 90-3641. Testing Facility: Huntington Life Sciences. East Milestone, NJ

a. Experimental Design

Malathion Technical (97% a.i.) was administered in the diet to groups of 90 male and female F344 rats at 0, 100/50, 500, 6000 or 12000 ppm [equivalent to respective mean values of 0, 4, 29, 359 and 739 mg/kg/day (males) and 0, 5, 35, 415 and 868 mg/kg/day (females)] for two years. Among the 90 rats/sex/group in the study, 10 rats/sex/group were scheduled for interim sacrifice at both 3 month and 6 month time intervals, primarily for ocular tissue assessments. A full 12 month interim sacrifice (not limited to ocular tissues) was performed. There were 55 rats/sex/group devoted to the full 2-year study. The low dose in the study was initially 100 ppm, but was reduced to 50 ppm in both sexes from the 3 month time point for the duration of the study due to the finding of statistically significant erythrocyte cholinesterase inhibition in females.

b. Discussion of Tumor Data (HED's Statistical Evaluation of Tumor Data, L. Brunsman **Attachment No. 9**; MRID Study Statistical Report, M. J. Nicolich **Attachment No. 10**).

Dosing-related increased mortality was observed in rats of both sexes (See Tables 3 and 4 of this document). Data from the DER show survivorships in the various groups were for males 67%, 75%, 53%, 26% and 0% and for females 69%, 74%, 75%, 62% and 36% for the respective 0, 100/50, 500, 6000 and 12000 ppm dose groups. The DER concluded that malathion exerted a dosing-related adverse effect on survivorship among males at the top three dose levels, albeit marginal at 500 ppm, and in females at the highest dose level only.
The study was classified Reserved pending review and discussion by the HED HAZID and Carcinogenicity Peer Review Committees.

In interpreting carcinogenicity findings (and other chronic findings) the study is complicated by competing toxicity and resulting high mortality primarily at 6000 and 12000 ppm in males and 12000 ppm in females. Chronic nephrotoxicity and leukemia were principal competing causes of mortality.

Table 3. Malathion - Fischer 344 Rat Study. (From Attachment 9: L. Brunsman HED Memo dated 7/16/97).

Male Mortality Rates[+] and Cox or Generalized K/W Test Results

| | Weeks | | | | | |
|---|---|---|---|---|---|---|
| Dose (ppm) | 1-26 | 27-53 | 54[i] | 54-78 | 79-106[f] | Total |
| 0 | 0/70 | 0/70 | 15/70 | 0/55 | 18/55 | 18/55 (33)** |
| 100/50 | 0/70 | 0/70 | 15/70 | 0/55 | 14/55 | 14/55 (25) |
| 500 | 0/70 | 0/70 | 15/70 | 3/55 | 23/52 | 26/55 (47) |
| 6000 | 0/70 | 0/70 | 15/70 | 1/55 | 38/52[a] | 39/53 (74)** |
| 12000 | 1/70 | 1/69 | 14/68 | 15/54 | 39/39 | 56/56 (100)** |

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[i]Interim sacrifices at week 14, 27 and 54. Only week 54 interim sacrifice animals are included in this analysis.

[f]Final sacrifice at week 105.

[a]Two accidental deaths at week 105, dose 6000 ppm.

( )Percent.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.



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Table 4. Malathion - Fischer 344 Rat Study (From Attachment 9: L. Brunsman HED Memo dated 7/16/97).

Female Mortality Rates[+] and Cox or Generalized K/W Test Results

| | Weeks | | | | | |
|---|---|---|---|---|---|---|
| Dose (ppm) | 1-26 | 27-53 | 54[i] | 54-78 | 79-106[f] | Total |
| 0 | 0/70 | 0/70 | 15/70 | 1/55 | 16/54 | 17/55 (31)** |
| 100/50 | 0/70 | 1/70 | 14/69 | 1/55 | 13/54 | 15/56 (27) |
| 500 | 0/70 | 0/70 | 15/70 | 2/55 | 12/53 | 14/55 (25) |
| 6000 | 0/70 | 1/70 | 15/69 | 1/54 | 19/53 | 21/55 (38) |
| 12000 | 0/70 | 1/70 | 15/70 | 4/55 | 30/51 | 35/55 (64)** |

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[i]Interim sacrifices at week 14, 27 and 54. Only week 54 interim sacrifice animals are included in this analysis.

[f]Final sacrifice at week 105.

( )Percent.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.



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1) Hepatocellular Tumors

Female rats had a significant increasing trend ($p < 0.01$) and a significant pair-wise comparison relative to the control group for combined liver adenomas/carcinomas at 6000 ppm ($p < 0.05$) and 12000 ppm ($p < 0.01$). See HED's statistical treatment of the data (**Attachment No. 9 and Table 5 of this document**). Increases observed at 100/50 and at 500 ppm though not statistically significant at the $p = 0.05$ criterion of significance are considered to be consequences of dosing with malathion. As presented in the DER of the study, the conclusion that the increases at the 100/50 ppm and 500 ppm levels are compound related is rationalized on the grounds that a) the zero incidence (particularly of carcinoma) in the control group is not unexpectedly low, b) further increased findings were observed at the higher doses and c) the hepatocellular tumor incidences (particularly carcinomas) in female F344 rats are very rare. Historical control data from Huntington Laboratory, accompanied by discussion of NTP historical data, is presented on pages 59-60 of the DER. (**Attachment No. 8**). It is very important to note that the Office of Science and Technology Policy (OSTP) (1985) as quoted on p. 61 of the DER emphasizes that slight increases in rare tumors, i.e., tumors with historical incidence of < 1%, "..... may be biologically significant and may be considered adequate evidence of carcinogenicity." Certainly a tumor incidence as rare as 0.07% or less as in the 1996 NTP data base for hepatocellular carcinoma in female F344 rats satisfies this criterion as a rare tumor type. The tumor types are not as rare in the Huntington historical data base, but that historical data is small, covering only 254 rats.

Among females there were no non-neoplastic hepatocellular findings such as hypertrophy or hyperplasia to suggest that 12000 ppm was an excessive dose. However, liver weight was increased in males and females at 6000 ppm and in females at 12000 ppm at terminal sacrifice, suggesting the liver as a target site.

Among male rats, there were no significant increases in hepatocellular tumors at any dose



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level (Attachment 9), however, high mortality at the 6000 and 12000 ppm dose levels may have precluded expression of this tumor type.

Table 5. Malathion - Fischer 344 Rat Study (From Attachment 9: L. Brunsman HED Memo dated 7/16/97).

Female Liver Tumor Rates+ and Peto's Prevalence Test Results (p values)

| | Dose (ppm) | | | | |
|---|---|---|---|---|---|
| | 0 | 100/50 | 500 | 6000 | 12000 |
| Adenomas (%) | 0/40 (0) | 1[a]/48 (2) | 1/43 (2) | 3/39 (8) | 3/29 (10) |
| p = | 0.007** | 0.240 | 0.168 | 0.032* | 0.008** |
| Carcinomas (%) | 0/41 (0) | 1/50 (2) | 1/44 (2) | 0/41 (0) | 3[b]/38 (8) |
| p = | 0.063 | 0.168 | 0.168 | - | 0.085 |
| Combined (%) | 0/41 (0) | 2/50 (4) | 2/44 (5) | 3/41 (7) | 6/38 (16) |
| p = | 0.002** | 0.134 | 0.085 | 0.032* | 0.003** |

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

[a]First liver adenoma observed at week 103, dose 100/50 ppm.

[b]First liver carcinoma observed at week 101, dose 12,000 ppm.

Note: Interim sacrifice animals are not included in this analysis. There were no liver tumors in any interim sacrifice animals.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

2) Nasal Tumors

In this study, two sections through the nasal turbinates were examined microscopically in each rat. For these particular tissues the FIFRA Guidelines require examination of control and high dose group animals only. In the event either tumors and/or hyperplasia is observed in the high dose group, lower dose groups must be likewise examined. In the nasoturbinal tissues, a carcinoma was found in one male rat in the 12000 ppm group and an adenoma was found in one male rat in the 6000 ppm group. Both of these tumors were of the olfactory epithelium. Tumors of the olfactory epithelium are exceedingly rare, historically, as discussed on pp. 61-63 of the DER (It is very important that Committee members read these pages of the DER, **Attachment No. 8**). The MRID study report acknowledges both of these tumors to be compound related effects of the test material. Although hyperplasia of the olfactory epithelium and other nasal tissue effects were prevalent in rats of both sexes at the 6000 and 12000 ppm dose groups, the principal reason offered in the text for calling these tumors compound related was their extreme rarity. The fact that they occurred in tissues so clearly affected by the test material certainly support their designation as compound related effects.

While not discussed in the MRID study report, two additional rare tumors were identified in histopathology slides of nasoturbinal sections. These are described as "squamous cell carcinoma of the alveolus of the root of a tooth". One such tumor was identified in each of two female rats, one in the 100/50 ppm group and one in the 12000 ppm group. Though appearing on nasoturbinal slides, these tumors lie close to the peripheries between the oral and nasal tissues and may be properly regarded as oral tissue tumors. As discussed in the DER of the study (pp. 63-65) regardless of the location, oral or nasal, these, like the tumors of the olfactory epithelium, are extremely rare and while not commented upon in the MRID study report, are concluded in the DER to be compound related. So in the nasal tissue sections there are four rare tumors in this one study that, collectively, appear in both sexes and across the



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entire dose range. Of added concern in this study, which in fact constitutes a deficiency, is the fact that nasoturbinal slides were not examined for all animals in the low dose group despite the facts, as discussed in the DER, that one of the rare tumors was seen in a low dose female rat and that hyperplasia of the olfactory epithelium was extensive in the 6000 and 12000 ppm dose groups. Only unscheduled death rats were examined in the 100/50 ppm group. It is a further requirement in the DER that the registrant discuss with the Agency examining additional nasoturbinal sections, beyond the two already examined.

3) Testicular Interstitial Cell Tumors

Testicular tissues were examined in all male groups. The incidence of this tumor type was very high, approaching 100%, in all groups, including the control. The MRID study report claims that this is a very common tumor in the F344 rat and that nearly all will develop this tumor if allowed to complete their normal life span (p. 68, **Attachment No. 8**). However, in this study all males did not complete their normal life span and yet developed the tumors. So the question may be one of decreased latency, or earlier tumor onset, as the result of dosing, a principal parameter in the defining of carcinogenicity. Hence, according to the MRID study report where the tumor incidence was analyzed by the Haseman statistic and Fisher's exact test (tests considered appropriate for this tumor) it was concluded that dosing with malathion is associated with increases in this tumor at all doses assayed based on Haseman's and Fisher's Tests; Haseman's at 12000 ppm and Fisher's at the 100/50,500 and 6000 ppm dose levels. By contrast, HED's independent statistical treatment (**Attachment No. 9 and Table 6 of this document**) found a highly positive dose trend ($p = 0.000$) and significantly increased incidences at the 12000 ppm ($p < 0.01$) and at the 500 and 6000 ppm ($p < 0.05$) dose levels. The incidence at 100/50 was not reportedly statistically significant by HED's analysis.

Given the importance of knowing whether the 100/50 ppm dose level is positive, resolution of the apparent differences arising out of differing statistical approaches to analysis is essential.



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Table 6. Malathion - Fischer 344 Rat Study (From Attachment 9: L. Brunsman HED Memo dated 7/16/97).

Male Testes Interstitial Cell Tumor Rates+ and Peto's Prevalence Test Results (p values)

| | Dose (ppm) | | | | |
|---|---|---|---|---|---|
| | 0 | 100/50 | 500 | 6000 | 12000 |
| Tumors (%) | 52/55 (95) | 52/55 (95) | 53/55 (96) | 52/53 (98) | 53[a]/54 (98) |
| p = | 0.000** | - | 0.037* | 0.032* | 0.004** |

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

[a]First testes interstitial cell tumor observed at week 54, dose 0 ppm, in a 54-week interim sacrifice animal. First testes interstitial cell tumor not in an interim sacrifice or accidental death animal observed at week 64, dose 12,000 ppm.

Note: Interim sacrifice and accidental death animals are not included in this analysis. Two animals in the 0 ppm dose group and five animals in the 12,000 ppm dose group of the 54-week interim sacrifice group had testes interstitial cell tumors. Two accidental death animals in the 6,000 ppm dose group had testes interstitial cell tumors.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.



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4) Thyroid Gland C-Cell Carcinoma

In the expanded incidence summary for thyroid C-cell carcinoma for males, the recorded incidences in the MRID study report were 1/69, 2/54, 6/53, 2/54 and 0/69 for the 0, 100/50, 500, 6000 and 12000 ppm groups. According to the MRID statistician's report (**Attachment No. 10**, p. 5347), the increase at 500 ppm is statistically significant by Fisher's Exact Test, and the dose-trend is positive. Excessive mortality at 6000 and 12000 ppm may have compromised expression of the tumor at those doses. (Note: HED's statistical analysis did not include thyroid C-cell carcinoma.)

5) Thyroid Gland Follicular Cell Tumors

As presented in HED's statistical analysis (**Attachment No. 9 and Table 7 of this document**) there was a positive dose trend among male rats for follicular cell adenomas and carcinomas combined. The incidences in question were 2/55(4%), 1/54(2%), 3/51(6%), 6/51(12%) and 4/43(9%) for the 0, 100/50, 500, 6000 and 12000 dose groups. Pairwise comparisons were not statistically significant, p = 0.077 for the 6000 ppm group. The finding at 6000 ppm, 12%, is well above the control 4%. Further increased expressions at 6000 and 12000 ppm may have been preempted by early and extensive mortality, particularly in the highest dose group. In the 1996 NTP historical control data base for oral feeding studies using the F344 rat, the incidence of follicular cell adenomas or carcinomas is 28/1347 (2.08%) for males. Among the 27 studies comprising this data base, the highest incidence was 4/50 (8%), which occurred one time. An incidence as high as 6% occurred in two of the 27 studies.

Table 7. Malathion - Fischer 344 Rat Study (From Attachment 9: L. Brunsman HED Memo dated 7/16/97).

Male Thyroid Tumor Rates[+] and Peto's Prevalence Test Results (p values)

| | Dose (ppm) | | | | |
|---|---|---|---|---|---|
| | 0 | 100/50 | 500 | 6000 | 12000 |
| Adenomas (%) | 2/55 (4) | 1/54 (2) | 1/51 (2) | 4/51 (8) | 4[a]/43 (9) |
| p = | 0.063 | - | - | 0.150 | 0.378 |
| Carcinomas (%) | 0/42 (0) | 0/45 (0) | 2/41 (5) | 2[b]/26 (8) | 0/0 (0) |
| p = | 0.196 | - | 0.085 | 0.162 | - |
| Combined (%) | 2/55 (4) | 1/54 (2) | 3/51 (6) | 6/51 (12) | 4/43 (9) |
| p = | 0.035* | - | 0.321 | 0.077 | 0.160 |

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

[a]First thyroid follicular cell adenoma observed at week 76, dose 12,000 ppm.

[b]First thyroid follicular cell carcinoma observed at week 100, dose 6,000 ppm.

Note: Interim sacrifice and accidental death animals are not included in this analysis. There were no thyroid follicular cell tumors in any interim sacrifice or accidental death animals.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.



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6) Pituitary Gland Pars Distalis Carcinoma

In the expanded incidence summary for pars distalis carcinoma for females the recorded incidences are 0/66(0%), 1/31(3.2%), 3/34(8.8%), 4/34(11.8%) and 1/69(1.8%) for the 0, 100/50, 500, 6000 and 12000 ppm groups, respectively. High mortality in females at 12000 ppm could have compromised expression of this tumor at that dose level. It appears there are dosing related increases at all other dose levels among the limited animals examined. All animals in all dose groups should have been examined for this tumor. HED's statistical treatment **(Attachment 9 and Table 8 of this document)** found the increases at 500 and 6000 ppm to be statistically significant, but without a positive trend presumably due to the low incidence at the high dose. According to the 1996 NTP historical control data base, the incidence of this tumor in the oral feeding studies in female F344 rats is 14/1340 (1.04%). In 27 historical studies presented in the data base, the highest incidence in any one study was 2/49(4%), which occurred three times (the other two actually being 2/50). This approaches being a rare tumor type so the incidences at 500 and 6000 ppm of 8.8% and 11.8%, respectively, are well beyond the highest historical incidences. In addition to the positive pair-wise comparisons for the 500 and 6000 ppm groups, HED's statistical analysis reported a positive pair-wise comparison for the 500 ppm group when adenomas and carcinomas were combined, again though without a positive dose time. The MRID statistician's report does not include pituitary gland pars distalis carcinomas for females.



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Table 8. Malathion - Fischer 344 Rat Study (From Attachment 9: L. Brunsman HED Memo dated 7/16/97).

Female Pituitary Pars Distalis Tumor Rates+ and Peto's Prevalence Test Results (p values)

| | Dose (ppm) | | | | |
|---|---|---|---|---|---|
| | 0 | 100/50 | 500 | 6000 | 12000 |
| Adenomas (%) | 25/51 (49) | 13/31 (42) | 20[a]/34 (59) | 17/33 (52) | 14/53 (26) |
| p = | 0.980 | - | 0.133 | 0.266 | - |
| Carcinomas (%) | 0/50 (0) | 1/30 (3) | 3[b]/32 (9) | 4/32 (12) | 1/49 (2) |
| p = | 0.778 | 0.319 | 0.029* | 0.027* | 0.369 |
| Combined (%) | 25/51 (49) | 14/31 (45) | 23/34 (68) | 21/33 (64) | 15/53 (28) |
| p = | 0.987 | - | 0.033* | 0.097 | - |

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

[a]First pituitary pars distalis adenoma observed at week 56, dose 500 ppm.

[b]First pituitary pars distalis carcinoma observed at week 79, dose 500 ppm.

Note: Interim sacrifice animals are not included in this analysis. There were no pituitary pars distalis tumors in any interim sacrifice animals.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.



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7) Mononuclear Cell Leukemia

Mononuclear cell leukemia and chronic nephropathy were principal and competing causes of early mortality in the study. The influence of these two chronic effects in premature mortality are discussed under the topic of mortality in the DER (**Attachment No. 8**, pp. 20-25). The DER suggests that while leukemia may not have increased in incidence with increasing dose, animals with leukemia tended to die sooner of the condition in a dose related manner. In other words, there was a dose related decreased latency in terms of death due to leukemia among animals harboring the malignancy. HED's statistical treatment found that in the case of females **(Attachment 9 and Table 9 of this document)**, there was an increased incidence of leukemia at 100/50 ppm by pair-wise comparison. Also, the increase at 500 ppm ($p = 0.059$) was close to being significant by the $p = 0.05$ criterion of significance. The dose trend was not positive. In the MRID statistician's report (**Attachment No. 10**), the increases at 100/50 and 500 ppm are reportedly both statistically significant. In the case of male rats there was a positive trend test. Careful reading of this statistician's report, p. 5345) would indicate that in the case of male rats, ".... the numbers of tumors (leukemia) in the higher doses were greater than expected, and were thus judged to have statistically more tumors (leukemia)."



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Table 9. Malathion - Fischer 344 Rat Study (From Attachment 9: L. Brunsman HED Memo dated 7/16/97).

Female Mononuclear Cell Tumor Rates[+] and Peto's Prevalence Test Results (p values)

| | Dose (ppm) | | | | |
|---|---|---|---|---|---|
| | 0 | 100/50 | 500 | 6000 | 12000 |
| Leukemia (%) | 9/55 (16) | 18/55 (33) | 15/55 (27) | 13/54 (24) | 10[a]/55 (18) |
| p = | 0.917 | 0.025* | 0.059 | 0.181 | 0.670 |

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor. Also excludes week 54 interim sacrifice animals.

[a]First mononuclear cell leukemia observed at week 47, dose 12,000 ppm.

Note: Interim sacrifice animals are not included in this analysis. There were no mononuclear cell leukemias in any interim sacrifice animals.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

c. Non-neoplastic Lesions:

Non-neoplastic lesions include those of the nasal mucosa and nasopharynx (several pathologies), males and females, 6000 and 12000 ppm; bilateral subacute-chronic inflammation/chronic nephropathy (high incidence in all study groups including controls), increased severity , males, 6000 and 12000 ppm, females, 500, 6000 and 12000 ppm; stomach (several pathologies), males and females, 6000 and 12000 ppm; increased parathyroid hyperplasia, males and females, all doses; other findings in various tissues (thyroid, lymph nodes, lungs, liver, spleen, adrenal gland, eyes) as summarized in the review, being more remarkable in males, and often extending across the top three doses in males and top two doses in females.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential:

This may be viewed as a philosophical question. The study has been classified as Reserved in the DER (**Attachment No. 8**) pending review by the HED HAZID and Cancer Peer Review Committees. In seeking to comment on this question of the adequacy of dosing, one might begin with the findings of mortality. In male rats, pre-term deaths were 33%, 25%, 47%, 74% and 100% and in females were 31%, 26%, 25%, 38% and 64% at the 0, 100/50, 500, 6000 and 12000 ppm dose levels, respectively. As a guide in this discussion we would cite OSTP (1985): "A negative test is ordinarily accepted by regulatory agencies .... if survival of all groups (per sex per dose) is no less than 50% at .... 104 weeks for rats." (p. 10414) This toxicologist is of the opinion that among females at doses up to and including 6000 ppm, i.e. at doses of 0, 100/50, 500 and 6000 ppm the study was adequate to assess carcinogenic potential, were it not for the fact that for many tissues the testing Guidelines require that all animals be examined histopathologically only in the control and highest dose groups. In the case of females, the 64% mortality exceeds the 50% figure. So to the extent that the mortality exceeded 50% in the highest dose group, and all animal tissues were not examined at 6000 ppm and below, the study is not fully adequate. If one ignores the 50% criterion

of acceptability and treats the 64% mortality as, say, minimally satisfactory, one must then accept positive carcinogenic findings at all doses including HDT as real, assuming there is no other evidence in the study, such as excessive compromises in body weight, to conclude an MTD was exceeded. In the case of females at 12000 ppm, body weight deficits (10-15%) were not excessive, but support that an MTD was reached. On the other hand, tumorigenic effects exhibiting a dose-related response at doses up to and including 6000 ppm, but not at 12000 ppm (e.g. pituitary pars distalis carcinoma findings in this study) cannot be dismissed as real, as female rats in the 12000 ppm group might not have been at full risk timewise. The bottom line is that positive statistically significant tumorigenic findings across all doses are to be accepted as positive, and those positive across all but the 12000 ppm group should also be accepted as real evidence of carcinogenicity, particularly if late occurring, given the fact that sufficient rats may not have been at risk till term at 12000 ppm.

In the case of males, similar reasoning applies if 100% mortality at 12000 ppm and 74% mortality at 6000 ppm are considered acceptable, i.e. all positive tumorigenic findings across all doses are to be considered positive, but findings at lower doses that are not displayed at higher doses should be considered real to the extent that sufficient animals at higher doses may not have been at risk due to premature death. Decrements in body weight gain in males were 3-13% at 6000 ppm and 12-32% at 12000 ppm, which supports that the MTD was not exceeded at 6000 ppm and possibly not at 12000 ppm in these groups even though mortality was high. A good example in this malathion study illustrating how early deaths may compromise expression of a particular tumorigenic response is that of leukemia. As presented on p. 25 of the DER (**Attachment No. 8**), the overall leukemia incidences were: 23/55, 16/55, 24/55, 18/55 and 1/55 for the respective 0, 100/50, 500, 6000 and 12000 ppm groups. In the 12000 ppm group, early deaths were beginning around week 57 (day 400) and all had either died of been sacrificed moribund by week 94 (day 658) in a 104 week (728 day) study. Death from competing toxicity (chronic nephropathy primarily) preempted expression of even the background incidence of leukemia, let alone any

possible further increase due to the test material had the animals survived to term. This phenomenon is perhaps not peculiar to leukemia expression only among potential carcinogenic findings. In like manner, early deaths beginning around week 86 (day 600) in the 6000 ppm group may have compromised the full potential of leukemia expression beyond the 18/55 incidence seen in that group. In the event malathion induced leukemia (or any other tumor) at the higher dose levels, it might not be evident until a normal life expectancy were achieved by saving animals from early death due to competing toxicity. In this study , where mortality was 100% at 12000 ppm and 74% at 6000 ppm, any evidence of a tumorigenic response at the lower doses only must be taken seriously. This very explanation may hold in the case of thyroid C-cell carcinoma response in this study, where incidences in males were 1/55, 2/54, 6/53, 2/54 and 0/55 at the same respective doses, and may be a good reason to accept the statistically significant increase at 500 ppm as real evidence of carcinogenicity in spite of the drop off in incidence at 6000 and 12000 ppm. It may also explain the lack of a dose response for thyroid tumors, or for that matter, any other tumorigenic response in males.

Overall to the extent that the study was negative for tumorigenicity, it cannot be accepted as a negative study in males due to high mortality at 6000 and 12000 ppm, i.e. dosing was inadequate to assess carcinogenic potential for the full two years.

3. Evaluation of Combined Chronic Toxicity/Carcinogenicity Study with Malaoxon in F344 Rats. 1996. (DER) (**Attachment No. 11**). There is no HED statistician's report for this study.

Reference: Daly, W. I.: "A 24-Month Oral Toxicity/Oncogenicity Study of Malaoxon in the Rat via Dietary Administration", April 2, 1996. MRID 43975201, Lab. Study No.: 93-2234, Testing Facility: Huntingdon Life Sciences, East Milestone, NJ.

a. Experimental Design

Malaoxon technical (96.4% a.i.) was administered in the diet to groups of 85 male and female F344 rats

at 0, 20, 1000 and 2000 ppm [equivalent to 0, 1, 57, and 114 mg/kg/day (males) and 0, 1, 68 and 141 mg/kg/day (females)] for 2 years. Ten rats/sex/group were sacrificed at 3 months, 6 months, and 12 months for interim evaluations and cholinesterase activity determinations. There were 55 rats/sex/group devoted to the full 2-year study.

b. Discussion of Tumor Data

The DER shows percent mortality by 24 months, males: 29, 35, 42 and 53; females: 13, 24, 44 and 49 at the 0, 20, 1000 and 2000 ppm dose levels, respectively (see table 2, p. 10 of the DER, **Attachment No. 11**). The DER affirms that mortality was significantly increased at 2000 ppm in males and at 1000 and 2000 ppm in females. However, the review also says the data suggests a possible dose related effect in females and males at 20 ppm (perhaps intending to say at 1000 ppm rather than 20 ppm in males). The dose trend for mortality for both males and females was $p \leq 0.01$. Table 2 of the DER also shows a positive trend for females by 18 months, where mortality was 0, 7, 13 and 22% at the 0, 20, 1000 and 2000 ppm dose levels. The increases at 1000 and 2000 ppm were statistically significant and the DER claims the 7% increase at 20 ppm to be a possible dosing related effect.

The study was classified Acceptable, and satisfies the Registration Standard Requirement for a repeat combined chronic toxicity/carcinogenicity study on malaoxon, a metabolite of malathion.

The DER of this study concluded that a treatment-related increase in tumor incidence was not observed in males or females after 105 weeks of treatment with malaoxon. The HED statistics team was not requested to perform any statistical analysis of tumor data in this study.

c. Non-Neoplastic Lesions:

Nasal lumen inflammation was seen in high dose males and in mid and high dose females. Nasal lumen epithelial hyperplasia was increased in mid and high dose females. Lung interstitium inflammation was increased in mid and high dose females and tympanic cavity inflammation was seen in mid and high dose early female decedents. Increased incidences of mineral deposits in the

stomach muscularis were seen in mid and high dose males.

d Adequacy of Dosing for Assessment of Carcinogenic Potential:

Employing the same logic for the malaoxon study as for the new malathion study (1996), there is a less remarkable effect on mortality in males and females as occurred in the malathion study. Mortality at term for males was 29%, 35%, 42% and 53%, and for females was 13%, 24%, 44% and 49% at the respective doses of 0, 20, 1000 and 2000 ppm. In this study, increased mortality was statistically significant in both sexes at 2000 ppm and in females at 1000 ppm. The survival in all groups essentially satisfies the 50% survival criterion established by OSTP (1985) for acceptability. There was no other evidence that an MTD had been exceeded and the DER (**Attachment No. 11**) concluded the study to be acceptable. However, as in the case of the new malathion study, elevated mortality at the top two doses in both sexes may have precluded full assessment of tumorigenic responses, especially for late occurring tumors, and must be evaluated on a case by case basis.

## E. Additional Toxicology Data

1. Evaluation of a Subchronic (13-week) Inhalation Study with Malathion in Sprague-Dawley Rats, 1994 (DER) (**Attachment No. 12**)

Reference: "A 13-Week Toxicity Study of Aerosolized Malathion Administered by Whole Body Inhalation Exposure to the Albino Rat." Report Date March 16, 1994, MRID No.: 43266601, Lab. Study No.: 90729. Testing Facility: Bio-Research Laboratories Ltd., Senneville, Quebec, Canada.

a. Experimental Design

Groups of 15 male and 15 female Sprague-Dawley rats were exposed by inhalation in whole body exposure chambers to malathion (96.4% a.i.) aerosols (no vehicle) at concentrations of 0, 0.1, 0.45 or 2.01 mg/L, 6 hrs/day, 5 days/week for 13 weeks.

b. Summary of Findings

Clinical signs such as urogenital staining, excess salivation and ungroomed fur were seen mostly at 2.01 mg/L, but occurred

sporadically also at 0.45 and 0.1 mg/L in both sexes. These clinical signs were consistent with decreases in cholinesterase activity. A dose-related increase in cholesterol levels, ranging from 5% to 33%, were seen in both sexes. Microscopic lesions of the nasal cavity and larynx, classified as slight to moderate, were observed in most animals of both sexes at all three exposure concentrations. Based on microscopic lesions of the nose and larynx in both sexes, this study provided a LOEL of 0.1 mg/L; a NOEL could not be identified. According to the DER, based on inhibition of cholinesterase activity in RBC and plasma in female rats (> 10%), this study provided a LOEL of 0.1 mg/L; a NOEL for cholinesterase inhibition could not be established. The DER deferred to the HED RfD Committee the resolution of these no NOEL issues. However, the study has not yet been considered by the RfD committee.

The study was rated core **minimum**.

2. **Metabolism**

Reference: V. Reddy, T. Freeman and M. Cannon, 1989. Disposition and Metabolism of 14C-Labeled Malathion in Rats (Preliminary and Definitive Study). Midwest Research Institute. Study No. MRI 9354-B. December 20, 1989. Unpublished. MRID 41367701.

Executive Summary: In a metabolism study in Sprague-Dawley rats, single doses of radiolabeled 14C-malathion (98% purity; SA = 90.0 uCi/mg) were administered by oral gavage to groups of 5 male and 5 female adult rats at dose levels of 40 mg/kg (low dose), 800 mg/kg (high dose) and 40 mg/kg following 15 days of daily oral gavage of non-radiolabeled malathion (94.6% purity) at a dose level of 40 mg/kg/day. The rats were then placed in metabolism cages and urine and feces were collected for 72 hours. Radioactivity in urine and feces was determined at 4, 8, 12, 24, 48 and 72 hours after dosing. In a preliminary study, it was determined that less than 1% of the radioactivity in similarly treated animals was eliminated in expired air. At 72 hours, the animals were sacrificed and major organs/tissues (including GI tract plus contents and residual carcass) were collected, weighed and analyzed for radioactivity. Whole blood, plasma and erythrocytes were also analyzed for radioactivity. In addition, individual and pooled urine and fecal samples were analyzed for biotransformation products (i.e. malathion and metabolites) at 0-24 hours and 24-48 hours after dosing.

More than 90% of the radioactivity in the 40 mg/kg low dose was excreted within 72 hours with most excretion occurring in

the first 24 hours and considerably less occurring during the remainder of the 72 hour period. Approximately 80-90% of the radioactivity in the administered dose was excreted in the urine with females excreting slightly more than males in the urine. Only minor differences in urine/fecal excretion ratios were observed between animals given 40 mg/kg (low dose), 800 mg/kg (high dose) and 40 mg/kg after 15 previous daily doses of malathion. At 72 hours, the highest concentration of radioactivity was observed in the liver, but less than 0.3% of the administered radioactivity was present in that organ. Radioactivity did not bioaccumulate in any of the organs/tissues analyzed. Although 8 radiolabeled metabolites were observed in urine, greater than 80% of the radioactivity in urine was represented by the diacid (DCA) and monoacid (MCA) metabolites. The remaining radiolabeled metabolites were identified as components of "peak A" and "peak B". It was determined that between 4 and 6% of the administered dose was converted to malaoxon, the active cholinesterase inhibiting metabolite of malathion.

This study is **ACCEPTABLE**.

3. **Mutagenicity**

Under the pre-1991 guidelines, the three acceptable studies [Salmonella typhimurium/Escherichia coli reverse gene mutation assay, in vivo bone marrow cytogenetic assay in rats, and an unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes (UDS)] satisfy the minimum requirements for performing single tests in the three major categories of genetic damage (i.e., gene mutation, structural chromosome aberrations and other genotoxic effects). The acceptable studies were negative. However, there is overwhelming confirmation from the published literature summarized for the Carcinogenicity Peer Review of Malathion held on February 7, 1990 (see Memorandum from K. Dearfield to J. Edwards, 1990) indicating that Malathion is genotoxic, producing structural damage to chromosomes in vitro and in whole animal studies with mice and hamsters. Similar conclusions were reached by Flessel et al., (1993) in the genetic toxicology review prepared for the California Department of Health Services. The positive mutagenicity studies, support the unambiguous evidence of liver tumor induction in male and female mice. Based on the overall results, there is a clear concern for somatic cell mutagenicity. The issue as to whether a mutagenicity concern exists for germinal cells can not be resolved until acceptable germinal cell assays have been submitted. It is, therefore, recommended that germinal cell assays be conducted in both rats and mice. The outcome of these additional studies will direct the course of further investigations.

References:

(a) Traul, K.A. (1987). Evaluation of CL 6601 in the Bacterial/Microsome Mutagenicity Test. Study No. 114 [MRID No. 40939302].

(b) Gudi, R. (1990). Acute Test for Chemical Induction of Chromosome Aberrations in Rat Bone Marrow Cells In Vivo with AC 6601. Study No. 0125-1531 [MRID No. 41451201].

(c) Pant, K.J. (1989). Test for Chemical Induction of Unscheduled DNA synthesis in Rat Primary Hepatocyte Cultures by Autoradiography with AC 6601. Study No. 0125-5100 [MRID No. 41389301].

(d) Dearfield, K.L. (1990). Carcinogenicity Peer Review of Malathion; Memorandum to J. Edwards, dated April 12, 1990.

(e) Flessel, P., Quintana, P.J.E. and Hooper, K. (1993). Genetic Toxicity of Malathion: A Review. Environ. Mol. Mutagen 22:7-17.

Summaries of Acceptable Studies

a) In a Salmonella typhimurium/Escherichia coli reverse gene mutation assay (MRID No. 40939302/Doc. No. 008032), malathion (95.4%) was negative in independent trials up to the highest dose tested (5000 μg/plate) with or without S9 activation.

b) In an in vivo bone marrow cytogenetic assay (MRID No. 41451201; Doc. No. 007965), malathion (94% a.i) was negative following the single oral gavage administration of 500-2000 mg/kg to male and female Sprague-Dawley rats. A dose-related reduction in mitotic indicies (MIs) was seen in the females of all treatment groups at 24 hours. Reduced MIs were also recorded for high-dose males and females at 48 hours.

c) In an in vitro primary rat hepatocytes unscheduled DNA synthesis (UDS) assay (MRID No. 41389301; Doc. No. 007864), malathion (94% a.i) was negative up to cytotoxic levels (≥ 0.12 μL/mL; ≈ 150 μg/mL).

Reviews of the Open Literature: An open literature review of the mutagenicity studies on malathion and malaoxon, the major metabolite formed by oxidation, was prepared for the Carcinogenicity Peer Review of Malathion held on February 7, 1990 (see Memorandum from K. Dearfield to J. Edwards, 1990). The overall assessment from this review indicated that

malathion is generally not mutagenic in bacteria and did not cause UDS or mitotic recombination. Equivocal results were produced in the only mammalian cell gene mutation assay found in the open literature (NTP, 1988). Similar conclusions were reached by Flessel et al., (1993) in the genetic toxicology review prepared for the California Department of Health Services. In contrast to the lack of an effect on the above endpoints, there is compelling evidence from the published studies summarized by EPA (Dearfield, 1990) and by Flessel et al., (1993) that malathion (of varying purities up to 99%) is clastogenic and induces sister chromatid exchanges (SCEs) in cultured mammalian cell lines. Confidence in the negative results from the in vivo rat bone marrow cytogenetic assay (MRID 41451201) is high because the data demonstrated that 94% malathion reached the target organ but failed to cause a positive response. Nevertheless, studies in the open literature contradict this finding. EPA (Dearfield, 1990) and Flessel et al., (1993) concluded that malathion at purity levels up to 95.5% was clastogenic and/or aneugenic in mice and/or hamsters when administered dermally, intraperitoneally or orally using single and/or multiple dosing protocols. Of the six published studies, only the assays performed with >99% test material produced negative results leading Flessel et al (1993) to conclude that impurities in commercial or technical grade Malathion may contribute to the clastogenic response. However, marked increases in liver tumors were observed in the 18-month mouse carcinogenicity study performed with 96.4% Malathion (MRID No. 43407201). Since humans are exposed to the commercial grade of malathion, the relevance of the negative bone marrow results in the absence of a 2-year bioassay with the purified test material can not be determined at this time. It was further noted that the positive bone marrow results in mice are consistent with the unambiguous data from the long-term studies showing that Malathion induced liver tumors in male and female mice (MRID No. 43407201). No assays with germinal cells have been submitted to the Agency and no conclusions can be drawn from the conflicting results reported in published germinal cell assays carried out with malathion.

## Mutagenicity Studies With Malathion Metabolite (Malaoxon)

No mutagenicity studies have been submitted to the Agency on malaoxon, the major metabolite of malathion. However, Malaoxon was included in the cited reviews of the open literature (K. Dearfield, 1990; Flessel et al., (1993). The consensus opinion from these reviews is that malaoxon is not mutagenic in bacteria but is a confirmed positive without S9 activation in the mouse lymphoma assay (increasing the incidence of both large and small colony mutants with a slight



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preference for small colony mutants) (Myhr and Caspary, 1991). malaoxon was not clastogenic in cultured Chinese hamster ovary (CHO) cells; however, the findings from the mouse lymphoma assay suggest that Malaoxon may induce both gene mutations and chromosome aberrations in this cell line. In the absence of S9 activation, malaoxon also caused SCEs in independently performed investigations with CHO cells.

References

Dearfield, K.L. (1990). Carcinogenicity Peer Review of Malathion; Memorandum to J. Edwards, dated April 12, 1990.

Flessel, P., Quintana, P.J.E. and Hooper, K. (1993). Genetic Toxicity of Malathion: A Review. Environ. Mol. Mutagen 22:7-17.

Myhr, B.C. and Caspary, W.J. (1991). Chemical Mutagenesis at the Thymidine Kinase Locus in L5178Y Mouse Lymphoma Cells: Results for 31 Coded Compounds in the National Toxicology Program. Environ. Mol. Mutagen 18:51-83.1

## F. Structure Activity Considerations.

Both Malathion and Malaoxon should be considered structural analogs of each other. Therefore, evidence of carcinogenicity/mutagenicity should be considered mutually relevant.

## G. Weight of the Evidence Considerations

Documents previously cited in this review contain lengthy and complex discussions/renderings of findings in the six carcinogenicity studies that were taken under consideration at the 1990 HED cancer peer review. The particular findings in all of these studies are consolidated in a summary listing in the April 12, 1990 Cancer Peer Review document (**Attachment No. 1**, p. 28) In summarizing here the most contemporaneous weight of the evidence based on the three new carcinogenesis studies, relationships that may exist to findings in the former studies will be cited. The noting of findings from the previous cancer peer review cannot portray adequately the earlier considerations. The former cancer peer review document and its supporting materials must be studied anew.

### 1. Carcinogenicity study of malathion in B6C3F1 mice (1994)

The principal carcinogenicity findings in this study were increased incidences of hepatocellular tumors in mice of both sexes. The increases were of high incidence in mice of both sexes at the highest dose, and there was no NOEL for this end point in males. This study was required in

the Registration Standard to address liver tumor increases in males of marginal statistical significance in the 1978 NCI study. The 1994 study on liver tumor incidences were more pronounced than in the NCI study in males, and in contrast to the NCI study findings, occurred in females. Adenomas as opposed to carcinomas were a more prevalent diagnosis in the recent study, but where mouse liver tumorigenesis is concerned, the distinction may not be definitive. See the January 13, 1997 letter of B. Dementi to D. Barolo for further discussion on this important subject. (**Attachment No. 4**). This same letter also discusses the importance of the mouse liver tumor model in assessing carcinogenicity. This study confirms mouse liver tumorigenicity of malathion, in both sexes

2. Carcinogenicity of Malathion in F344 Rats (1996)

Carcinogenic findings of concern identified in this study include hepatocellular tumors, nasal tumors, testicular interstitial cell tumors, thyroid C-cell carcinoma, thyroid parafollicular cell tumors, pituitary gland carcinoma and mononuclear cell leukemia.

In response to malathion treatment, the DER of this study concluded that liver tumors were increased in female rats at all doses. A NOEL for this effect was not identified for females. In male rats, liver tumor incidence was not increased, but competing toxicity and extensive mortality at the top two dose levels may have precluded expression of this tumor type as well as other tumor types in the study. There was no evidence of hepatocellular tumor response in the 1978 NCI F344 rat study.

Exceedingly rare tumors were identified in the nasoturbinate slides (where further tissue histopathology is called for) which are considered positive, compound related effects, across the entire dose range. A lengthy discussion in the study DER (**Attachment No. 8**, pp. 61-66) undergirds this conclusion.

In the recent study, malathion was associated with increased interstitial cell testicular tumors in male rats at all doses. Incidences of this tumor were high in all groups, controls included, but the statistical treatment of the data would indicate that decreased latency in the high dose groups accounted for high incidences in these groups despite early mortality. By the statistical methods used in the MRID study report, the Trend Test was positive at $p \leq 0.01$ and statistically significant increases were reported for all dose groups.



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HED's statistical analysis (Peto Test) yielded a positive dose trend and statistically significant increases at the top three dose levels. Dosing related increases of this tumor was not a finding in the earlier studies. However, in the new malaoxon (1996) study there are similar statistically significant findings indicating increases of this tumor type. More specifically, "The statistical analysis of male testicular interstitial cell tumors indicated a difference in the time corrected incidence at the 2000 ppm dose level at the 0.03 level by Cox's test and at the 0.01 level by the Gehan-Breslow test. There was no difference in incidence at the 2000 ppm dose level by the Fishers Exact Test. The incidence of testicular interstitial cell tumors at 1000 ppm was statistically significant by the Fishers Exact Test. A positive trend was obtained for this finding." (Malaoxon MRID, pp. 75-76) The reason the time corrected procedures as opposed to the Fishers Exact Test were positive at 2000 ppm is considered due to increased mortality (and decreased latency for this tumor type) at the 2000 ppm dose level, a finding qualitatively similar to that in the case of the recent malathion study. This malaoxon data was not analyzed by HED's statistics team. The NOEL for this tumorigenic response in the malaoxon study is therefore the lowest dose, 20 ppm.

In the 1996 malathion study, among male rats there was for thyroid gland C-cell carcinoma a positive dose trend and a statistically significant increased incidence at the 500 ppm dose level. However, again in this study mortality was high in the 6000 ppm group and extensive in the 12000 ppm group, which may have precluded full expression of the tumor in those groups. The 1978 NCI study of malathion in the F344 rat did not yield a positive response for C-cell tumors. The 1990 Cancer Peer Review document says in the case of the 1978 Osborne-Mendel rat study, "However, the Peer Review Committee noted the apparent increases in male C-cell adenomas/carcinomas....." (p. 6, Cancer Peer Review document, **Attachment No. 1**). The Cancer Peer Review document goes on to explain that adequate data was not available for proper in-house statistical analysis. Also, there is an indication of an increase of this tumor type in the 1980 Food and Drug Labs Sprague-Dawley rat study. As derived from a very qualified statistical analysis: "There was a significant trend, but no pair-wise comparison difference for thyroid parafollicular cell (C-cell) malignant tumors in female rats." (p. 11, Cancer Peer Review document, **Attachment No. 1**)

Thyroid gland C-cell adenomas and carcinomas combined was a positive finding in rats of both sexes in the 1979 NCI malaoxon study in the F344 rat. "In particular, the NTP reevaluation resulted in an increase in incidence of C-cell tumors in females that was significant at the high dose (p = 0.045, pair-wise comparison) and yielded a dose-related trend. For males, the NTP reported a positive finding for C-cell adenomas and carcinomas in the high dose group (p = 0.035, pair-wise comparison) and a positive trend." (p. 15, Cancer Peer Review document, **Attachment No. 1**). In the case of the new malaoxon (1996) F344 rat study, possible C-cell carcinoma increases cannot be entirely dismissed, though it is not a reported finding in the study review. Incidences of C-cell carcinoma as they appear in the summary table (p. 2188) from the MRID report are as follows: males: 1/65, 3/55, 3/55 and 0/63; females: 2/65, 0/55, 5/54 and 1/64 at the 0, 20, 1000 and 2000 ppm dose levels, respectively. As before, high mortality may have compromised expression of this tumor type. There is no HED statistical analysis of this data available.

In the new malathion (1996) study there was also a positive dose trend for thyroid gland follicular cell tumors in male rats. The 1990 Cancer Peer Review document indicates a positive finding for follicular cell neoplasms in Osborne-Mendel rats of both sexes. It was not a reported finding in the new malaoxon (1996) study.

Pituitary gland pars distalis carcinoma incidence was statistically significantly increased in female rats at 500 and 6000 ppm, but not at 12000 ppm. The dose trend was not positive, presumably because of the low incidence at 12000 ppm. High mortality at 12000 ppm may have precluded expression at that dose level. This is a borderline rare tumor type (1.04%) in the 1996 NTP historical data base for the F344 female rat, and the incidences of 8.8% and 11.8% at the 500 and 6000 ppm dose levels, respectively, exceed both the mean and highest incidences (4%) of this tumor in the NTP data base.

By HED's statistical treatment, mononuclear cell leukemia was increased by pair-wise comparison in female rats at the 100/50 ppm dose level, and increases at 500 ppm (p = 0.059) approached statistical significance on a p = 0.05 criterion basis. The dose trend was not positive. In the new malaoxon (1996) study, the MRID says, "The analysis of lymphoreticular system mononuclear cell leukemia in males indicated an increase in the dose related trend at the 0.03 level by Cox's test and the

Gehan-Breslow test. However, these differences were not statistically significant by a pair-wise comparison and were not dosing related in females. It should be noted, however, that mortality in females was excessive at 1000 and 2000 ppm dose levels, which may have compromised expression of leukemia in females. Also, mortality in males at 2000 ppm was high which may have compromised the effect somewhat in males as well. The leukemia incidence was not evaluated by HED's statistics team.

Among the earlier studies, in the malathion F344 rat study, an increased incidence in leukemia in the low dose group was noted. In that study, mortality was excessive in males at the highest dose which likely compromised expression of this malignancy at that dose level. In the 1979 malaoxon study in F344 rats, there was an increased incidence of lymphoma (hematopoietic system) among high dose males. A qualified calculation by HED revealed a positive Trend Test and positive Exact Test for Trend. The pairwise comparison yielded p = 0.059. It is noteworthy that in some cases, lymphoma and leukemia are combined by the NTP. (p. 17, Cancer Peer Review document, **Attachment No. 1**)

3. Mutagenicity Considerations

The available studies indicate that malathion is genotoxic, producing structural damage to chromosomes *in vitro* and in whole animal studies with mice and hamsters but not with rats. Hence, the positive mutagenicity studies support the evidence of a carcinogenic effect in mice. Based on the overall results, there is a clear concern for somatic cell mutagenicity. This assessment, which is primarily based on the information found in the published literature, provides strength to the weight-of-the-evidence evaluation for the carcinogenicity of malathion.

The limited information on malaoxon indicate that this metabolite of malathion is also not mutagenic in bacteria but causes gene mutation and possibly chromosome aberrations *in vitro* as well as SCEs in cultured mammalian cells.

G. Additional Issues of Concern

1. In the new malathion (1994) study in the B6C3F1 mouse (**Attachment No. 2**) a liver tumorigenic response was identified. Arguments are set forth in the 1/13/97 letter of B. Dementi to D. Barolo (**Attachment No. 4**) to justify an



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independent Pathology Work Group reassessment of the liver pathology in this study. This recommendation is supported by letters from Drs. Joseph Haseman (7/17/97) and Robert Maronpot (7/24/97) (**Attachment No. 6**).

2. A review of metabolic capabilities of the olfactory epithelium of the nasal passages is presented in the DER of the new malathion study (**Attachment No. 8**, pp. 50-52). This metabolic capability may well explain the dosing related histopathology findings in the olfactory epithelium in the recent studies. This metabolic capability of the olfactory epithelium is similar to that of liver tissue and could serve as a common explanation for the nasal and liver pathology findings in the new malathion F344 rat study. Hepatocellular tumorigenesis was also a finding in the malathion B6C3F1 mouse study, but unlike the rat study, nasal tissues were not examined in the mouse study. It is therefore recommended that the registrant be requested to assess nasal tissue histopathiology in the recent malathion mouse study, MRID 42407201. It is noteworthy that "sneezing" was a clinical sign in the 1978 NCI malathion study in the B6C3F1 mouse.

3. In the malathion subchronic (13-week) inhalation study in the Sprague-Dawley rat (MRID 43266601), the HED review (**Attachment No. 12**) identified no NOEL for microscopic lesions of the nose and larynx. At the exposure concentrations of 0, 0.1, 0.45 and 2.01 mg/L "A high incidence of degeneration and/or hyperplasia of the olfactory epithelium of the nasal cavity and hyperplasia of the larynx was seen in all groups exposed to malathion." (DER, p. 14) Committee members should read the histopathologic characterization of these findings in the DER (**Attachment No. 12**, p. 14) These lesions are very much the same as those in the new malathion F344 rat study. The Committee is requested to address the question of the need for further inhalation testing to identify a LOEL/NOEL, and time of onset of these nasal effects. The Committee is also requested to consider the need for a chronic/oncogenicity study by the inhalation route.

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## ATTACHMENTS

Attachment No. 1: April 12, 1990 Cancer Peer Review Committee Report with accompanying documents used in the February 7, 1990 Peer Review meeting. (p. 44)

Attachment No. 2: February 10, 1995 Data Evaluation Report (DER) of the Carcinogenicity Study with Malathion in B6C3F1 Mice, 1994 (MRID 43407201). (p. 378)

Attachment No. 3: May 8, 1997 HED Statistical Evaluation of the Carcinogenicity Study with Malathion in B6C3F1 Mice, 1994. (p. 446)

Attachment No. 4: January 13, 1997 Letter of Brian Dementi to Daniel Barolo. (p. 454)

Attachment No. 5: Individual Animal Data Sheets (3) from the Carcinogenicity Study with Malathion in B6C3F1 Mice, 1994 (MRID 43407201). (p. 466)

Attachment No. 6: Letters of Joseph Haseman (July 17, 1997) and Robert Maronpot (July 24, 1997) to Brian Dementi. (p. 470)

Attachment No. 7: IRDC Historical Control Data from the Carcinogenicity Study with Malathion in B6C3F1 Mice (Males) Including Hepatocellular Tumor Incidences (MRID 43407201). (p. 476)

Attachment No. 8: August 14, 1997 Data Evaluation Report (DER) of the Combined Chronic Toxicity/Oncogenicity Study with Malathion in F344 Rats, 1996 (MRID 43942901). (p. 478)

Attachment No. 9: July 16, 1997 HED Statistical Evaluation of the Combined Chronic Toxicity/Oncogenicity Study with Malathion in F344 Rats, 1996. (p. 640)

Attachment No. 10: Statistical Analysis of Time to Tumor Data from the Combined Chronic Toxicity/Oncogenicity Study with Malathion in F344 Rats, 1996 (MRID 43942901). (p. 650)

Attachment No. 11: July 2, 1997 Data Evaluation Record (DER) of the Combined ChronicToxicity/Oncogenicity Study with Malaoxon in F344 Rats, 1996 (MRID 43975201). (p. 656)

Attachment No. 12: April 27, 1995 Data Evaluation Record (DER) of the Subchronic (13-Week) Inhalation Study With Malathion in Sprague-Dawley Rats, 1994 (MRID 43266601). (p. 688)

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Attachment 1

FILE COPY

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

APR 12 1990

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Peer Review of Malathion

FROM: Kerry L. Dearfield, Ph.D. Kerry L Dearfield
Executive Secretary, Peer Review Committee
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

TO: Joanne Edwards
Review Manager
Special Review and Registration Division (H7508C)

The Health Effects Division Peer Review Committee met on February 7, 1990 to discuss and evaluate the weight-of-the-evidence on Malathion with particular reference to its carcinogenic potential. The Committee agreed to classify malathion as a Group D Carcinogen; that is, malathion is not classifiable as to human carcinogenicity. This decision was based on the inadequacy of the available studies to make a definitive determination of the carcinogenicity of malathion. The Committee reaffirmed the requirements of the Malathion Registration Standard that requires the Registrant to perform an additional mouse carcinogenicity study with malathion and an additional rat carcinogenicity study with malaoxon. The Committee also determined that the Registration Standard recommendation to perform a carcinogenicity study in combination with the required rat chronic study on malathion be made into a requirement that both be performed.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

| | |
|---|---|
| Penelope A. Fenner-Crisp | Penelope A. Fenner-Crisp |
| William L. Burnam | Wm L Burnam |
| Karl Baetcke | Karl Baetcke |
| Marcia Van Gemert | Marcia Van Gemert |

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John Quest

Esther Rinde

Kerry Dearfield

Richard Levy

Marion Copley

George Ghali

Richard Hill

Robert Beliles

Julie Du

Yin-Tak Woo

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Brian Dementi

Roger Gardner

3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Reto Engler

4. Other Attendees: Bruce Jaeger, HED
Bernice Fisher, HED
Hugh Pettigrew, HED
Linda Kutney, HED

B. Material Reviewed:

The material available for review consisted of 1) a draft Toxicology Branch I response to the Registrant's technical response to the Malathion Registration Standard; 2) selected pages from the Malathion Registration Standard (issued February, 1988); 3) reviews of carcinogenicity studies on malathion and malaoxon (consisting of memoranda and DER's); 4) journal publication Huff et al. (Environ. Res. 37: 154-173, 1985) on the National Toxicology

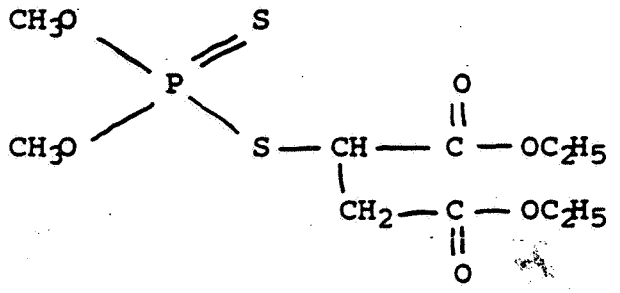
Program (NTP) reevaluation of malathion and malaoxon National Cancer Institute (NCI) rat carcinogenicity studies; 5) memorandum of E. McConnell, D.V.M. to J. Moore, D.V.M., June 14, 1984 with attached Summary Minutes of the NTP's Board of Scientific Counselors review of malathion; this is a status report on the NTP review of NCI malathion carcinogenicity studies; and 6) memorandum from A. Gross, Ph.D. (April 24, 1984) concerning the carcinogenicity of malathion.

This package was prepared by Brian Dementi, Ph.D., of Toxicology Branch I, Health Effects Division. The discussion on each of the individual carcinogenicity studies follows the presentations by Dr. Dementi. The material reviewed is attached to the file copy of this report.

C. Background Information: A chemical name for malathion is S-[1,2-bis(ethoxycarbonyl)ethyl]-O,O-dimethyl phosphorodithioate. In one of the submitted carcinogenicity studies, it is also named Cythion. Malathion is an organophosphate insecticide and miticide. It is used on a wide variety of food and non-food crops as well as for insect control for both outdoor and indoor situations. Its mode of activity is through cholinesterase inhibition. There are several basic producers of malathion in the United States.

The Chemical Abstracts Service (CAS) Registry number for malathion is 121-75-5 and the Tox Chem Number (or Caswell number) is 535. The CAS Registry number for malaoxon (this oxygen analogue of malathion is considered the active metabolite of malathion; the double bonded S in malathion is replaced by a double bonded O in malaoxon) is 1634-78-2.

Structure of Malathion:



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D. Evaluation of Carcinogenicity Evidence for Malathion:

There were four carcinogenicity studies reviewed using malathion as the test chemical (total of three rat studies using Osborne-Mendel, Fischer 344 and Sprague-Dawley rats and one in B6C3F1 mice). There were two carcinogenicity studies reviewed using malaoxon as the test chemical (one in Fischer 344 rats and one in B6C3F1 mice).

1. Malathion - Osborne-Mendel Rat Dietary Feeding Carcinogenicity Study

Reference: National Cancer Institute. 1978. Bioassay of malathion for possible carcinogenicity, CAS No. 121-75-5. Technical Report Series, No. 24, National Cancer Institute, Bethesda, MD. NCI-CG-TR-24. Assay performed at Gulf South Research Institute, New Iberia, LA. Report prepared at Tracor Jitco, Rockville, MD under NCI direction. Those responsible for the report at Tracor Jitco were M. Steinberg et al. Reviewed in Document # 000314.

Malathion (technical grade; purity ≥95%) was administered in the diet to groups of 50 male and 50 female Osborne-Mendel rats (from Battelle Memorial Institute, OH) at time weighted average dosage levels of 4700 or 8150 ppm per group for 80 weeks. Animals were then observed for an additional 29 to 33 weeks. Low dose animals received 8000 ppm in the diet for an initial 14 weeks, which was then adjusted to 4000 ppm for the remaining 66 weeks. High dose animals received 12,000 ppm for an initial 3 weeks, which was then adjusted to 8000 ppm for the remaining 77 weeks. Matched controls consisted of groups of 15 untreated rats of each sex (however, it is noted that matched controls are reported as 2 groups of 10 animals/sex for low dose matched controls and 5 animals/sex for high dose matched controls; the reason for this was that there was an abortive start to the high dose group and when it was reinitiated, the 5 high dose matched controls per sex were added to the original 10 matched controls per sex). Pooled controls consisted of the matched controls combined with 40 untreated male and 40 untreated female rats from similar bioassays of four other test chemicals. These other pooled controls came from bioassays performed at the same laboratory and overlapped the malathion bioassay by at least 1 year. All surviving rats were killed at 108 to 113 weeks.

The Peer Review Committee decided that the analyses that should be of primary importance would be the NTP reevaluation of the original NCI studies (this applies to the three NCI rat studies, two with malathion and one with malaoxon) (these analyses are found in the Huff et al., 1985 article and the E. McConnell memorandum to J. Moore). The Committee felt that the NTP reevaluation provided a more extensive evaluation than the original analysis. Also, there was a consensus of opinion concerning the examined tumors by a panel of expert pathologists.

a. Discussion of Tumor Data

The original NCI report indicated a statistically significant dose-related trend for follicular cell adenomas and carcinomas of the thyroid in female rats. However, there was no significance from a pair-wise comparison. The NCI concluded "there was no clear evidence of the association of the tumor incidence with the administration of malathion."

The NTP reevaluated tissues from these organs (thyroid gland as well as adrenal gland) as potential suggestive targets. They reaffirmed the original NCI conclusion by stating "under the conditions of these studies, there was no evidence of carcinogenicity in male or female Osborne-Mendel rats that received time-weighted average doses of 4700 or 8150 ppm malathion in their diet for 80 weeks." NTP examinations of the major sites of potential targets are shown in Tables 1 and 2. In particular, the NTP reevaluation diagnosed additional follicular cell adenomas in the control and low dose groups that eliminated the positive trend the NCI reported. It was noted by the Peer Review Committee that the NTP did not report hyperplasia incidence although the NTP Summary minutes attached to the McConnell memo states there was no hyperplasia in their C-cell arguments.

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Table 1: Malathion Osborne-Mendel Rat Study - Thyroid Findings in NTP Reevaluation

| | Sex | C1 | C2 | Low Dose | High Dose |
|---|---|---|---|---|---|
| No. Tissues Examined | M | 14 | 41 | 35 | 40 |
| | F | 14 | 41 | 44 | 42 |
| C-cell adenoma and carcinoma | M | 1 (7.1%) | 3 (7.3%) | 1 (2.9%) | 7 (17.5%) |
| | F | 2 (14.3%) | 10 (24.4%) | 2 (4.5%) | 5 (11.9%) |
| Follicular Cell Adenoma & Carcinoma | M | 2 (14.3%) | 8 (19.5%) | 9 (25.7%) | 12 (30.0%) |
| | F | 0 (0%) | 1 (2.4%) | 1 (2.3%) | 4 (9.5%) |

C1 = Matched Controls
C2 = Pooled Controls
(Incidence %)



52
48

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Table 2: Malathion Osborne-Mendel Rat Study - Pheochromocytoma Findings in NTP Reevaluation

| Males | C1 | C2 | Low Dose | High Dose |
|---|---|---|---|---|
| Adrenal Gland Pheochromocytoma | 0/14 (0%) | 2/50 (4%) | 0/46 (0%) | 5/44 (11%) |

C1 = Matched Controls
C2 = Pooled Controls
Number of Lesions/Number of tissues examined (Incidence %)

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The Registration Standard for malathion states "the Agency agrees with the NCI/NTP that malathion is not carcinogenic in Osborne-Mendel rats." Subsequent considerations by Toxicology Branch I/Health Effects Division and this Peer Review Committee raise doubt as to how definitive this study is to make a clear conclusion about the carcinogenicity of malathion. The NTP had concluded that under the conditions of this study, there was no evidence of carcinogenicity in male or female Osborne-Mendel rats. However, the Peer Review Committee noted the apparent increases in male C-cell adenomas/carcinomas, of male and female follicular cell adenomas/carcinomas and of male adrenal pheochromocytomas. A detailed independent statistical treatment of the data by statisticians supporting the Peer Review Committee could not be performed as there are not enough data presented (e.g. individual animal data, information about the "pooled" controls) in the NTP reevaluation. This makes it difficult for the Peer Review Committee to make an independent decision based on their own analyses of the data and come to a clear decision regarding the carcinogenicity of malathion in this study.

Although detailed statistical treatment of the NTP data was not able to be performed, it was pointed out that Toxicology Branch I calculated a trend for follicular cell adenomas/carcinomas for females ($p = 0.047$) when compared to matched controls (calculation by B. Dementi and H. Pettigrew (statistician in HED) based on the NTP reevaluated summary numbers). Also, the increasing trend is primarily due to carcinoma incidence (0/14, 0/41, 0/44 and 3/42 for matched controls, pooled controls, low and high dose groups, respectively). The "exact test for trend" was used for cases where relatively few tumors are found and in this instance, provided an increased trend for follicular cell carcinomas compared to pooled controls ($p = 0.034$; B. Dementi and H. Pettigrew calculation; however, $p = 0.071$ compared to matched controls). Carcinoma incidences are viewed with more concern than adenoma incidences.

b. Considerations of Study Adequacy for Assessment of Carcinogenic Potential

The Peer Review Committee questions the adequacy of this study to make definitive conclusions regarding the carcinogenicity of malathion and notes the following: 1) This study was performed before current methods for performing and evaluating an adequate study were in effect. This would introduce uncertainty as to the quality of the study with respect to contemporary guidelines. For example, a significant deviation from the current OPP Subdivision F Pesticide Assessment Guidelines (Series 83-2) is the length of dosing in this study where rats received malathion for 80 weeks instead of a 2 year period. 2) The number of concurrent control animals (15) is very small; this makes comparative analyses difficult when there is not a dramatic difference between tumor incidences. Analysis is also complicated by the use of a concurrent "matched" control group which was divided into two groups of 10 and 5 rats each; these two subgroups exhibited different weight gains. Furthermore, it cannot be concluded with certainty that if a larger concurrent control size had been used, the incidence of spontaneous tumors would rise proportionately. 3) The appropriateness of using "pooled" control animals as "concurrent" controls is unclear. It was in this regard that the historical control incidence from this laboratory was not available to the Peer Review Committee during these deliberations. 4) The NTP states that malathion had no significant effect on survival of male and female Osborne-Mendel rats. The Peer Review Committee noted there were suggestions of a dose-related decrease in survival for both male and female animals near the end of the study. While there were suggested decreases in survival at the end of the study, there were no pair-wise survival disparities between control and dose groups; thus an adjusted tumor analysis is not necessary.

2. Malathion - Fischer 344 Rat Dietary Feeding Carcinogenicity Study

Reference: National Cancer Institute. 1979. Bioassay of malathion for possible carcinogenicity, CAS No. 121-75-5. Technical Report Series, No. 192, National Cancer Institute, Bethesda, MD. NCI-CG-TR-192. Assay performed at Gulf South Research Institute, New Iberia, LA. Report prepared at Tracor Jitco, Rockville, MD under NCI direction. Those responsible for the report at Tracor Jitco were C.R. Angel et al. Reviewed in Document # 000314.

Malathion (manufacturer's assay; purity 95%) was administered in the diet to groups of 49 to 50 Fischer 344 rats of each sex (from NCI Frederick Cancer Research Center, MD) at doses of 2000 or 4000 ppm per group for 103 weeks. Animals were then observed for an additional 2 or 3 weeks. Matched controls consisted of 50 untreated rats per sex. All surviving rats were killed at 105 to 106 weeks.

a. Discussion of Tumor Data

The original NCI report stated "malathion was not carcinogenic in male or female rats, but the females may not have received a maximum tolerated dose." The NCI acknowledged that the increase in adrenal gland pheochromocytomas in low dose males was statistically significant by pair-wise comparison, but did not consider this to be associated with the administration of malathion. This conclusion was based on the lack of an effect at the high dose and the lack of a dose response effect.

The NTP reevaluated tissues from the male adrenal gland. They reaffirmed the original NCI conclusion by stating "under the conditions of these studies, there was no evidence of carcinogenicity in male or female Fischer 344 rats that were provided diets containing 2000 or 4000 ppm malathion for 103 weeks." However, the NTP suggested that two neoplasms appeared to be increased in the low dose males: pheochromocytoma of the adrenal gland (Table 3) and leukemia (Table 4; note the NCI did not comment on this lesion). However, these marginal increases were only significant by life-table analyses. The NTP suggested that life-table analyses are appropriate if the lesion is the cause of death. The NTP judges that the early deaths seen in this study (discussed below) are due to chemical toxicity. However, the increases in these two tumor types were not significant by incidental tumor tests or pair-wise tests. The NTP suggested that the reduced survival in the dosed groups made the overall interpretation of the data difficult.

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Table 3: Malathion Fischer 344 Rat Study - Pheochromocytoma Findings in NTP Reevaluation

| Males | Control | Low Dose | High Dose |
|---|---|---|---|
| Adrenal Gland Pheochromocytoma | 5/49 (10%) | 10/48 (21%) | 6/46 (13%) |

Number of Lesions/Number of tissues examined (Incidence %)

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Table 4: Malathion Fischer 344 Rat Study - Leukemia Findings in NTP Reevaluation

| Males | Control | Low Dose | High Dose |
|---|---|---|---|
| Hematopoietic System - Leukemia | 13/50 (26%) | 20/50 (40%) | 8/49 (16%) |

Number of Lesions/Number of tissues examined (Incidence %)

The Registration Standard for malathion states "the Agency agrees with the conclusions of the NCI/NTP, but notes that the dose levels employed in this study were approximately one-half of those employed in the NCI Osborne-Mendel rat study, and that therefore it is unlikely the maximum tolerated dose was reached in females." Subsequent considerations by Toxicology Branch I/Health Effects Division and this Peer Review Committee raise doubt as to how definitive this study is to make a clear conclusion about the carcinogenicity of malathion. The NTP had concluded that under the conditions of this study, there was no evidence of carcinogenicity in male or female Fischer 344 rats. However, the Peer Review Committee noted the apparent increases in male adrenal pheochromocytomas and male leukemia at the low dose. An independent statistical treatment of the data by statisticians supporting the Peer Review Committee could not be performed as there are not enough data presented (e.g. individual animal data) in the NTP reevaluation. The large decrease in survival of exposed males confounds the interpretation of potential tumor induction. This makes it difficult for the Peer Review Committee to make an independent decision based on their own analyses of the data and come to a clear decision regarding the carcinogenicity of malathion in this study.

b. <u>Consideration of Study Adequacy for Assessment of Carcinogenic Potential</u>

Other considerations about the adequacy of this study were made by the Committee: 1) While this study employed lower doses than the Osborne-Mendel rat study, there was a major problem with mortality in the Fischer 344 rats, especially in the males. Survival at 103 weeks for males was 54%, 28% and 0% for control, low- and high-dose groups, respectively. For females, the comparable figures were 64%, 52% and 50%. In males, it was noted there was not a great disparity in survival figures among groups at 90 weeks, so increased mortality rates appeared after this time. The large drop in survival in males confounds the observations found at the high dose where apparently less animals were at risk for tumor induction. It is not clear how this may have impacted upon a possible dose-response association. Despite the small decrease in survival for females, it was suggested that the top dose may not be high enough for a definitive assessment of carcinogenic potential. 2) The concurrent control incidence for pheochromocytomas in males tabulated by the NTP was discussed. In the NTP reevaluation, this incidence is reported to be 10% (5/49 animals). The original NCI review reported the incidence to be 4% (2/49 animals; three less than the NTP reevaluation). This latter value appears more in line with the historical control incidence from the testing laboratory of 3% (8/275 animals) among males. It is not known what the range of control values from separate studies in the historical database is from the testing laboratory. Therefore, the issue of the NTP concurrent control incidence and

what it means to the statistical evaluation of the tumors observed in this study is not resolved. 3) Like the Osborne-Mendel rat study, this study was performed before the current OPP Subdivision F Pesticide Assessment Guidelines (Series 83-2) were in place. This would introduce uncertainty as to the quality of the study with respect to contemporary guidelines. However, unlike the Osborne-Mendel rat study, this study employed a more appropriate number of concurrent control animals and dosing was performed over a 2 year period.

c. Non-Neoplastic Findings

Several significant non-neoplastic findings were noted. Stomach inflammation and ulceration were clearly increased in a dose-related fashion among males. Also, there were increased incidences of fatty metamorphosis and focal cellular changes of the liver for females and chronic inflammatory change of the kidney in females. These non-neoplastic findings help provide support for the Agency's decision to require a full 2 year chronic toxicity study in the Fischer 344 rat as detailed in the Registration Standard.

3. Malathion - Sprague-Dawley Rat Dietary Feeding Carcinogenicity Study

Reference: Rucci, G., P.J. Becci and R.A. Parent. May 13, 1980. The evaluation of the chronic toxicity effects of Cythion administered in the diet to Sprague-Dawley rats for 24 consecutive months. Unpublished study (No. 5436) prepared by Food and Drug Research Laboratories, Inc., Waverly, NY for Agricultural Division, American Cyanamid Co., Princeton, NJ. Reviewed in Document #s: 002504, 004208).

Malathion (technical Cythion; purity 92.1%) was administered in the diet to groups of 50 male and 50 female Sprague-Dawley rats (from Blue Spruce Farms, NY) at doses of 100, 1000 or 5000 ppm per group for 24 months. Matched controls consisted of 50 untreated rats per sex. Surviving animals were killed at the end of the 24 month period (104 weeks).

The malathion Registration Standard states "this study was determined by the Agency to be unacceptable for use as either a chronic rat toxicity study or as a rat oncogenicity (sic) study. An independent reevaluation of the microscopic slides from this study is required in order to determine the acceptability of this study." However, it is not clear that an independent review will resolve all the problems associated with this study (detailed below) and elevate it to an acceptable study.

a. Consideration of Study Adequacy for Assessment of Carcinogenic Potential

This study was determined by the Peer Review Committee to be insufficient to provide definitive evidence on the carcinogenicity of malathion. A final review of this study in a Data Evaluation Report prepared by R.B. Jaeger (July 17, 1987) concluded this study should be classified as invalid. Many reasons were provided for the invalid classification, including: the summary tables do not distinguish between animals killed at term and animals found dead, killed moribund or accidental deaths; pathology slides were not read "blind"; rather each pathologist had prior knowledge of the dose level administered; there is no indication of the numerical rating for the degree or severity of change observed by each pathologist; there were several pathologists involved which raises substantial concern for "consistency" and "uniformity", especially in light of several apparent discrepancies noted in the findings; animals which died, killed moribund or accidental deaths were not examined in a manner to preclude autolysis of tissue; animals in all groups suffered from substantial degrees of several illnesses, raising a question about good animal husbandry for this study; the substantial amount of geriatric changes in all groups makes it extremely difficult to separate or identify normal ageing processes from compound-related effects; kidney, pituitary, adrenal and thyroid weights were selectively screened and eliminated from the organ weight and organ-to-body weight comparisons if they were above or below pre-selected values; sufficient information for examining biochemical and clinical effects in a chronic bioassay were not obtained; and, use of chloroform to euthanize animals at termination of the study is not presently a common practice as it is a suspect carcinogen and may induce potential adverse effects on its own. These points serve to illustrate the substantial faults in the study design, conduct and reporting of this study.

b. Discussion of Tumor Data

Even though this study was found to be invalid, a statistical analysis was performed and some effects were noted (memorandum C.J. Nelson, July 21, 1987; it is realized that this is an analysis on unverified summary data and has not undergone secondary review). Uterus polyps in females had no significant trend, but both high and low dose groups were significantly different from controls by pair-wise comparison. There was a significant trend, but no pair-wise comparison difference for thyroid parafollicular cell (C-cell) malignant tumors in female rats. It was concluded by the Peer Review Committee however that there should not be much weight put upon these findings, although it was noted that C-cell tumors had been observed in the Osborne-Mendel rat study.

c. Non-Neoplastic Findings

Many chronic effects were noted in this study, some even at the low dose of 100 ppm. Statistical analysis (memorandum C.J. Nelson, July 21, 1987) revealed several significant effects which included: swollen liver and kidney glomerulosclerosis, prostate calcification, liver sinusoidal dilation, lymph node reticuloendothelial hyperplasia, pituitary cyst, and heart inflammation in male rats; kidney tubular casts, spleen extramedullary hematopoiesis, thymus cyst, uterus pyometra, kidney tubular dilation, and pancreas duct dilation in female rats. Although this study is unacceptable for a chronic toxicity study, these effects suggest concern for chronic non-neoplastic adverse effects induced by malathion.

4. Malathion - B6C3F1 Mouse Dietary Feeding Carcinogenicity Study

Reference: National Cancer Institute. 1978. Bioassay of malathion for possible carcinogenicity, CAS No. 121-75-5. Technical Report Series, No. 24, National Cancer Institute, Bethesda, MD. NCI-CG-TR-24. Assay performed at Gulf South Research Institute, New Iberia, LA. Report prepared at Tracor Jitco, Rockville, MD under NCI direction. Those responsible for the report at Tracor Jitco were M. Steinberg et al. Reviewed in Document # 000314.

Malathion (technical grade; purity ≥95%) was administered in the diet to groups of 50 male and 50 female B6C3F1 mice (from Charles River Breeding Laboratories, MA) at doses of 8000 or 16,000 ppm per group for 80 weeks. Animals were then observed for an additional 14 or 15 weeks. Matched controls consisted of groups of 10 untreated mice of each sex. Pooled controls consisted of the matched controls combined with 40 untreated male and 40 untreated female mice from similar bioassays of four other test chemicals. These other pooled controls came from bioassays performed at the same laboratory and overlapped the malathion bioassay by at least 1 year. All surviving mice were killed at 94 to 95 weeks.

a. Discussion of Tumor Data

The NCI concluded that under the conditions of this study, there was "no clear evidence" of an association between malathion administration and tumor incidence. The NCI report noted a possible increased incidence of hepatocellular carcinoma in male mice (see Table 5). Statistical treatment showed a dose-related trend ($p = 0.019$) when neoplastic nodules and hepatocellular carcinoma were combined and compared to pooled controls. The direct comparison between the high-dose group and the pooled control group for combined nodules and carcinoma revealed a significant difference ($p = 0.031$ Fisher's Exact Test); however, the NCI employed as its criterion of significance $p = 0.025$, based

on Bonferroni adjustments, and therefore did not consider this a positive finding. The NTP did not reexamine this study.

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Table 5: Malathion B6C3F1 Mouse Study - Liver Findings from NCI Evaluation

| Males | C1 | C2 | Low Dose | High Dose |
|---|---|---|---|---|
| Hepatocellular Carcinoma | 2/10 (20%) | 5/49 (10.2%) | 7/48 (14.6%) | 11/49 (22.5%) |
| Neoplastic Nodules & Hepatocellular Carcinoma | ---- | 8/49 (16.3%) | 7/48 (14.6%) | 17/49 (34.7%) |

C1 = Matched Controls
C2 = Pooled Controls
Number of Lesions/Number of tissues examined (Incidence %)

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The Registration Standard states "because of study design flaws and the questionable liver findings (i.e. dose-related trend (p = 0.019) and increased incidence of hepatocellular carcinomas at the high dose (p = 0.031)), another study in mice is required." Further considerations by Toxicology Branch I/Health Effects Division and this Peer Review Committee also raise doubt as to how definitive this study is to make a clear conclusion about the carcinogenicity of malathion. The NCI did not consider the pair-wise comparison (p = 0.031) significant based on Bonferroni adjustments. It was further noted that the current NTP practice for evaluating common tumors from data representative of the NTP/NCI historic database that Haseman's rule of thumb (Haseman et al., Fund Appl Toxicol 7: 573-584, 1986) would apply where p values of 0.01 or less denotes significance. However, from the NCI evaluation, there are not enough data presented (e.g. individual animal data, information about the "pooled" controls) to allow an independent statistical treatment of the data by statisticians supporting the Peer Review Committee. This makes it difficult for the Peer Review Committee to make an independent decision based on their own analyses of the data. Therefore, it is not entirely clear that the suggestive evidence of male hepatocellular carcinoma and combined neoplastic nodules/hepatocellular carcinoma can be totally dismissed. Furthermore, it was noted that a large proportion of the increased tumor numbers was due to an increase in carcinoma. Although detailed statistical treatment of the NCI data was not able to be performed, it was pointed out that Toxicology Branch I calculated a trend for hepatocellular carcinomas (p = 0.046; calculation by B. Dementi and H. Pettigrew (statistician in HED) based on the NCI summary numbers). Carcinoma



60
56

incidences are viewed with more concern than adenoma incidences.

b. Considerations of Study Adequacy for Assessment of Carcinogenic Potential

The Peer Review Committee made the following observations about the adequacy of this study to make definitive conclusions regarding the carcinogenicity of malathion: 1) The number of concurrent control animals (10) is a very small group; this makes comparative analyses difficult when there is not a dramatic difference between tumor incidences. It cannot be concluded with certainty that if a larger concurrent control size had been used, the incidence of spontaneous tumors would rise proportionately. 2) The appropriateness of using "pooled" control animals as "concurrent" controls is unclear. 3) It was noted that the historical control incidence data for hepatocellular carcinoma in this strain of mouse often is higher than that observed in the high-dose group seen in this study. 4) The Registrant points out that the dose levels employed in this study (8000 and 16,000 ppm) exceed the OPP accepted upper limit dose of 1.0 g/kg/day in mouse carcinogenicity studies. 5) The NCI report lists a number of signs of disease appearing "with increasing frequency in dosed animals", especially at weeks 72 and beyond to the end of the study. This raises questions about the general health of animals during a crucial period of the study. 6) This study was performed before the current OPP Subdivision F Pesticide Assessment Guidelines (Series 83-2) were in place. This would introduce uncertainty as to the quality of the study with respect to contemporary guidelines.

Since there were many uncertainties about the conduct of this study and the questionable liver findings, the Peer Review Committee endorsed the requirement for an additional mouse carcinogenicity study with malathion to help clarify any possible carcinogenic potential by malathion.

5. Malaoxon - Fischer 344 Rat Dietary Feeding Carcinogenicity Study

Reference: National Cancer Institute. 1979. Bioassay of malaoxon for possible carcinogenicity, CAS No. 1634-88-2. Technical Report Series, No. 135, National Cancer Institute, Bethesda, MD. NCI-CG-TR-135. Assay performed at Gulf South Research Institute, New Iberia, LA. Report prepared at Tracor Jitco, Rockville, MD under NCI direction. Those responsible for the report at Tracor Jitco were C.R. Angel et al. Reviewed in Document # 000314.

Malaoxon (synthesized by testing laboratory, purity >95%) was administered in the diet to groups of 50 male and 50 female Fischer 344 rats (from NCI Frederick Cancer Research Center, MD) at doses

of 500 or 1000 ppm per group for 103 weeks. Animals were then observed for up to an additional 2 weeks. Matched controls consisted of 50 untreated rats per sex. All surviving rats were killed at 103 to 105 weeks.

a. Discussion of Tumor Data

The original NCI report concluded that under the conditions of this study, malaoxon was not carcinogenic in Fischer 344 rats. The review did note a possible increase in thyroid C-cell adenomas or carcinomas in female rats at the high dose. However, this positive finding for C-cell tumors was questioned by reference to "historical" control data. The review states "the historical record of this laboratory shows an incidence of female F344 rats with C-cell adenomas or carcinomas of 16/223 (7%), compared with 0/50 in the control group, 1/49 (2%) in the low-dose group and 5/47 (11%) in the high-dose group of this study. This indicates that the incidence of C-cell tumors of the thyroid in female rats of the present study is comparable to that usually seen in control animals."

The NCI report also revealed an increased incidence of benign mammary gland tumors in low-dose females ($p = 0.026$). However, this increase was not considered to be significant as the NCI employed as its criteria of significance $p = 0.025$, based on Bonferroni adjustments, and therefore did not consider this a positive finding. The original NCI report also noted an increase in the incidence of adrenal gland pheochromocytoma in males, but reported these increases were not statistically significant.

The NTP reevaluated tissues from these organs (thyroid gland, adrenal gland) as potential suggestive targets. The NTP reevaluation revealed one difference from the original NCI review. The NTP concluded that there was equivocal evidence of carcinogenicity for male and female F344 rats based on findings for C-cell neoplasms of the thyroid gland (Huff et al., 1985). NTP examinations of the major sites of potential targets are shown in Table 6. In particular, the NTP reevaluation resulted in an increase in incidence of C-cell tumors in females that was significant at the high-dose ($p = 0.045$, pair-wise comparison) and yielded a dose-related trend. For males, the NTP reported a positive finding for C-cell adenomas and carcinomas in the high-dose group ($p = 0.035$, pair-wise comparison) and a positive trend. The NTP reevaluation also showed an increase in the incidence of mammary gland adenomas in low-dose females, but this increase was dismissed as related to malaoxon administration as the increase was not seen at the high dose and the incidence in the concurrent controls was unusually low. The subsequent NTP reevaluation resulted in a considerable revision in the incidence of pheochromocytoma from that in the original NCI report; however, the NTP did not indicate any statistically significant findings for



62
58

this tumor.

The NTP provided a detailed description of their reasoning for considering the equivocal evidence for C-cell neoplasms of the thyroid gland (Huff et al., 1985). Arguments for an associative effect by malaoxon, from the publication, are: (i) dose-response trends in both sexes, (ii) the incidences in the high-dose groups were increased, albeit marginally, in comparison to concurrent controls, (iii) the incidences exceed the historical rates observed in this species (male F344 rats, 196/2230, 8.8%; female F344 rats, 190/2265, 8.4%), and (iv) six carcinomas were found in the high-dose groups, compared with one in the controls. The arguments against this being a malaoxon related response are: (i) no corresponding increases were seen for hyperplasia (see Table 6 below), (ii) the neoplasms were microscopic in size and morphologically identical to naturally occurring tumors, (iii) no supporting effects were observed in Study II of malathion in F344 rats or in the Study I of malathion in Osborne-Mendel rats, both at higher doses, and (iv) the incidence in the concurrent control group was somewhat lower than the rates observed in Study II of malathion and the mean historic control.

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Table 6: Malaoxon Fischer 344 Rat Study - Findings in NTP Reevaluation

| | Sex | Control | Low Dose | High Dose |
|---|---|---|---|---|
| Thyroid C-cell hyperplasia | M | 8/49 (16%) | 11/45 (24%) | 8/49 (16%) |
| | F | 24/48 (50%) | 24/48 (50%) | 25/48 (52%) |
| Thyroid C-cell adenoma & carcinoma | M | 3/49 (6%) | 3/45 (7%) | 10/49 (20%) |
| | F | 4/48 (8%) | 7/48 (15%) | 11/48 (23%)* |
| Adrenal Pheochromocytoma | M | 5/50 (10%) | 6/50 (12%) | 10/49 (20.4%) |
| Mammary gland adenoma | F | 2/50 (4%) | 9/50 (18%) | 1/50 (2%) |
| Hematopoietic system lymphoma | M | 0/50 (0%) | 0/50 (0%) | 4/50 (8%) |

Number of Lesions/Number of tissues examined (Incidence %)
* may be 12/48 (25%) for females

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The Registration Standard for malathion requires that the Registrant perform an additional Fischer 344 rat study using malaoxon. The stated purpose of this additional study is to

clarify the carcinogenic potential of malaoxon and provide additional needed data on the effects of malaoxon on cholinesterase inhibition. Further considerations by Toxicology Branch I/Health Effects Division and this Peer Review Committee raise doubt as to how definitive this study is to make a clear conclusion about the carcinogenicity of malaoxon. Again, as in the Osborne-Mendel rat study, the NTP does not provide detailed data from which to perform independent statistical analyses. Therefore, it is not entirely clear that the suggestive evidence of male adrenal gland pheochromocytomas and of female mammary gland adenomas at the low dose can be totally dismissed.

The Peer Review Committee agrees with the NTP language regarding the equivocal evidence for the C-cell neoplasms of the thyroid gland; i.e. "equivocal evidence of carcinogenicity is demonstrated by studies that are interpreted as showing a chemically-related marginal increase of neoplasms." The Peer Review Committee made several comments on the detailed reasoning the NTP provided on the equivocal classification. 1) While the incidences of C-cell neoplasms are above the mean historical values provided by the NTP, it is not clear what the range of values were from the individual studies that made up the historical database. This may have some bearing on the significance of the increased thyroid findings. 2) It was noted that the thyroid C-cell rate for hyperplasia was not significantly elevated over control. 3) It was considered that the elevated number of carcinomas observed was a significant contribution to the possible chemical induced effect by malaoxon. For example, there was a positive trend for C-cell carcinomas alone for females ($p = 0.015$) although the pair-wise comparison was $p = 0.059$. 4) As regards to the possible low concurrent control group incidence, it was noted that the NTP incidence was similar to the original NCI incidence. 5) There was question as to whether it can be definitively concluded that there were no supporting effects seen in the two malathion rat carcinogenicity studies.

It was also noted in the NTP reevaluation (but not discussed by the NTP), there was an evident increase in the incidence of lymphoma (hematopoietic system) among high dose males. Although detailed statistical treatment of the NTP data was not able to be performed, it was pointed out that Toxicology Branch I calculated a trend for lymphoma ($p = 0.006$; calculation by B. Dementi and H. Pettigrew (statistician in HED) based on the NTP reevaluated summary numbers). The pair-wise comparison however was $p = 0.059$. The "exact test for trend" was used for cases where relatively few tumors are found and in this instance, provided an increased trend for lymphoma ($p = 0.0114$; B. Dementi and H. Pettigrew calculation). It was noted that in some cases, lymphoma and leukemia are combined by the NTP. Leukemia was suggested in the Fischer 344 rat malathion study.

Therefore, due to the uncertainty of the total findings in this malaoxon study, the Peer Review Committee reaffirms the Registration Standard requirement for an additional rat carcinogenicity study using malaoxon.

b. Consideration of Study Adequacy for Assessment of Carcinogenic Potential

Another consideration by the Peer Review Committee and Toxicology Branch I provides support for requiring an additional rat study with malaoxon. The percent survival at week 90 of the study for males and females, respectively, was 80% and 82% for controls, 82% and 90% for the low dose group, and 64% and 80% for the high dose group. For the male animals, the Peer Review Committee noted there were suggestions of a dose-related increase in mortality. However, there were no pair-wise survival disparities between control and dose groups; thus an adjusted tumor analysis in not suggested. Furthermore, while it is not clear if the NTP took the higher mortality into account in their deliberations, the NTP mentioned in their reevaluation that sufficient numbers of rats of each sex were at risk for the development of late appearing tumors.

c. Non-Neoplastic Findings

The NTP reports that in this study forestomach ulcers were observed at increased incidences in male and female rats (males: 3/47, 6%, control; 7/48, 14%, low dose; 9/48, 18%, high dose; and, females: 0/49, 0%, control; 1/48, 2%, low dose; 3/48, 6%, high dose). This is similar to the findings evaluated by the NTP in the malathion Fischer 344 rat study.

6. Malaoxon - B6C3F1 Mouse Dietary Feeding Carcinogenicity Study

Reference: National Cancer Institute. 1979. Bioassay of malaoxon for possible carcinogenicity, CAS No. 1634-88-2. Technical Report Series, No. 135, National Cancer Institute, Bethesda, MD. NCI-CG-TR-135. Assay performed at Gulf South Research Institute, New Iberia, LA. Report prepared at Tracor Jitco, Rockville, MD under NCI direction. Those responsible for the report at Tracor Jitco were C.R. Angel et al. Reviewed in Document # 000314.

Malaoxon (synthesized by testing laboratory, purity >95%) was administered in the diet to groups of 50 male and 50 female B6C3F1 mice (from NCI Frederick Cancer Research Center, MD) at doses of 500 or 1000 ppm per group for 103 weeks. Animals were then observed for up to an additional 2 weeks. Matched controls consisted of 50 untreated mice per sex. All surviving mice were killed at 103 to 105 weeks.

The NCI report concluded that under the conditions of this study, malaoxon was not carcinogenic in the B6C3F1 mouse. The OPP review of this study concurred with the NCI in this opinion and the malathion Registration Standard does not call for additional testing of malaoxon in the mouse.

E. Additional Toxicology Data on Malathion:

1. Acute Toxicity

Technical malathion is mildly toxic on an acute oral (Category III), dermal (Category III) and inhalation (Category III) basis. Technical malathion is only mildly irritating to the eye of rabbit (Category III) and slightly irritating after dermal exposure to rabbit (Category IV). Technical malathion is nonsensitizing by dermal application. No data are available on the acute delayed neurotoxicity of malathion in the hen and, since malathion is an organophosphate, this study is required.

2. Metabolism

According to the Malathion Registration Standard issued in February, 1988, there are data gaps in the chronic toxicology data base which includes a data gap for metabolism studies. The OPP has just recently received a study concerning malathion metabolism and it is currently undergoing review. It is considered that malaoxon is a metabolite of malathion. However, it is not clear how much malathion is metabolized to malaoxon. Since malaoxon is considered a metabolite of malathion, and may be responsible for some or all malathion toxic effects, malaoxon was also examined for carcinogenicity in long term bioassays.

3. Mutagenicity

According to the Malathion Registration Standard issued in February, 1988, there are no data available on the mutagenic potential of malathion. Studies are required in all of the following mutagenicity test areas: gene mutation, structural chromosomal aberrations, and other genotoxic effects. While there are no acceptable studies submitted to the OPP, there are many open literature articles concerning mutagenicity testing with malathion and malaoxon. Tables 7 and 8 present a listing of many of these tests (this is not prepared as an exhaustive or critical review).

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Table 7. Open Literature Mutagenicity Studies on Malathion

Gene Mutation Category

Salmonella assay
Haworth et al., Environ Mutagen 5 (Suppl 1): 3-142, 1983
Moriya et al., Mutat Res 116: 185-216, 1983
Waters et al., Basic Life Sci 21: 275-326, 1982
Other references
Results: all Negative

E. coli reverse mutation (WP2; WP2 uvr A)
Brusick et al., Mutat Res 76: 169-190, 1980
Waters et al., Basic Life Sci 21: 275-326, 1982
Moriya et al., Mutat Res 116: 185-216, 1983
Results: all Negative

Mouse lymphoma assay
NTP, 1988 Annual Plan
Result: Equivocal

Drosophila sex-linked recessive lethal assay
Waters et al., Basic Life Sci 21: 275-326, 1982
Velazquez et al., Environ Mutagen 9: 343-348, 1987
Results: all Negative

Structural Chromosomal Aberrations Category

In vitro mammalian cell aberrations
Galloway et al., Environ Mol Mutagen 10 (Suppl 10): 1-175, 1987
Result: CHO cells, Negative w/o activation, Positive w/act.
Ishidate et al., Mutat Res 195: 151-213, 1988
Results: CHL cells, Positive ± activation
human lymphocytes, Positive w/o activation
human hematopoietic B411-4 cells, Negative w/o act.

In vivo mammalian aberrations - bone marrow
Dulout et al., Mutat Res 122: 163-167, 1983
Result: Positive, one i.p. dose Balb/c mouse
Degraeve and Moustschen, Environ Res 34: 170-174, 1984
Result: Negative, one i.p. dose Q strain mouse
Degraeve et al., Arch Toxicol 56: 66-67, 1984
Result: Negative, 7 weeks drinking water, a low dose (8 ppm)
Dzwonkowska and Hubner, Arch Toxicol 58: 152-156, 1986
Result: weak Positive, one i.p. dose Syrian hamster
Salvadori et al., Mutat Res 204: 283-287, 1988
Result: one dose, Negative; multiple doses (5 days/2 weeks), Positive; Swiss Webster mice

Mouse micronucleus
Dulout et al., Mutat Res 105: 413-416, 1982
Result: Positive, cutaneous route; weak Positive, i.p. route

In vivo human - acute malathion intoxication
van Bao et al., Humangenetik 24: 33-57, 1974
Result: increase in breaks in lymphocytes

In vivo mammalian aberrations - germ cells
Degraeve and Moutschen, Environ Res 34: 170-174, 1984
Result: Negative, spermatogonia, one i.p. dose Q strain mouse

Degraeve et al., Arch Toxicol 56: 66-67, 1984
Result: Negative, spermatogonia and primary spermatocytes, 7 weeks - drinking water, 8 ppm, Q strain mouse
Salvadori et al., Mutat Res 204: 283-287, 1988
Result: one dose, primary spermatocytes, Negative
multiple doses, primary spermatocytes, Positive
Swiss Webster mice

Dominant lethal assay - mouse
3 reported Negative studies, but problems with each study

Note: Krause et al., Bull Environ Contam Toxicol 15: 458-462, 1976
Result: found slight damage to testicular tissues, but recovered (indicates malathion can reach germ cell area)

Other Genotoxic Effects Category

In vitro mammalian cells - SCE
Galloway et al., Environ Mol Mutagen 10 (Suppl 10): 1-175, 1987
Result: Positive ± activation, CHO cells
Nicholas et al., Mutat Res 67: 167-172, 1979
Result: Positive w/o activation, human fetal lung fibroblasts
Nishio and Uyeki, J Toxicol Environ Health 8: 939-946, 1981
Result: Positive w/o activation, CHO cells
Chen et al., Mutat Res 88: 307-316, 1981
Result: weak, but dose response Positive w/o act., V79 cells
Chen et al., Environ Mutagen 4: 621-624, 1982
Result: weak, but dose response Positive with act., V79 cells
Sobti et al., Mutat Res 102: 89-102, 1982
Result: Positive ± activation, human lymphoid cells (LAZ-007)

UDS in WI-38 cells
Waters et al., Basic Life Sci 21: 275-326, 1982
Result: Negative

Mitotic recombination in Saccharomyces
Waters et al., Basic Life Sci 21: 275-326, 1982
Result: Negative

Differential toxicity in DNA repair deficient strains of *E. coli* and *B. subtilis*
Waters et al., Basic Life Sci 21: 275-326, 1982
Result: Negative

Other:
Griffin and Hill, Mutat Res 52: 161-169, 1978
Result: induced in vitro breakage of plasmid DNA at a slow rate, but significantly greater than control rate

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Table 8. Open Literature Mutagenicity Studies on Malaoxon

Gene Mutation Category

Salmonella assay
Zeiger et al., Environ Molec Mutagen 11 (Suppl 12): 1-158, 1988
Result: Negative

Mouse lymphoma assay
Tennant et al., Science 236: 933-941, 1987
Result: Positive without activation

Structural Chromosomal Aberrations Category

Aberrations/CHO cells
Ivett et al., Environ Molec Mutagen 14: 165-187, 1989
Result: Negative

Other Genotoxic Effects Category

SCE/CHO cells
Ivett et al., Environ Molec Mutagen 14: 165-187, 1989
Result: Positive ± activation, but weak with activation
Nishio and Uyeki, J Toxicol Environ Health 8: 939-946, 1981
Results: Positive without activation (slightly greater than malathion

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These mutagenicity data suggest malathion has genetic activity. While malathion is generally negative in all point mutation assays, it is positive in all the available in vitro sister chromatid exchange assays. There are both positive and negative findings for structural chromosomal alterations. While there are negative aberration studies for malathion, there are sufficient positive findings in both in vitro and in vivo cytogenetic assays and in both somatic and germ cells to warrant a mutagenicity concern. This information provides support for a possible genetic component in the weight of evidence consideration for carcinogenicity. The limited amount of available malaoxon mutagenicity data appear similar to the malathion data.

4. Developmental and Reproductive Effects

A developmental study was performed with New Zealand rabbits. Twenty female rabbits per dose group were exposed via gastric intubation to single daily doses of vehicle (corn oil) or malathion on days 6 to 18 gestation. Dose levels used were 25, 50 and 100 mg/kg body weight per exposure. Animals were observed between days 0 to 20 of gestation. No adverse developmental effects were seen. Due to decreased dam body weight gain at 50 mg/kg/day and increases



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in mean percent of resorptions at the top two dose groups, the developmental No Observable Effect Level (NOEL) is 25 mg/kg/day and the maternal NOEL is also 25 mg/kg/day.

The Registration Standard for malathion states that a rat developmental toxicity study is required to support registration of products containing malathion. This study has just recently been submitted and is currently undergoing review. A reproduction study in the rat is also required to be performed and this study is currently in progress.

5. Structure-Activity Correlations

There was not a great deal of discussion regarding possible structure-activity correlations with malathion. It appears that as a general class organophosphates are not consistent in their actions, as evidenced by differences in toxicity, metabolism, distribution, etc. This may be due to the differences in chemical groups attached to the phosphate portion of the organophosphates. The variety of these groups may provide an explanation for the inconsistency in organophosphate SAR. However, it was noted that malaoxon is a metabolite and structural analogue of malathion. This suggests that malaoxon is an appropriate analogue and important metabolite for comparison to malathion. Several of the suggestive findings in the malathion rat carcinogenicity studies are apparent in the malaoxon rat carcinogenicity study. These included lesions in the thyroid and adrenal glands. Furthermore, some non-neoplastic findings were comparable, especially the occurrence of stomach ulceration. Finally, the genetic toxicity data appear to be similar between malathion and malaoxon, at least based on the limited amount of data available for malaoxon.

F. Weight of Evidence Considerations:

The Committee considered the following facts regarding the toxicology data on Malathion to be of importance in a weight-of-the-evidence determination of carcinogenic potential.

1) The major consideration of the Peer Review Committee was the inadequacy of the available studies to make definitive determinations on the carcinogenicity of malathion and malaoxon. There were many issues raised (e.g. concurrent controls, insufficient data to perform independent statistical analyses, performance not consistent with current standards, survival) concerning the adequacy of each study from which a firm conclusion regarding carcinogenicity could not be reached.

2) In addition to the equivocal evidence for carcinogenicity in the malaoxon Fischer 344 rat study for C-cell neoplasms of the thyroid gland for males and females, there are other suggestions of carcinogenic responses [illegible] of the 6 studies considered:

| | |
|---|---|
| Osborne-Mendel rat: (malathion) | C-cell [illegible]lasms of thyroid gland, male<br>follic[illegible] cell neoplasms of thyroid gland, male [illegible] female<br>pheochr[illegible]ocytoma of adrenal gland, male |
| Fischer 344 rat (malathion) | : pheochromocytoma of adrenal gland, male<br>leukemia, male |
| Sprague-Dawley rat: (malathion) | C-cell neoplasms of thyroid gland, female,<br>uterus polyps, female mammary tumors |
| B6C3F1 mouse (malathion) | : neoplastic nodules/hepatocellular carcinoma, male |
| Fischer 344 rat (malaoxon) | : equivo[illegible] evidence (NTP call) for C-cell neop[illegible]s of thyroid gland, male and female<br>pheochr[illegible]ytoma of adrenal gland, male<br>mammar[illegible]nd adenomas, female<br>lymphoma [illegible]ematopoietic), male |
| B6C3F1 mouse (malaoxon) | : no evidence of carcinogenicity |

3) While the NTP, in commenting upon the three rat studies they reexamined, does not attribute the appearance of the different tumors to malathion or malaoxon administration (outside of the equivocal evidence for C-cell neoplasms in the malaoxon rat study), in many instances, the same tumor types appear in different studies (see point above for specifics). Also, in several instances, more than one tumor type was suggested by the study.



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4) The mutagenicity data suggest that malathion has genetic activity. This information provides some support for a possible genetic component in the weight of evidence consideration for carcinogenicity. The limited amount of available malaoxon mutagenicity data appear similar to the malathion data.

5) The NTP has indicated in its memorandum to J. Moore (from E. McConnell) that the NTP is considering a further study of malathion using current "state-of-the-art" methods. There has been no further information on this intention. Furthermore, the NTP's Board of Scientific Counselors has recommended that there is need for a state of art carcinogenesis study for malathion (NTP Fiscal Year 1986 Annual Plan, NTP Publication No. NTP-86-086). This indicates that although they have no reason to believe malathion is carcinogenic, there is a perceived need for a state-of-the-art carcinogenicity study for malathion.

G. Classification of Carcinogenic Potential:

Criteria contained in the EPA Guidelines [FR51: 33992-34003, 1986] for classifying a carcinogen were considered.

The Peer Review Committee agreed to classify malathion as a Group D Carcinogen; that is, malathion is not classifiable as to human carcinogenicity. The Peer Review Committee decision was based on the inadequacy of the available studies to make definitive determinations on the carcinogenicity of malathion and malaoxon. The Committee agreed with the NTP reanalysis that there was no clear evidence of carcinogenicity due to malathion or malaoxon administration in most of these studies (the NTP concluded equivocal evidence in the malaoxon rat study). This is also consistent with past Agency positions. However, the Committee felt there were many issues regarding the adequacy of each study from which a firm conclusion on the carcinogenic potential of malathion could not be made.

In addition, while there may have been doubts about the significance of each tumor type in each of the individual studies, there was the suggestive appearance of similar tumors (e.g. C-cell neoplasms of the thyroid gland and pheochromocytomas of the adrenal gland) and of multiple tumors in more than one study. Also, there was some evidence from mutagenicity studies that a genetic component for malathion and malaoxon is possible. These points provided weight to the evidence of possible carcinogenic effects that could not be totally dismissed.

Because of the unresolved questions about the adequacy of the existing studies vis-a-vis current standards and the potential effects noted in these existing studies, additional studies need to be performed to address these concerns. The Committee reaffirmed the requirement of the Malathion Registration Standard that requires the Registrant to perform an additional mouse carcinogenicity study with malathion. The Committee also determined that the Registra-tion Standard recommendation to perform a carcinogenicity study in combination with a rat chronic study on malathion be made into a requirement. It is believed these studies using current standards are necessary for a more adequate assessment of malathion.

The Committee also reaffirmed the requirement to perform an additional rat carcinogenicity study with malaoxon. Since malaoxon was considered the metabolite of malathion that produces much of the effects of malathion, it was felt important to properly assess the carcinogenicity of malaoxon. Also, in several instances (e.g. Daminozide/UDMH, EBDCs/ETU), the quantitative risk assessment has been based on the metabolite where tumorigenicity may be more apparent at lower doses with the metabolite when it is the tumor inducing agent. For these reasons, a well conducted study on malathion's metabolite, malaoxon, would be required.

Attachment 8

[Malathion]

Carcinogenicity Study 83-5

EPA Reviewer: Brian Dementi, Ph.D., D.A.B.T.
Toxicology Branch I (7509C)
EPA Secondary Reviewer: Edwin Budd, M.S.
Registration Action Branch II (7509C)

Brian Dementi 8/14/97
Budd 8/14/97

**DATA EVALUATION RECORD**

Study Type: Combined Chronic/Oncogenicity Study; OPPTS 870.4300 [83-5]

DP BARCODE: D224174
P.C. CODE: 057701

Submission No.: S501871
Tox. Chemical No.: 535

Test Material (purity): Malathion; butanedioic acid, [(dimethoxyphosphinothioyl) thio] diethyl ester (97.1% a.i.)

Synonym: Mercaptosuccinic acid diethyl ester; S-ester with 0, 0, -dimethyldithiophosphate

Citation: Ira W. Daly, Ph.D., D.A.B.T., 27 February 1996. A 24-Month Oral Toxicity/Oncogenicity Study of Malathion in the Rat via Dietary Administration. Huntingdon Life Sciences, East Millstone, NJ, Study No. 90-3641, MRID 43942901, Unpublished.

Sponsor: Cheminova Agro A/S, P.O. Box 9, DK-7620, Lemvig, Denmark

Executive Summary: In a combined chronic toxicity/oncogenicity study (MRID 43942901), malathion (97.1% a.i.) was administered to 90 Fischer 344 rats/sex/dose via the diet for up to 24 months at dose levels of 0, 100/50 (100 ppm for first 3 months of study, 50 ppm for duration of study in both sexes due to finding of erythrocyte cholinesterase inhibition in females only at 3 month assay) 500, 6,000 or 12,000 ppm [equivalent to respective mean values of 0, 4, 29, 359 and 739 mg/kg/day (males) and 0, 5, 35, 415 and 868 mg/kg/day (females)].

The only clinical sign observed was yellow anogenital staining among females at 12000 ppm. **Increased mortality was seen in females at 12000 ppm and in males at 500, 6000 and 12000 ppm. All 12000 ppm males died or were sacrificed moribund by about 94 weeks.** Treatment related decrements in body weight gain were observed at 6000 and 12000 ppm in both sexes. **Food consumption was increased at 100 ppm in males for the first 3 months (prior to lowering of dose to 50 ppm).** At subsequent time points for males, and across all time points for females food consumption was increased, the LOEL = 6000 ppm and NOEL = 500 ppm. Among parameters for hematology, erythrocyte count was reduced in males at 12000 ppm, mean corpuscular hemoglobin concentration was decreased in males at 6000 and 12000 ppm; and

the following were observed in rats of both sexes at 6000 and 12000 ppm: increased platelet count, decreased mean corpuscular volume and mean corpuscular hemoglobin. Hence, for hematologic parameters overall, LOEL = 6000 ppm, NOEL = 500 ppm, both sexes. Among clinical chemistry parameters, erythrocyte cholinesterase inhibition, males, LOEL = 6000 ppm, NOEL = 500 ppm; females, at 3 months, the enzyme was inhibited at all doses, LOEL = 100 ppm. After 3 months, when lowest dose was reduced to 50 ppm, LOEL = 500 ppm, NOEL = 50 ppm. For plasma cholinesterase inhibition, males, LOEL = 500 ppm, NOEL = 50 ppm (100 ppm first 3 months); females, LOEL = 6000 ppm, NOEL = 500 ppm. For brain cholinesterase inhibition, LOEL = 6000 ppm, NOEL = 500 ppm, both sexes. **For inhibition of cholinesterase activity, for males the overall NOEL is 50 ppm (4 mg/kg/day) and the LOEL is 500 ppm (29 mg/kg/day) based on inhibition of plasma activity at 24 months. For females the overall (beyond 3 months) NOEL is 50 ppm (5 mg/kg/day) and the LOEL is 500 ppm (35 mg/kg/day) based on inhibition of erythrocyte activity.** Decreased aspartate aminotransferase, females, 12000 ppm; decreased alkaline phosphatase, males and females, 6000 and 12000 ppm; elevated blood urea nitrogen, males, 12000 ppm; elevated cholesterol, males and females, 6000 and 12000 ppm; elevated gamma-glutamyl transpeptidase, males and females, 6000 and 12000 ppm. Ocular effects testing inconclusive. Organ weight effects: increased kidney and liver weights, males and females, 6000 and 12000 ppm; thyroid/parathyroid weight increased (males), decreased (females) 6000 and 12000 ppm; increased spleen weight, males, 6000 and 12000 ppm; increased heart weight, males, 6000 ppm (term). In males, increases in liver and thyroid/parathyroid weights may have extended to 500 ppm. **Microscopic findings: non-neoplastic: nasal mucosa and nasopharynx (several pathologies), males and females, 6000 and 12000 ppm; bilateral subacute-chronic inflammation/chronic nephropathy (high incidence in all study groups including controls), increased severity, males, 6000 and 12000 ppm, females, 500, 6000 and 12000 ppm**; stomach (several pathologies), males and females, 6000 and 12000 ppm; increased incidence parathyroid hyperplasia, males and females, all doses; other findings in various tissues (thyroid, lymph nodes, lungs, liver, spleen, adrenal gland, eyes) as summarized in the review, being more remarkable in males, and often extending across the top three doses in males and top two doses in females; neoplastic: **treatment-related increased combined hepatocellular adenomas/carcinomas, females at all doses, incidences: 0/55 (0%), 2/55 (3.6%), 2/55 (3.6%), 3/55 (5.5%) and 6/55 (10.9%) for the 0, 100/50, 500, 6000 and 12000 ppm groups, respectively; rare tumors (one in each of four dose groups) on nasoturbinal slide preparations considered compound related effects: males, carcinoma 12000 ppm, adenoma 6000 ppm; females, squamous cell carcinoma 100/50 and 12000 ppm. Other tumor types observed included testes interstitial cell tumors significant at all doses with possibly decreased latency**; significant trend in thyroid follicular cell adenomas and/or carcinomas, males; significant

trend and positive pairwise comparison at 500 ppm for thyroid c-cell carcinoma, males; significant difference in pair-wise comparison, mononuclear cell leukemia, 100/50 ppm, females; significant difference in pair-wise comparisons, pituitary pars distalis carcinomas, 500 and 6000 ppm, females; significant difference in pair-wise comparison, pituitary pars distalis adenomas and/or carcinomas combined, 500 ppm, females. Tumorigenic responses may have been compromised by high mortality in males at 6000 and 12000 ppm and in females at 12000 ppm.

The classification and acceptability of this chronic/oncogenicity study in the F344 rat is **reserved** pending review and discussion by the HED Hazid and Carcinogenicity Peer Review Committee.

Detailed Summary of Results:

**Clinical Signs**

There were no clinical signs in the study in either sex at any dose level except yellow anogenital staining among females at the 12000 ppm dose level. This clinical effect was of high incidence and consistently expressed throughout the study.

**Mortality**

Increased mortality among males was observed in a dosing-related manner across the 500, 6000 and 12000 ppm dose levels. Among females, increased mortality was seen at the 12000 ppm dose level only. A principal contributor to the increased mortality observed among male rats at 500 ppm was that attributed to leukemia. It should be noted that while the incidence of leukemia at the 500 dose level was not increased relative to the control group incidence, leukemia as a cause of death was increased in that group. Evidently, leukemia laden male rats in the 500 ppm group, under the added burden of the test material, were more likely than controls with the disease to succumb to the condition before term.

**Body Weight**

Treatment-related decrements in body weight gain were observed at 6000 and 12000 ppm for rats of both sexes.

**Food Consumption**

Increased food consumption was observed in rats of both sexes. Among males at time points prior to the reduction in dosage level from 100 ppm to 50 ppm, there were statistically significant dosing-related increases of 2-7% in food consumption frequently observed across all doses, particularly during weeks 5-12, i.e.,

there was no NOEL. This may be an appetitive (behavioral) effect in males during the first 3 months, prior to reduction of malathion in the diet. Following the reduction in dosage level in group 2 from 100 to 50 ppm (both sexes), there were, over the remainder of the study time, few instances where the low dose group differed from the control group for male rats, and increases in food consumption were variable at the higher doses being generally confined to the top two dose levels. For males, for the bulk of the study period, the LOEL was 6000 ppm and the NOEL was 500 ppm for increased food consumption. For females, the LOEL was 6000 ppm and the NOEL 500 ppm for increased food consumption.

**Hematology**

The following were small but statistically significant findings: reduced hemoglobin and hematocrit at one or more intervals at 6000 and 12000 ppm in both sexes, where the effect in males was more remarkable in terms of degree of lowering of and endurance in time; erythrocyte count reduction was observed at 12000 ppm at 12 months in males only; platelet count increases at 6000 and 12000 ppm in rats of both sexes; decreases in mean corpuscular volume and mean corpuscular hemoglobin at 6000 and 12000 ppm in rats of both sexes; decreased mean corpuscular hemoglobin concentration in males only at 6000 and 12000 ppm; and increased total leukocyte counts in rats of both sexes at 12000 ppm at the 12 month time point only. Hence, for hematologic parameters overall, LOEL = 6000 ppm for both sexes and NOEL = 500 ppm for both sexes.

**Clinical Chemistry**

**Cholinesterase**

At the 3-month time point, <u>erythrocyte</u> cholinesterase was statistically significantly inhibited among females at all dose levels. Inhibitions were 25%, 30%, 58% and 66% at 100, 500, 6000 and 12000 ppm dose levels, respectively. In consideration of the lack of a NOEL at this time point, the dosage level for the low dose group was reduced from 100 ppm to 50 ppm in both sexes. At subsequent time points <u>erythrocyte</u> cholinesterase for females was significantly inhibited in a dose-related manner at 6000 and 12000 ppm at 6 months, and at 500, 6000 and 12000 ppm at 12 months and at term. Inhibitions at term were 27%, 44% and 52% at 500, 6000 and 12000 ppm, respectively. Inhibition at the top three dose levels tended to be less at term than at 3 months suggesting some degree of adaptive recovery. Among males erythrocyte cholinesterase was considered inhibited only at 6000 and 12000 ppm.

In conclusion, erythrocyte cholinesterase inhibition LOEL = 6000 ppm, NOEL = 500 ppm for males. For females, LOEL = 100 ppm. A NOEL was not identified at 3 months. At subsequent time intervals (excepting 6 months), LOEL = 500 ppm, NOEL = 50 ppm for females. The data are not sufficiently definitive to conclude a NOEL for the study for females, given the statistically significant 25% inhibition at 100 ppm on a shallow dose-response curve between 100 and 12000 ppm. Furthermore, even though erythrocyte cholinesterase was not inhibited among females at the 6 month and subsequent time intervals at 50 ppm, there is no certainty the enzyme would not have been inhibited at 50 ppm during the first 3 months of study, particularly in view of the shallow dose response and the propensity for the enzyme to recover for a period from a initial inhibition as it did after 6 months at 500 ppm. Accordingly, the finding establishes the requirement for a 3-month study to define a NOEL for erythrocyte cholinesterase inhibition in the female F344 rat.

Plasma cholinesterase inhibition among male rats was significant at 3, 6 and 12 months at both of the top dose levels. At term it was inhibited at 500 ppm (29%) and at 6000 ppm (64%) (there were no male survivors at 12000 ppm). Among females, the plasma cholinesterase was inhibited across time by 38-61% at 6000 ppm and by 70-89% at 12000 ppm. Hence, for plasma cholinesterase inhibition, LOEL = 500 ppm, NOEL = 50 ppm (100 for first 3 months) (males) and LOEL = 6000 ppm, NOEL = 500 ppm (females).

Brain cholinesterase was significantly inhibited only at 6000 and 12000 ppm in rats of both sexes. At 6000 ppm, inhibition among males across the four time points ranged 11-31% and in females ranged 12-18%. The respective inhibitions at 12000 ppm ranged 15-19% (first 3 time points) for males and 28-67% (28-49% over the first 3 time points) for females. Hence, for brain cholinesterase inhibition, LOEL = 6000 ppm and NOEL = 500 ppm for rats of both sexes.

Additional clinical chemistry parameters considered affected and the lowest dose level at which the effect was observed include: aspartate aminotransferase, decreased among females at 12000 ppm; alkaline phosphatase, decreased among males and females at 6000 ppm; blood urea nitrogen, elevated among males at 12000 ppm; cholesterol, elevated among males and females at 6000 ppm; gamma-glutamyl transpeptidase, elevated among males and females at 6000 ppm.

**Urinalysis**

Urine pH values tended to decline variably by about one pH unit with increasing dose essentially at the top two dose levels at various assay time intervals in both sexes.

**Ocular Testing**

The ocular effects component of this study which involved opthalmoscopy, electroretinogrphy (ERG), fundic photos and histopathology of the retina did not reveal any effects identifiable as compound-related. ERGs were more remarkably impaired in all dose groups than in the controls, but there were no clear dosing-related effects. Variability in ERGs was so great that a dosing-related effect if present could have gone undetected. The F344 rat is considered a poor model for detecting ocular effects by ERG and thus this component of the overall study does not satisfy as a negative ocular effects study.

**Pathology**

Organ Weights: For the interim (12-month) sacrifice, increases in the weights of the following organs are considered treatment related: males (6000 and 12000 ppm) - kidneys, liver, spleen and thyroid/parathyroid; females (6000 and 12000 ppm) - kidneys and liver.

At terminal sacrifice, heart weight was increased among males at 6000 ppm; kidney and liver weights were increased in males at 6000 ppm and in females at 6000 and 12000 ppm. There were small nonstatistically significant increases in liver weight by all modes of expression among 500 ppm males. Whether this is a treatment-related effect is uncertain. Also uncertain is a nonstatistically significant increase in testes/epididymides weight at 6000 ppm. Thyroid/parathyroid weight was increased for males at 6000 ppm and considered likely to be increased at 500 ppm, and decreased among females at 12000 ppm and considered likely so at 6000 ppm.

Macroscopic Findings: Increased incidences of irregular surfaces of the kidneys were observed for males at 500, 6000 and 12000 ppm and for females at 12000 ppm. Increased incidences of emaciation were observed for males and females at 6000 and 12000 ppm.

Microscopic Findings:

**Nonneoplastic Findings**

Both the respiratory and olfactory epithelia of the nasal mucosa exhibited microscopic alterations in both sexes at 6000 and 12000 ppm. Effects seen in both types of epithelia include hyperplasia, subacute (chronic active)/chronic inflammation and dilated glands. In addition, there were findings in the olfactory epithelium of degeneration, epithelial cysts, replacement of the olfactory epithelium by ciliated and

nonciliated columnar epithelial cells and hyperplasia of both of the latter replacement epithelia. These effects were not observed in either the 50 or 500 ppm dose groups. A probable reason for the more remarkable effects observed for olfactory epithelium than the respiratory epithelium is that the former is richly endowed with a wide spectrum of metabolic capabilities not unlike those of the liver, that would potentially activate a xenobiotic such as malathion.

There were also increased incidences of hyperplasia of the respiratory epithelium of the nasopharynx in rats of both sexes at 6000 and 12000 ppm dose levels.

Bilateral subacute-chronic inflammation/chronic nephropathy was observed at high incidence in all groups in both sexes. However, the severity of this finding was increased in males and females at 6000 and 12000 ppm and in females at 500 ppm as well.

Several microscopic compound-related findings were identified in the stomach. These effects (e.g., congestion, edema, ulcers, acute/subacute inflammation, subacute (chronic active)/chronic inflammation, squamous cell hyperplasia, hyperkeratosis) were observed in males and females at 6000 and 12000 ppm. The bulk of these were observed in rats that died prior to term, and in the opinion of the study author were likely due to decreased food intake in moribund animals.

Congestion was a finding in many tissues (e.g., liver, thyroid, brain, heart, lung, forestomach, pancreas, pituitary, harderian gland, lacrimal gland, sternal marrow, femoral marrow) of both males and females, but were generally more remarkable in males than in females. In females the effect was primarily in the 12000 ppm group and extending into the 6000 ppm group, while in males the effect was universal at 6000 and 12000 ppm in the tissues identified and frequently extended to the 500 ppm group. These findings were generally observed in decedents and to the extent congestion was a finding at 500 ppm, it may be a corollary to increased mortality in that group.

There were certain other non-neoplastic microscopic findings in various tissues (thyroid, lymph nodes, lungs, liver, spleen, adrenal gland, eyes) as summarized on p. 49 of this review generally more remarkably so in males than in females, and often extending across the top three doses in males and top two doses in females.

**Neoplastic Findings**

Neoplastic findings of the liver were identified in this study. The effects were evidently dosing-related only in the case of

female rats. Among male rats, combined incidences of hepatocellular adenomas and carcinomas were 3/55 (5.5%), 4/55 (7.3%), 4/55 (7.3%), 3/55 (5.5%) and 1/55 (1.8%) at 0, 100/50, 500, 6000 and 12000 ppm dose levels, respectively. The corresponding incidences among females were 0/55 (0%), 2/55 (3.6%), 2/55 (3.6%), 3/55 (5.5%) and 6/55 (10.9%). While there were no statistically significant increases in liver neoplasia in the case of males, high mortality among male rats in the 6000 and 12000 ppm groups may have precluded expression of a tumorigenic response, particularly if late occurring. Since the next lowest dose level tested (500 ppm) was substantially lower than the 6000 ppm dose level (more than 10-fold lower), malathion was not tested for potential carcinogenicity at adequate dose levels for males in this study. Hence, this study does not satisfy as a negative study for liver carcinogenicity among male rats, or for that matter for carcinogenicity at any other anatomic site among male F344 rats. This constitutes a major study deficiency.

In the case of females, increased mortality at 12000 ppm also constitutes a study deficiency. The combined hepatocellular tumorigenic findings for females at all dose levels are considered positive, compound-related findings. At 6000 and 12000 ppm, the findings are statistically significant as claimed in the study report. The findings at 100/50 and 500 ppm are considered positive in view of the effects at the two higher dose levels (suggesting the liver as a target organ) in concert with the facts that hepatocellular adenomas and carcinomas in female F344 rats are rare (i.e. < 1%) as defined by Office of Science and Technology Policy (1985). The most recent NTP (1996) historical control data for the F344 female rat reveals incidences for hepatocellular adenomas of 8/1351 (0.59%) and for carcinomas of 1/1351 (0.07%).

Nasoturbinal tissue tumors that occurred in male rats, i.e., one carcinoma in the 12000 ppm group and one adenoma in the 6000 ppm group, are considered to be positive compound-related tumorigenic effects. Though not identified as statistically significant by any particular method of statistical analysis, the findings are considered positive on the basis of the extremely rare historical incidence and the clear evidence of nasoturbinal tissue being a "target tissue" in this study. There were also identified in the nasoturbinal tissue slides, one incidence each in the 100/50 and 12000 ppm female groups of squamous cell carcinomas of the squamous epithelium lining the alveolus of a tooth. These are also extremely rare tumors, whether viewed as nasal or oral tissues, as documented in the NTP (1996) historical data and thus again for the reason of rarity are likewise considered compound-related findings. Further, in view of the extensive hyperplasia of nasoturbinal tissues in rats of both sexes at the higher doses and the rare tumorigenic findings, there is according to the

FIFRA Guidelines an outstanding requirement for histopathologic examination of nasoturbinal tissue slides in all study groups not yet examined. Two nasoturbinal sections per rat are considered inadequate to properly evaluate the potential tumorigenic response particularly if rare. The registrant should discuss with the Agency a protocol for adequate histopathologic assessment of possible nasoturbinal tissue effects.

Although similar and high incidences of interstitial cell testicular tumors were observed in all the control and treated male groups in this study, the latency or time to tumor appeared to be decreased in the treated groups. A NOEL for increased interstitial cell testicular tumors at earlier than normal time points (decreased latency) was not identified.

Other tumorigenic findings showing increased incidences as discussed in this review which await independent HED statistical analyses include the following: leukemia (both sexes); thyroid gland follicular cell adenoma and carcinoma in males; thyroid gland c-cell carcinoma in males; pituitary gland, pars distalis, adenomas and carcinomas in females.

Compliance: Signed and dated GLP, quality assurance, data confidentiality and flagging statements were provided.

# I. MATERIALS AND METHODS

## A. MATERIALS

1. Test Material: Malathion
   Description: clear yellow liquid - stored in steel drums at room temperature.
   Lot Nos.: 11029-01 and 30628-01 (p.19)
   Purity: 97.1% a.i.
   Stability of compound: Stable as analyzed before, during and after the study period.
   CAS. No.: 121-75-5
   Supplier: Cheminova Agro A/S, Lemvig, Denmark

2. Vehicle: None

3. Test Animals:
   Species: Rat
   Strain: CDF (F-344) CrlBR
   Age at study initiation: 48 days
   Mean weight at study initiation: males, 140 grams; females, 103 grams.
   Source: Charles River Laboratories, Kingston, NY
   Housing: Individually in stainless steel cages.
   Diet: Certified Rodent Chow, No. 5002 (meal); Purina Mills Inc., St. Louis, MO. Test diets were prepared weekly by admixture with test material and fed ad libitum.
   Water: Ad libitum; by automated watering system.
   Environmental conditions:
   - Temperature: 67-76° F (19-24° C)
   - Humidity: 22-84%
   - Air Changes: Not provided.
   - Photoperiod: 12 hour light/dark cycle
   - Acclimation Period: 20 days

## B. Study Design

1. In life dates: Start: 12/30/92 End: 1/10/95

2. Animal Assignment

Animals were assigned into 5 groups of 90 animals per sex (Table 1) by a computerized random sort program so that body weight means for each group were comparable.

| TABLE 1: STUDY DESIGN | | | |
|---|---|---|---|
| A: Main Study | | | |
| Test Group | Conc. in Diet (ppm) | Main Study (24 Months) | |
| | | Male | Female |
| Control | 0 | 55 | 55 |
| Low (LDT) | 50/100 | 55 | 55 |
| Mid (MDT) 1 | 500 | 55 | 55 |
| Mid (MDT) 2 | 6,000 | 55 | 55 |
| High (HDT) | 12,000 | 55 | 55 |

| B: Interim Sacrifices | | | | | | | |
|---|---|---|---|---|---|---|---|
| Test Group | Conc. in Diet (ppm) | 3 Months | | 6 Months | | 12 Months | |
| | | Male | Female | Male | Female | Male | Female |
| Control | 0 | 10 | 10 | 10 | 10 | 15 | 15 |
| Low (LDT) | 50/100 | 10 | 10 | 10 | 10 | 15 | 15 |
| Mid (MDT) 1 | 500 | 10 | 10 | 10 | 10 | 15 | 15 |
| Mid (MDT) 2 | 6,000 | 10 | 10 | 10 | 10 | 15 | 15 |
| High (HDT) | 12,000 | 10 | 10 | 10 | 10 | 15 | 15 |

The 3-month and 6-month interim sacrifices (10 rats of each sex per group) were incorporated primarily for ocular tissue pathologic examination. All rats from all dose groups received complete macroscopic examination. All rats from the control and 12000 ppm groups received only ocular tissue microscopic examinations at the 3-month and 6-month interim sacrifices.

3. Dose Selection Rationale: Not provided in report.

It should be noted that the original dose chosen for the low dose group, 100 ppm, resulted in



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statistically significant erythrocyte cholinesterase inhibition in females when assayed following the first ~~the~~ three months of dosing. In pursuit of a NOEL for cholinesterase inhibition, the dietary concentration for the low dose group was reduced to 50 ppm for rats of both sexes for the duration of the study.

4. Diet Preparation and Analysis

Fresh diet was prepared weekly by mixing appropriate amounts of malathion (in three stages: mortar and pestle, Hobart mixer and twin shell mixer) with Certified Rodent Chow No. 5002 (meal) (Purina Mills Inc., St. Louis, Mo.) to yield final designated concentrations. Prior to the initiation of the study, mock batches of treated diets at the 100 ppm (low concentration) and 12000 ppm (high concentration) were prepared and analyzed for achievement of homogeneity. Later in the study, at about Week 20, a 50 ppm batch was similarly prepared for homogeneity analysis. For assessments of homogeneity, nine samples were taken from each diet preparation (three each from the top, middle and bottom portions), and analyzed. As disclosed in Appendix O of the Study Report, "The mean values of each of the three levels were within ± 10.0% of each other, as well as within 15% of nominal. These values indicated that an acceptable mixing procedure for malathion in rodent diet was utilized. (p. 5552 of the Study Report). We concur with the conclusion that satisfactory mixing was achieved. Stability of malathion in the prepared diets was determined in a previous study (Pharmaco LSR study no. 92-3806). The diets were determined to be stable for 14 days at room temperature. Prepared diets were stored in clear polyethylene bags inside of dark plastic buckets with lids, at room temperature.

On 2/23/93, 10/6/93 and 7/7/95, the technical grade malathion was assayed for % purity as compared with analytical grade malathion (p. 5551 and 5554 of the study report). On each occasion two samples were assayed in duplicate. The % purity (mean) on the three respective occasions were 96.4%, 96.8% and 98.2%. The mean of these three assays is 97.1%.

In order to confirm concentration levels of malathion in the diet over the course of the study, all five dietary levels were assayed in duplicate weekly for the first 8 weeks and once every 2 weeks for the following 8 weeks. For the remainder of the study, assays were performed every 4 weeks. The results of the assays, as disclosed in Appendix 0, indicate that diets employed in the study were within 15% of the nominal concentration and duplicate samples were within 10% of each other (pp. 5553-5554 of the Study Report.)

5. Animals Received Fresh Diet [Weekly]: "Test diets were prepared weekly and were offered to the animals *ad libitum*." (p. 27 of Study Report.)

6. Statistics: The following is a statement regarding statistical methods as it appears in the Study Report:

"Body weight, body weight change, body weight change from Week 0, food consumption, hematology and clinical chemistry parameters, electroretinogram values, terminal organ and body weights and organ/body weight and organ/brain weight ratios, survivorship and time-to-tumor incidences were analyzed. Mean values of all dose groups were compared to control at each time interval. Statistically significant differences from control are indicated on mean tables of appendices.

"The time-to-tumor analyses were performed using the Thomas, Breslow and Gart analyses which tests for both tumor incidence (chi-square and Fisher tests) and time-to-tumor (Kaplan-Meier curves, Cox's Tests and the Gehan-Breslow/Kruskal-Wallis Analyses). The chi-square and Fisher Exact Tests consider only simple incidence in a pairwise manner; each treated group is compared to control.

"Cox's test and the Gehan-Breslow/Kruskal-Wallis Analyses are based on incidence and survival. They separately perform a multiple comparison test, a test for trend, and a series of pairwise tests with each treated group compared to control. The Haseman test is a test that divides the study into time segments, tests each segment for tumor

incidence differences, then pools the results for an overall test of differences." (pp. 48-49)

C. METHODS:

1. Observations:

Animals were inspected twice daily for signs of toxicity and mortality. Detailed examinations (including palpation) were conducted weekly.

2. Body Weight:

Animals were weighed three times pretest, weekly for weeks 1-14, once every two weeks during weeks 16-26 and monthly thereafter and at term.

3. Food Consumption and Compound Intake:

Food consumption for each animal was determined and mean daily diet consumption was calculated as grams of food/kg body weight/day. Compound intake (mg/kg/day) values were calculated as time-weighted average from the consumption and body weight gain data.

4. Ophthalmoscopic Examination:

Performed pretest (all animals) and at 3(35/sex/group), 6(25/sex/group), 12 (all animals) and 24 months (all animals).

5. Electroretinogram Examinations (ERG):

From among 35 satellite rats/sex/group, electroretinographic (ERG) evaluations were performed on 7 rats/sex/group at pretest and at months 3, 6 and 12 (p. 26 of study report). ERG evaluations were also performed on 5 rats/sex/group at 24 months using oncogenicity animals, i.e., chosen from among survivors of the 55 rats/sex/group originally designated for the principal study (p. 24 of the study report). The Materials and Methods section of the study report indicates (p. 35) that methodology and references for the techniques employed are to be found in Appendix A of the study report. In Appendix A (p. 105 of the study report) under the Electroretinographic Parameters the Reference and Description of test procedures says simply: "LKC Technologies, Epic II, Photic Stimulator, Model PS22. Photographs were taken on anesthetized animals." The study report provides no further information as to details of the testing procedure followed.

6. Hematology and Clinical Chemistry Parameters:

Blood was collected from 10 rats/sex/group (satellite animals) at months 6 and 12. The month 12 rats were also used for cholinesterase assays. Blood was collected from 10 rats/sex/group (oncogenicity animals) at month 18 and at term.

Blood was collected from these animals via venipuncture of the orbital sinus under light $CO_2/O_2$ anesthesia. Animals were fasted overnight prior to blood collection. The CHECKED (X) parameters were examined.

| a. Hematology | | | |
|---|---|---|---|
| x | Hematocrit (HCT) | x | Leukocyte differential count* |
| x | Hemoglobin (HGB) | x | Mean corpuscular HGB (MCH) |
| x | Leukocyte count (WBC) | x | Mean corpusc. HGB conc. (MCHC) |
| x | Erythrocyte count (RBC) | x | Mean corpusc. volume (MCV) |
| x | Platelet count | x | Reticulocyte count |
| | Blood clotting measurements | | |
| | **Time** | | |
| | Thromboplastin | | |
| | Thromboplastin | | |
| | Clotting | | |
| | Prothrombin | | |

* Minimum required for carcinogenicity studies (only on Cont. and HDT unless effects are observed based on Subdivision F Guidelines

b. Clinical Chemistry*

| | ELECTROLYTES | | OTHER |
|---|---|---|---|
| x | Calcium | x | Albumin |
| x | Chloride | x | Blood creatinine |
| | Magnesium | x | Blood urea nitrogen |
| x | Phosphorus | x | Total cholesterol |
| x | Potassium | x | globulin/(calculated) |
| x | Sodium | x | A/G ratio |
| | **ENZYMES** | x | Glucose (fasting) |
| x | Alkaline phosphatase (ALK) | x | Total bilirubin |
| | Cholinesterase (ChE)* | x | Direct bilirubin |
| x | Creatine phosphokinase | x | Total serum protein (TP) |
| | Lactic acid dehydrogenase (LDH) | | Triglycerides |
| x | Serum alanine amino-transferase (also SGPT) | | Serum protein electrophorese |
| x | Serum aspartate amino-transferase (also SGOT) | | |
| x | Gamma glutamyl transferase (GGT) | | |
| | Glutamate dehydrogenase | | |

* See statement below on cholinesterase.

Cholinesterase assays were performed on 10 rats/sex/dose at 3, 6 and 12 months (satellite animals) and at 24 months (oncogenicity animals). In addition, erythrocyte assays were performed on selected animals in the control and low dose (100/50 ppm) groups six weeks after the dose level was reduced from 100 to 50 ppm. These animals were not fasted prior to blood collection. Plasma, erythrocyte and brain cholinesterase activity was determined on a Hitachi 717 Boehringer Mannheim Diagnostics Automatic Analyzer using a modified Ellman method (kinetic).

7. Urinalysis*

Urine was collected from fasted, water-deprived animals (approx. 2 hours) at months 6, 12, 18 and term. The Checked (X) parameters were examined.

| | | | |
|---|---|---|---|
| x | Appearance | x | Glucose |
| | Volume | x | Ketones |
| x | Specific gravity | x | Bilirubin |
| x | pH | x | Blood |
| x | Sediment (microscopic) | | Nitrate |
| x | Protein | | Urobilinogen |

* Not required for carcinogenicity studies based on Subdivision F Guidelines.

Also, 16-hour urine volume samples were collected from animals not deprived of food or water, also at months 6, 12, 18 and term.

8. Sacrifice and Pathology:

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination . The (XX) organs, in addition were weighed.

| SACRIFICE AND PATHOLOGY | | | | | |
|---|---|---|---|---|---|
| X | DIGESTIVE SYSTEM | X | CARDIOVASC./HEMAT. | X | NEUROLOGIC |
| | | x | Aorta* | xx | Brain* |
| x | Tongue | xx | Heart* | x | Periph. nerve* |
| x | Salivary glands* | x | Bone marrow* | x | Spinal cord (3 levels)* |
| x | Esophagus* | x | Lymph nodes* | | |
| x | Stomach* | xx | Spleen* | x | Pituitary* |
| x | Duodenum* | x | Thymus* | x | Eyes (optic n.) |
| x | Jejunum* | | | | |
| x | Ileum* | | | | |
| x | Cecum* | | | | |
| x | Colon*Rectum* | | | | |
| x | Rectum | | | | |
| x | Liver*+ | | | | |
| | Gall bladder* | | | | |
| x | Pancreas* | | | | |
| X | RESPIRATORY | X | UROGENITAL | X | GLANDULAR |
| x | Trachea* | xx | Kidneys*+ | xx | Adrenal glands* |
| x | Lung | x | Urinary bladder* | x | Lacrimal gland |
| x | Nose (2 levels) | xx | Testes*+ | x | Mammary gland* |
| | Pharynx | xx | Epididymides | xx | Parathyroids*++ |
| | Larynx | x | Prostate | xx | Thyroids*++ |
| | | x | Seminal vesicle | | |
| | | xx | Ovaries*+ | | |
| | | x | Uterus* | | |
| | | | | X | OTHER |

| SACRIFICE AND PATHOLOGY | | | | | |
|---|---|---|---|---|---|
| | RESPIRATORY (cont.) | | UROGENITAL (cont.) | x<br><br>x<br>x | Bone<br>Skeletal muscle*<br>Skin<br>All gross lesions and masses |

* Required for carcinogenicity studies based on Subdivision F Guidelines.
\+ Organ weight required in chronic studies.
++ Organ weight required for non-rodent studies.

In addition to the above, the following tissues were specifically identified as examined histopathologically: harderian glands, oviducts, retina, vagina and zymbal's gland.

## II. RESULTS

### A. OBSERVATIONS:

#### 1. Clinical Signs of Toxicity:

An examination of Appendix C "Physical Observations" (pp. 141-217 of the study report) reveals little evidence of dosing-related clinical signs of toxicity except yellow anogenital staining most notably among females at the 12000 ppm dose level and less so among males at the same dose level. At times this sign extended in a less remarkable manner to females of the 6000 ppm group. There is the absence of the spectrum of cholinergic signs, though mortality was extensive among males and females at 12000 and among males at 6000 ppm as well.

#### 2. Mortality:

Percent survivorship is reproduced below as it appears on p. 50 of the study report.

| (%) Percent Survivorship | | | | | | | |
|---|---|---|---|---|---|---|---|
| Group | Dose Level (ppm) | Month 12 | | Month 18 | | Termination (Month 24) | |
| | | Male | Female | Male | Female | Male | Female |
| I | 0 | 100 | 100 | 100 | 98 | 67 | 69 |
| II | 100/50 | 100 | 98 | 100 | 98 | 75 | 74 |
| III | 500 | 100 | 100 | 95 | 96 | 53 | 75 |
| IV | 6000 | 100 | 98 | 98 | 96 | 26 | 62 |
| V | 12000 | 96 | 98 | 71 | 91 | 0 | 36 |

Based upon statistical analyses of survivorship data as performed and reported in the study report, mortality was concluded in the study report to be significantly increased at the p = .01 level at 6000 ppm and 12000 ppm among males and at 12000 ppm among females.

A matter of concern is whether the 53% survivorship for males at 500 ppm relative to 67% in the control group constitutes evidence of enhanced mortality at this dose level. While there were 90 rats/sex/group at the beginning of the study, interim sacrifices by year one reduced the number in each group by 35 rats, leaving, by design, 55 rats/sex/group to continue on for the second year. However, 1 rat in group 5 died spontaneously prior to the one year time point (see p. 119 of the study report, copy included here in Exhibit 1). So the number of male rats per group post year one were 55 for all but group 5 for which the number at risk post year one was 54. Among these animals, the number of unscheduled deaths during year two were 18 (33%), 14 (25%), 26 (47%), 41 (74.5%) and 54 (100%) for groups 1 through 5 in the respective order. An independent statistical treatment of this data yields p = 0.0000 for Cochran-Armitage Trend Test and Fisher's exact p values versus control of 0.2646, 0.0846, 0.0000 and 0.0000 for groups 2, 3, 4 and 5, respectively. In view of these findings which include a <u>remarkably</u> significant trend test and Fisher's exact comparisons for the two high dose groups, while the p value for the 500 ppm dose group did not achieve statistical significance at the $p \leq 0.05$ level, the level of significance that was observed, p = 0.0864 is sufficient to indicate as likely an adverse effect on survival at 500 ppm.

Furthermore, according to Haseman et al (1990), "normal 2-year survival rates in NTP carcinogenicity studies are approximately 66% for untreated male F344 rats and 73% for untreated female F344 rats." (p. 556). Thus, control survival rate in this study is consistent with historical control survival data for the F344 rat. The unscheduled deaths in the 500 ppm male group is close to being 50% in excess of that of the contemporaneous and historical control values. For these reasons it is considered appropriate to identify 500 ppm was an effect level for increased mortality among male F344 rats. For increased mortality, LOEL = 500 ppm, NOEL = 50 ppm (males); LOEL = 12000 ppm, NOEL = 6,000 ppm (females).

The study report says that in males, the early deaths were beginning around day 400 (month 14) for the 12000 ppm dose group and around day 600 (month 20) for the 6000 ppm dose group. Independent inspection of the data shows that extra deaths in 500 ppm male group occurred late in the study. In the case of females, deaths were toward the end of the animal's normal lifespan (p. 53 of study report).

According to the study report (p. 50), chronic nephropathy and mononuclear cell leukemia were the two most common causes of moribundity and/or death among males and females at 6000 and 12000 ppm dose levels. This assessment requires some clarification and comment. Tabulated below (p. 25) are the incidences of leukemia and nephropathy for the various dose groups during the second year of the study as compiled from tables of data from various pages of the study report, specifically from pages 2734, 2775, 2810 and 2858. An examination of this table shows that for Group 5 males, leukemia was not a meaningful contribution to moribundity or mortality, although nephropathy was the major cause of death, i.e., death among 47 of 56 unscheduled deaths was attributed to nephropathy. In Group 4 males, both leukemia and nephropathy were major contributors to unscheduled deaths, where together they accounted for 36 of the 41 unscheduled deaths. For all groups of male rats, nephropathy was observed in essentially all animals, but it was only in Groups 4 and 5 that nephropathy was claimed in large number as the cause of death.

Examination of page 2810 of the study report (Exhibit 1) where unscheduled deaths is concerned, the frequency and severity of nephropathy for males tended to increase with increasing dose across Groups 3-5 relative to Groups 1 and 2; and for females across Groups 4 and 5 relative to Groups 1-3. Among terminal sacrificed animals (p. 2734 of the study report, Exhibit 1) interpretation is more difficult

because of high mortality in groups 4 and 5 and elevated mortality in group 3 males. It is also difficult to interpret for females due to high mortality in Group 5, but there appears to be a dose-related trend toward proportionally higher grade of nephropathy across Groups 3-5 relative to Groups 1 and 2. In the hope of clarifying this somewhat, a table which consolidates the data for nephropathy for terminal sacrifices and unscheduled death animals has been prepared and included in Exhibit 1.

The combined data for females suggest that while nephropathy across dose groups affects nearly all animals, a trend (dosing-related) toward higher graded nephropathy is evident for Groups 3-5 relative to Groups 1-2. In other words, there appears to be a malathion related increase in severity of nephropathy for Group 3 (500 ppm) and becoming progressively more severe in Groups 4 (6000 ppm) and 5 (12000 ppm). The study report claims severity among females increased only at 6000 and 12000 ppm.

Among females, leukemia incidence (p. 21) does not appear to be affected by dosing, although the incidence in Group 5 may have been higher had mortality not been increased in that group, i.e., animals in that group would have been at greater risk of developing leukemia had they lived longer. Mortality from leukemia was essentially the same in all groups (female) and cannot be said to have contributed disproportionately to the increased mortality in Group 5 females.

The problem of competing toxicity is more evident among males, particularly where Groups 3 and 4 are concerned. Leukemia incidence was low in Group 5 probably due to premature mortality from nephropathy. One cannot say what effect malathion would have had on leukemia incidence and leukemia related mortality in Group 5 had there not been the fatal kidney effects. The same can be said of group 4 where the leukemia incidence (18/55) may also have been compromised by increased mortality. Male Group 3 poses a particular challenge for interpretation. Leukemia incidence was not increased relative to the control group, but it was an increased cause of death, i.e., death was attributed to leukemia in 14 group 3 males as opposed to 7 males in the control group.

Furthermore, in consideration of the percentage of male rats in each group diagnosed with leukemia that died of the condition, namely, 7/23 (30%), 7/16 (44%) 14/24 (58%), 13/18(72%) and 1/1 (100%) for groups 1 through 5, respectively, there is evidence of dosing-related increased

mortality from leukemia among those rats harboring that condition. Rats with leukemia are more likely to die of leukemia as the result of a competing dosing related toxicologic burden of the test material. This effect appears to be evident at 500 ppm and 6000 ppm and perhaps so at 50 ppm, and constitutes supporting evidence of chronic toxicity of the test material at these lower doses.

In summary, for males chronic nephropathy (not leukemia) was the most common cause of death in the 12000 ppm group. At 6000 ppm, chronic nephropathy and leukemia were the two most common causes of death (and presumably moribundity) among males. Also, among males, 500 ppm apparently was an effect level in terms of increased mortality (unscheduled deaths), with leukemia (and not chronic nephropathy) as an increased cause of unscheduled deaths. The incidence of leukemia among males of the 500 ppm group was not increased. It is uncertain whether the incidence of leukemia (18/55) in the 6000 ppm male group would be the expected incidence for that group when adjusted for the increased mortality and the resulting decreased time of exposure. The fact that greater percentages of male rats with leukemia died of the condition in groups 3 and 4 (and possibly group 2 as well) is supporting evidence for a malathion related chronic toxicity effect at these doses.

For females, chronic nephropathy was the principal cause of the increased deaths in the 12000 ppm group. Neither the incidence of leukemia nor deaths due to leukemia was elevated in that group. Yet, for females, 500 ppm appears to be an effect level in terms of increased severity (not incidence) of chronic nephropathy.

| Incidences (mortality) of Leukemia and Nephropathy | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Males | | | | | Females | | | | |
| Dose Group | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| No. Examined | 55 | 55 | 55 | 55 | 56 | 55 | 55 | 55 | 55 | 55 |
| a) Terminal Sacrifice | 37 | 41 | 29 | 14 | 0 | 38 | 41 | 41 | 34 | 20 |
| b) Unscheduled Deaths | 18 | 14 | 26 | 41 | 56 | 17 | 14 | 14 | 21 | 35 |
| No. with Leukemia (Death Due To) | 23(7) | 16(7) | 24(14) | 18(13) | 1(1) | 9(5) | 18(4) | 15(6) | 12(5) | 10(7) |
| No. With Chronic Nephropathy (Death Due To)* | 54(2) | 54(2) | 54(4) | 55(23) | 55(47) | 49(0) | 53(1) | 52(1) | 54(2) | 53(20) |
| No. of Deaths Due to Leukemia or Chronic Nephropathy | 9 | 9 | 18 | 36 | 48 | 5 | 5 | 7 | 7 | 27 |

* For ranking of severity of chronic nephropathy, see Exhibit 1, a) terminal sacrificed animals, p. 2734 of Study Report and b) unscheduled deaths, p. 2810 of Study Report.



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B. BODY WEIGHT:

An inspection of mean body weight change data as presented in Appendix A (pp. 399-445 of the study report) discloses a generally consistent decrement of body weight gain ranging upward in time for males of about 3-13% at 6000 and 12-32% at 12000 ppm dose levels. Among females, there were consistent decrements of about 10-15% at 12000 ppm throughout the bulk of the study period with numerical inhibitions of about 4-5% observed at 6000 ppm which were at certain time intervals (e.g. 0-10 and 0-20 weeks) statistically significant. See table below of selected body weight gain data as taken from the study report. The study report says "The mean body weights and body weight gains of male and female animals at 6000 and 12000 ppm dose levels were reduced compared to the controls throughout most of the study. Differences from controls were statistically significant in the males and females at 12000 ppm throughout the treatment period. At 6000 ppm, differences from controls were statistically significant in the males from week 13 through termination and in females from week 3 through week 50." (p. 62 of study report). It is reasonable therefore to conclude that for body weight effects, LOEL (M,F) = 6000 ppm; NOEL (M,F) = 500 ppm.

**Mean Body Weight Change (g) from Week 0**
**(Selected Increments from Corresponding Table in Study Report, pp. 423-433)**

| Weeks | Males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 50/100 | 500 | 6000 | 12000 | 0 | 50/100 | 500 | 6000 | 12000 |
| 0-5 | 94.3 | 93.1 | 94.9 | 92.6 | 82.4** | 43.3 | 44.5 | 45.2 | 42.1 | 39.2** |
| 0-10 | 134.2 | 135.1 | 137.6 | 130.0 | 118.3** | 59.7 | 60.5 | 62.0 | 56.3* | 53.4* |
| 0-20 | 174.8 | 177.3 | 179.3 | 166.3** | 155.7* | 77.3 | 79.8 | 79.9 | 73.4* | 69.7** |
| 0-30 | 201.6 | 202.7 | 205.8 | 192.1** | 175.6** | 88.9 | 91.1 | 93.0 | 85.0 | 79.0** |
| 0-50 | 228.5 | 225.6 | 227.9 | 211.3* | 193.8* | 106.9 | 109.5 | 110.5 | 101.8 | 89.2** |
| 0-82 | 237.8 | 231.0 | 233.5 | 206.7** | 162.0** | 137.2 | 141.7 | 144.2 | 131.8 | 117.2** |

* $p \leq 0.05$ Dunnett's Test
** $p \leq 0.01$



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C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. Food consumption:

Inspection of mean food consumption data as expressed in g/kg/day (pp. 446-457 of the study report reproduced here as Exhibit 2) discloses the following:

**Males**: Possible early treatment-related increases in food consumption at all doses. For example, by week 4, there were statistically significant increases in food consumption of 2.6% at 6000 and 3.3% at 12000 ppm dose levels. On weeks 5 and 7 through 12 there were statistically significant increases in food consumption of approximately 2-7% in all dose groups, except the increase for week 12 at 500 ppm, which did not achieve statistical significance. It is noteworthy that the table for mean percent differences in food consumption presented on p. 63 of the study report (p. 63 included in Exhibit 2) used in support of the Registrant's arguments, begins with data as late as week 14, and does not disclose the earlier time point effects or food consumption. The low dose (group II) effects mentioned above preceded the reduction in dosage level from 100 to 50 ppm, which took place after week 16. The increased food consumption during the earlier period of the study tended to be a dosing-related effect. Throughout the bulk of the study, beyond week 18, food intake was statistically significantly elevated in the high dose group, and somewhat later in the study, weeks 74 through 86, significant increases were again frequently evident at 6000 ppm. Hence, for males there was no NOEL, though an effect was not observed in the low dose group following the adjustment in dosage concentration from 100 to 50 ppm. One cannot be certain that effects on food intake would not have been evident early in the study had 50 ppm been the initial low dose concentration.

**Females**: Food consumption at 12000 ppm was generally increased, often with statistical significance at various weekly intervals. From week 78 to the end of the study the effect was most pronounced, where increases were 12-26% at various weekly intervals relative to control values. Food consumption at lower doses was not so remarkably affected except in the 6000 ppm group late in the study, post week about 78, where increases were of sufficient magnitude and statistically significant often enough to conclude that increased food consumption was a treatment-related



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effect. However, during the first five weeks of study, food consumption among females of the 100 ppm group was elevated by 3-5%, and was statistically significant at all but one of the six weekly time point assessments involved. Similar elevations of no greater magnitude were seen in other dose groups during these early weeks. Between weeks 6 and 16, before dosing was reduced in the low dose group to 50 ppm, there were no clear dosing related effects at any dose level, although group 4 tended to be lower at times after week 18. Hence, for males and females, LOEL = 6000 ppm; NOEL = 500 ppm for the bulk of the study period, however, a NOEL cannot be established for either sex during the earlier periods before the dose level change was made.

2. Compound Intake:

Mean test substance intake, based upon food consumption data, body weight and nominal dose levels, as tabulated in the study report (p. 64) is reproduced below:

| Mean Test Substance Intake Values (mg/kg/day) | | | | |
|---|---|---|---|---|
| **Group** | **Dose Level (ppm)** | **Weeks** | **Male** | **Female** |
| I | 0 | 1-102 | 0 | 0 |
| II | 100 | 1-16 | 7 | 8 |
| | 50 | 18-102 | 2 | 3 |
| | 100/50 | 1-102 | 4 | 5 |
| III | 500 | 1-102 | 29 | 35 |
| IV | 6000 | 1-102 | 359 | 415 |
| V | 12000 | 1-102 | 739 | 868 |

D. OPHTHALMOSCOPIC EXAMINATION:

An examination of results as presented in Appendix D (pp. 218-312 of the study report) does not disclose any treatment related effects of malathion. Furthermore, this was the conclusion of the study pathologist for ophthalmoscopic effects, Dr. Lionel Rubin. It is interesting that retinal degeneration (see summary incidence for males, p. 226 of the study report) as

assessed ophthalmoscopically occurred with greater incidence among rats examined after 52 weeks than after 104 weeks. For example, among Group II male rats, there were 8 incidences of retinal degeneration, at 52 weeks, yet after 104 weeks there was but one rat with retinal degeneration even though 7 of 8 rats observed with retinal degeneration at week 52 were among those examined at 104 weeks. Evidently, 7 rats with retinal degeneration at week 52 no longer had the condition at week 104 as assessed ophthalmoscopically. It appears therefore that retinal degeneration observed after 52 weeks was essentially reversed during the second year. To the extent that the retina is considered to be a neural tissue, restoration of degeneration with continued dosing is somewhat puzzling and unexpected.

E. ELECTRORETINOGRAPHIC EXAMINATION:

The results of ERG testing are summarized as follows. Mean values for a- and b-amplitudes (uV) and latencies (msec) for all study groups as evaluated at pretest (P) and months 3, 6, 12 and 24 are appended as Exhibit 3 (pp. 325-326 of the study report). In discussing this data, the study author evaluated changes in these parameters over the first 12 months by pairing mean data at each time point (3, 6 and 12 months) with that of the previous time point for each study group.

Since ERG data taken at term (24 months) were on different animals for which there were no earlier time point or pretest data, comparisons were made with mean values for all other pretest animals assessed. The particular discussion in question by the study author is located on page 55-61 and 314-322 (Exhibit 4) of the study report.

A fundamental hypothesis of the study author is that in the course of time, and particularly in the ageing F344 rat there is a progressive drop-out or decline in the number of retinal neurons that are responsive to a flash of light as used in ERG testing. If there were a test material enhancement of this decline in retinal neurons, there would be superimposed on the age-related decline a dose-related decline as well. Such an effect of the test material is not apparent in this study. For example, at the 12-month time point, b-wave and a-wave amplitudes expressed as percentages of pretest values, as tabulated in the study report (p. 56) are reproduced below. The percentages presented in this table have been confirmed from data elsewhere in the

study as presented in Exhibit 4. Inspection of these tables confirms the study authors conclusion that after 12 months there is no dosing-related effect on a- or b-wave amplitudes. Similarly reproduced below are tables of percentage changes in a- and b-wave at 24 months, where mean pretest values for all animals tested were used for comparison. Again, there is no evidence of a dosing-related effect.

| 12 month b-wave amplitudes expressed as a percent of pretest values | | | |
|---|---|---|---|
| **Group** | **Dose Level (ppm)** | **Male** | **Female** |
| I | 0 | 62 | 83 |
| II | 100/50 | 98 | 93 |
| III | 500 | 71 | 88 |
| IV | 6000 | 62 | 111 |
| V | 12000 | 59 | 93 |

| 12 month a-wave amplitudes expressed as a percent of pretest values | | | |
|---|---|---|---|
| **Group** | **Dose Level (ppm)** | **Male** | **Female** |
| I | 0 | 70 | 65 |
| II | 100/50 | 93 | 85 |
| III | 500 | 57 | 45 |
| IV | 6000 | 61 | 98 |
| V | 12000 | 61 | 92 |

| 24 month mean b-wave amplitudes expressed as a percent of mean pretest values for the entire pretest population | | | |
|---|---|---|---|
| Group | Dose Level (ppm) | Male (189.4 uV*) | Female (135.4 uV*) |
| I | 0 | 62 | 87 |
| II | 100/50 | 49 | 22 |
| III | 500 | 13 | 22 |
| IV | 6000 | 52 | 62 |
| V | 12000 | | 51 |
| * pretest mean | | | |

| 24 month mean a-wave amplitudes expressed as a percent of mean pretest values for the entire pretest population | | | |
|---|---|---|---|
| Group | Dose Level (ppm) | Male (189.4 uV*) | Female (87.4 uV*) |
| I | 0 | 37 | 71 |
| II | 100/50 | 51 | 20 |
| III | 500 | 15 | 2 |
| IV | 6000 | 35 | 44 |
| V | 12000 | | 29 |
| * pretest mean | | | |

It is noteworthy that while there is no evidence of a progressive compromise of a- or b- wave response with increasing dose, the responses for all dose groups appear more remarkably impaired than the control. One would have to conclude at least that variability in a- and b-wave testing results is too great for the test model to detect what could be meaningful compromises in ERG responsiveness that may have resulted from dosing. The study author concludes "The lack of dose response and the high degree of variability of the data make a compound related effect unlikely." (p. 57 of study report). An alternative conclusion is that variability was so remarkable that a dosing-related effect is rendered indeterminable.

Additional independent comparisons made between ERG responses of various dose groups and control values for a- and b-wave amplitude and latency at various time intervals taken from mean electroretinogram values as presented in the study report (pp. 325-326), (Exhibit 3) serves to confirm the basic conclusion that there are no progressive dosing related effects. However, there is also confirmation of the finding that dose groups as a whole were less responsive, and the data too variable to be useful in detecting meaningful effects of the test material, if occurring.

In addition to ERG a- and b-wave amplitude and latency data, the incidence of abnormal electroretinograms was discussed in the study report. Among rats at 24-months (termination), for example, the tabulation of incidences of abnormal ERGs presented in the study report (p. 58) is reproduced below:

| Group | Dose Level (ppm) | Total Incidence | Male Incidence | Female Incidence |
|---|---|---|---|---|
| I | 0 | 1/9 | 1/4 | 0/5 |
| II | 100/50 | 4/8 | 2/4 | 2/4 |
| III | 500 | 7/9 | 3/4 | 4/5 |
| IV | 6000 | 3/8 | 1/4 | 2/4 |
| V | 12000 | 2/4 | - | 2/4 |

As discussed by the study author (p. 320), the above incidences exclude animals in which unilateral disease indicates the animal is not capable of providing a response to stimulation with white light as employed in ERG testing. These data also do not reveal any dose-related increases in abnormal ERGs in rats of either sex, but in terms of total incidences, all dose groups had higher incidences of abnormal ERGs. The study report affirms (p. 58) that the number of abnormal or nonrecordable ERGs of treated rats is greater in each treated group than in the control. Again, the high variability in abnormal ERGs would be expected to mask meaningful compound-related effects.

In reference to those rats that received ERG testing at termination, the study report claims the following: "There was no significant difference in the histologic appearance of the retina between untreated (Group I)

animals and high-dose (Group V females and Group IV males) at termination (24 months). The histology of the fellow eyes (i.e., the left eye rather than the ERG-tested right eye) was compared in high-dose and control animals. Most (4/5) Group I males had a peripheral retinal degeneration of a slight to moderate degree. Four of five Group IV males had peripheral retinal degeneration and one rat had generalized retinal degeneration." (p. 61 of the study report). The male rats in question as identified in the report of Dr. Lionel Rubin were untreated (Group I): 1010, 1012, 1015, 1024 and 1043, and Group IV: 4013, 4020, 4029, 4039 and 4050. (pp. 352-353 of the study report) An independent reading of the individual histopathology sheets for the identified rats discloses that in Group I, rats 1012 and 1015 were negative for retinal degeneration while rats 1010, 1024 and 1043 exhibited retinal "degeneraion/atrophy" of a slight to minimal degree that was "focal" for rat 1024 and "multifocal" for rats 1010 and 1043. For Group IV rats, rat 4013 exhibited "marked" retinal degeneration/atrophy while the other four rats in this group exhibited slight to minimal, focal or multifocal retinal degeneration/ atrophy. Thus among controls there were 3/5 (not 4/5) with slight to minimal retina degeneration (the term peripheral does not appear on the pathology sheets) while among Group IV rats 5/5 were affected, one with "marked" retinal degeneration/atrophy. The term "generalized" retinal degeneration does not appear.

It is noteworthy that during ERG testing, rat # 4013 was "incapable of responding to stimulation with light due to complete cataract and unilateral retinopathy", according to the report of Dr. Rubin. (p. 353 of the study report) The other rats in Group IV yielded ERG data. So when comparing histopathology of the retinas for the very male animals that received ERGs at term, there is some level of concern that the dosed group is more remarkably affected. However, this is not reflected in a comparison of retinal degeneration/ atrophy for male Groups I versus IV in the study-wide histopathology assessment as disclosed in the overall summaries of microscopic postmortem findings, pp. 2924-25 of the study report.

With regard to females examined by ERG at term, the study report says: "In the untested eyes of female rats subjected to electroretinography, all Group I animals (5/5) had peripheral retinal degeneration histologically. In four of five Group V female rats

the preparation of the retinas was good; in the fifth rat there were sufficient fracture artifacts of the tissue to make interpretation difficult. Two of the four Group V female rats had peripheral retinal degeneration; the other two had no significant retinal degeneration." (p. 61 of the study report).

The histopathology data in concert with ERG data support a conclusion that the test model is inadequate to detect meaningful chronic effects of the test material on visual parameters assayed.

Data from this study were provided to Dr. William Boyes for evaluation. His conclusions are appended (Exhibit 5).

F. BLOOD WORK:

1. Hematology:

Hematology parameters which exhibited statistically significant alterations considered treatment related are tabulated in the Results and Discussion Section of the study report (pp. 65-66) and reproduced here as Exhibit 6, appended. An inspection of the tabulations of data in conjunction with the mean findings for all hematologic parameters as presented in the study report (pp. 494-541) confirms the adequacy of the reproduced table to convey positive findings. Actually, the only hematologic parameter assayed that did not respond to treatment was reticulocyte count. From Exhibit 6, it can be seen that the following were small but statistically significant findings: reduced hemoglobin and hematocrit at one or more time intervals at 6000 and 12000 ppm in both sexes, where the effect in males was more remarkable in terms of degree of lowering of and endurance in time; erythrocyte count reduction was observed at 12000 ppm at 12 months in males only; platelet count increases at 6000 and 12000 ppm in rats of both sexes; decreases in mean corpuscular volume and mean corpuscular hemoglobin at 6000 and 12000 ppm in rats of both sexes; decreased mean corpuscular hemoglobin concentration in males only at 6000 and 12000 ppm; and increased total leukocyte counts in rats of both sexes at 12000 ppm at the 12 month time point only. Hence, for hematologic parameters overall, LOEL = 6000 ppm (M,F); NOEL = 500 ppm (M,F).

2. Clinical Chemistry:

a. Cholinesterase:

Cholinesterase data (plasma, erythrocyte, brain) are adequately tabulated in summary form in the Results and Discussion Section of the study report (p. 69) and is reproduced here as Exhibit 7 for reference purposes. Examination of mean cholinesterase data as presented in Appendix J of the study report (pp. 566-714) supports the tabulation of cholinesterase data reproduced here as Exhibit 7.

Cholinesterase data as summarized in Exhibit 7 discloses the following:

1) Erythrocyte Cholinesterase: at the 3-month time point statistically significant inhibition was observed among females at all dose levels. The inhibition for females relative to mean values of the contemporaneous control were: 25% at 100 ppm, 30% at 500 ppm, 58% at 6000 ppm and 66% at 12000 ppm. The inhibition of erythrocyte cholinesterase among females was not only statistically significant at each dose level, but exhibited a dose-response across all doses. In consideration of the finding of erythrocyte cholinesterase inhibition among females at 100 ppm, the lowest test dose, the Registrant elected to reduce the concentration of malathion in the diet at the low dose level to 50 ppm for dosing beyond month 3. This reduction was effected for both sexes even though the enzyme among males was inhibited only at 6000 and 12000 ppm dose level at 3 months . At the subsequent time points erythrocyte cholinesterase for females was significantly inhibited in a dose related manner at 6000 ppm and 12000 ppm dose levels at 6 months and at 500, 6000 and 12000 ppm dose levels at 12 months and term. Inhibitions at term were 27% at 500 ppm, 44% at 6000 ppm and 52% at 12000 ppm. Inhibition at the top three doses tends to be less at term than at 3 months, indicating some degree of adaptive recovery.

Among males, erythrocyte cholinesterase was inhibited only at 6000 and 12000 ppm, but consistently so at these dose levels. For example, at the 3-month time point, the enzyme was inhibited 48% at both 6000 and 12000 ppm and at the 12-month time point, was inhibited 45% at 6000 ppm and 58% at 12000 ppm.

Hence, for erythrocyte cholinesterase inhibition LOEL = 6000 ppm, NOEL = 500 ppm for males.

For females, LOEL = 100 ppm. A NOEL was not identified at 3 months. At subsequent time intervals (excepting 6 months), LOEL = 500 ppm, NOEL = 50 ppm for females. The data are not sufficiently definitive to conclude a NOEL for the study for females, given the statistically significant 27% inhibition at 100 ppm on a shallow dose-response curve between 100 and 12000 ppm. Furthermore, even though erythrocyte cholinesterase was not inhibited among females at the 6 month and subsequent time intervals at 50 ppm, there is no certainty the enzyme would not have been inhibited at 50 ppm during the first 3 months of study, particularly in view of the shallow dose response and the propensity for the enzyme to recover for a period from an initial inhibition as it did after 6 months at 500 ppm. Accordingly, the finding identifies the need for a 3-month study, employing a larger number of animals, to define with more certainty the NOEL for erythrocyte cholinesterase inhibition in the female F344 rat.

2) Plasma Cholinesterase : in reference to Exhibit 7, at the 3, 6 and 12 month time points, among males, plasma cholinesterase was inhibited only at the 6000 and 12000 ppm dose level, where inhibition ranged 17-27% at 6000 ppm and 43-53% at 12000 ppm. However, at terminal sacrifice, the enzyme was inhibited 29% at 500 ppm and by 64% at 6000 ppm. Among females, the enzyme was not inhibited at doses up to and including 500 ppm. The enzyme was statistically significantly inhibited at 6000 ppm by 38-61% across the four time intervals and at 12000 ppm by 70-89% across the four time points. Hence, for plasma cholinesterase inhibition, LOEL = 500 ppm, NOEL = 50/100 ppm for males and LOEL = 6000 ppm; NOEL = 500 ppm for females.

3) Brain Cholinesterase: Brain cholinesterase was statistically significantly inhibited only at 6000 and 12000 ppm in rats of both sexes. At 6000 ppm, inhibition among males across the four time points ranged 11-31% and in females ranged 12-18%. The respective inhibitions at 12000 ppm ranged 15-19% (first 3 time points) for males and 28-67% (28-49% over the first 3 time points) for females. Hence, for brain cholinesterase inhibition, LOEL = 6000 ppm and NOEL = 500 ppm for rats of both sexes.

b. Other Clinical Parameters:

From among the various clinical parameters assayed, the study report consolidated in tabular form those for which there may be treatment related effects (pp. 75-76), reproduced here as Exhibit 8. An examination of mean values for all clinical chemistry parameters as presented in Appendix J of the study report (pp. 566-714) confirms the tabulated findings to represent essentially all of the possibly treatment related effects. In reference to Exhibit 8, the following were noteworthy effects of dosing with malathion:

Aspartate Aminotransferase:

Among females there were statistically significant reductions in enzyme activity at 500, 6000 and 12000 ppm at the 12 month time point. The respective magnitudes of reduction at the three dose levels were 41%, 40% and 50%. These effects might be due simply to an inordinately high value for the control group (132 IU/L) at week 12. Control values at other time points were uniform and considerably lower. The enzyme was also depressed 42% (statistically significant) and 28% (non-statistically significant) at 18 months in the 12000 and 6000 ppm groups, respectively. There were no remarkable effects for the enzyme among male rats at the various time points and dose levels.

Alanine Amino Transferase:

Among females at 12 months there were statistically significant decreases in this enzyme of about 36% in all three of the top dose levels. The lack of a dose response and the inordinately high control group value of 86 IU/L at 12 months as contrasted with the other time points renders questionable the significant findings at the top three dose levels. There were no noteworthy effects for this enzyme among male rats.

Alkaline Phosphatase:

Responses in males and females were remarkably similar. At the 6 months time point, the enzyme was decreased by 24-28% at 6000 ppm and by 29-36% at 12000 ppm; at 12 months, the same respective

dose group inhibitions were 25-29% and 27-45%. At the 18 months time point the enzyme was significantly inhibited in males and females by 30-40% in the 12000 ppm dose group. For reduced alkaline phosphatase activity, LOEL = 6000 ppm; NOEL = 500 ppm (M,F).

Blood Urea Nitrogen (BUN):

The findings for this parameter are somewhat difficult to interpret. Among males, the small but statistically significant reductions of 12-15% at 6000 and 12000 ppm during the first 12 months are of uncertain interpretation. However, the statistically significant 90% increase in this parameter at month 18 in the 12000 ppm group is likely a manifestation of deterioration of the health of the group of rats that resulted in their total mortality prior to term. Among females, the similar minor decrease of 11-14% during the first 12 months are considered to be of questionable importance. For increased BUN, LOEL = 12000 ppm, NOEL= 6000 ppm (M); LOEL > 12000 ppm; NOEL = 12000 ppm (F).

Cholesterol:

This parameter was elevated in a dose-related manner among rats of both sexes at the 6000 and 12000 ppm dose levels. Among males at 6, 12, 18 and 24 months (term), the parameter was increased, respectively, by 65%, 43%, 46% and 139%, at 6000 ppm, where all but the 18 month increases were reportedly statistically significant. Increases in the same respective order at 12000 ppm were 109%, 124%, 227% and N/A. All increases were reported to be statistically significant. Among females, the increases at 6, 12, 18 and 24 months (term) for the 6000 ppm dose group were 22%, 20%; 83% and 30%, respectively, where only the latter increase was not reported to be statistically significant. The same respective increases for the 12000 ppm group were 48%, 32%, 127% and 63%, all of which values were statistically significant. Hence, for increased cholesterol levels, LOEL = 6000 ppm (M, F); NOEL = 500 ppm (M, F).

Gamma-Glutamyl Transferase:

An inspection of the tabulated data discloses highly variable, and often times remarkable, increases in the enzyme at the various time points in both the 6000 and 12000 ppm groups of both sexes. Hence, LOEL = 6000 ppm, NOEL = 500 ppm (M,F).

The following are additional findings identified in Appendix J not presented in the Table (Exhibit 8) which were perhaps more equivocal in nature; reduced albumin in males at 6000 and 12000 ppm at 12 and 18 months; reduced albumin/globulin ratio in males at 6000 and 12000 ppm at 18 months; increased globulin and decreased albumin/globulin ratio in females at 6000 and 12000 ppm at 18 months; increased creatine kinase in males at 500 and 6000 ppm at term; decreased total protein in males at 6000 ppm at term; decreased total protein and albumin in females at 6000 and 12000 ppm at term and decreased albumin/globulin ratio in females at 12000 ppm at term. All of these effects were essentially limited to the two high dose levels except increased creatine kinase in males which also occurred at 500 ppm.

G. URINALYSIS:

An inspection of individual urinalysis data presented in Appendix K (pp. 715-754 of the study report) reveals few remarkable findings of dosing-related effects. Following is a tabulation of mean urine pH values as computed from the individual data.

| Mean pH | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Dose Group (ppm) | 6 Mo. | | 12 Mo. | | 18 Mo. | | 24 Mo. | |
| | M | F | M | F | M | F | M | F |
| 0 | 7.5 | 7.75 | 6.85 | 6.91 | 7.30 | 7.20 | 7.05 | 6.70 |
| 50 | 7.25 | 7.28 | 6.83 | 6.85 | 7.00 | 7.00 | 7.15 | 6.85 |
| 500 | 7.15 | 7.28 | 6.65 | 7.15 | 7.20 | 6.75 | 7.15 | 6.95 |
| 6000 | 6.8 | 7.35 | 6.75 | 6.70 | 6.90 | 6.60 | 6.0 | 6.70 |
| 12000 | 6.25 | 6.65 | 6.80 | 6.00 | 6.80 | 7.20 | - | 6.50 |

There is an indication in the data of a drop in pH with increasing dose for rats of both sexes, particularly males, at the 6 month time point. Whether this finding early in the study is related to dosing or is a random finding is uncertain. Malathion is a diethyl ester of a dicarboxylic acid, which upon hydrolysis yields carboxyl groups which in turn could explain decreased pH. The data also indicate an increase of cloudiness of urine in rats of both sexes, particularly at the highest dose level. Beyond these observations, the study report claims that there were no treatment related urinalysis findings, an observation supported by independent inspection of the data as presented.

H. SACRIFICE AND PATHOLOGY:

1. Organ Weights:

For reference purposes, mean absolute organ weight and relative organ weight values for the interim (12-month) and terminal sacrifices are reproduced here (Exhibit 9) from pp. 756-771 of Appendix L of the study report.

Noteworthy findings relative to control values (i.e., percentage changes) are as follows:

a. Interim Sacrifice:

Brain: Statistically significantly increased on a body weight basis by 11% in males and 9% in females at 12000 ppm. These increases in both sexes are considered the consequence of reduced body weight. Adrenals: Statistically significantly increased on a body weight basis by 17% in males and a non-statistically significant increase of 10% in females at 12000 ppm. These effects are considered consequences of body weight decline in both sexes. Heart: Statistically significantly increased on a body weight basis by 7% at 6000 ppm and 10% at 12000 ppm among males and 7% at 12000 ppm among females. Kidneys: Absolute organ weight was statistically significantly increased by 14% at 6000 ppm and 29% at 12000 ppm among males and by 9% and 13% among females, respectively. Relative to body weight, the respective kidney weight increases were 19% and 45% among males and 12% and 24% among females. Similarly on a brain weight basis, kidney weights were increased by 13% and 31% among males and by

9% and 15% among females. Liver: Liver weights were statistically significantly increased for males on all three bases of assessment at the two highest doses. These respective increases at 6000 ppm and 12000 ppm were: absolute, 22% and 36%; relative to body weight, 23% and 53%; relative to brain weight, 23% and 37%. For females, liver weight was statistically significantly increased on all three bases at 12000 ppm. These increases were 16% (absolute), 28% (relative to body weight) and 18% (relative to brain weight). At 6000 ppm, increases occurred on all three bases of expression, but achieved statistical significance only on the relative to body weight basis. These increases were 8% (absolute), 11% (relative to body weight) and 8% (relative to brain weight). Spleen: Among male rats, spleen weight was statistically significantly increased at 12000 ppm by 23% (absolute weight), 38% (relative to body weight) and 24% (relative to brain weight). The respective increases for males at 6000 ppm were 9%, 14% and 9%, where only the increases on the relative to body weight basis was reportedly statistically significant. Spleen weight for females was not remarkably altered on any basis of expression. Testes/Epididymides, there were no remarkable effects on absolute or relative to brain weight modes of expression. Statistically significant increases on a body weight basis of 7% and 13% at the 6000 ppm and 12000 ppm dose levels are considered expressions of body weight reduction. Ovaries: There were no remarkable effects. Thyroid/Parathyroid: Among male rats, statistically significant increases in all three modes of expression were found at the top two dose levels. At 12000 ppm, the increases were 25% (absolute), 40% (relative to body weight) and 27% (relative to brain weight). The respective increases at 6000 ppm were 15%, 20% and 15%. Among females, there were no remarkable effects on absolute and relative to brain weight modes of expression. Increases of 12% (non-significant) at 12000 ppm and 14% at 6000 ppm were observed on the relative to body weight basis.

In conclusion, for the interim (12-month) sacrifice, the following organ weight increases are considered treatment related. **Males**: (6000 ppm and 12000 ppm), kidneys, liver, spleen and

thyroid/parathyroid; **females**: (6000 ppm and 12000 ppm), kidneys and liver.

b. Terminal Sacrifice:

Brain: There were no remarkable effects on brain weight for male rats in the 6000 ppm dose group. (recalling there were no surviving male rats of the 12000 ppm group). Among females, there was a statistically significant 24% increase relative to body weight at 12000 ppm, considered to be a reflection of the remarkable decline in body weight at that dose level. Brain weight among females was not remarkably affected at 6000 ppm. Adrenals: There were no remarkable effects for rats of either sex. There was a large variability in adrenal weight data, making it more difficult to detect any effect of treatment that may have occurred. Heart: Among male rats of the 6000 ppm dose group there were statistically significant increases in heart weight of 17% (relative to body weight) and 10% (relative to brain weight) and a non-statistically significant increase in absolute heart weight of 6%. The concerted effects of a 6% increase in absolute weight and the decline of body weight and a modest brain weight decline explains the significant relative heart weight increases. The effect on heart weight is here considered an effect of treatment at 6000 ppm. Among females, there were no remarkable treatment related effect on heart weight. Kidneys: Among males, kidney weight was statistically significantly increased at 6000 ppm by all three modes of expression, 11% (absolute), 22% (relative to body weight and 15% (relative to brain weight). Among females, kidney weight was statistically significantly increased at the top two doses by all three modes of expression. The respective increases at 6000 and 12000 ppm were 22% and 37% (absolute), 28 and 72% (relative to body weight) and 23% and 41% (relative to brain weight). Liver: Among male rats, liver weight was statistically significantly increased at 6000 ppm, 34% (absolute), 47% (relative to body weight) and 38% (relative to brain weight). Although not statistically significant, the respective increases at the 500 ppm dose level were 8%, 10% and 7%. For females, liver weight was statistically significantly increased at 6000 ppm and 12000 ppm, the respective increases being 30%

and 31% (absolute), 35% and 61% (relative to body weight) and 31% and 35% (relative to brain weight). Non-statistically significant increases at 500 ppm were 7% (absolute), 4% (relative to body wight) and 7% (relative to brain weight). Spleen: There were no remarkable effects of treatment at any dose level for rats of either sex. Testes/Epididymides: At 6000 ppm there were non-statistically significant weight increases of 11% (absolute), 21% (relative to body weight) and 15% (relative to brain weight). Ovaries: Very remarkable decline in ovary weight at all doses suggests control values were excessively high (note high standard deviation for the control). Thyroid/parathyroid: Among males there were statistically significant increases at 6000 ppm of 19% (absolute), 29% (relative to body weight) and 22% (relative to brain weight). Respective non-statistically significant increases at 500 ppm were 98%, 82% and 77% (note the high standard deviation for the absolute weight mean value). For females, respective decreases in weight at 12000 ppm were 26%, 8% and 24%. Of these values, only the change relative to body weight (i.e., 8%) was statistically significant. At 6000 ppm respective decreases were 8%, 6% and 8%, all of which were statistically significant. There were non-statistically significant decreases at 50 ppm and 500 ppm, which may be indicative of inordinately high control weight (standard deviation for the control group was considerably greater than for the other groups).

In conclusion, at terminal sacrifice, heart weight was increased among males at 6000 ppm. Kidney weight and liver weight were increased in males at 6000 ppm and in females at 6000 ppm and 12000 ppm. There was a small non-statistically significant increase in liver weight among 500 ppm males by all three modes of expression. Whether this is treatment related is uncertain. Also uncertain is a non-statistically significant increase in testes/epididymides at 6000 ppm. Thyroid/parathyroid weight was increased for males at 6000 ppm, and likely so at 500 ppm, and decreased among females at 12000 ppm and likely so at 6000 ppm.

2. Gross Pathology:

a. Three-Month Interim Sacrifice:

Ten rats/sex/group were sacrificed at the three month time point into the study with primary intent to address ocular effects findings. However, each rat received a complete postmortem macroscopic examination. Inspection of Appendix M, Table 1A (pp. 2604-2608 of the study report) does not reveal any noteworthy macroscopic findings except perhaps 2 of 10 females with ovarian cyst in the 12000 ppm group and 1 of 10 in the 6000 ppm group, with none in lower dose groups or control.

b. Six-Month Interim Sacrifice:

Ten rats/sex/group were sacrificed at the six-month interim sacrifice, again with primary intent directed toward the visual system. Inspection of Appendix M, Table 1B (pp. 2609-2613 of the study report) does not reveal any noteworthy findings.

c. Twelve-Month Interim Sacrifice:

Fifteen rats/sex/group were sacrificed at the twelve month time point. An examination of macroscopic findings as presented in Appendix M , Table 1C (pp. 2614-2619 of the study report) does not disclose any clear treatment related findings. The following are noted. There were a number of abnormalities of the eyes, but were random in nature and not evidently treatment related. One in 14 male rats of the 12000 ppm group exhibited irregular surface of the kidneys. One in 15 females in both the 6000 ppm and 12000 ppm groups had liver "module(s)/mass(es)."

d. Terminal (24-month) Sacrifice:

Rats surviving to term were sacrificed. An examination of macroscopic findings as presented in Appendix M, Table 1D (pp. 2620-2627 of the study report) reveals the following. Irregular surfaces of the kidneys where the following incidences were found: 1/37 (3%); 2/41 (5%); 4/29 (14%) and 4/14 (29%) among males of the respective 0, 100/50, 500 and 6000 ppm dose groups and among females 0/38 (0%); 0/41 (0%); 3/41 (7%); 2/34 (6%)

of the same respective dose groups plus 2/20 (10%) of the 12000 ppm dose group. Enlarged liver was observed among 0/37 (0%); 1/41 (2%); 2/29 (7%) and 3/14 (21%) male rats of the 0, 50/100, 500 and 6000 ppm dose groups. Liver enlargement was not a reported finding among females of any group. Macroscopic findings were not reported for the nose/turbinates of any animal in any dose group.

e. Unscheduled Deaths:

The following are noteworthy findings derived from an inspection of Appendix M, Table 1E (pp 2628-2636 of the study report). Incidences for irregular surfaces of the kidneys were for male rats: 2/18 (11%); 3/14 (21%); 2/26 (8%); 19/41 (46%) and 23/56 (41%) for the 0, 50/100, 500, 6000 and 12000 ppm groups, respectively. For females, the respective incidences were 1/17 (6%); 0/15 (0%); 1/14 (7%); 2/21 (10%) and 13/35 (37%). Rats described as emaciated, presented in the same order, males: 0/18 (0%); 3/14 (21%); 4/26 (15%); 7/41 (17%); and 23/56 (41%), females: 2/17 (12%); 5/15 (33%); 5/14 (36%); 8/21 (38%) and 14/35 (40%).

f. Overall Macroscopic Postmortem Findings:

The following are noteworthy findings from inspection of Appendix M, Table 1F (pp. 2637-2646 of the study report). Incidences for irregular surfaces of the kidneys, males: 3/90 (3%); 5/90 (6%); 6/90 (7%); 23/90 (26%) and 24/90 (21%) at the 0, 50/100, 500, 6000 and 12000 ppm dose levels. The respective incidences for females: 1/90 (1%); 0/90 (0%); 4/90 (4%); 4/90 (4%) and 15/90 (17%). Emaciated incidences in the same respective order, males: 0/90, 4/90, 4/90, 7/90 and 23/90, females: 2/90, 5/90, 5/90, 8/90 and 15/90.

In conclusion, with respect to macroscopic findings, the following appear to be treatment related. Increased incidences of irregular surfaces of the kidneys for males at the 500, 6000 and 12000 ppm dose groups and for females at 12000 ppm. Increased incidence of emaciation were observed for males and females at 6000 and 12000.

3. Microscopic Findings:

a. Three-Month Interim Sacrifice

Ten rats/sex/group were sacrificed at the three-month time point with the specific purpose of evaluating ocular tissues. Generally, such tissues were examined microscopically in the control and 12000 ppm dose groups. Inspection of Appendix M, Table II A (pp. 2648-2653 of the study report) did not reveal any remarkable microscopic findings.

b. Six-Month Interim Sacrifice

Ten rats/sex/group of the control and 12000 ppm groups sacrificed at the six-month time point were examined microscopically for ocular tissue effects. Inspection of Appendix M, Table III B (pp. 2654-2660 of the study report) yielded the following noteworthy findings: one female rat in the 12000 ppm group exhibited lens degeneration and one exhibited retinal degeneration/atrophy. Individual animal data sheets disclose the same rat accounted for both of the findings.

c. Twelve-Month Interim Sacrifice

Fifteen rats/sex/group were sacrificed at the twelve-month time point. All rats in the control and 12000 ppm groups were examined microscopically for effects in all tissues. Lower dose level groups were also examined sequentially as considered needed. The following are noteworthy findings: plasma cell hyperplasia of the mesenteric lymph node in three of the 15 high dose group females versus none in the control. Subacute-chronic inflammation/chronic nephropathy was observed in 6 of 15 high dose group females versus none in the control. While in males the effect was found in all males of the high dose group versus 11/15 in the control. The high dose group male findings were of higher order of severity than in the control. These findings support that tissues in the lower dose groups of both sexes should have been

examined for the assessment of possible dosing-related early onset of this effect. Testicular maturation arrest/degeneration/atrophy of the germinal epithelium (unilateral) was observed in 3 of 14 high dose group males versus 1 of 15 in the control group. The one observed in the control group and one of the three in the high dose group were classified as "minimal," the lowest rating in terms of severity. However, the remaining two in the high dose group were of higher order of severity. Also, in both of the rats, oligospermia was noted. An effect not observed in any of the controls or in any other high dose group of rats examined. As it turns out, after terminal (2-year) sacrifice, testicular degeneration/atrophy was observed in virtually all rats of all study groups, control included. This finding at 12 months may indicate an earlier onset of this condition in dosed animals. Hence, rats at the lower dose group levels should have been examined for this lesion at the 12-month time point. Retinal degeneration/atrophy (unilateral) was observed in 3 of 14 male rats of the high dose group versus 1 of 15 in the control group. One of the three in the high dose group was rated severe (the highest rating). This particular rat exhibited degeneration of the lens, also noted as severe. However, among females, there was one rat in the control with this lesion that was rated moderate while none was observed in the high dose group.

Microscopic examination of nasal turbinate sections revealed many dosing-related findings such that rats in the 500 and 6000 ppm dose groups were examined in addition to those of the 12000 ppm and control groups. There are several findings that appear elevated in incidence in rats of both sexes in both dose groups 4 and 5. These are delineated as follows: nasal turbinate, Section 2: nasal mucosa (respiratory)-hyperplasia; nasal mucosa (olfactory)-epithelium degeneration. Nasal turbinate, Section 4: nasal mucosa (olfactory)-epithelial degeneration, epithelial

hyperplasia, epithelial cysts and olfactory epithelium replaced by ciliated and non-ciliated epithelial cells. In addition, in nasal turbinate Section 2, among male rats only of groups 4 and 5 there were increased incidences of subacute (chronic active)/chronic inflammation of the nasal mucosa (olfactory).

The effects observed as described above were clearly evident in both groups 4 and 5, but appear to be confined to these two dose levels in both sexes, as characterized.

d. Terminal Sacrifice

Non-neoplastic Findings:

Findings which the study author considers related to malathion treatment include those of nasoturbinal and nasopharyngeal tissues, the kidneys and stomach (p. 84 of the study report). Those particular findings will be evaluated first.

1) Nasal Tissues:

For purposes of facilitating discussion of nasal tissue effects, appended as Exhibit 10 is a copy of the "Consolidated Nasoturbinal Tissue Findings", as reproduced from pp. 86-88 of the study report. An inspection of the "Expanded Incidence Summary Data for nasal tissue effects (pp. 2932-2943 of the study report) reveals that data were obtained for two sectionings of the nasal passages (sections 2 and 4), and these data from the two sectionings are combined to form the table of consolidated findings. One must refer to individual animal data to confirm the consolidated findings.

Both the respiratory and olfactory epithelia of the nasal mucosa were adversely affected in rats of both sexes at the two high dose levels. Effects seen in both types of epithelia include hyperplasia, subacute (chronic active)/chronic inflammation and dilated glands. However, there are other endpoints for which there were increased

incidences among both sexes at the top two dose levels that involve only the olfactory epithelium. These include degeneration of the olfactory epithelium, epithelial cysts (considered as an aspect of the degenerative process), the olfactory epithelium being replaced by ciliated and nonciliated columnar epithelial cells, and hyperplasia of the ciliated and nonciliated columnar epithelial cells which replaced the olfactory epithelium. This is interpreted to mean that in the case of the nasal mucosa, the olfactory epithelium (a neural tissue) is being compromised and replaced by a nonneural tissue as a result of test material at the top two dose levels.

For these particular effects there is no clear evidence to indicate that the nasal mucosa was affected in rats of either sex at the doses of 500 or 100/50 ppm, i.e., NOEL = 500 ppm.

Since anatomically the olfactory epithelium is located essentially posterior to the respiratory epithelium in the nasal passages, section 4 discloses more effects on the olfactory epithelium, while section 2 is more expressive of effects occurring in the respiratory epithelium. According to Boorman et al (1990), a publication cited on p. 93 of the study report, it is customary at NTP to take three sections of the nasal passages, while according to these same authors, some laboratories take four sections. Given that remarkable nasal tissue effects were found in this study of malathion, an outstanding question is whether two sections should be considered adequate. More will be said about this in the discussion of neoplastic effects.

With regard to the nasal tissue findings, we are unable to say whether the effects are the result of systemic exposure via blood supply to nasal tissues or local, i.e., resulting from inhalation of compound laden food particles during feeding, or the concerted effects of both types of exposure. We are more inclined to conclude the effects are due to systemic exposure. Bogdanffy et

al (1987) cite information indicating that nasal passages are highly vascularized, receiving up to 0.9% of cardiac output. Also, Morgan (1994) says that the nose has a rich and complex vascular system and that for noninhaled materials, and possibly certain inhaled gases or vapors, vascular delivery of toxic chemicals is probably a significant cause of nasal lesions. He also says that local blood perfusion and/or regional metabolism combine to produce patterns of tissue damage.

There are possible reasons why the olfactory epithelium may be more vulnerable than the respiratory epithelium to compound induced degeneration as observed in this study of malathion. The olfactory epithelium is a neuronal tissue consisting of sustentacular, sensory and basal cells. The sensory cells are bipolar neurons interposed between the sustentacular cells. The basal cells are considered to be the stem cells for the regenerating olfactory neuroepithelium. Olfactory neurons in contrast to other neurons, are capable of regeneration. Also Bowman's glands are located in the olfactory epithelium. [Boorman et al (1990)]. There are publications which show that the respiratory tract epithelium as a whole contains cytochrome P-450 activity, which is particularly rich in the olfactory epithelium. [Reed et al (1993)] As studied in the dog, Dahl et al (1982) reports activity of cytochrome P-450 in the olfactory epithelium comparable to that of the liver. They suggest that high activity in the olfactory epithelium may play a role in the removal of odorants from the olfactory tissue and may be important in chemical-induced tumorigenicity.

Bogdanffy et al (1987) say that preferential degeneration of the olfactory epithelium, as opposed to the respiratory epithelium, by certain types of compounds may be due to high levels of carboxylesterases located in the olfactory epithelium. (Apparently such activity in the olfactory region is localized in Bowman's gland and sustentacular tissue as

opposed to neurons.) Specifically, they say, for example, that acute inhalation exposure of rats or mice (of either sex in either species) to certain esters results in selective degeneration of the olfactory epithelium characterized by necrosis of the neuronal cell layer. They view the effect to be systemic even though exposure was via inhalation. The rationale for the selective degeneration of the olfactory epithelium is the effect of acids generated via carboxylesterase hydrolysis of the esters. Olfactory neurons they say may be "exquisitely" sensitive to acids. Also, Trela et al (1992) claim, based upon their own studies on the nasal tissue effects of dimethyl adipate in concert with the published findings of others on dimethyl glutarate and dimethyl succinate, that esters of such diacids exert a selective degenerative effect on the olfactory epithelium (as opposed to the respiratory epithelium), attributable to the location of carboxylesterases within the olfactory epithelium. These findings are particularly relevant in the review of this study on malathion, since this agent is a diethylester of a dibasic acid. Malathion is a diethyl phosphorodithioate derivative of succinic acid. It is well known that carbylesterases located in the liver and plasma of the rat will catalyze hydrolysis of the malathion ester groups to yield mono- and dibasic acids that no longer inhibit acetylcholinesterase, i.e., this is the mechanism of detoxification of malathion in the cholinergic sense. Thus from these publications there are reasons to explain the more selective effect observed in the malathion study in terms of degeneration of the olfactory epithelium, i. e., possible metabolism by cytochrome P-450 and hydrolysis by carboxylesterase, effects like those expected to occur in the liver.

2) <u>Nasopharyngeal Hyperplasia</u>:

The tabulated incidences of nasopharyngeal hyperplasia of the respiratory epithelium as presented in the study report (p. 89) are reproduced here as Exhibit 11. There were

increased incidences in both males and females at 6000 ppm and among females at 12000 ppm. High mortality among males of the 12000 ppm group serves to explain the lack of a finding among that group. The NOEL for this finding is 500 ppm in rats of both sexes.

3) Kidneys:

Incidences of bilateral subacute-chronic inflammation/chronic nephropathy as summarized in the study report (p. 90) are reproduced as Exhibit 12. This particular nephropathy was of high incidence in all groups, control included. In terms of incidence there appears to be no particular treatment related effect in rats of either sex. However, upon consideration of the aspect of severity of the findings, there was evident a treatment related increased effect, for which the NOEL was 500 ppm in male rats and 100/50 ppm for female rats. The study author says that ". . . treatment with malathion appeared to have exacerbated the severity", but considers the severity to have increased only at the top two dose levels. The study author also says "This finding correlated with the surface irregularities of the kidneys which were noted macroscopically and with the increased kidney weight observed at necropsy" (p. 90 of the study report).

4) Stomach:

According to the study report "When examined by light microscopy, squamous cell hyperplasia and hyperkeratosis of the epithelium covering the forestomach (non-glandular portion) were seen in numerous decedents from the 6000 ppm and 12000 ppm dose levels and in a small number of those from the 0, 100/50, and 500 dose levels. Congestion, edema, erosions/ulcers and acute to chronic inflammation were also seen in one or more of the affected animals" (p. 91 of the study report). An inspection of the Expanded Incidence Summary for Microscopic Findings for the Stomach, as reproduced here from page 2885-2889 of the study report,

Exhibit 13, discloses the following treatment related effects at 6000 and 12000 ppm:

Congestion of the forestomach (M,F),forestomach edema (M,F), erosion(s)/ulcer(s)/necrosis of the forestomach (M,F), acute/subacute inflammation of the forestomach (M, F) subacute (chronic active)/chronic inflammation of the forestomach (M,F), basal cell hyperplasia of the forestomach (M),(F at 12000 ppm only), squamous cell hyperplasia of the forestomach (M,F), hyperkeratosis of the forestomach (M,F). The bulk of the findings were in rats that did not survive to terminal sacrifice. The study report attributes these findings to likely decreased food intake in moribund animals in concert with continued proliferation of the squamous epithelium covering the forestomach, such that the forestomach becomes prominent and cornified. Regardless of the explanation for these changes in the forestomach, the effect appears to be limited to the 6000 and 12000 ppm groups for both sexes. Gastric erosions and ulcers in the rats of the higher dose groups are attributed by the study report to stress (p. 92 of the study report). The NOEL is 500 ppm (M,F) for these effects on the forestomach.

Further inspection of the "expanded incidence summary of microscopic postmortem findings" (pp. 2867-2954) for additional non-neoplastic findings discloses the following (dose groups in which findings were identified are numbered 2, 3, 4, 5, representing the 100/50, 500, 6000 and 12000 ppm dose levels, respectively):

<u>Congestion</u>: This histopathologic end point was a reported finding in all study groups for many tissues including brain, thyroid gland, thymus gland, salivary gland, mediastinal lymph node, liver, kidney, forestomach, pituitary gland, adrenal gland, harderian gland, sternal marrow, femoral marrow, nasal mucosa and possibly others. Among certain of these tissues, histopathology was performed on all animals

in all groups in accordance with Guideline requirements. From among those listed examples include thyroid, lung, liver, forestomach. In other cases only the control and high dose group animals were examined and then sequentially the next lower dose groups as evidently considered necessary by the study pathologist in search of a NOEL. As examples, such tissues include brain, heart, pancreas, pituitary, harderian gland, lacrimal gland. In certain cases where we were not satisfied that a NOEL had been secured, we estimated (conservatively) what the incidences of congestion might have been at a lower dose(s) by combining incidences obtained for unscheduled deaths (which the guidelines require for all such animals) with assumed zero incidences of congestion for all terminally sacrificed animals.

The overall results of the assessment of data supports the conclusion that congestion was a positive (or predicted positive) finding for the tissues listed below for the dose groups indicated.

| **Dose Groups Positive for Increased Tissue Congestion relative to Controls** | | |
|---|---|---|
| | **Males** | **Females** |
| Liver | 4,5 | 5 |
| Kidneys | 4,5 | 4,5 |
| Thyroid | 3,4,5 | 5 |
| Brain | 3,4,5 | 5 |
| Heart | 3,4,5 | 4,5 |
| Lung | 4,5 | 4,5 |
| Forestomach | 4,5 | 4,5 |
| Pancreas | 3,4,5 | - |
| Pituitary | 4,5 | 5 |
| Harderian Gland | 3,4,5 | 4,5 |
| Lacrimal Gland | 3,4,5 | 4,5 |
| Sternal Marrow | 3,4,5 | 4,5 |

| | | |
|---|---|---|
| Femoral Marrow | 3,4,5 | 4,5 |

These findings show increased incidences of congestion in certain tissues in the top three dose groups(males) and top two dose groups (females), which are probably correlates of increased mortality in these groups evident in the mortality data. The findings in the case of group 3 males is of particular interest as it is consistent with the conclusion of increased mortality in that group.

Continuation of findings: thyroid (follicular cyst) 5 (M,F); parathyroid (hyperplasia) 2, 3, 4, 5 (M,F) mediastinal lymph node(lymphoid cell depletion/atrophy) 3, 4, 5 (M); lungs alveolar collapse 4, 5 (M); liver (spongiosis hepatis) 4, 5 (M), 5 (F); spleen (lymphoid cell depletion/atrophy) 4, 5 (M,F); mesenteric lymph node (lymphoid cell depletion/atrophy) 4, 5, (M), 5 (F); adrenal glands (bilateral cortex zona glomerulose - vesiculated/vacuolated) 4, 5 (M); eyes (cornea, mineral deposits 3, 4, 5 (M), 5 (F); eyes(bilateral-cornea, neutrophilic infiltrate) 4, 5 (M), 5 (F).

In commenting on these various findings the study report claims: " Numerous tissues and organs from the decedents were congested. These animals were either not exsanguinated (found dead or accidental death) or were incompletely exsanguinated (sacrificed in extremis) prior to postmortem examination. In the decedents from the treatment groups, this finding was not considered to be related to the dietary administration of malathion." (p. 92 of study report). It appears reasonable that rats in the moribund condition would be expected to have multiple and varied tissue evidence of congestion. However, to the extent that premature mortality itself occurred in this study particularly as evidenced at the two highest dose levels, the finding of congested tissues would not be unanticipated and, hence, at least an indirect effect of treatment with malathion. To the extent that increased incidences of congested tissues is indicative of animals in extremis as a result of treatment, while the bulk of the findings

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were in the 6000 and 12000 ppm groups, congestion was of such frequency in the 500 ppm (group 3), particularly in males, to support a conclusion that 500 ppm is an effect level. This also supports increased mortality as an effect level at 500 ppm in males.

The study report also advises that "Also considered to be stress associated was the lymphoid cell depletion/atrophy in the thymus and spleen and the mediastinal and mesenteric lymph nodes in a number of the animals which were killed in extremis or were found dead," (p. 92 of study report).

It is to be noted there was no NOEL for parathyroid gland hyperplasia. (Exhibit 14) In rats of both sexes the incidence was essentially constant across all doses, i.e., no dose response, but high mortality at 12000 ppm in males and females and at 6000 ppm in males as well may have preempted a dose response. Parathyroid hyperplasia may be a corollate of kidney chronic nephropathy which was a principal cause of mortality in this study. Parathyroid harmone increases the movement of calcium from bone into extracellular fluid in the regulation of extracellular calcium concentration. It also increases renal tubular calcium resorption.

b. Neoplastic Findings:

1) Liver: According to the study report, "Hepatocellular adenomas and carcinomas were seen in a small number of males and females from the treatment and/or control groups." (Note: these were not seen in the control female group.) "Among the females, the incidence was statistically significantly increased for both neoplasms at the 12000 ppm dose level and for the incidence of hepatocellular adenoma at the 6000 ppm dose level. At these dose levels, the increased incidences of both neoplasms were considered to be attributed to the dietary administration of malathion."

(p. 94 of the study report). A tabulation of the findings as presented on p. 95 of the study report is reproduced below.

| Dose Levels (ppm) | 0 | 100/50 | 500 | 6000 | 12000 |
|---|---|---|---|---|---|
| **MALES** | | | | | |
| Hepatocellular Adenoma | 2/70 (2.9%) | 2/55 (3.6%) | 3/55 5.5%) | 2/55 (3.6%) | 1/70 (1.4%) |
| Hepatocellular Carcinoma | 1/70 (1.4%) | 2/55 (3.6%) | 1/55 (1.8%) | 1/55 (1.8%) | 0/70 (0%) |
| **FEMALES** | | | | | |
| Hepatocellular Adenoma | 0/70 (0%) | 1/55 (1.8%) | 1/55 (1.8%) | 3/55[a,c] (5.5%) | 3/70[b] (4.3%) |
| Hepatocellular Carcinoma | 0/70 ()%) | 1/55 (1.8%) | 1/55 (1.8%) | 0/55 (0%) | 3/70[b] (4.3%) |

a. $p \leq 0.05$ (Fisher Exact Test, Haseman Test, and Cox's Test).
b. $p \leq 0.05$ (Haseman Test, Cox's Test and Gehan-Breslow).
c. $p \leq 0.01$ (Gehan-Breslow).

There is no apparent increased tumorigenic response among males. It is important to note, however, that among males there were no survivors at term in the 12000 ppm group, and survival was reduced to 26% at term in the 6000 ppm group as compared with 67% survival in the control group and 75% survival in the low dose (100/50 ppm) male group. Hence, while not discussed in the report, it is fair to say that male rats, particularly in the 6000 and 12000 ppm groups were not as at risk as control and lower dose groups for the full exposure period, due to competing toxicity of the test material. Therefore it cannot be affirmed that males were adequately evaluated at the 6000 and 12000 ppm dose levels. Among females, there was clearly a tumorigenic response of the liver at 6000 and 12000 ppm. At 12000 ppm, the combined incidences of adenomas and carcinoma was 8.6% versus 0% in the control group, a finding, which is clearly statistically significant. It would actually be more appropriate to express all incidences on a 55 rat/group basis since 15 rats each in the control and high dose groups were

actually eliminated at the 12 month interim sacrifice. Under that circumstance, the combined incidence at 12000 ppm would be 10.9% versus 0% in the control, an even more remarkable finding. It is also noteworthy, that survival at terminal sacrifice in the 12000 ppm female group was compromised relative to the control group, and hence, the potential for a tumorigenic response in the group was also compromised by reduced survivorship. There was no evidence from liver histopathology in terms of non-neoplastic findings (e.g., hepatocellular hypertrophy or hyperplasia) for female rats to suggest that the 12000 ppm was an excessive dose with respect to the liver. However, liver weight was increased at 6000 and 12000 ppm for female rats.

From the standpoint of historical control data, the study report claims that: "In the NTP historical control data, the incidence of hepatocellular adenoma in 1979 comparable, untreated F344 females was 2.3% with a range of 0-10%; the incidence of hepatocellular carcinoma was 0.2% with a range of 0-2%. In this laboratory, in six previous studies (254 control females), the incidence of hepatocellular adenoma was 1.6% with a range of 0-5.4%; the incidence of hepatocellular carcinomas was 1.1% with a range of 0-2.4%." (p. 94 of the study report) These findings are tabulated below as reproduced from p. 95 of the study report. No historical data were provided on combined incidence. **NOTE**: In Appendix P (p. 5596 of the study report) presenting HLS historical data, study dates for Study Code Nos. 8, 58 and 59 require editing.

NOT

| Historical Control Data (Hepatocellular Tumors in Female F344 Rats) | | | | |
|---|---|---|---|---|
| **Type of Tumor** | **NTP** | | **HLS** | |
| | **Mean** | **Range** | **Mean** | **Range** |
| Hepatocellular Adenoma | 2.3% | 0-10% (n = 1900) | 1.6% | 0-5.4% (n = 254) |
| Hepatocellular Carcinoma | 0.2% | 0-2% (n = 1900) | 1.1% | 0-2.4% (n = 254) |

The source of the NTP historical information mentioned above is given in the study report as Haseman et al (1990). An independent inspection of that publication reveals that for control female F344 rats in feeding studies, the incidence of "neoplastic nodules" (taken to mean adenomas) of the liver is 45/1979 (2.3%) and for "carcinomas" of the liver is 3/1979 (0.15%). In a more recent publication of the NTP historical data, National Institute of Environmental Health Sciences (1996), the historical incidence in oral feeding studies among female F344 rats is "hepatocellular adenoma" 8/1351 (0.59%) and "hepatocellular carcinoma" 1/1351 (0.07%). It is noteworthy that in this particular publication of the NTP data base, the single incidence of carcinoma in 1351 feeding study control females was actually the only incidence of hepatocellular carcinoma recorded in a total of 3621 control female F344 rats when other types of chronic studies, i.e., gavage (corn oil and water vehicle), oral (water vehicle), inhalation (air), are included. It is reasonable to conclude that spontaneous hepatocellular carcinomas of the liver of female F344 rats is extremely rare in the NTP data base. A spokesman at NIEHS who worked on the 1996 NIEHS publication advised (oral communication) that the 1996 publication provides a more contemporary window of historical control data than does Haseman et al (1990). Dr. Joseph Haseman rendered via personal communication the opinion that the more recent NTP data is more appropriate than the 1990 data for use with a study as recent as 1995. He also advised that the term neoplstic nodules is not strictly adenomas only. The more recent (1996) data is limited to hepatocellular adenomas and is of lower incidence.

In conclusion, for males there was no clear evidence of a treatment related tumorigenic effect on the liver. However, high mortality in the 6000 and 12000 ppm groups may have compromised the risk factor of developing such tumors, particularly if late occurring.

Among females, there was a treatment related statistically significant carcinogenic effect at 6000 and 12000 ppm, the expression of which may also have been somewhat compromised at 12000 ppm due to increased

unscheduled deaths in that group. The increased incidences of adenomas and carcinomas, 3.6% combined, in both of the lower dose groups are also considered to be results of treatment. This is rationalized on the grounds that 1) The zero incidence (particularly of carcinoma) in the control group is not unexpectedly low; 2) further increased findings were observed at the higher doses, i.e., 5.5% at 6000 ppm and 10.9% at 12000 ppm; and 3) the hepatocellular tumor incidences (particularly carcinoma) in female F344 rats are very rare. In support of the conclusion is the following comment on the interpretation of rare tumors from the Office of Sciences and Technology Policy (1985).

"The spontaneous tumor incidence can be of considerable importance in the interpretation of results from carcinogenicity studies. If, for example, the effect of a chemical is to double or triple the background tumor incidences, tissue sites with low spontaneous tumor rates are more likely to yield false-negative results than are sites with high spontaneous tumor rates. For example, if a tumor is a rare tumor even a slight increase in incidence may be biologically significant and may be considered adequate evidence of carcinogenicity (53). This factor must be taken into account in the overall evaluation of the data. For such tumors (e.g., with spontaneous tumor rates of 1% or less) the utilization of historical control data may be particularly useful in increasing the sensitivity of the study for detecting carcinogenic effects." (P. 10418)

It is noteworthy that microscopic examination of the liver did not disclose any attendant dosing related increased hypertrophy, hyperplasia, necrosis or inflammation that might provide a corollary to neoplasia. Just how important such findings might be to the interpretation of increased incidences of rare tumors is perhaps open to discussion.

2) Nasoturbinal Tissues: The study report says the following: "Neoplasms which were considered to be related to treatment with malathion were seen in the nasoturbinal tissues and liver. In the nasoturbinal

tissues, an adenoma was observed in one male (animal number 4033) from the 6000 ppm dose level and a carcinoma was observed in one male (animal number 5040) from the 12000 ppm dose level. Spontaneous neoplasms of the nasoturbinal tissues are rare in F344 rats. In untreated dietary and corn oil control animals from eight recent NTP studies only six were identified from nearly 4000 control males and none occurred in a similar number of control females (citing Boorman et al, 1990). None have been observed in this laboratory in six previous studies (238 control males and 241 control females." (p. 93 of the study report) The historical NTP data discussed in Boorman, et al (1990) is published in Haseman et al (1990), mentioned previously.

An inspection of the summary table of microscopic findings in the study report confirms the finding of the two tumors in male rats. However, both tumors occurred in the olfactory region of the nasal mucosa. (individual animal data, pp. 3805 and 4100 of the study report) An independent reading of Boorman et al (1990) confirms nasal tumors as rare among NTP historical control. However, the claim of some six tumors among nearly 4000 control males is with reference to the respiratory epithelium (confirmed by personal communication with the principal author and inspection of Haseman, et al (1990). Boorman et al (1990) and Haseman, et al (1990) claim/identify zero incidence of tumors of the olfactory epithelium from among nearly 4000 control male rats, and none among a similar number of control females. In fact, Boorman et al (1990) says "Neoplasms of the olfactory epithelium have occurred in F344 rats exposed to certain carcinogens, but have not been observed in controls." (p. 332) So the finding in this study of two such tumors of the olfactory epithelium is exceedingly rare indeed, and heretofore unique to carcinogens.

Since the histopathology sheets for the two nasal tumor bearing rats in question say no more than the tumors are located in the olfactory epithelium, it would be important for the registrant to say just what kinds of tumors (i.e.,

of what tissue) these are. For example, are they of the neurons or other tissues of the olfactory epithelium.

In support of a conclusion that the neoplastic findings in question are treatment-related, we would cite here the quotation of the Office of Science and Technology Policy (1985) p. 10418 reproduced on p. 54 of this review.

The above cited background incidence for nasoturbinal tissues among male rats (6/4000) is about 0.15% (or 0% in the case of tumors of the nasal olfactory epithelium) while the incidence in this study was 1/55 or 1.8% in each of two dose groups, specifically the 6000 and 12000 ppm dose groups.

Thus, the finding of the two rare nasal passage tumors in this study viewed in light of the position of OSTP (1985) on the assessment of rare tumors serve to support the study author's conclusion that these are test material related responses. A question to be posed at this point is whether additional nasal sections should be examined to help confirm the absence of such effects at lower doses. More on this will follow.

Further inspection of the expanded incidence summary of microscopic findings in the study report discloses two additional rats (female in this case) with malignant tumors identified in nasal turbinate Section 4 (p. 2943 of the study report). These are described as "squamous cell carcinoma arising from the squamous epithelium lining the alveolus of a tooth." One was seen in a female rat, No. 5503, from the high dose group and the other in a female rat, No. 2546, of the low dose group. It is noteworthy that only fourteen rats (unscheduled deaths) were examined in the low dose group, so the incidence for this tumor is one in fourteen. In the high dose group, 65 rats were examined (includes early sacrifice animals). Such tumors are also evidently rare tumors. According to the NTP data base, again as reported in Haseman et al (1990), the incidence of squamous cell carcinoma of the oral mucosa (any site) of the F344 rat is one in nearly 4000 untreated or corn oil gavage female F344 control rats. The likelihood that that particular

finding was of the alveolus of the root of a tooth is probably low. Furthermore, these squamous cell carcinomas being located in the alveolus of the root of a tooth, as identified in the nasoturbinal sections, places the tumors in very close proximity to the nasal passages (more will be presented on this subsequently). To the extent that these tumors may possibly have a nasal tissue etiology or involvement the same NTP data shows no incidence of squamous cell carcinomas of the nasal passages among nearly 4000 female F344 rats. So whether these tumors be classified as oral or nasal, the incidence is exceedingly rare. The registrant is invited to submit any additional information that might be used to further define the historical incidence of the carcinomas in question.

A few comments are indicated to facilitate an appreciation of the anatomic closeness of the alveolar tissue of the root of the tooth to the nasal tissues. The study revealed a high incidence of periodontal disease among rats of both sexes in all study groups. There are published works which indicate that the periodontal disease process may violate separations between nasal and oral cavities perhaps making it more difficult to say how tumors in the alveolus may arise. For example, Morgan (1991) in discussing approaches to the identification and reading of nasal lesions in toxicology studies says: "Nasal lesions may result from 'spontaneous' or treatment-related dental problems. The roots of the incisor teeth lie very close to the lateral recess of the lateral meatus (45), and periodontal lesions in this region can readily involve the adjacent nasal tissues. Lesions of the molar teeth, buccal cavity, and even the skin may also lead to nasal involvement, at which point it is critical to identify the primary target site. Thus, awareness of the potential role of aging-related nasal lesions as well as changes in adjacent tissues can be of considerable value to the toxicologic pathologist during interpretation of toxic responses." (p. 341)

Further along, this author also says: "Feron and Woutersen (21) reported that periodontal inflammation

can be associated with nasal lesions in aging rats. In our laboratory, periodontal disease has been observed around the roots of the incisor teeth in aging rats and mice. In some cases, these lesions were severe, and led to the development of rhinitis and granulomas that partially obstructed the nasal airway in the regions adjacent to the lateral recess of the lateral meatus. The close physical relationship between the incisor roots and this lateral recess (45) provides a ready means of progression from periodontitis to rhinitis. The fine membranes of bone separating the lateral recess of the lateral meatus from the periodontal ligament are frequently structurally altered during this process, with localized bone dissolution permitting passage of the inflammatory process into the adjacent nasal tissue." (p. 343)

This discussion is introduced here to illustrate the close proximity of the nasal cavity and that of the alveoli of roots of teeth. It is therefore difficult for this reviewer to appreciate the level of distinction that may exist between the etiology of neoplasms of the two regions, particularly when periodontal diseases may be a complicating variable. It is necessary for pathologists to provide more descriptive information on the location of tumors of the alveolus of the teeth and the conditions of surrounding tissue.

We believe that to the extent the two neoplasms of the nasal olfactory epithelium are to be considered compound related because of infrequent incidence among historical controls, the two squamous cell carcinomas of the alveolus of roots of teeth should also be considered compound related. These latter findings should also have been discussed in the text of the study report.

Of added concern is the fact that all rats in the low dose group were not examined histopathologically for nasal tissue effects. The FIFRA Guidelines for carcinogenicity testing (83-2) reads under the <u>histopathology</u> section, "The following histopathology should be performed": "Target organs in all animals" [paragraph (e)(11)(i)(C)] and "If a significant difference is observed in hyperplastic, preneoplastic or neoplastic lesions between

the highest dose and control groups, microscopic examination should be made on that particular organ or tissue of all (emphasis added) animals in the study" [paragraph (e)(11)(iii)]. In this particular study hyperplasia of the nasal respiratory and olfactory epithelia was observed at the top two dose levels. In addition, there was an adenoma of the olfactory epithelium of a male rat at the penultimate dose level and a carcinoma of the olfactory epithelium of a male rat of the highest dose group. Add to this the finding of two squamous cell carcinomas of the alveolus of the roots of teeth, one in a low dose group female and one in a high dose group female, we must conclude there was an incumbency on the performing laboratory to have examined the nasoturbinal slides (2 and 4) not only of the 14 male and 14 female rats of the low dose groups that died early, but for all animals in that group.

Inspection of individual animal histopathology data sheets for the low dose female group shows that of the 14 rats to be examined for nasal tissue effects, one data sheet (#2503) claims the nasal tissue to be missing, three (#s 2525, 2545, 2552) claim "extensive postmortem autolysis" and one (2541) claims moderate postmortem autolysis". It could therefore be argued that of 14 rats (unscheduled deaths) in question, only 9 could be expected to provide fully adequate histopathologic information, one of which did show the squamous cell carcinoma of the alveolus of the tooth.

The fact that nasal tissue sections for all rats in the low dose group were not similarly examined is considered a deficiency.

Further to these particular findings more than two sections of the nasal mucosa should be examined. For instance, Morgan(1991) says that if nasal lesions are expected, it is recommended that a series of 6-8 section levels from selected regions be prepared for laboratory rats. The registrant should discuss with EPA examining additional segments of the nasal epithelium.

3) Testes: Testicular interstitial cell tumor incidence was extensive in all groups. The appended Exhibit 15 contains selected pages from the Expanded Incidence Summary of the study report which incorporate incidences of unilateral and bilateral interstitial cell hyperplasia and interstitial cell tumors for (a) the 12-month interim sacrifice, (b) unscheduled deaths, (c) terminal sacrifice and (d) consolidated findings.

At the 12-month time point, 15 rats in both the control and high dose groups were examined histopathologically. Incidences of hyperplasia were essentially equivalent in control and high dose groups. The combined incidences for unilateral and bilateral interstitial cell tumors were 2 (13%) in the control and 5 (33%) in the high dose groups. Among unscheduled deaths, incidences of hyperplasia appeared to be unaffected at any dose level. The combined incidences of unilateral and bilateral interstitial cell tumors were 83%, 79%, 92%, 98% and 96% for groups 1 through 5 in the given order. So among rats dying early in the study after year one, there were numerical increases in groups 3 through 5 versus groups 1 and 2. At terminal sacrifice, incidences of rats with unilateral or bilateral tumors were 100% in groups 1 through 4. Of course, no male rats in group 5 survived to terminal sacrifice.

The tabulation of consolidated incidence data for rats exhibiting combined unilateral and bilateral interstitial cell tumors from among the approximately 55 rats per group at risk post-year one were 54/55 (98%), 52/55 (94.5%), 53/55 (96%), 54/55 (98%) and 53/55 (96%).

The overall consolidated incidences of combined tumors do not illustrate a compound-related effect. However, among unscheduled deaths there appear to be increased incidences in groups 3 through 5 even though rats in groups 4 and 5 died at earlier time points. There is a possibility that the test material provokes an earlier onset of this type of tumor at the high dosages.

Proliferative lesions of the interstitial cells in the testis as disclosed in Boorman et al (1990) for the F344 rat is reproduced below.

| Sacrifice Interval[a] | | | | |
|---|---|---|---|---|
| (%)[b] | 3 Mo. | 9 Mo. | 15 Mo. | 24 Mo. |
| Hyperplasia | 0 | 3 | 33 | 54 |
| Adenoma | 0 | 0 | 80 | 83[c] |

a. Rats were 6 weeks old at start of study.
b. Percentage of animals with a lesion in one or both testes; n = 30 per group except at 24 months, when n = 100.
c. Represents both early death and terminal sacrifice animals.

In comparing the above data with that of the malathion study, there is an obviously greater incidence of hyperplasia and a lesser incidence of adenoma in the historical data at the 24 month time point than in the study under review. It remains possible, that the incidence of tumors in the current study was increased in groups 3 through 5 as evaluated on the basis of unscheduled deaths. The study author claims that "statistically, the dietary administration of malathion was considered to be associated with increased incidence of this tumor." (p. 95 of the study report) However, he goes on to say that this is a very common tumor in the F344 rat and that nearly all will develop this tumor if allowed to complete their natural life span. We should note, however, that in this study that particular condition was not met and many male rats did not live out their normal life spans, but nonetheless developed the tumors, suggesting decreased latency, a critical parameter in defining carcinogenicity. The study report also cites the high historical control incidence for the tumor, but again that historical data is for animals having normal survival and may not be appropriate to employ in this case as a reason to say there was no real effect of the test material. Presented in the study is a "statistical analysis of time to tumor data" (pp. 5345-5346 of the study report) reproduced here as Exhibit 15, in which it is concluded: "The material is associated with increased interstitial cell

testicular tumors in male rats at all (emphasis added) doses measured based on Haseman's and Fisher's test; Haseman's at the 12000 ppm dose group; Fisher's at the 50, 500 and 6000 ppm groups." (p. 5346 of study report).

4) Thyroid c-cells: In the expanded incidence summary for thyroid c-cell carcinoma for males the recorded incidences are 1/69, 2/54, 6/53, 2/54 and 0/69 for the control, 100/50, 500, 6000 and 12000 ppm groups, respectively. According to the statistician's report (p. 5347 of the study report), the increase at 500 ppm is statistically significant by Fisher's exact test, and the dose trend is positive. Excessive mortality at 6000 and 12000 ppm among males may have compromised expression of the tumor at those doses.

5) Pituitary Gland Pars Distalis Carcinoma: In the expanded incidence summary for pars distalis carcinoma for females the recorded incidences are 0/66 (0%), 1/31 (3.2%), 3/34 (8.8%), 4/34 (11.8%) and 1/69 (1.8%) for the 0, 100/50, 500, 6000 and 12000 ppm groups, respectively. High mortality in females at at 12000 ppm could have compromised expression of this tumor in that group. It appears there are increases at all other dose levels, manifesting a dose-response. According to the 1996 NTP historical control data base, the incidence of this tumor (described as "Pituitary Gland: Pars Distalis or Unspecified Site") in the oral feeding studies in female F344 rats is 14/1340 (1.04%). In 27 historical studies presented in this data base, the highest incidence in any study was 2/49 (4%), which occurred three times (the other two actually being 2/50). The study MRID submission did not report statistical analysis for this carcinoma, but did report a positive trend for adenoma. (Exhibit 16) Given the high incidence of this tumor in the 6000 ppm dose group, it could be argued that all animals in all dose groups should have been examined.

One must also conclude by inference from the statistical analysis of time to tumor data (Exhibit 16) that, in the case of males, two of the eight tumor types mentioned as

first analyzed were causes of death in this study, namely, lymphoreticular system mononuclear cell leukemia and pituitary pars distalis adenomas, and therefore were not analyzed secondarily by an incidental context method. Hence, the two tumorigenic responses were significantly increased when corrected for survivorship. We should also note from the statement in Exhibit 15 that among females mononuclear cell leukemia was statistically significantly increased in the 100/50 and 500 ppm dose groups, but with no dose response, according to the statistician.

Based upon an examination of tumor incidence summary data as tabulated in the study report, the following tumorigenic responses are being analyzed independently by the HED Statistics Team: mononuclear cell leukemia (male and female); liver adenomas, carcinomas, combined (male and female), interstitial cell tumors, one-year only data for "early onset"; thyroid follicular cell adenomas, carcinomas, combined (male only); pituitary, pars distalis adenomas, carcinomas, combined (male and female).

## III. DISCUSSION

A. The critical findings in this study might be commented on as follows. At the time of initiation of the study, dosage levels employed were 0, 100, 500, 6000 and 12000 ppm. However, due to the finding of significant erythrocyte cholinesterase inhibition at 100 ppm among female rats at the 3-month time point, the decision was made at that time to reduce the low dose level from 100 ppm to 50 ppm for rats of both sexes. Subsequent to this reduction the LOEL for erythrocyte cholinesterase inhibition proved to be 500 ppm and the NOEL 50 ppm for females, while the respective values for males were 6000 ppm and 500 ppm. It could be argued that the study did not identify a true NOEL for females, as one cannot be certain that 50 ppm would have been a NOEL for the first three months of the study given the tendency for some degree of adaptation to occur after longer time periods of testing. In the case of plasma cholinesterase inhibition, for males the LOEL was 500 ppm and the NOEL 50 ppm (100 ppm during the first 3 months), while the respective values for females was 6000 ppm and 500 ppm. For brain cholinesterase inhibition, the LOEL was 6000 ppm and the NOEL 500 ppm for rats of both sexes. Cholinesterase inhibition in general exhibited a very shallow dose

response. Despite the finding of cholinesterase inhibition in both sexes as indicated, there were no obvious cholinergic clinical signs among males at any dose level. Among females, the only reported clinical sign was anogenital staining, evident throughout the study period at the 12000 ppm dose level only. To the extent that this was a cholinergic sign, the fact that it occurred in females only may be a reflection of the greater degree of brain cholinesterase inhibition at 12000 ppm in females, 28-49% over the first three time points, than in males, 15-19% over the corresponding period of the study.

Increased mortality was observed among males in a dosing-related manner across the 500, 6000 and 12000 ppm dose levels. Increased mortality was observed among females at the 12000 ppm dose level only. Given the lack of cholinergic clinical signs among males even at the highest dose level and given the small inhibition of brain cholinesterase at the high dose level of 15-19%, as determined over the first 18-months of study, the elevated mortality that embraced the top three dose levels cannot likely be assigned in an essential way to cholinesterase inhibition, but was due to other chronic toxic effects of malathion. Specifically, increased mortality appears to have been primarily due to the effects of leukemia, chronic nephrotoxicity and the concerted effects of various other collective toxicologic burdens of the test material, which may indeed have included cholinesterase inhibition. It should be noted that among males at 500 ppm, while leukemia was a contributor to the increased mortality, the incidence of leukemia was not increased in the study group relative to that of the control group, suggesting that leukemia bearing animals were more vulnerable to other toxicologic effects of the test material. However explained, malathion exposure at the high dose level resulted in complete demise of male rats before term and resulted in increased mortality among males at the 6000 and 500 ppm dose level as well. The NOEL for mortality among male rats in this study was 50 ppm, an extremely low value given past findings with malathion. It is likely, however, that a higher NOEL for increased mortality for male rats, considerably closer to the 500 ppm dose level, could have been identified had such a dose been tested. Nevertheless, malathion by chronic exposure proved to be more toxic to male F344 rats than one might have anticipated. The MTD for male rats was exceeded. The fact that increased mortality occurred among females at 12000 ppm also indicates the MTD was exceeded for females. Since the mortality in both sexes was evidently the result of chronic exposure, it is unlikely that shorter term dose range-finding studies would have anticipated this outcome. However, in the 1979 National Cancer Institute study in the F344 rat (MRID 43269) where doses of 0, 2000 and 4000 ppm of malathion (American Cyanamid 95% t.a.i.) were used, mortality was extensive at week 103, particularly among male rats.

With regard to the influence of extent of mortality on the acceptability of a carcinogenicity study, Office of Science and Technology Policy (1985) says: "A negative test is ordinarily accepted by regulatory agencies if: .... survival of all groups (per sex per dose) is no less than 50% ... at 104 weeks for rats". (p. 10414). By this criteria of acceptability, the finding among male rats of 100% and 74% mortality at the high and penultimate doses, respectively, would preclude the validity of the study to the extent that it is negative for carcinogenicity, at least in male rats. Among females, survival in all groups well exceeded 50% except the highest dose group where survival was 36%, and should be considered acceptable for females, or certainly equivocally so. As related to this interpretation, the FIFRA Subdivision F Guidelines for chronic toxicity/oncogenicity studies says: "At the termination of the experiment at 18 months in mice and 24 months in rats the survival in any groups should not fall below 25%", and further along "The highest dose level in rodents should elicit signs of toxicity without substantially altering the normal life span due to effects other than tumors". "For rodents, the incidence of fatalities in low and intermediate dose groups and in the controls should be low to permit a meaningful evaluation of the results." (p. 138)

Generally, various clinical chemistry and hematology parameters were altered in both males and females at the 6000 ppm and 12000 ppm dose levels, which serve to confirm a burden of the test material on the system at these doses. In addition, certain of the parameters were altered at 500 ppm, including decreased aspartate aminotransferase activity, alanine aminotransferase activity and blood urea nitrogen in females at 500 ppm. Decreased blood urea nitrogen may have extended to the 100/50 ppm level in females. So there is evidence of effects on biochemical parameters for certain at 500 ppm and above, and possibly at 50 ppm.

The ocular effects testing component of this study which involved ophthalmoscopy, electroretinography (ERG) and histopathology of the retina did not yield any unequivocal effects of the test material. However, variability in ERG data was so great that if a compound-related effect of significant proportions occurred, it could have been missed. The F344 rat is considered a poor model for such testing. Effective testing for ocular toxicity remains outstanding.

With regard to pathology, organ weight data indicates that in male rats kidney, liver, spleen, heart and thyroid/parathyroid and in females kidney and liver may be target organs as evidenced by increased weight at the top two dose levels. Increased liver and thyroid/parathyroid weights may have extended to the 500 ppm dose level in males, and increased testes/epididymides weight

may have occurred at 6000 ppm. Decreased thyroid/parathyroid weight occurred in females at 12000 ppm and likely so at 6000 ppm.

Macroscopic examinations revealed increases of irregular surfaces of the kidney for male rats at 500, 6000 and 12000 ppm and in females at 12000 ppm. These findings correlate with increased mortality.

Microscopically there were no non-neoplastic correlates for the increased liver weight observed in either sex. Microscopic examination of the kidney revealed bilateral subacute-chronic inflammation/chronic nephropathy of high incidence in all groups. In terms of incidence there was no dosing related effect, but in terms of severity there was a NOEL for males of 500 ppm and for females 100/50 ppm. This microscopic finding does appear to provide a correlate of the macroscopic kidney effects with chronic nephropathy as a factor in excessive mortality.

Another outstanding finding in the study was that of nasal tissue effects (hyperplasia, degeneration, replacement of the olfactory epithelium by ciliated and non-ciliated columnar epithelial cells) and nasopharyngeal hyperplasia. These effects were of high incidence in males and females at 6000 ppm and 12000 ppm. It is noteworthy that the olfactory epithelium as opposed to the respiratory epithelium was particularly affected in this study. Certain literature references as identified and discussed in the review suggest that unique effects on the olfactory epithelium might not be so surprising. The olfactory epithelium has certain metabolic capabilities not unlike those of the liver, and might be expected to metabolize a diester such malathion in a way similar to that of the liver. So it is quite likely that the microscopic lesions noted of the nasal epithelium result in some degree as the result of chemical modifications of malathion.

Squamous cell hyperplasia and hyperkeratosis of the epithelium covering the forestomach were seen in numerous decedents from the 6000 ppm and 12000 ppm dose levels of both sexes.

Congestion was a histopathologic finding for many tissues as discussed in this review. While the bulk of the findings were in the 6000 ppm and 12000 ppm dose group, congestion was of such frequency in the 500 ppm dose group, particularly in males, as to support a conclusion that 500 ppm is an effect level among males. This may be a corollary to increased mortality at 500 ppm. Among male rats in particular, tissues exhibiting increased incidence of congestion among tissues of the 500 ppm dose group were brain, salivary glands, kidneys (females also possibly affected), femoral marrow?

With regard to neoplastic findings, the study report as written acknowledges that among female rats there were statistically significant increases of hepatocellular tumors at 6000 ppm and 12000 ppm. Specifically, at 12000 ppm the combined incidence of hepatocellular adenomas and carcinomas was 10.9% and at 6000 ppm 5.5% versus 0% in the control group. We in addition conclude that increased combined incidences of hepatocellular adenomas and carcinomas in the 500 ppm (3.6%) and 100/50 ppm (3.6%) groups were positive extensions to these dose levels of the tumorigenic effects of malathion. The conclusion that one hepatocellular adenoma and one carcinoma in each of the two lowest dose groups are positive findings rests with the rarity of spontaneous hepatocellular tumors in female F344 rats in concert with the positive findings at the higher doses. In the most recent publication of the National Toxicology Program's historical data, NIEHS (1996), the historical incidences in oral feeding studies among female F344 rats are 8/1351 (0.59%) for hepatocellular adenoma and 1/1351 (0.07%) for hepatocellular carcinoma. It is noteworthy that in this particular publication of the NTP data base, the single incidence of carcinoma in 1351 feeding study control females was actually the only incidence of hepatocellular carcinoma recorded in a total of 3621 control female F344 rats when other types of chronic studies, i.e., gavage (corn oil and water vehicle), oral (water vehicle), inhalation (air), are used.

Among nasoturbinal tissues, there was one carcinoma reported in a male rat in the 12000 ppm group and one adenoma reported for a male rat in the 6000 ppm group. The study report acknowledges these two tumors to be related to treatment with malathion. The rationale for acknowledging these to be treatment-related rests with the very low incidence of such tumors in the F344 rat in concert with the fact that extensive non-neoplastic changes were seen in the nasoturbinal tissues, as discussed previously. More specifically, both of the tumors were of the olfactory epithelium. As noted in the discussion on non-neoplastic findings, various publications indicate that the olfactory epithelium of the rat is endowed with metabolic capabilities very similar to those of the liver. It might be anticipated therefore that the olfactory epithelium and the liver may respond similarly to a carcinogen. As discussed in this review, the NTP historical control data base for the F344 rat does not disclose a single incidence of tumor of the olfactory epithelium from among nearly 4000 control male rats, and none among a similar number of control females. So in view of the rarity of the tumor type, the finding of two in this one study merits a positive interpretation.

Also identified in the nasal turbinate sections were two additional tissues (female in this case) with malignant tumor, one appearing in the 12000 ppm group and the other in the 100/50 ppm group. These are described as

"squamous cell carcinomas arising from the squamous epithelium lining the alveolus of a tooth".

These tumors are also very rare historically. According to Haseman et al (1990), the incidence of squamous cell carcinoma of the oral mucosa (any site) of the F344 rat is one in nearly 4000 untreated or corn oil gavage female F344 controls. The likelihood that this finding was of the alveolus of the root of a tooth is probably low. To the extent that the tumors may possibly have a nasal tissue etiology or involvement, the same NTP data shows no incidence of squamous cell carcinomas of the nasal passages among the nearly 4000 control female F344 rats in the data base. So whether these tumors be classified as oral or nasal, the incidence is exceedingly rare, and in view of the rarity, the two same such findings observed in this study are concluded to be compound-related. The registrant should submit further information characterizing the anatomic location of these two tumors. Of additional concern is the fact that all rats in the low dose group were not examined histopathologically for nasal tissue effects. As explained in this review, the FIFRA Guidelines call for histologic examinations of rats from <u>all</u> dose groups for those tissues ordinarily examined in the control and high dose groups only, when hyperplasia and/or tumors are identified in the high dose group. The fact that nasal tissue sections for all rats in the low dose groups were not examined is considered a study deficiency. Furthermore, as discussed in the review more than two sections of the nasal mucosa should be examined.

Testicular interstitial cell tumor incidence was extensive in all groups including the control. This is not a surprising finding in itself as this tumor has a high historical incidence. The study report itself claims that "Statistically, the dietary administration of malathion was considered to be associated with increased incidence of this tumor" (p. 95 of the study report), but qualifies this on the grounds that nearly all will develop this tumor if allowed to complete their natural lifespan. However, that particular condition was not met and many male rats did not live out their normal lifespan, but nonetheless developed the tumors. This suggests decreased latency, a critical parameter in defining carcinogenicity. Furthermore, in the study report a "Statistical Analysis of Time to Tumor Data" is provided where the study statistician concluded "The material is associated with increased interstitial cell testicular tumors in male rats at all doses measured . . ." (p. 5346 of the study report). One must conclude from this treatment of the data that a NOEL was not identified for increases of this tumor type.

As mentioned under the discussion of mortality, leukemia was a principle cause of early mortality in this study, although an increased incidence of leukemia was evidently not a dosing-related effect. However, as explained in

this review, among male rats of the 0, 100/50, 500 and 6000 ppm groups, there was a dosing-related increased mortality from leukemia among those rats harboring the condition. Evidently, rats with leukemia are more likely to die of leukemia as the result of a competing dosing-related toxicologic burden of the test material. This effect appears to be evident at 500 ppm and 6000 ppm, and perhaps so at 100/50 ppm, and at least constitutes an element of supporting evidence of chronic toxicity of the test material at all doses.

B. Study Deficiencies

1. A NOEL for erythrocyte cholinesterase inhibition among female rats was not identified for the first three months of testing. Although following reduction of dosage level in the low dose group from 100 ppm to 50 ppm at the three month time point yielded a subsequent (six months) 50 ppm NOEL, it cannot be claimed that 50 ppm was a NOEL during the first three months. Additional testing is necessary in the female F344 rat in order to identify a three month NOEL for erythrocyte cholinesterase inhibition.

2. The MTD was exceeded in both sexes as evidenced by increased mortality at 500, 6000 and 12000 ppm in males and at 12000 ppm in females. Among females, the lower doses of 100/50, 500 and 6000 ppm may satisfy Guideline requirements (although 6000 ppm may not be an MTD) in terms of MTD, but among males there is the deficiency of adequate dosing at and below an MTD to satisfy Guideline requirements.

3. A NOEL was not identified for increased food consumption particularly among males during the first three months, i.e., prior to reduction in dose from 100 ppm to 50 ppm. There is no certainty that 50 ppm would not have elicited the same effect during the first three months of testing, since this may be a period of adaption. This could be a cholinergic appetitive effect of malathion.

4. A NOEL was not identified for the microscopic finding of hyperplasia of the parathyroid gland for rats of either sex.

5. A NOEL was not identified for increased incidences of hepatocellular adenomas/carcinomas in female rats.

6. While there were no significant increases in liver tumor neoplasia in the case of males, high mortality in the 6000 and 12000 ppm groups may have precluded expression of a tumorigenic response, particularly if

late occurring. Since the next lowest dose level tested (500 ppm) was substantially lower than the 6000 ppm dose level (more than 10 fold lower), malathion was not tested for potential carcinogenicity at adequate dose levels for males in this study. Hence this study does not satisfy as a negative study for liver carcinogenicity among male F344 rats, or for that matter for carcinogenicity at any other anatomic site among male rats. This constitutes a major study deficiency. In the case of females, increased mortality at 12000 ppm also constitutes a study deficiency in terms of assessing carcinogenicity.

7. The absence of histopathologic examination of nasoturbinal tissue sections for the low dose (100/50 ppm) group for rats of both sexes is a study deficiency. In view of extensive hyperplasia of the olfactory epithelium at the 6000 and 12000 ppm doses in both sexes, in concert with the finding of rare tumors of the olfactory epithelium in male rats at 6000 ppm (one adenoma) and 12000 ppm (one carcinoma), there is under FIFRA Guideline requirements incumbency to examine all dose groups. The need for this is further reinforced by the finding of rare squamous cell carcinomas that appeared one each in the 12000 ppm and 100/50 ppm (unscheduled death animal) in the nasoturbinal tissue slides of female rats. These latter tumors may strictly be viewed as oral cavity tumors, but even that assessment requires some clarification. Not only should nasoturbinal tissue slides be examined in the low dose groups for tumors in question, but the number of sections taken should be increased from two per rat in each study group to five or six sections per rat.

8. A NOEL for decreased latency of interstitial cell testicular tumors was not identified. Although similar and high incidences of interstitial cell testicular tumors were observed in all the control and treated male groups in this study, the latency or time to tumor appeared to be decreased in the treated groups.

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Attachment 2

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D

# DATA EVALUATION REPORT

Study Type: Carcinogenicity Study, Mice
EPA Subdivision F Guideline 83-2(b)

Test Material: Malathion (CAS No. 121-75-5)

Tox. Chem. No.: 535

PC Code No.: 057701

MRID No.: 434072-01 (Original Report, 5 volumes, 1454 pages)

Study Title: 18-Month Oral (Dietary) Oncogenicity Study in Mice

Testing Laboratory: International Research and Development Corporation (IRDC)
Mattawan, Michigan

Lab. Project ID.: 668-001

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Study Completion Date: October 12, 1994

Sponsor: Cheminova Agro A/S
Lemvig, Denmark

## I. EXECUTIVE SUMMARY:

In an 18-month carcinogenicity study, technical grade malathion (96.4% pure) was administered in the diet to groups of 65 male and 65 female B6C3F1 BR strain mice at dose levels of 0 (control), 100 ppm, 800 ppm, 8000 ppm or 16000 ppm (equivalent to 0, 17.4, 143, 1476 or 2978 mg/kg/day in males and to 0, 20.8, 167, 1707 or 3448 mg/kg/day in females). Ten mice/sex/group were sacrificed at 12 months and the remaining survivors were sacrificed at

18 months. Mortality, clinical signs of toxicity, body weights and food consumption were monitored at appropriate times during the study. Hematological examinations and determinations of plasma, erythrocyte and brain cholinesterase activity levels were made on 8-10 mice/sex/group at 9 (erythrocyte cholinesterase only), 12 and 18 months. Necropsy examinations were performed on all animals and organ weight determinations were made on all animals at the 12-month interim and 18-month terminal sacrifices. Histopathological examinations were made on a complete set of organs/tissues from all animals that died or were sacrificed in extremis during the study and on all control and 16000 ppm animals at the 12 month and 18 month scheduled sacrifices. Histopathological examinations were also performed on a more limited set of organs/tissues from the other dose level groups at the scheduled sacrifices.

At 16000 ppm and 8000 ppm in both males and females, treatment related effects included decreased absolute body weights throughout the entire duration of the study (14.3-20.0% decrease in males and 9.7-16.1% decrease in females at 18 months), decreased food consumption (2.0-5.9% in males and 5.4-12.5% in females), decreased plasma cholinesterase activity levels at 12 and 18 months (≥ 86%), decreased erythrocyte cholinesterase activity levels at 9, 12 and 18 months (≥ 61%) and decreased brain cholinesteraase activity levels at 18 months (37-43%) at 16000 ppm only. Mortality rates, clinical signs of toxicity and hematological parameters were not affected by treatment with malathion at any dose.

A treatment-related increased incidence of hepatocellular tumors was observed in both male and female mice in this study at 8000 ppm and 16000 ppm. For male mice which died during the final 6 months of the study or were killed at the terminal sacrifice at 18 months, the percent incidences of hepatocellular adenomas were 1.9%, 7.3%, 3.6%, 21.8% and 94.1%; of hepatocellular carcinomas were 0.0%, 10.9%, 5.5%, 10.9% and 2.0%; and of combined hepatocellular adenomas/carcinomas were 1.9%, 18.2%, 9.1%, 32.7% and 96.1% for the 0 (control), 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively. For female mice which died during the final 6 months of the study or were killed at the terminal sacrifice at 18 months, the percent incidences of hepatocellular adenomas were 0.0%, 1.8%, 0.0%, 17.0% and 80.8%; of hepatocellular carcinomas were 1.8%, 0.0%, 3.7%, 1.9% and 3.8%; and of combined hepatocellular adenomas/carcinomas were 1.8%, 1.8%, 3.7%, 18.9% and 84.6% for the 0 (control), 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively.

The dose-related increases in hepatocellular adenomas and in combined adenomas/carcinomas at 8000 ppm and 16000 ppm in both males and females were attributed to treatment with malathion. Although some hepatocellular carcinomas were also observed in nearly all of the malathion treated groups, the incidences were not dose-related and overall the relationship of these carcinomas to treatment with malathion was equivocal. The increased numbers of hepatocellular tumors observed in this study in both males and females were due primarily to increased numbers of adenomas.

Regarding the male mice in this study, more hepatocellular tumors (adenomas, carcinomas and combined adenomas/ carcinomas) were observed at 100 ppm than at 800 ppm. Thus, a dose-related response was not observed at the lower dose levels of 100 ppm and 800 ppm, whereas a dose-related response was observed at the higher dose levels of 8000 ppm and 16000 ppm. How to interpret the increased numbers of hepatocellular tumors at 100 ppm, but not at 800 ppm, for the male mice in this study is not clear at this time.

The increased tumor incidences in the livers of both males and females at 16000 ppm and 8000 ppm were accompanied by concurrent observations of masses, nodules and foci in the livers of these animals at the terminal sacrifice and also by increased liver weights and highly increased incidences of hepatocellular hypertrophy in the livers at 12 and 18 months. The data for hepatocyte hypertrophy was quite remarkable in that an extremely steep dose-response curve was observed for both males and females in this study. Thus, in the control, 100 ppm and 800 ppm groups, no case of hepatocellular hypertrophy was observed in any animal at any time during the entire duration of this study whereas at 8000 ppm and 16000 ppm, a $\geq$ 50% incidence was observed at 12 months and a 100% incidence at 18 months.

Other findings were observed in this study that appeared to be related to treatment, but their biological significance was uncertain. These findings included the following: decreased vacuolation in the convoluted tubules of the kidneys in males; increased mineralization of the kidneys in females; decreased fibrous osteodystrophy of the femur and sternum in females; and early disappearance of the "x zone" in the the adrenal cortex of females.

The NOEL for cholinesterase inhibition for both sexes was estimated to be 100 ppm for plasma and erythrocyte cholinesterase and 8000 ppm for brain cholinesterase. Although there was some decrease in cholinesterase activity at these doses, the decreases were not statisticaly significant and the data were considered to be too variable to conclude that the inhibition seen was really related to

treatment. Cholinesterase activities were assayed using a Boehringer Mannheim Diagnostics kit and a Technicon AutoAnalyzer I. The NOEL for systemic effects was 800 ppm. The LEL was 8000 ppm, based on decreased body weights and food consumption in males and females, increased liver weight in males and females and increased hepatocellular hypertrophy in males and females. The biological significance of the decreased vacuolization of convoluted tubules in the kidney in males, increased mineralization of the kidney in females, decreased fibrous osteodystrophy in females and early disappearance of the "x zone" of the adrenal cortex in females at this and other doses is uncertain.

This study is classified as Core Guideline and satisfies the guideline requirement for a carcinogenicity study in mice, Guideline 83-2(b).

NOTE--The 2 highest dose levels used in this study, 16000 ppm (equivalent to 2978 mg/kg/day in males and 3448 mg/kg/day in females) and 8000 ppm (equivalent to 1476 mg/kg/day in males and 1707 mg/kg/day in females), both exceeded the limit dose of 1000 mg/kg/day which is oftentimes used by EPA to establish an upper dose level for carcinogenicity studies in mice and rats. In this instance, however, EPA required that the highest dose levels in this particular study be 16000 ppm and 8000 ppm in order to duplicate the dose levels used in a previously conducted 1978 National Cancer Institute (NCI) carcinogenicity study in B6C3F1 mice in which the results were equivocal.

II. DETAILED REVIEW OF STUDY

A. Test Material: Malathion, technical grade (CAS # 121-75-5). Description: Clear amber liquid; Product no. 30 0791; Batch no. 11029-01; 96.4% ± 0.3% purity; obtained from Cheminova Agro A/S (Lemvig, Denmark); storage conditions: not given.

Samples of the test material were collected at 28, 56 and 76 weeks and assayed for storage stability of the bulk chemical. Means ± S.D. of analyses, expressed as percent of target concentration, were 99.8 ± 4.09%, 98.4 ± 3.29% and 92.9 ± 1.09% for weeks 28, 56 and 76 respectively. These results indicate that the bulk chemical was stable under the conditions of storage over the duration of the study.

B. Test Animals: Mice, B6C3F1 BR strain, males and females. Description: obtained from Charles River Laboratories (Portage, Michigan); 4 weeks old when received (2/3/92); acclimated 4 weeks prior to commencement of treatment (3/2/92); all animals were given detailed physical examinations prior to treatment; a pretest viral screen was conducted on 5 animals/sex (results were negative); the mean weight of males was about 23 gm and of females was about 20 gm in each of the various groups at the commencement of treatment.

Environment: All mice were individually housed (since receipt) in wire-mesh cages in an environmentally controlled room under standard environmental conditions; temperature and humidity were monitored and recorded continuously; 12 hour light/dark cycle; diet and water (IRDC well water supply, analyzed quarterly for contaminants) were available ad libitum during the acclimation period and entire treatment period; water was provided via an automatic watering system.

C. Study Design: Animals were randomized and assigned to treatment groups as shown below "utilizing a block randomization procedure in which animals were stratified by body weight." Main study animals (55/sex/group) were given control or treated diet mixtures for 18 months; interim kill animals (10/sex/group) were sacrificed at 12 months.

| Dose Level Malathion (ppm) | Main Study | | Interim Kill | |
|---|---|---|---|---|
| | Males | Females | Males | Females |
| 0 | 55 | 55 | 10 | 10 |
| 100 | 55 | 55 | 10 | 10 |
| 800 | 55 | 55 | 10 | 10 |
| 8000 | 55 | 55 | 10 | 10 |
| 16000 | 55 | 55 | 10 | 10 |



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Treatment was commenced on 3/2/92. The interim kill was on 3/1/93 to 3/5/93. The last days of treatment and terminal sacrifices were on the same days viz. 8/30/93 to 8/31/93, 9/1/93 to 9/3/93 and 9/7/93 to 9/9/93.

Based on food consumption and body weight data, the mean consumption of test material for each of the treatment groups was later calculated in units of mg/kg/day for the entire duration of the study. For males, the mean consumption of test material was 0.0, 17.4, 143, 1476 and 2978 mg/kg/day for the control, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively. For females, the mean consumption of test material was 0.0, 20.8, 167, 1707 and 3448 mg/kg/day for the control, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively.

Note--During week 6, control animals were inadvertently given the low dose (100 ppm) diet mixture for 2 days and low dose (100 ppm) animals were given control diet for the same 2 days. This mistake in dosing would not be expected to affect the study results.

D. Rationale for Selection of Dose Levels: In a Data-Call-In notice dated 6/15/92, EPA required that the 2 highest dose levels in this study be 8000 ppm and 16000 ppm. These 2 dose levels duplicated dose levels used in a 1978 National Cancer Institute (NCI) 80-week carcinogenicity study in B6C3F1 mice (NCI-CG-TR-24). In this NCI study, possibly increased incidences of hepatocellular carcinomas and of combined neoplastic nodules and hepatocellular carcinomas were observed in male mice at 16000 ppm. There was also a dose-related trend in male mice for combined neoplastic nodules and hepatocellular carcinomas when compared to pooled controls. The results in this study, however, were equivocal and a clear association between liver neoplasms and malathion could not be established. In addition, study design flaws, uncertainties about the conduct of the study, and lack of sufficient detail to allow independent statistical analyses of the data further compromised the usefulness of this study. Hence, EPA required a new study to be performed under similar conditions in order to resolve the question of possible carcinogenicity of malathion in B6C3F1 mice.

E. Diet Preparation and Analyses of Diet Mixtures: The test material was diluted in acetone and then incorporated into ground Certified Rodent Chow #5002 (Purina Mills, Inc.; St. Louis, Missouri). Control diets were prepared with acetone only. Fresh diet mixtures and control diets were prepared weekly and stored in closed, polyethylene containers under refrigeration (see results of stability analyses below).

Homogeneity and Stability: Prior to commencement of treatment, the low (100 ppm) and high (16000 ppm) diet mixtures were sampled as follows.

1. Triplicate sets of samples from each side of the containers at the top, middle and bottom were taken (3 x 6 = 18 total samples).

2. One set of samples (6 samples) was assayed immediately for malathion content (homogeneity analysis).

3. The remaining 2 sets of samples (6 samples each) were both stored in standard containers at room temperature in the laboratory and then assayed at 7 days or at 14 days (stability analysis). Additional refrigerated samples were also assayed at 7 days or at 14 days.

Results for Homogeneity: The mean concentration of malathion in the low diet mixture for the 6 samples was 101 ppm (range: 95.5 - 107 ppm). The mean percent of target concentration and percent coefficient of variation were 100% ± 4.5%. The mean concentration of malathion in the high diet mixture for the 6 samples was 16000 ppm (range: 14900 - 17100 ppm). The mean percent of target concentration and percent coefficient of variation were 98% ± 4.4%. These data indicate satisfactory homogeneity of the low and high diet mixtures.

Results for Stability: Assays for malathion content in the low (100 ppm) diet mixture subjected to room temperature in the laboratory for 7 or 14 days indicated mean concentrations of 87% and 83%, respectively, of the day 0 concentration. Assays of similar samples kept under refrigeration for 7 or 14 days indicated means of 88% and 95% respectively. Both of these analyses were repeated about 2 months later. At this time, assays for malathion content in the low (100 ppm) diet mixture subjected to room temperature in the laboratory for 7 or 14 days indicated mean concentrations of 89% and 92%, respectively, of the day 0 concentration. Assays of similar samples kept under refrigeration in a sealed, stainless steel container for 7 or 14 days indicated means of 96% and 100% respectively.

Assays for malathion content in the high (16000 ppm) diet mixture subjected to room temperature in the laboratory for 7 or 14 days indicated mean concentrations of 100% and 98%, respectively, of the day 0 concentration. Assays of similar samples kept under refrigeration for 7 or 14 days indicated means of 102% and 103% respectively.

Results for the low diet mixture indicated moderate loss of test material over a 7 day interval (up to 13%) and over a

14 day interval (up to 17%). Since shorter storage durations and refrigeration were observed to decrease losses, actual diet mixtures in the study were prepared weekly and stored under refrigeration.

Concentration Analyses: All diet mixtures, including the control diet, were assayed for malathion content weekly for the first 8 weeks and monthly thereafter. The control diet and low (100 ppm) diet mixture were also assayed for weeks 9, 10, 11, 13, 14 and 15.

Results: For the entire duration of the study (analyses for weeks 1 to 76), the mean concentrations ± S.D. of malathion in the diet mixtures were 0, 98.4 ± 4.88, 763 ±36.0, 8030 ± 303 and 15900 ± 908 ppm. In terms of percent of target concentrations, these mean concentrations corresponded to 0%, 98%, 95%, 100% and 99% respectively. The vast majority of individual analyses were between 90% and 110%. The extreme range of values for individual samples was 84% to 117%. These data indicate that satisfactory concentrations of test material in the diet mixtures were achieved at all dosage levels throughout the entire duration of the study.

F. Quality Assurance, GLP Compliance and EPA Flagging Statements: Quality assurance inspections were conducted throughout the study from 2/6/92 to 10/11/94. The Quality Assurance statement was signed on 10/12/94. The GLP Compliance statement was signed, but not dated. The EPA Flagging Statement was signed, but not dated, and stated that "this study meets or exceeds the criteria numbered 1,2" [1.: an incidence of neoplasms in male or female animals which increases with dose; 2.: a statistically significant ($p \leq 0.05$) incidence of any type of neoplasm in any test group (male or female animals at any dose level) compared to concurrent control animals of the same sex].

G. Statistical Evaluation: Quoted from p. 19 of the report.

"Body weight, food consumption and clinical pathology laboratory values and organ weights (absolute and relative) were analyzed using analysis of variance (one-way classification) and Bartlett's test for homogeneity of variance. Treatment groups were compared to the control group, by sex, using the appropriate t-statistic (for equal or unequal variance) as described by Steel and Torrie[1]. Dunnett's[2] multiple comparison tables or pairwise comparisons with a Bonferroni correction[3] were used to determine the significance of differences. Nonparametric analyses were conducted as appropriate by transforming the data into ranks prior to analysis, as described by Conover and Iman[4]. All statistical analyses were performed with $p \leq 0.05$ and $p \leq 0.01$ as levels of significance."

"Analysis of tumor incidence data was performed as described by Huff[5] . These procedures include life table tests, the Hoel-Walburg 'incidental tumor' tests, Fisher's exact tests and Cochran-Armitage trend tests."

See References [1] - [5] on p. 33 of the report.

H. Observations and Results:

1. Mortality: All animals were observed twice each day for mortality and morbidity.

   Results: The fates of all animals in the study are presented in Table 1. For males, in the first 39 weeks of the study, 4 unscheduled deaths occurred in the 16000 ppm group whereas during the same period, no unscheduled deaths occurred in the control or any other treatment group. After 39 weeks, however, there were considerably fewer mortalities in the 8000 ppm and 16000 ppm groups than in the control, 100 ppm or 800 ppm groups. At the terminal sacrifice, 48 - 54 males per group were subjected to postmortem procedures. The pattern of deaths in the male groups did not indicate a relationship between mortality and the test material. For females, few unscheduled deaths occurred during the study in any group and no relationship to treatment with the test material was evident. At the terminal sacrifice, 51 - 55 females per group were subjected to postmortem procedures.

   Conclusion: Treatment with malathion had no apparent effect on the rate of mortality for either males or females in this study.

2. Clinical Signs: All animals were observed twice each day for clinical signs of toxicity. The onset and duration of signs were recorded. In addition, all animals were given a detailed clinical examination, including palpation for masses, once each week.

   Results: Clinical signs and palpable masses were summarized and listed for individual animals in the study report. There were no clinical signs in either males or females that could be attributed to treatment with malathion. The recorded signs were few and were typical for mice of this strain and age. Decreased incidences of hair loss in males at 8000 ppm and 16000 ppm and in females at 16000 ppm were not considered to be biologically meaningful. It should be particularly noted that clinical signs of toxicity ordinarily associated with cholinesterase poisoning (e.g. tremors, hyperactivity, salivation, malaise, etc.) were not

observed at any time in either the male or female mice in this study. Externally palpable masses were also few in number and could not be related to treatment with the test material.

Conclusion: The observed clinical signs and palpable masses in males and females in this study could not be directly related to treatment with malathion. Clinical signs of toxicity ordinarily associated with cholinesterase poisoning were not observed in this study in either males or females at any time.

3. Body Weights: Body weights for all mice were recorded prior to treatment, weekly for the first 14 weeks of the study, biweekly for weeks 14 - 26, and monthly thereafter.

Results: Mean absolute body weights for males and females at selected weeks during the study are presented in Table 2. Also, graphical representations of mean absolute body weights for males and females are presented in Figure 1 (copied from p. 34 and p. 35 of the study report). See pp. 35-36 in this DER. Changes in body weight gains were not presented in the study report (and are not considered by TB-I to be necessary for this particular study). For males, starting at week 1 and continuing for the entire duration of the study, absolute body weights were significantly decreased ($p \leq 0.01$) in a dose-related manner for both the 8000 ppm and 16000 ppm groups when compared to the control group. Mean body weights were not significantly decreased for male mice at 100 ppm or 800 ppm. At 78 weeks, the percentage decreases in mean absolute body weights for male mice, compared to the control group, were 14.3% and 20.0% for the 8000 ppm and 16000 ppm groups respectively. For females, starting at week 0 and continuing for the entire duration of the study, absolute body weights were also significantly decreased ($p \leq 0.01$) in a dose-related manner for both the 8000 ppm and 16000 ppm groups when compared to the control group. Mean body weights were not significantly decreased for female mice at 100 ppm or 800 ppm. At 78 weeks, the percentage decreases in mean absolute body weights for female mice, compared to the control group, were 9.7% and 16.1% for the 8000 ppm and 16000 ppm groups respectively.

Conclusion: Statistically significant ($p \leq 0.01$) dose-related decreases in mean absolute body weights for both males and females throughout the entire duration of the study at 8000 ppm and at 16000 ppm were clearly related to treatment with malathion.

4. Food Consumption: Food consumption for all mice was recorded weekly for the first 14 weeks of the study, biweekly for weeks 14 - 26, and monthly thereafter.

Results: Mean food consumption values for males and females at selected weeks during the study are presented in Table 3. Also, graphical representations of mean food consumption data for males and females are presented in Figure 2 (copied from p. 36 and p. 37 of the study report). See pp. 37-38 in this DER. For males, for the first 3 weeks of the study, mean food consumption for the 16000 ppm group was reduced considerably, possibly due to poor palatability of the diet mixture or to lack of appetite resulting from lower plasma and erythrocyte cholinesterase activity levels presumably present in these animals at that time (see 10. below). Food consumption for the 16000 ppm males then approached that of the control group for weeks 4-26. After 26 weeks and for the remainder of the study, however, food consumption was significantly decreased ($p \leq 0.01$) in a dose-related manner for both the 8000 ppm and 16000 ppm groups when compared to the control group. Mean food consumption was not affected for the male 100 ppm and 800 ppm groups. For the entire duration of the study, the percent differences from the control group for male mice were 0.0%, +2.0%, -2.0% and -5.9% for the 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively. For females, the pattern of food consumption was quite similar to that observed for the males, except that the period of initial reduction for the 16000 ppm females lasted about 13 weeks. As for males, after 26 weeks and for the remainder of the study, food consumption was significantly decreased ($p \leq 0.01$) in a dose-related manner for both the 8000 ppm and 16000 ppm groups when compared to the control group. Mean food consumption was not affected for the female 100 ppm and 800 ppm groups. For the entire duration of the study, the percent differences from the control group for female mice were 0.0%, 0.0%, -5.4% and -12.5% for the 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively.

Conclusion: Statistically significant ($p \leq 0.01$) decreases in food consumption were observed for 16000 ppm males and females during the first 3 weeks and 13 weeks of the study respectively. These decreases were possibly due to poor palatability of the diet mixture or to lack of appetite resulting from lower plasma and erythrocyte cholinesterase activity levels presumably present in these animals at that time. After 26 weeks and for the remainder of the study, however, statistically significant ($p \leq 0.01$) dose-related

decreases in food consumption for both males and females were observed at 8000 ppm and at 16000 ppm. These latter decreases were attributed to treatment with malathion.

5. Consumption of Test Material: Based on food consumption and body weight data, the mean consumption of test material for each of the treatment groups was calculated in units of mg/kg/day for the entire duration of the study. For males, the mean consumption of test material was 0.0, 17.4, 143, 1476 and 2978 mg/kg/day for the control, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively. For females, the mean consumption of test material was 0.0, 20.8, 167, 1707 and 3448 mg/kg/day for the control, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively.

6. Water Consumption: Not performed.

7. Ophthalmoscopy: Not performed.

8. Hematology: Samples of whole blood were collected from the orbital sinus of 8-10 randomly selected mice/sex/group at 12 and at 18 months. Animals were not fasted prior to blood collection. The following parameters were determined:

| | |
|---|---|
| Total RBC | Total WBC |
| Hemoglobin | Differential white count |
| Hematocrit | Platelets |
| Mean Cell Volume (MCV) | |
| Mean Cell Hemoglobin (MCH) | |
| Mean Cell Hemoglobin Conc (MCHC) | |

Results: For male mice, there were no statistically significant differences between treated groups and the control group for any of the hematology parameters examined at either 12 or 18 months, except for an increased MCHC in 16000 ppm males ($p \leq 0.01$) at 12 months. This increase was not considered to be biologically meaningful because no other differences in erythrocyte parameters were observed in these same animals. Similarly, for female mice, there were no statistically significant differences between treated groups and the control group for any of the hematology parameters examined at either 12 or 18 months, except for a decreased MCV in 16000 ppm females ($p \leq 0.01$) at 12 and 18 months. Again, these decreases were not considered to be biologically meaningful because no other differences in erythrocyte parameters were observed in these same animals.

Conclusion: There was no apparent effect on any of the hematology parameters examined in either male or female mice as a result of treatment with malathion.

9. Clinical Chemistries: Other than cholinesterase activity determinations (see below), no clinical chemistry assays were performed on the animals in this study.

10. Cholinesterase Activity Determinations: Samples of whole blood were collected by cardiac puncture from the 8-10 mice/sex/group that were sacrificed at the 12 month interim sacrifice. At 18 months, whole blood was collected from the orbital sinus of 10 mice/sex/group. As much as possible, blood was collected from the same randomly selected animals that were used for the hematology examinations. The animals were not fasted prior to blood collection. Cholinesterase activity determinations were performed for plasma, erythrocyrte and brain at these times (i.e. at 12 and 18 months). In addition, at 9 months, blood was collected from 10 randomly selected mice/sex/group and assayed for erythrocyte cholinesterase activity only. For all cholinesterase activity determinations, a Technicon AutoAnalyzer I was used in accordance with the analytical methodology decribed in the following reference:

Cholinesterase Reagent Set package insert (1981).
CAT # 124117.
Boehringer Mannheim Diagnostics, Houston, TX.

No further description of the analytical methodology utilized for the cholinesterase activity determination was provided in the study report.

Results: Residual cholinesterase activities for plasma, erythrocyte and brain for males and females at 12 and 24 months and for erythrocyte activity (only) for males and females at 9 months are presented in Table 4. Residual cholinesterase activity, as used here, is defined as "percent of cholinesterase activity remaining as compared to the mean of the control group for the same sex". Results for males and females were very similar. Plasma cholinesterase activity was decreased in a dose-related manner for both males and females at 12 and 18 months. The decreases at 8000 ppm and 16000 ppm were considerable ($\geq$ 86%; $p \leq 0.01$) and were clearly related to treatment with malathion. The decreases at 800 ppm (18-36%) were not statistically significant in males but were statistically significant ($p \leq 0.05$) in females. It is likely, however, that the



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decreases in males, although not statistically significant, were nevertheless related to the test material. At 100 ppm, plasma cholinesterase inhibition was considered to be equivocal.

Erythrocyte cholinesterase activity was also decreased in a dose-related manner for both males and females at 9, 12 and 18 months. The decreases at 8000 ppm and 16000 ppm were considerable ($\geq$ 61%; $p \leq 0.05$) and were clearly related to treatment with malathion. Some of the decreases at 800 ppm (35-58%) were also statistically significant in males and females. Effects at 800 ppm, therefore, were also related to treatment with the test material. At 100 ppm, although not statistically significant in either males or females, erythrocyte values at 18 months were decreased 15% in males and 31% in females. It is possible that these decreases at 100 ppm at 18 months were also related to treatment with malathion but the data is equivocal.

Brain cholinesterase activity was decreased for both males and females at 12 and 18 months at 16000 ppm. The decreases (20-43%) were not statistically significant in males or females at 12 months but were statistically significant ($p \leq 0.05$) in both sexes at 18 months. It is likely, however, that the decreases at 12 months, although not statistically significant, were nevertheless related to treatment with the test material. At 8000 ppm, nonsignificant decreases in brain cholinesterase activity were observed in both males and females at 18 months (20-23%). These decreases may also be related to the test material, but again the data is equivocal.

<u>Conclusion</u>: Plasma cholinesterase activity was decreased in both males and females in a dose-related manner at 800 ppm, 8000 ppm and 16000 ppm. The decreases at 8000 ppm and 16000 were particularly substantial ($\geq$ 86%) and were significant ($p \leq 0.01$). Erythrocyte cholinesterase activity was also decreased in both males and females in a dose-related manner at 800 ppm, 8000 ppm and 16000 ppm. The decreases at 8000 ppm and 16000 were particularly substantial ($\geq$ 61%) and most were significant ($p \leq 0.01$). It was difficult to tell whether or not there was any true reduction in plasma or erythrocyte cholinesterase activity in either sex at 100 ppm or in brain cholinesterase in either sex at 8000 ppm (even though decreases were part of a dose-related trend) because the decreases were not statistically significant and there was a large degree of variability associated with them (coefficients of

variation generally ranged from 20 to 46%). Although the data are equivocal, 100 ppm could be considered as an approximate NOEL for plasma and erythrocyte cholinesterase inhibition (both sexes) and 8000 ppm could be considered as an approximate NOEL for brain cholinesterase inhibition (both sexes) in this study.

11. Urinalyses: Not performed.

12. Necropsy: All animals in this study, regardless of time of death, were given a complete postmortem examination under the direct supervision of a pathologist, in accordance with standard gross dissection and necropsy procedures. Animals sacrificed at the scheduled sacrifice times of 12 and 18 months and animals sacrificed *in extremis* were euthanized by carbon dioxide inhalation overdose.

Results:

0 - 12 Months: Negative. No gross effects were observed in animals that died or were sacrificied during the first 12 months of the study that could be attributed to treatment with malathion.

12-Month interim Sacrifice: Negative. No gross effects were observed in animals that were sacrificed at 12 months (8-10 mice/sex/group) that could be atributed to treatment with the test material.

12 - 18 Months: Negative. No gross effects were observed in animals that died or were sacrificed during the last 6 months of the study that could be attributed to treatment with malathion. It might be recalled, however, that for the 8000 ppm and 16000 ppm groups, only 2 males and 1 female died during this period of the study.

Terminal Sacrifice: Selected macroscopic observations for male and female mice sacrificed at 18 months are presented in Table 5. For both males and females, gross effects attributed to treatment with malathion were observed in the liver. No macroscopic changes related to the test material were noted in any other organ/tissue.

For males, increased numbers of masses were observed in the livers of all malathion treatment groups when compared to the number in the control group. The incidences were 0, 8, 4, 5 and 18 for the 0, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm



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groups respectively. Increased numbers of liver nodules were also observed in the 8000 ppm and 16000 ppm male groups when compared to the control group. The incidences were 5, 2, 3, 10 and 19 for the 0, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively. An increased incidence of focus/foci (tan/yellow) was also observed in the livers of the 16000 ppm male group. The incidences were 0, 0, 1, 2 and 18 for the 0, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively. A very small increase in enlarged hepatic lymph nodes was also noted in malathion treated groups at 800 ppm, 8000 ppm and 16000 ppm. The relationship of this finding to treatment with malathion is uncertain. Finally, a decreased incidence of alopecia was observed in 8000 ppm and 16000 ppm males (data not shown). There is probably no biological significance related to this observation.

For females, an increased number of masses was observed in the livers of the 16000 ppm group when compared to the control group. The incidences were 1, 0, 3, 2 and 10 for the 0, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively. Increased numbers of liver nodules were also observed in the 8000 ppm and 16000 ppm female groups when compared to the control group. The incidences were 1, 2, 0, 9 and 29 for the 0, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively. An increased incidence of focus/foci (tan/yellow) was also observed in the livers of the 16000 ppm female group. The incidences were 0, 0, 0, 2 and 9 for the 0, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively. The significance of the small increase at 8000 ppm is uncertain. Decreased numbers of female mice with alopecia, clear cysts in the ovary and dilated uterus were also noted at 16000 ppm. There is probably no biological significance related to these latter observations.

Conclusion: Increased numbers of liver masses, compared to control groups, were observed in the 100 ppm, 800 ppm, 8000 ppm and 16000 ppm male groups and the 16000 ppm female group at the terminal sacrifice. Also at the terminal sacrifice, increased numbers of liver nodules were observed in the 8000 ppm and 16000 ppm male and female groups. Finally, an increased incidence of liver focus/foci (tan/yellow) was observed in the 16000 ppm male and female groups at the terminal sacrifice. No other macroscopic changes were noted in



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any organ/tissue that were attributed to the test material.

13. Organ Weights, Organ/Body Weight Ratios and Organ/Brain Weight Ratios: For all animals sacrificed at the scheduled sacrifice times of 12 and 18 months, the following organs were weighed and organ/body weight and organ/brain weight ratios calculated.

| | |
|---|---|
| adrenal (2) | kidney (2) |
| brain | liver |
| ovary (2) | lung |
| testes (2) | spleen |
| heart | |

Results: See Tables 6 and 7. For male mice, dose-related statistically significant ($p \leq 0.01$) increases in absolute liver weights, liver/body weight ratios and liver/brain weight ratios were observed at 12 and 18 months for the 8000 ppm and 16000 ppm groups. The percent increases in absolute liver weights at 18 months were 19% and 40% for the 8000 ppm and 16000 ppm groups respectively. These increased liver weights were attributed to treatment with the test material. Decreases in absolute brain weight at 12 and 18 months and in kidney and heart weights at 18 months for the 16000 ppm group were also noted. Increased lung/body weight ratios and testes/body weight ratios at 18 months for 8000 ppm and 16000 ppm males were considered to be the result of the significantly decreased body weights ($p \leq 0.01$) and not related to treatment with malathion. Other differences, such as increased kidney weights at 12 months for the 100 ppm group, were not considered to be biologically significant.

For female mice, statistically significant ($p \leq 0.01$) increases in absolute liver weights, liver/body weight ratios and liver/brain weight ratios were observed at 12 months in the 16000 ppm group. At 18 months, absolute liver weights, liver/body weight ratios and liver/brain ratios were also increased, but only the increase for liver/body weight ratio was statistically significant ($p \leq 0.01$). The percent increase in absolute liver weight at 18 months was 13% for the 16000 group. It is likely, however, that the increased liver weights at 16000 ppm at both 12 and 18 months are related to treatment with malathion. Decreases in absolute brain and spleen weights for the 8000 ppm and 16000 ppm groups and in heart and lung weights for the 16000 ppm group were also noted at 18 months. Although statistically significant increases in kidney weight values were frequently observed in all malathion treated female groups at 12 and/or 18 months, these



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differences were either not dose-related or were probably due to the significantly decreased ($p \leq 0.01$) body weights at 8000 ppm and 16000 ppm. Therefore, the increased kidney weights in female mice were not attributed to treatment with the test material.

Conclusion: Treatment-related increases in liver weights were observed in male mice at 8000 ppm and 16000 ppm and in female mice at 16000 ppm at 12 and 18 months. None of the other changes in absolute or relative organ/body weight ratios were considered to be related to treatment with the test material.

14. Fixing and Processing of Tissues/Organs: The following tissues/organs were collected from all animals at the time of necropsy and were fixed in phosphate-buffered neutral formalin.

Adrenal (2)
Aorta
Bone (femur)
Bone marrow (femur)
Bone marrow smear (2)
Brain (fore, mid and hind)
Eye including optic N. with contiguous Harderian gl (2)
Gallbladder
Gastrointestinal tract:
- esophagus
- stomach (glan & nonglan)
- duodenum
- jejunum
- ileum
- cecum
- colon
- rectum

Gonads:
- ovary (2)
- testis with epididymis (2)

Gross lesions
Heart
Kidney (2)
Lacrimal gl (exorbital)(2)
Larynx
Liver (2 lobes examined; 3 sections collected)
Lung with bronchi (2)
Lymph Nodes: mandibular, mediastinal, mesenteric and regional when applicable
Mammary gland (females only)
Oviduct (2)
Pancreas
Pituitary
Prostate & Seminal Vesic (2)
Salivary gl, mandib/subling
Sciatic nerve
Skeletal muscle (thigh)
Skin
Spinal cord (cer,thor & lum)
Spleen
Sternum
Thymus
Thyroid/parathyroid (2)
Tissue masses
Tongue
Trachea
Urinary bladder
Uterus (both horns)
Uterus, cervix
Vagina

Following fixation, representative samples of or the whole of the tissues/organs listed above were trimmed, sectioned, blocked in paraffin, and stained (H & E) according to standard histologic techniques.

15. Microscopic Examination: All the tissues/organs listed above were microscopically examined from all mice in the control and high dose (16000 ppm) groups at the interim and terminal sacrifices and from all mice which died or were sacrificed in extremis during the study. In addition, at the interim and terminal sacrifices, the following tissues/organs were examined from all mice in the 100 ppm, 800 ppm and 8000 ppm groups: liver, kidney (2), lung and adrenal (2). At the terminal sacrifice (only), samples of bone (femur and sternum) were also examined for all female mice in the 100 ppm, 800 ppm and 8000 ppm groups. All tissue masses and all gross lesions from all animals in the study were also examined. Gradable lesions were graded by a 4 step grading system of trace, mild, moderate and severe.

NONNEOPLASTIC FINDINGS

Results:

0 - 12 Months, Including 12-Month Interim Sacrifice: Selected nonneoplastic microscopic findings for male and female mice which died or were sacrificed in extremis during the first 12 months of the study or were sacrificed at the 12-month interim sacrifice are presented in Table 8. For males, effects attributed to treatment with malathion were observed in the liver and kidney. For females, treatment related effects were observed in the liver and adrenal cortex. No microscopic changes related to the test material were noted in any other organ/tissue during the first 12 months of the study.

For males, hepatocyte hypertrophy was observed in the livers of numerous animals at 8000 ppm and 16000 ppm whereas none was observed at lower doses or in the control group. The incidences were 0/11, 0/10, 0/10, 7/10 and 12/14 for the 0, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively. Also, the average severity score was increased at 16000 ppm (2.3) as compared to the score at 8000 ppm (1.0). In the kidney, the incidence of vacuolation in the convoluted tubules, a normal finding in male mice at 12 months and older, was clearly decreased at 8000 ppm and 16000 ppm. The incidences were 11/11, 10/10, 10/10, 1/10 and 0/14 for the 0, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively. Also in the kidney, an increased incidence of mineralization in the 16000 ppm group

(6/14) as compared to the control group (3/11) was observed. Chronic interstitial nephritis, a common finding in older male mice, was also noted in several mice in all groups, but was not considered to be related to treatment with malathion. Microscopic findings in the adrenal cortex of male mice were negative.

Hepatocyte hypertrophy was also observed in the livers of numerous female mice at 8000 ppm and 16000 ppm. The incidences and average severity scores were very similar to those observed for male mice. The incidences were 0/10, 0/10, 0/11, 6/12 (average score = 1.0) and 13/13 (average score = 2.2) for the 0, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively. Microscopic findings in the kidney were negative. In the adrenal cortex, however, a region described as the "x zone", which is present only in females and normally disappears in older mice, was observed to have disappeared sooner than expected in the 8000 ppm and 16000 ppm mice. This "x zone" was present in 10/10, 8/10, 9/11, 1/12 and 0/13 female mice for the 0, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively. The early disappearance of the "x zone" in the adrenal cortex of female mice is considered to be related to treatment with malathion. Also in the adrenal cortex, A cell hyperplasia, a normal finding in female mice of 12 months and older, was observed in nearly all control and malathion treated mice. In addition, cysts were frequently observed in the uterus of control and malathion treated mice, but were not considered to be treatment related.

For both males and females, the following additional lesions were frequently reported in the control and 16000 ppm treatment groups, but were not attributed to treatment with the test material (data not shown in Table 8): brain/ mineralization, Harderian gland/chronic inflammation, urinary bladder/mononuclear cell infiltration.

<u>12 - 18 Months, Including 18-Month Terminal Sacrifice</u>: Selected nonneoplastic microscopic findings for male and female mice which died or were sacrificed <u>in extremis</u> after 12 months or were sacrificed at the 18-month terminal sacrifice are presented in Table 9. For males, effects attributed to treatment with the test material were observed in the liver and kidney. For



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females, treatment related lesions were noted in the liver, kidney and bone (femur and sternum).

For males, hepatocyte hypertrophy was observed in all malathion treated mice at 8000 ppm and 16000 ppm whereas none was observed at 100 ppm or 800 ppm or in the control group. The incidences were 0/54, 0/55, 0/55, 55/55 (average score = 2.1) and 51/51 (average score = 3.1) for the 0, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively. These lesions were clearly induced by treatment with malathion. Slightly increased incidences of mononuclear cell infiltration in the portal areas and of necrosis were also reported in the 8000 ppm and 16000 ppm groups. Mononuclear cell foci in the parenchyma was reported with about equal frequency in the control and treatment groups. In the kidney, a decreased incidence of vacuolation in the convuloted tubules was again observed in the 8000 ppm and 16000 ppm groups (as during the first 12 months of the study). Incidences were 54/54, 55/55, 55/55, 33/55 and 0/51 for the 0, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively. Mineralization of the kidney and chronic interstitial nephritis were also observed in most male kidneys in the control and all treatment groups and were considered to be normal findings in male mice of this age. In the adrenal cortex, an increase in brown degeneration was noted in the 8000 ppm and 16000 ppm groups. The incidence of this lesion, however, was not dose related and when considered together with the frequent observation of this same finding in females, where there was no relationahip to treatment with malathion, the increase in males was considered to be most likely also not related to treatment with malathion. A cell hyperplasia was also frequently observed in the adrenal cortex, but was not attributed to treatment with the test material.

Also for males, increased incidences of mineralization in the lungs (a normal finding) and of brown pigment in the mandibular lymph node (not observed in other lymph nodes) were observed in the 16000 ppm group. The possible relationship of either of these findings to treatment with malathion, although somewhat suggestive based solely on numerical incidences, is equivocal. Incidences of other lesions in other organs listed in Table 9 were included in the table only for purposes of comparison with similar lesions in the

female groups. None of these lesions in male mice were considered to be related to treatment with malathion.

For females, as with males, hepatocyte hypertrophy was observed in all 8000 ppm and 16000 ppm animals, but in none of the animals at 100 ppm or 800 ppm or in the control group. Incidences were 0/55, 0/55, 0/54, 53/53 (average score = 1.7) and 52/52 (average score = 3.1) for the 0, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively. These lesions were induced by the test material. Mononuclear cell infiltration in the portal areas, mononuclear cell foci in the parenchyma and necrosis occurred at similar incidences in the control and all treatment groups. In the kidney, a dose-related increased incidence of mineralization was observed in the 8000 ppm and 16000 ppm mice. This increase in kidney mineralization was considered to be treatment related. Chronic interstitial nephritis, observed in numerous control and treated mice, was not related to treatment. In the adrenal cortex, the "x zone" had disappeared in nearly all mice, as is normal. A cell hyperplasia and brown degeneration were also frequently reported, but were not attributed to treatment with malathion.

A dose-related decreased incidence of fibrous osteodystrophy of the bone (femur) was observed in all malathion treated female groups. Incidences were 23/55, 14/55, 7/54, 3/53 and 2/52 for the 0, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively. A decreased incidence was also observed in the bone (sternum) for the 16000 ppm group. Incidences were 51/55, 48/55, 50/54, 50/53 and 10/52 for the 0, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively. These decreased incidences of fibrous osteodystrophy in the femur and sternum were considered to be related to treatment with the test material. The biological significance of this observation is uncertain.

Additional lesions in female mice that were increased in the 16000 ppm group were the following: increased acinar atrophy in the lacrimal gland, increased mineralization and peribronchial lymphoid infiltration in the lung (also increased at 8000 ppm), increased hemorrhage and brown pigment in the mandibular lymph node,

and increased dilatation in the uterus (also increased at 8000 ppm). Although increased incidences of these lesions in the 8000 ppm and/or 16000 ppm groups suggest a possible treatment related effect, the relationship to treatment is nevertheless considered to be equivocal because many of these lesions occur normally in older mice. Many mice in the control and treatment groups had cysts in the uterus and/or uterine cervix. These cysts were not related to treatment.

The following additional lesions were frequently reported for both males and females in the control and 16000 ppm groups, but were not attributed to treatment with malathion (data not shown in Table 9): brain/mineralization, Harderian gland/chronic inflammation, urinary bladder/mononuclear cell infiltration, mandibular salivary gland/ mononuclear cell infiltration, skin/acanthosis.

Conclusion: The following nonneoplastic microscopic findings in the liver were attributed to treatment with malathion. The biological significance of the findings in the kidney, adrenal cortex and bone are uncertain.

Liver
--hepatocyte hypertrophy in nearly all treated male and female mice at 8000 ppm and 16000 ppm, regardless of time of death or sacrifice

Kidney
--decreased incidence of vacuolation in the convoluted tubules in male mice at 8000 ppm and 16000 ppm, regardless of time of death or sacrifice

--increased incidence of mineralization in female mice at 8000 ppm and 16000 ppm at the 18-month terminal sacrifice

Adrenal Cortex
--early disappearance of the "x zone" in female mice at 8000 ppm and 16000 ppm at the 12-month interim sacrifice

Bone (femur and sternum)
--dose-related decreased incidence of fibrous osteodystrophy in the femur in female mice at 100 ppm, 800 ppm, 8000 ppm and 16000 ppm at the 18-month terminal sacrifice



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--decreased incidence of fibrous osteodystrophy in the sternum in female mice at 16000 ppm at the 18-month terminal sacrifice.

NEOPLASTIC FINDINGS

Results:

0 - 12 Months, Including 12-Month Interim Sacrifice: Only a few tumors were observed in either male or female mice during the first 12 months of this study. Other than 1 hepatocellular adenoma observed in 1 male mouse in the 16000 ppm group at the 12-month interim sacrifice, none of these tumors were attributed to treatment with malathion.

12 - 18 Months, Including 18-Month Interim Sacrifice: Treatment-related liver tumors were observed in both male and female mice in this study. No other types of tumors were observed in either male or female mice that were related to treatment with the test material. A slightly increased incidence of cystadenomas in the ovary of 16000 ppm females during the last 6 months of the study (0/54, 0/10, 0/14, 0/11 and 3/51 for the control, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively), in the absence of any other pathological findings in the ovary, was not considered to be related to treatment with malathion. All other tumors that were noted in both male and female mice in this study were few in number and not dose-related.

Incidences of hepatocellular adenomas, hepatocellular carcinomas and combined hepatocellular adenomas and carcinomas for the male and female mice in this study are presented in Table 10 and Table 11 respectively (p. 53 and p. 54 respectively). Some incidences presented in these tables may appear different than those presented in summary tables in the study report because animals with both an adenoma and a carcinoma were counted twice in the study report (i.e. as one adenoma and one carcinoma) whereas in Tables 10 and 11 in this DER these animals were counted only once (as one carcinoma). Also, although the data for liver tumors were statistically analyzed in the study report, the analysis was based on incidences which "double counted" animals with both an adenoma and a carcinoma (as explained above). For the purpose

of statistical analyses, it has been standard practice in HED to count these animals only once. Hence, statistical analyses of the liver tumor data in the study will be provided by HED in a separate report.

Historical control data for liver tumors in B6C3F1 mice for studies conducted at International Research and Development Corporation are presented in Table 12 (p. 55). It should be noted that the percent incidences presented in Table 12 are based on incidences per number of animals examined microscopically only at the terminal sacrifice, and therefore are most comparable to the percent incidences in Tables 10 and 11 for the period 12 months - termination. Even here, however, the data are not strictly comparable because the data in Tables 10 and 11 also include the few animals that died or were sacrificed in extremis during the last 6 months of the study.

For males, a hepatocellular adenoma was noted in one 16000 ppm animal at the 12-month interim sacrifice and a hepatocellular carcinoma in one 800 ppm animal found dead at 15 months. All other liver tumors were observed at the 18-month terminal sacrifice. For the last 6 months of the study (including the terminal sacrifice), a dose-related increased incidence of hepatocellular adenomas was observed at 8000 ppm and 16000 ppm. In percentage units, incidences of 1.9%, 7.3%, 3.6%, 21.8% and 94.1% were observed for the control, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively. The study report also indicated that 35 male mice at 16000 ppm had multiple adenomas present whereas multiple adenomas were not present in any other group of male mice. It should be noted that the percent incidences in the control (1.9%), 100 ppm (7.3%) and 800 ppm (3.6%) groups were far below the percent incidence range reported for the male historical control animals for 18 month studies (14.3-21.7%). For hepatocellular carcinomas during the same period, percent incidences were 0.0%, 10.9%, 5.5%, 10.9% and 2.0% for the control, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively. Although increased at all doses compared to the concurrent control group, the response was not dose-related. The percent incidence at 100 ppm (10.9%) and at 8000 ppm (10.9%), however, did exceed the upper historical control percent incidence for 18-month studies



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(0.0-6.4%). For combined adenomas and carcinomas during the last 6 months of the study, the percent incidences were 1.9%, 18.2%, 9.1%, 32.7% and 96.1% for the control, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively. For the male mice in this study, more hepatocellular tumors (adenomas, carcinomas and combined adenomas/ carcinomas) were observed at 100 ppm than at 800 ppm. Thus, a dose-related response was not observed at the lower dose levels of 100 ppm and 800 ppm, whereas a dose-related response was clearly observed at the higher dose levels of 8000 ppm and 16000 ppm. How to interpret the increased numbers of hepatocellular tumors at 100 ppm, but not at 800 ppm, for the male mice in this study is not clear at this time.

For females, no liver tumors were found in any animals until a hepatocellular adenoma was observed in one animal that died following blood collection just prior to the terminal sacrifice at 18 months. All other liver tumors were observed at the 18-month terminal sacrifice. For the last 6 months of the study (including the terminal sacrifice), a dose-related increased incidence of hepatocellular adenomas was observed at 8000 ppm and 16000 ppm. The percent incidences were 0.0%, 1.8%, 0.0%, 17.0% and 80.8% for the control, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively. One mouse at 8000 ppm and 23 mice at 16000 ppm had multiple adenomas present. The incidences at 8000 ppm (17.0%) and 16000 ppm (80.8%) exceeded the upper historical control percent incidence for 18 month studies (0.0-10.6%). For hepatocellular carcinomas during the same period, relatively few were observed (not more than 2 in any dose group) and they were not dose-related. Percent incidences were 1.8%, 0.0%, 3.7%, 1.9% and 3.8% for the control, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively. The percent incidence at 800 ppm (3.7%) and at 16000 ppm (3.8%) exceeded the upper historical control percent incidence for 18 month studies (0.0-2.3%). For combined adenomas and carcinomas during the last 6 months of the study (including the terminal sacrifice), the percent incidences were 1.8%, 1.8%, 3.7%, 18.9% and 84.6% for the control, 100 ppm, 800 ppm, 8000 ppm and 16000 ppm groups respectively.

Conclusion: For both male and female mice in this study, the dose-related increased incidences of hepatocellular adenomas and of combined adenomas/carcinomas at 8000 ppm and 16000 ppm were attributed to treatment with malathion. For male mice, however, it is noted that the percent incidences of hepatocellular adenomas in the control, 100 ppm and 800 ppm groups were far below the percent incidence range reported for the male historical control animals for 18 month studies. Regarding hepatocellular carcinomas, the data are equivocal. Incidences at 100 ppm and 8000 ppm in males and at 800 ppm and 16000 ppm in females were above concurrent control incidences and also above the upper historical control percent incidences for 18 month studies. However, for both males and females, the numbers of hepatocellular carcinomas were rather low in all groups and the incidences were not dose-related. The increased numbers of hepatocellular tumors observed in this study in both males and females were due primarily to increased numbers of adenomas.

For the male mice in this study, more hepatocellular tumors (adenomas, carcinomas and combined adenomas/carcinomas) were observed at 100 ppm than at 800 ppm. Thus, a dose-related response was not observed at the lower dose levels of 100 ppm and 800 ppm. How to interpret the increased numbers of hepatocellular tumors at 100 ppm, but not at 800 ppm, for the male mice in this study is not clear at this time.

Since all the hepatocellular tumors observed in this study in both male and female mice, except for 2 tumors in male mice and 1 tumor in a female mouse, were observed at the terminal sacrifice, there was no apparent decreased time to tumor for the malathion-treated mice in this study.

## III. SUMMARY OF STUDY RESULTS AND DISCUSSION

### A. Summary

1. Malathion was administered in the diet to male and female B6C3F1 mice at dose levels of 0 (control), 100 ppm, 800 ppm, 8000 ppm and 16000 ppm for 18 months.

2. At 16000 ppm (equivalent to 2978 mg/kg/day in males and 3448 mg/kg/day in females), the following treatment-related effects were observe

   a. decreased absolute body weights in males and females



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--statistically significant dose-related decreases throughout entire duration of study
--decreased 20.0% in males and 16.1% in females at 18 months

b. decreased food consumption in males and females
--statistically significant dose-related decreases after 26 weeks
--equivocal decreases prior to 26 weeks
--decreased 5.9% in males and 12.5% in females for entire duration of study

c. decreased plasma cholinesterase activity in males and females
--statistically significant dose-related decrease at 12 and 18 months
--$\geq$ 93 %

d. decreased erythrocyte cholinesterase activity in males and females
--statistically significant dose-related decrease at 9, 12 and 18 months
--$\geq$ 67%

e. decreased brain cholinesterase activity in males and females
--statistically significant decrease at 18 months
--37-43%

f. increased liver weights in males and females
--statistically significant dose-related increase at 12 and 18 months in males
--increase at 12 and 18 months in females was not consistently statistically significant
--absolute liver weight increased 40% in males and 13% in females at 18 months

g. increased hepatocellular hypertrophy in liver of males and females
--in nearly all animals at 12 months and in all animals at 18 months

h. decreased vacuolation in convoluted tubules in kidney of males
--in all animals at 12 and 18 months

i. increased mineralization in kidney of females
--at 18 months

j. decreased fibrous osteodystrophy in femur and sternum of females
--at 18 months

k. early disappearance of "x zone" in adrenal cortex of females
--at 12-month interim sacrifice

l. increased hepatocellular adenomas in livers of males and females

--dose-related increase during last 6 months of study
--94.1% incidence in males and 80.8% incidence in females

m. equivocal increase in hepatocellular carcinomas in females
--3.8% incidence during last 6 months of study

n. increased combined hepatocellular adenomas/ carcinomas in males and females
--dose-related increase during last 6 months of study
--96.1% incidence in males and 84.6% incidence in females

3. At 8000 ppm (equivalent to 1476 mg/kg/day in males and 1707 mg/kg/day in females), the following treatment-related effects were observed.

a. decreased absolute body weights in males and females
--statistically significant dose-related decreases throughout entire duration of study
--decreased 14.3% in males and 9.7% in females at 18 months

b. decreased food consumption in males and females
--statistically significant dose-related decreases after 26 weeks
--decreased 2.0% in males and 5.4% in females for entire duration of study

c. decreased plasma cholinesterase activity in males and females
--statistically significant dose-related decrease at 12 and 18 months
--$\geq$ 86%

d. decreased erythrocyte cholinesterase activity in males and females
--statistically significant dose-related decrease at 9, 12 and 18 months
--$\geq$ 61%

e. increased liver weights in males
--statistically significant dose-related increase at 12 and 18 months
--absolute liver weight increased 19% at 18 months

f. increased hepatocellular hypertrophy in liver of males and females
--in nearly all animals at 12 months and in all animals at 18 months



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g. decreased vacuolation in convoluted tubules in kidney of males

h. increased mineralization in kidney of females
   --at 18 months

i. decreased fibrous osteodystrophy in femur of females
   --at 18 months

j. early disappearance of "x zone" in adrenal cortex of females
   --at 12-month interim sacrifice

k. increased hepatocellular adenomas in livers of males and females
   --dose-related increase during last 6 months of study
   --21.8% incidence in males and 17.0% incidence in females

l. equivocal increase in hepatocellular carcinomas in males
   --10.9% incidence during last 6 months of study

m. increased combined hepatocellular adenomas/carcinomas in males and females
   --dose-related increase during last 6 months of study
   --32.7% incidence in males and 18.9% incidence in females

3. At 800 ppm (equivalent to 143 mg/kg/day in males and 167 mg/kg/day in females), the following treatment-related effects were observed.

a. decreased plasma cholinesterase activity in males and females
   --non-significant dose-related decrease at 12 and 18 months in males (23-24%)
   --statistically significant dose-related decrease at 12 and 18 months in females (18-36%)

b. decreased erythrocyte cholinesterase activity in males and females
   --dose-related decrease at 9, 12 and 18 months in males (37-44%, statistically significant only at 12 months)
   --dose-related decrease at 9, 12 and 18 months in females (35-58%, statistically significant at 9 and 18 months)

c. decreased fibrous osteodystrophy in femur of females
   --at 18 months

d. equivocal increase in hepatocellular carcinomas in females

--3.7% incidence during last 6 months of study

e. equivocal increase in combined hepatocellular adenomas/carcinomas in males and females
   --increased during last 6 months of study
   --9.1% incidence in males and 3.7% incidence in females

4. At 100 ppm (equivalent to 17.4 mg/kg/day in males and 20.8 mg/kg/day in females), the following treatment-related effects were observed.

   a. decreased fibrous osteodystrophy in femur of females (biological significance is uncertain)
      --at 18 months
   b. equivocal increase in hepatocellular carcinomas in males
      --10.9% incidence at 18 months
   c. equivocal increase in combined hepatocellular adenomas/carcinomas in males
      --increased during last 6 months of study
      --18.2% incidence in males

B. Discussion

The design, conduct and reporting of this study were satisfactory and in accordance with the Subdivision F Guidelines 83-2(b) for carcinogenicity studies in mice and in compliance with the FIFRA Good Laboratory Practice (GLP) standards. The study is classified as Core Guideline.

The 2 highest dose levels used in this study, 16000 ppm (equivalent to 2978 mg/kg/day in males and 3448 mg/kg/day in females) and 8000 ppm (equivalent to 1476 mg/kg/day in males and 1707 mg/kg/day in females), both exceeded the limit dose of 1000 mg/kg/day which is oftentimes used by EPA to establish an upper dose level for carcinogenicity studies in mice and rats. In this instance, however, it should be recalled that EPA required that the highest dose levels in this particular study be 16000 ppm and 8000 ppm in order to duplicate the dose levels used in a previously conducted 1978 NCI carcinogenicity in B6C3F1 mice in which the results were equivocal.

At the highest dose levels used in this study (16000 ppm and 8000 ppm), life-threatening effects were not observed in either males or females as evidenced by the

very few deaths which occurred during the 18 month in-life phase of the study and the lack of any differences in survival rates between control and malathion-treated groups. This finding is noteworthy because these same animals did have substantially decreased mean plasma and erythrocyte cholinesterase activity levels at 12 and 18 months and also highly increased incidences of hepatocellular adenomas and combined hepatocellular adenomas/carcinomas at 18 months. Clearly, neither of these conditions had any effect on survival in this study.

Levels of plasma cholinesterase activity at 12 and 18 months were ≤ 7% of the control level for 16000 ppm animals and ≤ 14% of the control level for 8000 ppm animals. Levels of erythrocyte cholinesterase activity at these times were ≤ 33% of the control level for 16000 ppm animals and ≤ 39% of the control level for 8000 ppm animals. In addition, at 18 months, brain cholinesterase activity for 16000 ppm animals was 57-63% of the control level and for 8000 ppm animals was 77-80% of the control level. It is also noteworthy that in spite of these low plasma, erythrocyte and brain cholinesterase activity levels that no clinical signs of toxicity ordinarily associated with poisoning by cholinesterase inhibitor compounds (such as tremors, hyperactivity, salivation, malaise etc.) were observed in these animals at any time during the study.

At 16000 ppm and 8000 ppm, absolute body weights were significantly decreased in a dose-related manner in both males and females throughout the study. At 16000 ppm, body weights for males and females were decreased 20.0% and 16.1% respectively at 18 months and at 8000 ppm, body weights were decreased 14.3% and 9.7% for males and females respectively at 18 months. Food consumption was also reduced in 16000 ppm animals during the first several weeks of the study, but returned to control levels by 13 weeks. It is thought that this initial reduction in food intake may have been due to poor palatability of the diet mixture or possibly to lack of appetite resulting from the low plasma and cholinesterase activity levels presumably present in these animals at that time. After 26 weeks, however, food consumption in both 16000 ppm and 8000 ppm males and females was observed to decrease in a dose-related manner and was apparently treatment-related. The decreases in food consumption were probably not sufficient in magnitude to account fully for the decreased body weights observed in these same animals.

Of most concern in this study was the treatment-related and dose-related increased incidence of hepatocellular tumors observed in both male and female mice at 16000 ppm and 8000 ppm. For mice which died during the last 6 months of the study or were killed at the terminal sacrifice at 18 months, the percent incidences of combined adenomas/carcinomas at 16000 ppm were 96.1% and 84.6% in males and females respectively and at 8000 ppm were 32.7% and 18.9% in males and females respectively. The large majority of these tumors in both males and females were hepatocellular adenomas, which were clearly related to treatment with malathion. Although hepatocellular carcinomas were also observed in these groups and in other treated groups, the incidences were not dose-related and overall the relationship of these carcinomas to treatment with malathion was equivocal. It might be noted that the authors of the study report did not consider the hepatocellular carcinomas observed in the mice in this study to be test substance-related.

Regarding the male mice in this study, more hepatocellular tumors (adenomas, carcinomas and combined adenomas/ carcinomas) were observed at 100 ppm than at 800 ppm. Thus, a dose-related response was not observed at the lower dose levels of 100 ppm and 800 ppm, whereas a dose-related response was clearly observed at the higher dose levels of 8000 ppm and 16000 ppm. How to interpret the increased numbers of hepatocellular tumors at 100 ppm, but not at 800 ppm, for the male mice in this study is not clear at this time.

As would be expected, the increased tumor incidences in the livers of both males and females at 16000 ppm and 8000 ppm were accompanied by concurrent observations of masses, nodules and foci in the livers of these animals at the terminal sacrifice and also by increased liver weights and highly increased hepatocellular hypertrophy in the livers at 12 and 18 months. The data for hepatocyte hypertrophy was quite remarkable in that an extremely steep dose-response curve was observed for both males and females in this study. Thus, in the control, 100 ppm and 800 ppm groups, <u>no</u> case of hepatocellular hypertrophy was observed in any animal at any time during the entire duration of this study. At 16000 ppm and 8000 ppm, however, the incidence of hepatocellular hypertrophy during the first year of the study (including the 12-month interim sacrifice) was $\geq$ 70% in males and $\geq$ 50% in females and during the last 6 months of the study (including the 18-month terminal sacrifice) was <u>100%</u> in both males and females.



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Other findings were observed in this study that appeared to be related to treatment, but their biological significance was uncertain. These findings included the following: decreased vacuolation in the convoluted tubules of the kidneys in males; increased mineralization of the kidneys in females; decreased fibrous osteodystrophy of the femur and sternum in females; and early disappearance of the "x zone" in the the adrenal cortex of females.

The NOEL for cholinesterase inhibition for both sexes was estimated to be 100 ppm for plasma and erythrocyte cholinesterase and 8000 ppm for brain cholinesterase. The NOEL for systemic effects was 800 ppm, based on decreased body weights and food consumption in males and females, increased liver weight in males and females and increased hepatocellular hypertrophy in males and females. The biological significance of the decreased vacuolization of convoluted tubules in the kidney in males, increased mineralization of the kidney in females, decreased fibrous osteodystrophy in females and early disappearance of the "x zone" of the adrenal cortex in females at this and other doses is uncertain.

HED Info/ Cancer Assessment Review Committee - Malathion

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Table 1. Fates of Animals on Study -- Male and Female Mice Given Malathion in the Diet for 78 Weeks [1]

| Males | 0 ppm | 100 ppm | 800 ppm | 8000 ppm | 16000 ppm |
|---|---|---|---|---|---|
| Unscheduled [2] Deaths (Weeks) | | | | | |
| 0 - 13 | 0 | 0 | 0 | 0 | 1 |
| 14 - 26 | 0 | 0 | 0 | 0 | 1 |
| 27 - 39 | 0 | 0 | 0 | 0 | 2 |
| 40 - 52 | 3 | 0 | 0 | 0 | 0 |
| 53 - 65 | 2 | 1 | 4 | 0 | 1 |
| 66 - 78 | 2 | 3 | 3 | 1 [3] | 0 |
| Interim Sacrifice 52 weeks | 8 | 10 | 10 | 10 | 10 |
| Terminal Sacrifice 78 weeks | 50 | 51 | 48 | 54 | 50 [4] |
| Total | 65 | 65 | 65 | 65 | 65 |

| Females | 0 ppm | 100 ppm | 800 ppm | 8000 ppm | 16000 ppm |
|---|---|---|---|---|---|
| Unscheduled [2] Deaths (Weeks) | | | | | |
| 0 - 13 | 0 | 0 | 0 | 1 | 1 |
| 14 - 26 | 0 | 0 | 0 | 1 [5] | 1 |
| 27 - 39 | 0 | 0 | 1 | 0 | 1 [5] |
| 40 - 52 | 0 | 0 | 0 | 0 | 0 |
| 53 - 65 | 0 | 0 | 1 | 0 | 0 |
| 66 - 78 | 0 | 3 | 1 | 0 | 1 [3] |
| Interim Sacrifice 52 weeks | 10 | 10 | 10 | 10 | 10 |
| Terminal Sacrifice 78 weeks | 55 | 52 | 52 | 53 [4] | 51 |
| Total | 65 | 65 | 65 | 65 | 65 |

(1) Data extracted from Table 1 (p. 38) and Appendix C (pp. 253-273) of study report.
(2) Includes animals found dead, sacrificed <u>in extremis</u> and died prior to sacrifice <u>in extremis</u> except as noted in footnotes below.
(3) Died following blood collection (at 77+ weeks).
(4) One died following blood collection at terminal sacrifice.
(5) Accidental death.

Table 2. Mean Absolute Body Weights (gm) for Male and Female Mice Given Malathion in the Diet for 78 Weeks [1]

| Males<br>Week of Study | 0 ppm | 100 ppm | 800 ppm | 8000 ppm | 16000 ppm |
|---|---|---|---|---|---|
| 0 | 23 | 23 | 23 | 23 | 22 |
| 1 | 23 | 24 | 23 | 22 ** | 21 ** |
| 3 | 25 | 25 | 25 | 24 ** | 22 ** |
| 5 | 26 | 26 * | 26 | 25 | 24 ** |
| 7 | 26 | 27 | 27 | 26 ** | 24 ** |
| 9 | 27 | 27 | 27 | 26 ** | 25 ** |
| 11 | 28 | 28 | 28 | 27 ** | 25 ** |
| 13 | 28 | 28 | 28 | 27 ** | 25 ** |
| 26 | 31 | 31 | 30 | 28 ** | 27 ** |
| 39 | 32 | 32 | 31 | 29 ** | 28 ** |
| 51 | 34 | 35 | 33 | 30 ** | 28 ** |
| 64 | 34 | 35 | 34 | 30 ** | 29 ** |
| 78 | 35 | 34 | 34 | 30 ** | 28 ** |

| Females<br>Week of Study | 0 ppm | 100 ppm | 800 ppm | 8000 ppm | 16000 ppm |
|---|---|---|---|---|---|
| 0 | 20 | 20 | 20 | 20 * | 20 ** |
| 1 | 21 | 21 | 21 | 20 ** | 18 ** |
| 3 | 23 | 23 | 22 | 21 ** | 19 ** |
| 5 | 24 | 24 | 24 | 22 ** | 20 ** |
| 7 | 24 | 25 | 25 | 23 ** | 21 ** |
| 9 | 25 | 26 ** | 25 | 24 ** | 22 ** |
| 11 | 26 | 26 | 26 | 24 ** | 22 ** |
| 13 | 26 | 26 | 26 | 24 ** | 22 ** |
| 26 | 28 | 28 | 28 | 26 ** | 24 ** |
| 39 | 29 | 29 | 29 | 26 ** | 24 ** |
| 51 | 31 | 31 | 32 | 27 ** | 25 ** |
| 64 | 31 | 31 | 31 | 28 ** | 25 ** |
| 78 | 31 | 31 | 31 | 28 ** | 26 ** |

[1] Data extracted from Table 3 (pp. 56-63) of study report.

* Significantly different from the control group ($p \leq 0.05$).
** Significantly different from the control group ($p \leq 0.01$).



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Table 3. Mean Food Consumption (gm/animal/day) for Male and Female Mice Given Malathion in the Diet for 78 Weeks [1]

| Males<br>Week of Study | 0 ppm | 100 ppm | 800 ppm | 8000 ppm | 16000 ppm |
|---|---|---|---|---|---|
| 1 | 4.9 | 5.0 ** | 5.2 ** | 5.0 | 3.9 ** |
| 3 | 5.1 | 5.2 * | 5.2 | 5.2 | 4.7 ** |
| 5 | 5.0 | 5.0 | 5.1 | 5.3 ** | 4.8 ** |
| 7 | 5.2 | 5.2 | 5.4 | 5.4 | 5.2 |
| 9 | 5.2 | 5.3 | 5.4 * | 5.6 ** | 5.0 ** |
| 11 | 5.0 | 5.1 | 5.3 ** | 5.3 * | 5.1 |
| 13 | 5.4 | 5.3 | 5.5 | 5.6 | 5.4 |
| 26 | 5.0 | 5.9 | 5.0 | 5.1 | 4.8 |
| 39 | 5.1 | 5.0 | 5.1 | 4.9 ** | 4.6 ** |
| 51 | 5.1 | 5.2 | 5.1 | 4.7 ** | 4.7 ** |
| 64 | 5.3 | 5.2 | 5.1 | 4.7 ** | 4.6 ** |
| 78 | 5.1 | 5.2 | 5.1 | 4.7 ** | 4.6 ** |

| Females<br>Week of Study | 0 ppm | 100 ppm | 800 ppm | 8000 ppm | 16000 ppm |
|---|---|---|---|---|---|
| 1 | 5.2 | 5.4 ** | 5.3 | 5.2 | 3.9 ** |
| 3 | 5.5 | 5.6 | 5.6 | 5.5 | 4.5 ** |
| 5 | 5.4 | 5.6 | 5.7 | 5.5 | 4.6 ** |
| 7 | 5.8 | 5.8 | 5.7 | 5.7 | 5.1 ** |
| 9 | 5.8 | 5.8 | 5.8 | 5.6 | 5.2 ** |
| 11 | 5.6 | 5.7 | 5.7 | 5.5 | 5.2 ** |
| 13 | 6.0 | 5.9 | 6.0 | 5.7 * | 5.7 * |
| 26 | 5.5 | 5.5 | 5.5 | 5.0 ** | 4.9 ** |
| 39 | 5.5 | 5.5 | 5.5 | 5.0 ** | 4.7 ** |
| 51 | 5.7 | 5.5 | 5.6 | 5.2 ** | 5.0 ** |
| 64 | 5.8 | 5.4 | 5.6 | 4.8 ** | 4.7 ** |
| 78 | 5.6 | 5.4 | 5.4 | 4.8 ** | 4.4 ** |

[1] Data extracted from Table 4 (pp. 64-71) of study report.

* Significantly different from the control group ($p \leq 0.05$).
** Significantly different from the control group ($p \leq 0.01$).

Table 4. Residual Cholinesterase Activity [1] for Plasma, Erythrocyte and Brain for Male and Female Mice Given Malathion in the Diet for 18 Months [2]

| Males | 0 ppm | 100 ppm | 800 ppm | 8000 ppm | 16000 ppm |
|---|---|---|---|---|---|
| Plasma | | | | | |
| 12 mo. | 100% | 94% | 77% | 14% ** | 7% ** |
| 18 mo | 100 | 110 | 76 | 10 ** | 5 ** |
| Erythrocyte | | | | | |
| 9 mo. | 100 | 108 | 63 | 29 * | 29 * |
| 12 mo. | 100 | 92 | 63 * | 35 ** | 31 ** |
| 18 mo. | 100 | 85 | 56 | 10 ** | 8 ** |
| Brain | | | | | |
| 12 mo. | 100 | 110 | 112 | 96 | 76 |
| 18 mo. | 100 | 101 | 93 | 77 | 63 ** |

| Females | 0 ppm | 100 ppm | 800 ppm | 8000 ppm | 16000 ppm |
|---|---|---|---|---|---|
| Plasma | | | | | |
| 12 mo. | 100% | 100% | 82% * | 11% ** | 6% ** |
| 18 mo | 100 | 88 | 64 * | 8 ** | 4 ** |
| Erythrocyte | | | | | |
| 9 mo. | 100 | 100 | 61 ** | 39 ** | 33 ** |
| 12 mo. | 100 | 102 | 65 | 35 ** | 31 ** |
| 18 mo. | 100 | 69 | 42 * | 8 ** | 8 ** |
| Brain | | | | | |
| 12 mo. | 100 | 104 | 98 | 109 | 80 |
| 18 mo. | 100 | 90 | 97 | 80 | 57 ** |

(1) Percent of cholinesterase activity remaining as compared to mean of control group for the same sex.

(2) Data extracted from Table 7 (pp. 88-91) of study report.

* Significantly different from the control group ($p \leq 0.05$).
** Significantly different from the control group ($p \leq 0.01$).

Table 5. Selected Macroscopic Observations at Terminal Sacrifice for Male and Female Mice Given Malathion in the Diet for 18 Months [1]

| Males | 0 ppm | 100 ppm | 800 ppm | 8000 ppm | 16000 ppm |
|---|---|---|---|---|---|
| No. Exam. | 50 | 51 | 48 | 54 | 50 |
| Liver | | | | | |
| Mass | 0 | 8 | 4 [2] | 5 | 18 |
| Nodule | 5 | 2 | 3 | 10 | 19 |
| Focus/foci (tan/yellow) | | | | | |
| --trace | 0 | 0 | 0 | 1 | 4 |
| --mild | 0 | 0 | 1 | 1 | 13 |
| --moderate | 0 | 0 | 0 | 0 | 1 |
| Lymph Node, Hepatic | | | | | |
| Enlarged | 0 | 0 | 2 [3] | 1 | 1 |

| Females | 0 ppm | 100 ppm | 800 ppm | 8000 ppm | 16000 ppm |
|---|---|---|---|---|---|
| No. Exam. | 55 | 52 | 52 | 53 | 51 |
| Liver | | | | | |
| Mass | 1 | 0 | 3 | 2 | 10 |
| Nodule | 1 | 2 | 0 | 9 | 29 |
| Focus/foci (tan/yellow) | | | | | |
| --trace | 0 | 0 | 0 | 1 | 6 [4] |
| --mild | 0 | 0 | 0 | 1 | 3 |
| --moderate | 0 | 0 | 0 | 0 | 0 |
| Lymph Node, Hepatic | | | | | |
| Enlarged | 0 | 0 | 0 | 0 | 1 |

(1) Data extracted from Table 8 (pp. 92-104) of study report.

(2) One additional mass was found in a mouse which died at 12 - 18 months.

(3) One additional enlarged hepatic lymph node was found in a mouse which died at 12 - 18 months.

(4) One additional focus/foci (tan/yellow; trace) was found in a mouse which died at 12 - 18 months.

Table 6. Mean Organ Weights, Organ/Body Weight Ratios and Organ/Brain Weight Ratios for Male Mice Given Malathion in the Diet for 12 Months and for 18 Months [1]

| Males | 0 ppm | 100 ppm | 800 ppm | 8000 ppm | 16000 ppm |
|---|---|---|---|---|---|
| **12 Months** | | | | | |
| Body Weight | 31 | 32 | 33 | 28 ** | 29 ** |
| Brain | | | | | |
| Absolute | 0.47 | 0.47 | 0.48 | 0.45 | 0.45 * |
| Brain/BW | 14.9 | 14.6 | 14.6 | 16.0 | 15.6 |
| Kidney | | | | | |
| Absolute | 0.69 | 0.78 * | 0.77 | 0.63 | 0.64 |
| Kidney/BW | 2.21 | 2.38 * | 2.35 | 2.21 | 2.23 |
| Kidney/Brain | 1.5 | 1.7 * | 1.6 | 1.4 | 1.4 |
| Liver | | | | | |
| Absolute | 1.62 | 1.71 | 1.78 | 1.98 ** | 2.38 ** |
| Liver/BW | 5.15 | 5.19 | 5.42 | 6.95 ** | 8.30 ** |
| Liver/Brain | 3.45 | 3.69 | 3.73 | 4.37 ** | 5.32 ** |
| **18 Months** | | | | | |
| Body Weight | 34 | 34 | 34 | 30 ** | 28 ** |
| Brain | | | | | |
| Absolute | 0.47 | 0.47 | 0.47 | 0.46 | 0.46 ** |
| Brain/BW | 13.9 | 13.9 | 13.9 | 15.5 ** | 16.1 ** |
| Kidney | | | | | |
| Absolute | 0.84 | 0.86 | 0.88 | 0.79 | 0.68 ** |
| Kidney/BW | 2.46 | 2.51 | 2.62 | 2.66 | 2.38 |
| Kidney/Brain | 1.8 | 1.8 | 1.9 | 1.7 | 1.5 ** |
| Liver | | | | | |
| Absolute | 1.90 | 2.09 | 1.96 | 2.26 ** | 2.66 ** |
| Liver/BW | 5.59 | 6.15 | 5.82 | 7.51 ** | 9.38 ** |
| Liver/Brain | 4.05 | 4.46 | 4.21 | 4.87 ** | 5.85 ** |
| Heart | | | | | |
| Absolute | 0.22 | 0.23 | 0.22 | 0.21 | 0.18 ** |
| Heart/BW | [illegible] | 6.77 | 6.62 | 6.95 | 6.40 |
| Heart/Brain | 4.76 | 4.90 | 4.76 | 4.48 | 3.99 ** |
| Lung | | | | | |
| Absolute | 0.27 | 0.28 | 0.27 | 0.28 | 0.28 |
| Lung/BW | 8.01 | 8.32 | 8.00 | 9.46 ** | 9.71 ** |
| Lung/Brain | 5.81 | 6.00 | 5.79 | 6.09 | 6.06 |
| Testes | | | | | |
| Absolute | 0.21 | 0.21 | 0.22 | 0.22 | 0.21 |
| Testes/BW | 6.30 | 6.30 | 6.46 | 7.29 ** | 7.56 ** |
| Testes/Brain | 4.55 | 4.54 | 4.64 | 4.70 | 4.72 |

(1) Data extracted from Table 9 (pp. 105-128) of study report.

* Significantly different from the control group ($p \leq 0.05$).
** Significantly different from the control group ($p \leq 0.01$).

Table 7. Mean Organ Weights, Organ/Body Weight Ratios and Organ/Brain Weight Ratios for Female Mice Given Malathion in the Diet for 12 Months and for 18 Months [1]

| Females | 0 ppm | 100 ppm | 800 ppm | 8000 ppm | 16000 ppm |
|---|---|---|---|---|---|
| **12 Months** | | | | | |
| Body Weight | 30 | 31 | 30 | 27 ** | 26 ** |
| Brain | | | | | |
| Absolute | 0.48 | 0.49 | 0.48 | 0.47 | 0.46 |
| Brain/BW | 16.3 | 15.7 | 16.1 | 17.5 | 18.2 ** |
| Kidney | | | | | |
| Absolute | 0.50 | 0.54 * | 0.55 * | 0.50 | 0.51 |
| Kidney/BW | 1.66 | 1.75 | 1.82 * | 1.88 ** | 1.99 ** |
| Kidney/Brain | 1.0 | 1.1 | 1.2 | 1.1 | 1.1 |
| Liver | | | | | |
| Absolute | 1.55 | 1.68 | 1.56 | 1.66 | 1.92 ** |
| Liver/BW | 5.22 | 5.39 | 5.22 | 6.21 ** | 7.56 ** |
| Liver/Brain | 3.23 | 3.45 | 3.24 | 3.55 | 4.16 ** |
| **18 Months** | | | | | |
| Body Weight | 31 | 31 | 31 | 28 ** | 26 ** |
| Brain | | | | | |
| Absolute | 0.50 | 0.49 | 0.50 | 0.48 ** | 0.46 ** |
| Brain/BW | 16.2 | 16.0 | 15.9 | 17.3 * | 17.8 ** |
| Kidney | | | | | |
| Absolute | 0.58 | 0.59 | 0.63 ** | 0.59 | 0.55 |
| Kidney/BW | 1.88 | 1.94 | 2.03 ** | 2.15 ** | 2.16 ** |
| Kidney/Brain | 1.2 | 1.2 | 1.3 ** | 1.2 ** | 1.2 |
| Liver | | | | | |
| Absolute | 1.93 | 1.77 | 1.96 | 1.92 | 2.18 |
| Liver/BW | 6.19 | 5.76 | 6.26 | 6.90 | 8.51 ** |
| Liver/Brain | 3.95 | 3.64 | 3.97 | 4.04 | 4.79 |
| Heart | | | | | |
| Absolute | 0.19 | 0.19 | 0.19 | 0.18 | 0.15 ** |
| Heart/BW | 6.16 | 6.26 | 6.07 | 6.50 | 5.84 |
| Heart/Brain | 3.84 | 3.95 | 3.84 | 3.78 | 3.29 ** |
| Lung | | | | | |
| Absolute | 0.29 | 0.31 | 0.27 | 0.27 | 0.24 ** |
| Lung/BW | 9.54 | 10.45 | 8.76 | 9.92 | 9.39 |
| Lung/Brain | 5.95 | 6.46 | 5.52 | 5.79 | 5.29 |
| Spleen | | | | | |
| Absolute | 0.14 | 0.17 | 0.14 | 0.11 * | 0.08 ** |
| Spleen/BW | 4.68 | 5.29 | 4.64 | 3.84 | 3.24 |
| Spleen/Brain | 2.92 | 3.48 | 2.93 | 2.24 | 1.82 |

[1] Data extracted from Table 9 (pp. 105-128) of study report.

* Significantly different from the control group ($p \leq 0.05$).
** Significantly different from the control group ($p \leq 0.01$).



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Table 8. Selected Nonneoplastic Microscopic Findings for Male and Female Mice Given Malathion in the Diet for 18 Months [1]

0 - 12 MONTHS

| MALES | 0 ppm | | 100 ppm | | 800 ppm | | 8000 ppm | | 16000 ppm | |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 - 12 Mo [2] | DOS | SAC | DOS | SAC | DOS | SAC | DOS | SAC | DOS | SAC |
| | 3 | 8 | 0 | 10 | 0 | 10 | 0 | 10 | 4 | 10 |
| **Liver** | | | | | | | | | | |
| No. Exam. | 3 | 8 | 0 | 10 | 0 | 10 | 0 | 10 | 4 | 10 |
| Hepatocyte hypertrophy | 0/[illegible] | | 0/10 | | 0/10 | | 7/10 (1.0) [3] | | 12/14 (2.3) | |
| **Kidney** | | | | | | | | | | |
| No. Exam. | 3 | 8 | 0 | 10 | 0 | 10 | 0 | 10 | 4 | 10 |
| Mineralization | 3/11 (1.0) | | 0/10 | | 0/10 | | 0/10 | | 6/14 (1.0) | |
| Nephritis, interstitial | 3/11 (1.0) | | 6/10 (1.2) | | 7/10 (1.0) | | 4/10 (1.0) | | 7/14 (1.0) | |
| Vacuolation, conv tubules | 11/11 (2.5) | | 10/10 (1.8) | | 10/10 (2.4) | | 1/10 (1.0) | | 0/14 | |
| **Adrenal Cortex** | | | | | | | | | | |
| No. Exam. | 3 | 8 | 0 | 10 | 0 | 10 | 0 | 10 | 4 | 10 |
| A cell hyperplasia | 3/11 (1.0) | | 6/10 (1.0) | | 1/10 (1.0) | | 5/10 (1.0) | | 1/14 (1.0) | |
| x zone present [4] | 0/11 | | 0/10 | | 0/10 | | 0/10 | | 0/14 | |

| FEMALES | 0 ppm | | 100 ppm | | 800 ppm | | 8000 ppm | | 16000 ppm | |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 - 12 Mo [2] | DOS | SAC | DOS | SAC | DOS | SAC | DOS | SAC | DOS | SAC |
| | 0 | 10 | 0 | 10 | 1 | 10 | 2 | 10 | 3 | 10 |
| **Liver** | | | | | | | | | | |
| No. Exam. | 0 | 10 | 0 | 10 | 1 | 10 | 2 | 10 | 3 | 10 |
| Hepatocyte hypertrophy | 0/10 | | 0/10 | | 0/11 | | 6/12 (1.0) | | 13/13 (2.2) | |

Table 8. Cont.

0 - 12 MONTHS

FEMALES (Cont.)

| | | | | | |
|---|---|---|---|---|---|
| **Kidney** | | | | | |
| No. Exam. | 0 10 | 0 10 | 1 10 | 2 10 | 3 10 |
| Mineralization | 0/10 | 0/10 | 0/11 | 0/12 | 1/13 (1.0) |
| Nephritis, interstitial | 3/10 (1.0) | 1/10 (1.0) | 3/11 (1.0) | 3/12 (1.0) | 3/13 (2.0) |
| Vacuolation, conv tubules | 0/10 | 0/10 | 0/11 | 0/12 | 0/13 |
| **Adrenal Cortex** | | | | | |
| No. Exam. | 0 10 | 0 10 | 1 10 | 2 10 | 3 10 |
| A cell hyperplasia | 10/10 (2.0) | 10/10 (1.9) | 11/11 (1.4) | 12/12 (1.1) | 11/13 (1.7) |
| x zone present | 10/10 | 8/10 | 9/11 | 1/12 | 0/13 |
| **Uterus** | | | | | |
| No. Exam. | 0 10 | 0 8 | 1 7 | 2 3 | 3 10 |
| Cyst | 9/10 (2.0) | 8/8 (2.3) | 7/8 (3.0) | 3/5 (2.7) | 8/13 (1.6) |

(1) Data extracted from Table 10 (pp. 129-178) of study report.

(2) Includes mice that died or were sacrificed in extremis during 0 - 12 months (DOS) and mice sacrificed at 12 month interim sacrifice (SAC).

(3) Numbers in parentheses indicate average severity score as follows: trace = 1, mild = 2, moderate = 3, severe = 4.

(4) Males do not have a "x zone" in the adrenal cortex.

Table 9. Selected Nonneoplastic Microscopic Findings for Male and Female Mice Given Malathion in the Diet for 18 Months [1]

12 MONTHS - TERMINATION

| MALES | 0 ppm | | 100 ppm | | 800 ppm | | 8000 ppm | | 16000 ppm | |
|---|---|---|---|---|---|---|---|---|---|---|
| | DOS | SAC | DOS | SAC | DOS | SAC | DOS | SAC | DOS | SAC |
| 12 Mo-Term [2] | 4 | 50 | 4 | 51 | 7 | 48 | 1 | 54 | 1 | 50 |
| **Liver** | | | | | | | | | | |
| No. Exam. | 4 | 50 | 4 | 51 | 7 | 48 | 1 | 54 | 1 | 50 |
| Hepatocyte hypertrophy | 0/54 | | 0/55 | | 0/55 | | 55/55 (2.1) | | 51/51 (3.1) | |
| Mononuclear cell infilt, portal | 0/54 | | 2/55 (1.0) | | 0/55 | | 4/55 (1.0) | | 4/51 (1.5) | |
| Mononuclear cell foci parenchyma | 5/54 (1.0) | | 4/55 (1.0) | | 7/55 (1.0) | | 9/55 (1.2) | | 4/51 (1.3) | |
| Necrosis | 2/54 (1.5) | | 2/55 (2.0) | | 1/55 (2.0) | | 3/55 (2.0) | | 5/51 (2.0) | |
| **Kidney** | | | | | | | | | | |
| No. Exam. | 4 | 50 | 4 | 51 | 7 | 48 | 1 | 54 | 1 | 50 |
| Mineralization | 45/54 (1.0) | | 50/55 (1.0) | | 50/55 (1.0) | | 55/55 (1.0) | | 50/51 (1.0) | |
| Nephritis, interstitial | 49/54 (1/0) | | 51/55 (1.0) | | 47/55 (1.0) | | 52/55 (1.0) | | 43/51 (1.0) | |
| Vacuolation, conv tubules | 54/54 (2.2) | | 55/55 (1.9) | | 55/55 (2.1) | | 33/55 (1.0) | | 0/51 | |
| **Adrenal Cortex** | | | | | | | | | | |
| No. Exam. | 4 | 50 | 4 | 51 | 7 | 48 | 1 | 54 | 1 | 50 |
| A cell hyperplasia | 31/54 (1.2) | | 38/55 (1.0) | | 38/55 (1.0) | | 38/55 (1.0) | | 39/51 (1.2) | |
| x zone present [4] | 0/54 | | 0/55 | | 0/55 | | 0/55 | | 0/51 | |
| Degeneration, brown | 1/54 | | 6/55 | | 1/55 | | 17/55 | | 7/51 | |

Table 9. Cont.

12 MONTHS - TERMINATION

MALES (Cont.)

| | | | | | |
|---|---|---|---|---|---|
| **Bone, Femur** | | | | | |
| No. Exam. | 4 50 | 4 0 | 7 0 | 1 0 | 1 50 |
| Fibrous osteo-dystrophy | 0/54 | 0/4 | 0/7 | 0/1 | 0/51 |
| **Bone, Sternum** | | | | | |
| No. Exam. | 4 50 | 4 0 | 7 0 | 1 0 | 1 49 |
| Fibrous osteo-dystrophy | 0/54 | 0/4 | 0/7 | 0/1 | 0/50 |
| **Lacrimal Gland** | | | | | |
| No. Exam. | 4 50 | 4 0 | 7 0 | 1 0 | 1 50 |
| Acinar atrophy | 0/54 | 0/4 | 0/7 | 0/1 | 0/51 |
| Mononuclear cell infilt | 18/54 (1.0) | 0/4 | 1/7 (1.0) | 0/1 | 18/51 (1.1) |
| **Lung** | | | | | |
| No. Exam. | 4 50 | 4 51 | 7 48 | 1 54 | 1 50 |
| Mineralization | 10/54 (1.0) | 10/55 (1.0) | 12/55 (1.0) | 15/55 (1.0) | 28/51 (1.0) |
| Peribronchial lymph infilt | 0/54 | 0/55 | 0/55 | 0/55 | 0/51 |
| Perivascular mononuc infil | 1/54 (1.0) | 3/55 (1.0) | 2/55 (1.0) | 2/55 (1.0) | 5/51 (1.2) |
| **Lymph Node, Mandibular** | | | | | |
| No. Exam. | 4 50 | 3 0 | 7 0 | 1 0 | 1 49 |
| Hemorrhage | 15/54 (1.0) | 0/3 | 1/7 (2.0) | 1/1 (1.0) | 9/50 (1.4) |
| Pigment, brown | 5/54 (1.2) | 1/3 (1.0) | 1/7 (1.0) | 0/1 | 12/50 (1.1) |



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Table 9. Cont.

12 MONTHS - TERMINATION

| FEMALES | 0 ppm | | 100 ppm | | 800 ppm | | 8000 ppm | | 16000 ppm | |
|---|---|---|---|---|---|---|---|---|---|---|
| 12 Mo-Term (2) | DOS | SAC | DOS | SAC | DOS | SAC | DOS | SAC | DOS | SAC |
| | 0 | 55 | 3 | 52 | 2 | 52 | 0 | 53 | 1 | 51 |
| **Liver** | | | | | | | | | | |
| No. Exam. | 0 | 55 | 3 | 52 | 2 | 52 | 0 | 53 | 1 | 51 |
| Hepatocyte hypertrophy | 0/55 | | 0/55 | | 0/54 | | 53/53 (1.7) | | 52/52 (3.1) | |
| Mononuclear cell infilt, portal | 8/55 (1.0) | | 7/55 (1.3) | | 4/54 (1/0) | | 5/53 (1.0) | | 7/52 (1.0) | |
| Mononuclear cell foci parenchyma | 19/55 (1.0) | | 21/55 (1.0) | | 12/54 (1.1) | | 18/53 (1.1) | | 24/52 (1.0) | |
| Necrosis | 1/55 (1.0) | | 0/55 | | 0/54 | | 0/53 | | 0/52 | |
| **Kidney** | | | | | | | | | | |
| No. Exam. | 0 | 55 | 3 | 52 | 2 | 52 | 0 | 53 | 1 | 51 |
| Mineralization | 1/55 (1.0) | | 6/55 (1.0) | | 8/54 (1.0) | | 32/53 (1.0) | | 36/52 (1.0) | |
| Nephritis, interstitial | 27/55 (1.0) | | 18/55 (1.0) | | 27/54 (1.0) | | 33/53 (1.0) | | 29/52 (1.0) | |
| Vacuolation, conv tubules | 0/55 | | 0/55 | | 0/54 | | 0/53 | | 0/52 | |
| **Adrenal Cortex** | | | | | | | | | | |
| No. Exam. | 0 | 55 | 3 | 52 | 2 | 52 | 0 | 53 | 1 | 51 |
| A cell hyperplasia | 55/55 (2.2) | | 53/55 (1.9) | | 54/54 (1.9) | | 53/53 (1.8) | | 52/52 (2.2) | |
| x zone present | 2/55 | | 1/55 | | 1/54 | | 0/53 | | 0/52 | |
| Degeneration, brown | 23/55 (1.0) | | 48/55 (1.0) | | 42/54 (1.0) | | 25/53 (1.0) | | 16/52 (1.0) | |



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Table 9. Cont.

12 MONTHS - TERMINATION

FEMALES (Cont.)

| | | | | | |
|---|---|---|---|---|---|
| **Bone, Femur** | | | | | |
| No. Exam. | 0 55 | 3 52 | 2 52 | 0 53 | 1 51 |
| Fibrous osteo-dystrophy | 23/55 (1.2) | 14/55 (1.2) | 7/54 (1.3) | 3/53 (1.0) | 2/52 (1.0) |
| **Bone, Sternum** | | | | | |
| No. Exam. | 0 55 | 3 52 | 2 52 | 0 53 | 1 51 |
| Fibrous osteo-dystrophy | 51/55 (1.7) | 48/55 (1.6) | 50/54 (1.8) | 50/53 (1.5) | 10/52 (1.0) |
| **Lacrimal Gland** | | | | | |
| No. Exam. | 0 55 | 3 0 | 2 0 | 0 0 | 1 51 |
| Acinar atrophy | 3/55 (2.0) | 0/3 | 0/2 | 0/0 | 10/52 (1.7) |
| Mononuclear cell infilt | 52/55 (1.5) | 0/3 | 1/2 (1.0) | 0/0 | 47/52 (1.6) |
| **Lung** | | | | | |
| No. Exam. | 0 55 | 3 52 | 2 52 | 0 53 | 1 51 |
| Mineralization | 8/55 (1.0) | 6/55 (1.0) | 4/54 (1.0) | 18/53 (1.0) | 26/52 (1.0) |
| Peribronchial lymph infilt | 1/55 (1.0) | 0/55 | 0/54 | 7/53 (1.6) | 6/52 (1.5) |
| Perivascular mononuc infil | 12/55 (1.1) | 8/55 (1.5) | 17/54 (1.1) | 10/53 (1.4) | 9/52 (1.2) |
| **Lymph Node, Mandibular** | | | | | |
| No. Exam. | 0 55 | 3 1 | 2 0 | 0 1 | 1 51 |
| Hemorrhage | 5/55 (1.0) | 0/4 | 1/2 (2.0) | 0/1 | 12/52 (1.1) |
| Pigment, brown | 5/55 (1.0) | 0/4 | 0/2 | 0/1 | 12/52 (1.0) |



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Table 9. Cont.

12 MONTHS - TERMINATION

FEMALES (Cont.)

| | | | | | |
|---|---|---|---|---|---|
| **Uterus** | | | | | |
| No. Exam. | 0 55 | 3 42 | 2 38 | 0 39 | 1 51 |
| Cyst | 55/55 (2.7) | 43/45 (2.9) | 36/40 (2.8) | 30/39 (2.9) | 33/52 (2.1) |
| Dilatation | 1/55 (3.0) | 4/45 (4.0) | 6/40 (3.3) | 12/39 (3.7) | 17/52 (3.0) |
| **Uterus, Cervix** | | | | | |
| No. Exam. | 0 55 | 3 0 | 2 0 | 0 1 | 1 51 |
| Cyst | 53/55 (2.5) | 2/3 (1.5) | 1/2 (2.0) | 0/1 | 46/52 (2.2) |

(1) Data extracted from Table 10 (pp. 129-178) of study report.

(2) Includes mice that died or were sacrificed in extremis during 13 - 18 months (DOS) and mice sacrificed at 18 month terminal sacrifice (SAC).

(3) Numbers in parentheses indicate average severity score as follows: trace = 1, mild = 2, moderate = 3, severe = 4.

(4) Males do not have a "x zone" in the adrenal cortex.

Table 10. Liver Tumors in Male Mice Given Malathion in the Diet for 18 Months [1] [2]

| MALES | 0 ppm | 100 ppm | 800 ppm | 8000 ppm | 16000 ppm |
|---|---|---|---|---|---|
| 0 - 12 Months [3] | | | | | |
| Hepatocellular adenoma | 0/11 (0.0%) | 0/10 (0.0%) | 0/10 (0.0%) | 0/10 (0.0%) | 1/14 (7.1%) |
| 12 Months - Termination [4] | | | | | |
| Hepatocellular adenoma | 1/54 (1.9%) | 4/55 (7.3%) | 2/55 (3.6%) | 12/55 (21.8%) | 48/51[6] (94.1%) |
| Hepatocellular carcinoma | 0/54 (0.0%) | 6/55 (10.9%) | 3/55 (5.5%) | 6/55 (10.9%) | 1/51 (2.0%) |
| Combined | 1/54 (1.9%) | 10/55 (18.2%) | 5/55 (9.1%) | 18/55 (32.7%) | 49/51[6] (96.1%) |
| 0 - Termination [5] | | | | | |
| Hepatocellular adenoma | 1/65 (1.5%) | 4/65 (6.2%) | 2/65 (3.1%) | 12/65 (18.5%) | 49/65[6] (75.4%) |
| Hepatocellular carcinoma | 0/65 (0.0%) | 6/65 (9.2%) | 3/65 (4.6%) | 6/65 (9.2%) | 1/65 (1.5%) |
| Combined | 1/65 (1.5%) | 10/65 (15.4%) | 5/65 (7.7%) | 18/65 (27.7%) | 50/65[6] (76.9%) |

(1) Data extracted from Table 11 (pp. 179-234) of study report.

(2) Mice with both an adenoma and a carcinoma were listed only one time in this table (under carcinoma).

(3) Includes mice that died or were sacrificed in extremis during 0 - 12 months (DOS) and mice sacrificed at 12 month interim sacrifice (SAC).

(4) Includes mice that died or were sacrificed in extremis during 13 - 18 months (DOS) and mice sacrificed at 18 month terminal sacrifice (SAC).

(5) Includes all mice in study.

(6) 35 of these mice had multiple adenomas present.



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Table 11. Liver Tumors in Female Mice Given Malathion in the Diet for 18 Months [1] [2]

| FEMALES | 0 ppm | 100 ppm | 800 ppm | 8000 ppm | 16000 ppm |
|---|---|---|---|---|---|
| 0 - 12 Months [3] | | | | | |
| Hepatocellular adenoma | 0/10 (0.0%) | 0/10 (0.0%) | 0/11 (0.0%) | 0/12 (0.0%) | 0/13 (0.0%) |
| 12 Months - Termination [4] | | | | | |
| Hepatocellular adenoma | 0/55 (0.0%) | 1/55 (1.8%) | 0/54 (0.0%) | 9/53[6] (17.0%) | 42/52[7] (80.8%) |
| Hepatocellular carcinoma | 1/55 (1.8%) | 0/55 (0.0%) | 2/54 (3.7%) | 1/53 (1.9%) | 2/52 (3.8%) |
| Combined | 1/55 (1.8%) | 1/55 (1.8%) | 2/54 (3.7%) | 10/53[6] (18.9%) | 44/52[7] (84.6%) |
| 0 - Termination [5] | | | | | |
| Hepatocellular adenoma | 0/65 (0.0%) | 1/65 (1.5%) | 0/65 (0.0%) | 9/65[6] (13.8%) | 42/65[7] (64.6%) |
| Hepatocellular carcinoma | 1/65 (1.5%) | 0/65 (0.0%) | 2/65 (3.1%) | 1/65 (1.5%) | 2/65 (3.1%) |
| Combined | 1/65 (1.5%) | 1/65 (1.5%) | 2/65 (3.1%) | 10/65[6] (15.4%) | 44/65[7] (67.7%) |

(1) Data extracted from Table 11 (pp. 179-234) of study report.

(2) Mice with both an adenoma and a carcinoma were listed only one time in this table (under carcinoma).

(3) Includes mice that died or were sacrificed in extremis during 0 - 12 months (DOS) and mice sacrificed at 12 month interim sacrifice (SAC).

(4) Includes mice that died or were sacrificed in extremis during 13 - 18 months (DOS) and mice sacrificed at 18 month terminal sacrifice (SAC).

(5) Includes all mice in study.

(6) 1 of these mice had multiple adenomas present.

(7) 23 of these mice had multiple adenomas present.

Table 12. Historical Control Data for Liver Tumors in B6C3F1 Mice for Studies Conducted at International Research and Development Corporation from 1983 Through 1992 [1]

| MALES | Percent Incidence (Range) [2] |
|---|---|
| 18-Month Studies [3] | |
| Hepatocellular adenoma | 14.3 - 21.7% |
| Hepatocellular carcinoma | 0.0 - 6.4% |
| 24-Month Studies [4] | |
| Hepatocellular adenoma | 9.5 - 29.6% |
| Hepatocellular carcinoma | 6.4 - 11.6% |
| FEMALES | |
| 18-Month Studies [3] | |
| Hepatocellular adenoma | 0.0 - 10.6% |
| Hepatocellular carcinoma | 0.0 - 2.3% |
| 24-Month Studies [4] | |
| Hepatocellular adenoma | 0.0 - 11.6% |
| Hepatocellular carcinoma | 0.0 - 11.1% |

(1) Data extracted from Appendix O (pp. 1398-1451) of study report.

(2) Percent incidence based on incidence/number of animals examined microscopically from terminal necropsy.

(3) Control group data from 5 studies (2 dietary, 1 gavage, 1 drinking water, 1 IP injection); 50-60 mice/sex initiated on study.

(4) Control group data from 7 studies (all dietary); 50-80 mice/sex initiated on study.

HED Info - Cancer Assessment Review Committee

Malathion

Page _____ is not included in this copy.

Pages 208 through 219 are not included in this copy.

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_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

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✓ FIFRA registration data.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Attachment #11

E

DATA EVALUATION REPORT

MALAOXON

STUDY TYPE: COMBINED CHRONIC/ONCOGENICITY FEEDING - RAT (83-5)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Biomedical and Environmental Information Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 97-01

| | |
|---|---|
| Primary Reviewer: Andrew A. Francis, M.S., D.A.B.T. | Signature: Robert H. Ross for A.A. Francis<br>Date: 4-1-97 |
| Secondary Reviewers: S. Milanez, Ph.D. | Signature: S. Milanez<br>Date: 4-1-97 |
| Robert H. Ross, M.S., Group Leader | Signature: Robert H. Ross<br>Date: 4-1-97 |
| Quality Assurance: Susan Chang, M.S. | Signature: S S Chang<br>Date: 4-1-97 |

Disclaimer

This Review may have been altered subsequent to signing by the contractor's signature above.

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

656 220

EPA Reviewer: E. Budd, M.S. E. Budd, Date 6/18/97
Review Section III, Toxicology Branch I (7509C)
EPA Secondary Reviewer:
M. Copley, D.V.M., D.A.B.T. Marion Copley, Date 7/2/97
Toxicology Branch I (7509C)

# DATA EVALUATION RECORD

STUDY TYPE: Combined Chronic/Oncogenicity Feeding - Rat
OPPTS 870.4300 [§83-5]

DP BARCODE: D226123
P.C. CODE: 057701

SUBMISSION CODE: S505315
TOX. CHEM. NO.: 535

TEST MATERIAL (PURITY): Malaoxon (96.4%)

SYNONYMS: Butanedioic acid, [(dimethoxy-phosphinyl)thio], diethyl ester

CITATION: Daly, I. (1996) A 24-month oral toxicity/oncogenicity study of malaoxon in the rat via dietary administration. Final report. Huntingdon Life Sciences, Mettlers Road, P.O. Box 2360, East Millstone, New Jersey. Study No: 93-2234, April 2, 1996. MRID 43975201. Unpublished.

SPONSOR: Cheminova Agro A/S, P.O. Box 9, DK-7620, Lemvig, Denmark.

EXECUTIVE SUMMARY: In a combined chronic toxicity/oncogenicity study (MRID 43975201), malaoxon (96.4% a.i.) was administered to 85 Fischer 344 CDF® (F-344)/CrlBR rats/sex/dose in the diet for up to 104-105 weeks at dose levels of 0, 20, 1000, or 2000 ppm (equal to 0, 1, 57, or 114 mg/kg/day in males and 0, 1, 68, or 141 mg/kg/day in females). Ten animals/sex/group were sacrificed at 3 months, 6 months, and 12 months for interim evaluations and cholinesterase activity determinations. Standard parameters were examined. Full histopathological examinations were performed on control and high dose animals at 12 and 24 months and on all animals that died or were sacrificed during the study. Additional tissues, as appropriate, also were examined from other dose groups.

Mortality was significantly increased in high dose males (control, 29%; high dose, 53%, $p \leq 0.01$) and in mid and high dose females (control, 13%; mid dose, 44%, $p \leq 0.01$; high dose, 49%, $p \leq 0.01$). Body weights were decreased in the high dose males and females throughout most of the study (-1.4% to -7.1% in males and -4.0% to -8.8% in females). The mean terminal body weight of high dose males was decreased by 14% compared to the control group ($p \leq 0.05$). The mean terminal body weight of high dose females was decreased by 11% but did not reach statistical significance. Food intake was consistently greater in both sexes at the high dose and increased sporadically at the mid dose throughout the study. Treatment-related yellow ano-genital staining was observed in high dose males and females. Increased incidences of emaciated rats were seen especially among the early decedent females.

Foreign material (food, hair) and cellular debris were found in the nasal cavity of high dose males and mid and high dose females. Nasal lumen inflammation was seen in high dose males (control, 24%, high dose, 56%, $p \leq 0.01$) and in mid and high dose females (control, 9%;

mid dose, 28%, $p \leq 0.05$; high dose, 46%, $p \leq 0.001$). Nasal lumen epithelial hyperplasia was increased in mid and high dose females (control, 6%; mid dose, 46%, $p \leq 0.001$; high dose, 36%, $p \leq 0.001$). Lung interstitium inflammation was increased in mid and high dose females (control, 26%; mid dose, 53%, $p \leq 0.01$; high dose, 58%, $p \leq 0.001$), and tympanic cavity inflammation was seen in mid and high dose early female decedents (control, 0; mid dose, 85%, $p \leq 0.001$; high dose, 74%, $p \leq 0.001$). Increased incidences of mineral deposits in the stomach muscularis were seen in mid and high dose males (control, 4%; mid dose, 24%, $p \leq 0.01$; high dose, 46%, $p \leq 0.001$) and females (control, 0; mid dose, 9%, $p \leq 0.05$; high dose, 42%, $p \leq 0.001$). The mean liver and kidney weights were increased by 22% and 10%, respectively, ($p \leq 0.01$) in high dose males at 12 months, and the mean adrenal weight was increased in high dose males by 13% at 24 months ($p \leq 0.05$). The mean spleen weight was decreased by 51% in high dose females at 24 months ($p \leq 0.01$). No enzyme or microscopic changes were found to correlate with the organ weight changes.

The plasma cholinesterase activity was decreased in males by 74%-91% and in females by 82%-96% compared to the controls after 3, 6, 12, and 24 months of Malaoxon treatment at the mid and high doses ($p \leq 0.05$ or 0.01). The red cell cholinesterase activity was decreased by 54%-66% in males and 45%-66% in females at the mid and high doses. The red cell cholinesterase activity was also decreased by 21% in males and 19% in females at 6 months of treatment at 20 ppm ($p \leq 0.01$). Brain cholinesterase activity was decreased 11%-74% compared to controls in high dose males at all time points and at the mid dose by 30% at 24 months ($p \leq 0.01$). It was decreased by 61%-78% in high dose females at all time points and by 5%-14% at the mid dose after 3, 6, and 12 months of treatment in females ($p \leq 0.05$ or 0.01).

A NOEL was not determined for cholinesterase activity inhibition in this study. The LOEL is 20 ppm (1 mg/kg/day) for males and females based on the 19-21% inhibition of red blood cell cholinesterase activity after 6 months of treatment. A NOEL of 20 ppm (1 mg/kg/day) and a LOEL of 1000 ppm (57 mg/kg/day for males, 68 mg/kg/day for females) for systemic toxicity were defined. In females, the systemic LOEL was based on increased mortality, and microscopic changes in the nasoturbinal tissues, lung interstitium, and tympanic cavity. In males, the systemic LOEL was based on mineral deposits in the stomach muscularis.

At the doses tested, there <u>was not</u> a treatment-related increase in tumor incidence after 105 weeks of treatment with malaoxon.

This combined chronic toxicity/oncogenicity study in the rat is acceptable, and does satisfy the guideline requirement for a combined chronic toxicity/oncogenicity study (83-5)in rats.

<u>COMPLIANCE</u>: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

# I. MATERIALS AND METHODS

## A. MATERIALS

### 1. Test material: Malaoxon

Description: colorless liquid
Lot/Batch #: 279-ABB-036 used in the diet
Purity: 96.4%
Stability of compound: Stable at room temperature, protected from light.
CAS #: Not supplied
Source: Cheminova Agro A/S (Lemvig, Denmark)

### 2. Vehicle and/or positive control

Test substance was mixed with food.

### 3. Test animals

Species: rat
Strain: Fischer 344 CDF® (F-344)/CrlBR
Age and weight at study initiation: males and females, about 42 days; weights- males, 101-134 g; females, 80-110 g
Source: Charles River Laboratories, Kingston, New York 12484
Housing: rats were housed doubly by sex during the initial week and singly thereafter in elevated stainless steel wire-mesh cages. The racks were rotated in the animal room every 2 weeks beginning on Sept. 2, 1993.
Diet: Purina Certified Rodent Chow® #5002 (meal). ad libitum
Water: tap water ad libitum
Environmental conditions:
Temperature: 66-79° F.
Humidity: 21-77%.
Air changes: not supplied
Photoperiod: 12 hours light/12 hours dark
Acclimation period: 14 days

## B. STUDY DESIGN

### 1. In life dates

Start: May 20, 1993; end: May 24, 1995.

2. Animal assignment

Animals were assigned randomly within weight limitations to the test groups in Table 1.

TABLE 1: Study design

| Test Group | Dose to Animal[a] ppm (mg/kg/day) | | Main Study 24 Months No. Animals | | Satellite Studies | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 3 Months No. Animals | | 6 Months No. Animals | | 12 Months No. Animal. | |
| | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female |
| Control | 0 | 0 | 55 | 55 | 10 | 10 | 10 | 10 | 10 | 10 |
| Low | 20 (1) | 20 (1) | 55 | 55 | 10 | 10 | 10 | 10 | 10 | 10 |
| Mid | 1000 (57) | 1000 (68) | 55 | 55 | 10 | 10 | 10 | 10 | 10 | 10 |
| High | 2000 (114) | 2000 (141) | 55 | 55 | 10 | 10 | 10 | 10 | 10 | 10 |

Taken from pp. 22-24, and p. 48 MRID 43975201.
[a]Target dose in ppm (calculated compound consumption, average for 102 weeks).

3. Dose selection

The dose selections were based on the results from a previous range-finding study conducted at Hungingdon Life Sciences (Study Number 92-3816), a description of which was not provided.

4. Diet preparation and analysis

Diets were prepared weekly by mixing appropriate amounts of test substance with Purina Certified Rodent Chow® #5002 (meal). In preparing the dietary mixtures and dose calculations, the test substance was assumed to be 100% malaoxon. The diets were then stored at room temperature in opaque containers. After mixing, samples were taken in triplicate from the top, middle, and bottom of the container for homogeneity analyses. Samples of 23.9 ppm and 3080 ppm dietary mixtures were tested for stability for up to 14 days exposed to air at room temperature in a separate study (Pharmaco LSR Study Number 93-8167). Two samples from each dietary level were assayed weekly for the first 8 weeks, once every 2 weeks for the next 8 weeks, then at monthly intervals until the end of the study for dietary level confirmation. The analyses were performed utilizing gas chromatography with a flame photometric detector and a phosphorus filter. The limit of detection measured with an analytical standard was 0.400 ng.

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Results -
Homogeneity Analysis: Analysis of samples taken from the top, middle, and bottom of the low and high concentration dietary mixtures, a total of 12 samples for each dose level, resulted in mean concentrations of malaoxon that were 93-106% of the target low dose and 90.8-102% of the target high dose.

Stability Analysis: dietary mixtures containing 23.9 ppm and 3080 ppm malaoxon were prepared and stored at room temperature. Samples wee analyzed in duplicate on days 0, 4, 7, and 14. The low concentration was 113% of the dietary concentration on day 0, and 91.2% on day 14. Analysis of the high concentration gave levels 96.5% of the prepared concentration on day 0, and 100% on day 14. The analyzed concentrations varied from the nominal concentrations by less than 15%. The stored malaoxon stock was analyzed after 1 year and after study termination (~2 years). The pre-study analysis indicated a purity of 92.5% malaoxon, at one year it was 94.3%, and at the post study analysis it was 102%.

Concentration Analysis: The overall means of the samples taken throughout the study as percent of target concentrations for the low, mid, and high doses, respectively are: 99.6% ± 9.24, 102% ± 6.01, and 102% ± 6.27. The individual low dose samples at week 16 were found to be 115% and 116% of the target concentration. At week 76, the samples were 121% and 127% of nominal. The low dose dietary mixture was remixed for this week, and upon reanalysis, was 116% of the target concentration. All other samples were within ± 15% of the target concentrations.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics

Body weight, body weight change, food consumption, hematology and clinical chemistry parameters, terminal organ and body weights, relative weights (to body and to brain), [illegible] survival incidences were analyzed. Mean values [illegible] all dose groups were compared to the control at each time interval for all in-life data. The time to tumor analyses were performed using the Thomas, Breslow and Gart analyses, which test for both tumor incidence (chi-square and Fisher tests) and time to tumor (Kaplan-Meier curves, Cox's Tests, and the Gehan-Breslow/Kruskal-Wallis analyses). The chi-square and Fisher Exact Tests consider only simple incidence in a pairwise manner; the mean incidences of the necropsy findings in each treated group was compared to control. Cox's test and the Gehan-Breslow/Kruskal-Wallis Analyses are based on incidence and survival, and were used to indicate decreasing trends in survival with increasing dose. They separately perform a multiple comparison test, a test for trend, and a series of pairwise tests with each treated group compared to the control.

C. METHODS

1. Observations

All animals were inspected twice a day for mortality and gross signs of toxicologic or pharmacologic effects. Each animal was given a detailed physical examination, including palpation, twice before initiation of treatment and weekly throughout the treatment periods.

2. Body weight

Animals were weighed twice prior to initiation of treatment, weekly through treatment week 14, once every two weeks from week 16 through week 26, and once a month for the remainder of the treatment periods.

3. Food consumption and compound intake

Food consumption was measured weekly beginning one week prior to the study initiation through treatment week 14, once every two weeks from week 16 through week 26, then monthly through the remainder of the treatment periods. Weekly averages were reported and food intake was calculated as g/kg/day. The average test substance intake (mg/kg/day) was calculated from food consumption and body weight values. Food efficiency (body weight gain in kg/food consumption in kg per unit time X 100) values were not calculated by the study authors.

4. Ophthalmoscopic examination

The eyes of up to 10 animals per group in the 3, 6, 12-month groups in the satellite study were examined prior to study initiation and after 12 months of treatment. In the main study, up to 55 rats per group were examined prior to study initiation, after 12 months of treatment, and at study termination (24 months). Eyes were dilated prior to the examinations with Opticyl and Tropicamide (1%). The lids, lacrimal apparatus, and conjunctiva were examined grossly. The cornea, anterior chamber, lens, iris, vitreous humor, retina, and optic disc were examined with an indirect ophthalmoscope. The anterior segment, lens, and anterior vitreous were also examined by slit lamp biomicroscopy using retro illumination and optical section when indicated.

5. Blood was collected for hematology and clinical analysis from the orbital sinus of fasted animals under light $CO_2$ anesthesia. Ten animals/sex/group in the satellite study were utilized at months 6 and 12. The same animals were used at both time intervals if possible; animals that died during the study were replaced with animals from the main study. Ten rats/sex/group were also bled in the main study at 18 months and at study termination. The same animals were used for both determinations if possible.

An additional 10 animals/sex/group were bled for cholinesterase determinations after 3, 6, 12, and 24

months of treatment. Rats were not fasted prior to blood collection for cholinesterase determinations. After bleeding, the animals were sacrificed and the brain was excised and also subjected to cholinesterase activity determinations (right hemisphere). Plasma, erythrocyte and brain cholinesterase activity was determined on a Hitachi 717, Boehringer Mannheim Diagnostics Automatic Analyzer using a modified Ellman method (kinetic).

The CHECKED (X) parameters were examined.

a. Hematology

| | | | |
|---|---|---|---|
| X | | X | |
| X | Hematocrit (HCT) | X | Leukocyte differential count* |
| X | Hemoglobin (HGB) | X | Mean corpuscular HGB (MCH) |
| X | Leukocyte count (WBC) | X | Mean corpusc. HGB conc. (MCHC) |
| X | Erythrocyte count (RBC) | X | Mean corpusc. volume (MCV) |
| X | Platelet count | X | Reticulocyte count |
| | Blood clotting measurements | | |
| | (Thromboplastin time) | | |
| | (Thromboplastin time) | | |
| | (Clotting time) | | |
| | (Prothrombin time) | | |

*Minimum required for carcinogenicity studies (only on control and high dose groups unless effects are observed) based on Subdivision F Guidelines).

b. Clinical Chemistry

| | | | |
|---|---|---|---|
| X | ELECTROLYTES | X | OTHER |
| X | Calcium | X | Albumin |
| X | Chloride | X | Blood creatinine |
| | Magnesium | X | Blood urea nitrogen |
| X | Phosphorus | X | Total Cholesterol |
| X | Potassium | X | Globulins |
| X | Sodium | X | A/G ratio |
| | | X | Glucose |
| | ENZYMES | X | Total bilirubin |
| X | Alkaline phosphatase (ALK) | X | Direct bilirubin |
| X | Cholinesterase (ChE) | X | Total serum protein (TP) |
| X | Creatine phosphokinase | | Triglycerides |
| | Lactic acid dehydrogenase (LDH) | | Serum protein electrophoresis |
| X | Serum alanine amino-transferase (also SGPT) | | |
| X | Serum aspartate amino-transferase (also SGOT) | | |
| X | Gamma glutamyl transferase (GGT) | | |
| | Glutamate dehydrogenase | | |

6. Urinalysis

Urine was collected over 16 hour intervals from 10 animals/sex/group using metabolism cages at 6 months, 12 months, 18 months, and at study termination. Freshly voided samples (2-hour) were collected for analysis at the same time intervals from the same animals. The rats were fasted and water deprived for the 2-hour samples, but the

rats had water and food *ad libitum* during the 16-hour sample periods. The CHECKED (X) parameters were evaluated.

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

*Required for chronic studies.

7. Sacrifice and pathology

All animals that died spontaneously, were killed in a moribund condition, or sacrificed by exsanguination under $CO_2$ anesthesia at the scheduled termination of the 3-, 6-, 12- or 24-month studies were subjected to gross pathological examination. Brain tissue from the 3, 6, 12, and 24-month animals was analyzed for cholinesterase activity. The CHECKED (X) tissues were collected and preserved in 10% neutral buffered formalin for histological examination. All tissues listed from the control and high dose groups at the 12- and 24-month scheduled sacrifices, and from all animals that died or were killed moribund during the study were examined microscopically. The adrenals, kidneys, liver, lungs, lymph nodes, mammary glands, testes, nasal turbinates, spleen, stomach, and thyroids/parathyroids were evaluated from all animals in the low and middle dose groups at 24 months. The nasal turbinates were also examined from all low and middle dose animals at 12 months. The (XX) organs from all animals at the 12- and 24-month scheduled necropsies, in addition, were weighed.

| X | DIGESTIVE SYSTEM | X | CARDIOVASC./HEMAT. | X | NEUROLOGIC |
|---|---|---|---|---|---|
| | Tongue | X | Aorta* | XX | Brain*• |
| X | Salivary glands* | XX | Heart* | X | Periph.nerve* |
| X | Esophagus* | X | Bone marrow* | X | Spinal cord (3 levels)* |
| X | Stomach* | X | Lymph nodes* | X | Pituitary* |
| X | Duodenum* | XX | Spleen* | X | Eyes (optic n.)* |
| X | Jejunum* | X | Thymus* | | |
| X | Ileum* | | | | GLANDULAR |
| X | Cecum* | | UROGENITAL | XX | Adrenal gland* |
| X | Colon* | XX | Kidneys*• | X | Lacrimal gland |
| X | Rectum* | X | Urinary bladder* | X | Harderian gland |
| XX | Liver*• | XX | Testes*• | X | Mammary gland* |
| X | Gall bladder* | XX | Epididymides | XX | Parathyroids* |
| X | Pancreas* | X | Prostate | XX | Thyroids* |
| | | X | Seminal vesicle | X | Preputial gland |
| | RESPIRATORY | XX | Ovaries*• | X | Zymbal's gland |
| X | Trachea* | X | Oviducts | | OTHER |
| X | Lung* | X | Uterus*/cervix | X | Bone* |
| X | Nose | X | Vagina | X | Skeletal muscle* |
| | Pharynx | | | X | Skin* |
| | Larynx | | | X | All gross lesions and masses* |

* Required for carcinogenicity studies based on Subdivision F Guidelines.
• Organ weight required in chronic studies.

## II. RESULTS

### A. OBSERVATIONS

#### 1. Toxicity

The only clinical sign that appeared to be treatment related was the increase in yellow ano-genital staining seen during the last 6 months of treatment. The maximum weekly incidences in males for yellow ano-genital staining from week 90 through week 105 were: 4/50 (8%), 3/44 (7%), 6/42 (14%), and 10/27 (37%, $p \leq 0.05$); in females the maximum incidences were: 5/52 (10%), 3/42 (7%), 7/42 (17%), and 12/28 (43%, $p \leq 0.01$) for the control, low, middle, and high doses, respectively. Other clinical signs frequently seen in all dose groups especially during the last year of the oncogenicity study included unilateral lacrimation and chromodacryorrhea (bloody tears). These signs were seen less frequently and in fewer animals in the mid and high dose groups than in the control groups. The highest incidence seen for chromodacryorrhea in each group of males from treatment week 90 to study termination was 18/50 (36%), 14/44 (32%), 10/40 (25%), and 6/27 (22%, N.S.); the highest incidence seen in each group of females during the same time interval was 20/54 (37%), 20/50 (40%), 9/34 (26%), and 7/40 (18%, $p \leq 0.01$) for the control, low, middle, and high doses, respectively. (Fisher Exact test by the reviewer. Values taken from Appendix C, pp. 121-179, MRID 43975201.)

2. Mortality

The cumulative mortality at various times during the treatment period for each dose level and the overall percent survival in the main study are given in Table 2. At the 18-month timepoint, the cumulative mortality at the high dose was 9% ($p \leq 0.05$) in males and 22% ($p \leq 0.01$) in females compared to 0% for both male and female controls. Cumulative mortality at the middle dose was 4% (N.S.) in males and 13% ($p \leq 0.01$) in females. Cumulative mortality at the low dose was 9% ($p \leq 0.05$) in males and 7% (N.S., but $p = 0.059$) in females. A positive dose-related trend ($p \leq 0.01$) was evident for females, but not for males. At termination of the study at 24 months, the overall mortality at the high dose was ~53% ($p \leq 0.01$) in males and ~49% ($p \leq 0.01$) in females compared to ~29% and ~13% for male and female controls, respectively. The overall mortality at the middle dose was ~42% in males (N.S.) and ~44% ($p \leq 0.01$) in females. A positive dose-related trend ($p \leq 0.01$) was evident for both males and females. The percent survival satisfied the requirements for a 2-year chronic/oncogenicity study in rats.

At both 18 and 24 months, the increased mortality at 2000 ppm for males and females and at 1000 ppm for females is considered to be treatment-related. Further, the data suggests a possible dose-related effect in 20 ppm females at 18 and 24 months and in 20 ppm males at 24 months, but not at 18 months. The most common causes of death and/or moribundity were dose-related purulent inflammation of the lungs (at 1000 ppm and 2000 ppm) and mononuclear cell leukemia (which was not dose-related).

TABLE 2: Mortality at various intervals in the 24-month study with Malaoxon

| Time period months | Cumulative percent mortality | | | |
|---|---|---|---|---|
| | Control | 20 ppm | 1000 ppm | 2000 ppm |
| | Males | | | |
| 0-12 | 0 | 2 | 2 | 0 |
| 0-18 | 0 | 9* | 4 | 9* |
| 0-24 | 29** | 35 | 42 | 53** |
| | Females | | | |
| 0-12 | 0 | 2 | 3 | 3 |
| 0-18 | 0** | 7 | 13** | 22** |
| 0-24 | 13** | 24 | 44** | 49** |

Data extracted from pp. 43-45, MRID 43975201.
* $p \leq 0.05$, Significantly different from controls.
**$p \leq 0.01$, Significantly different from controls.

NOTE--All statistics in this table were performed by the HED statistics team, EPA.

B. BODY WEIGHT

Group mean body weight gains at various intervals in the study are given in Table 3. The mean weekly body weights at the high dose were slightly, but significantly, lower than the control from week 1 through week 38 in males and throughout the study for females. The terminal body weight for high dose males was about 14% ($p \leq 0.05$) lower than the control body weight and about 11% lower than the control group in high dose females. The decreased terminal body weight was linearly related to the dose in both sexes. The overall body weight gain in the 24-month study was decreased by about 12% in high dose males and females compared to the control groups. However, the weight gain was not decreased in males at 1000 ppm and was decreased by only about 5% in females at 1000 ppm.

The decreased body weights and body weight gains at 2000 ppm for males and females are considered to be treatment-related.

TABLE 3: Mean body weight gains at various intervals in the 105-week Malaoxon study.

| Time period weeks | Group mean body weight gain (grams) | | | |
|---|---|---|---|---|
| | Control | 20 ppm | 1000 ppm | 2000 ppm |
| | Males | | | |
| 0-12 | 164.6 | 167.0 | 162.2 | 157.4 |
| 12-50 | 86.3 | 89.7 | 90.0 | 87.9 |
| 50-102 | -13.8 | -1.8 | -11.9 | -33.3 |
| 0-50 | 250.9 | 256.7 | 252.2 | 245.3 |
| 0-102 | 237.1 | 258.5 | 240.3 | 212.0 |
| Terminal body weight | 354 | 343 | 356 | 305* |
| | Females | | | |
| 0-12 | 75.3 | 73.3 | 73.0 | 67.0 |
| 12-50 | 41.2 | 47.3 | 46.0 | 37.8 |
| 50-102 | 41.5 | 41.2 | 26.8 | 35.2 |
| 0-50 | 116.5 | 120.6 | 119.0 | 104.8 |
| 0-102 | 158.0 | 161.8 | 145.8 | 140.0 |
| Terminal body weight (24 Mo.) | 252 | 255 | 240 | 224[L] |

Data extracted and calculated from Appendix E, pp. 249-260, and Appendix J, pp. 3939-3946, MRID 43975201.
[L]$p \leq 0.01$ Response is linearly related to dose.
*$p \leq 0.05$ Significantly different from control.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

The time-weighted average food consumption levels during various representative weekly time intervals in the study are given in Table 4. The food consumption decreased during the first week of treatment at the mid and high doses in both sexes, but was significantly increased in males and females at 2000 ppm compared to the control group at all but 10 weekly determinations from week 1 to week 102 (~3% to ~12%, $p \leq 0.05$ or 0.01). Weekly food consumption was also significantly increased in males at 1000 ppm at all but 14 weekly determinations and in females at all but 16 weeks after the first treatment week (~2% to ~6%, $p \leq 0.05$ or 0.01).

The changes in food consumption at 2000 ppm and 1000 ppm for males and females are considered to be treatment-related.

TABLE 4: Mean food consumption at selected weeks in the 105-week study with Malaoxon

| Week | Mean food consumption (g/kg/day) | | | |
|---|---|---|---|---|
| | Control | 20 ppm | 1000 ppm | 2000 ppm |
| | Males | | | |
| 1 | 116.0 | 117.0 | 112.1** | 100.0** |
| 12 | 58.8 | 59.8 | 60.5* | 61.0** |
| 24 | 51.9 | 51.3 | 50.9 | 51.5 |
| 34 | 50.2 | 49.3 | 49.2 | 49.2 |
| 46 | 44.9 | 45.4 | 46.9** | 48.0** |
| 62 | 41.0 | 43.9** | 42.1* | 42.1* |
| 74 | 43.8 | 43.4 | 45.8** | 46.9** |
| 86 | 41.6 | 42.5 | 43.3 | 46.6** |
| 102 | 45.0 | 43.8 | 43.0 | 46.1 |
| | Females | | | |
| 1 | 121.0 | 122.3 | 119.3 | 105.3** |
| 12 | 72.7 | 74.6 | 74.5 | 78.4** |
| 24 | 63.0 | 62.0 | 61.7 | 64.3 |
| 34 | 64.2 | 66.4** | 66.4** | 67.3** |
| 46 | 58.1 | 58.6 | 60.0** | 62.1** |
| 62 | 50.4 | 50.2 | 49.9 | 53.5** |
| 74 | 53.1 | 51.4 | 55.6** | 57.3** |
| 86 | 49.8 | 48.8 | 52.6* | 55.8** |
| 102 | 56.3 | 55.9 | 59.8** | 63.0** |

Data extracted from Appendix E, pp. 293-302, MRID 43975201.
*p ≤ 0.05, **p ≤ 0.01, significantly different from controls.

2. Compound consumption

The time-weighted average compound consumption for each study group calculated from the body weights and food intake is given in Table 1.

3. Food efficiency

The food efficiency was not calculated for this study; however, the observations of decreased weight gain at 2000 ppm for males and females with increased weekly food consumption would result in a decrease in food efficiency.

D. OPHTHALMOSCOPIC EXAMINATION

No treatment-related increases of ocular signs or abnormalities were found during the ophthalmoscopic examinations. The most common finding in males was corneal scars at 104 weeks, which occurred in 45%-76% of animals examined. The highest incidence was seen in the control group. In females, corneal dystrophy at 104 weeks was the most common finding occurring in 54%-84% of animals examined. The highest incidence in females was in the 1000 ppm group, but the incidences were not dose-related.

E. BLOOD WORK

1. Hematology

Although some changes in hematological parameters were identified as statistically significant, they were minor and of little or no biological significance. There were no consistently altered hematology parameters in males. In females, the mean corpuscular volume was significantly decreased at 2000 ppm compared to the control group (about 2% at 6, 12, and 18 months, and about 5% at study termination, $p \leq 0.05$ and 0.01). The mean corpuscular hemoglobin was slightly decreased in high dose females at 6 months (about 2%, $p \leq 0.01$), and the mean corpuscular hemoglobin concentration was increased at 12 months (about 1%, $p \leq 0.01$) compared to the controls. Although these changes were statistically significant, they were very slight, within the normal range of historical controls, and not biologically significant. (Values were calculated from Appendix F, pp.337-344, MRID 43975201.)

2. Clinical chemistry

The only biologically relevant blood clinical chemistry parameters that were altered in the study as a result of treatment with malaoxon were the plasma and erythrocyte cholinesterase activities. The brain cholinesterase activity was also decreased with treatment. The plasma, erythrocyte, and brain cholinesterase activities at different time points in the treatment period are given in Tables 5A, 5B, and 5C. The cholinesterase activities in plasma, erythrocytes, and brain were decreased throughout the study at 1000 and 2000 ppm. The decreases were dose-related and statistically significant ($p \leq 0.05$ or 0.01) at the mid and high doses at most time points.

Cholinesterase activity in plasma was decreased at the various time points during treatment compared to control levels by 74%-81% in males and 82%-87% in females at 1000 ppm, and by 83%-91% in males and 90%-96% in females at 2000 ppm. Cholinesterase activity in red blood cells was decreased by 54%-66% in males and 45%-62% in females at 1000 ppm, and by 56%-65% in males and 54%-66% in females at 2000 ppm. Brain cholinesterase activity was decreased by 2%-30% in males and 5%-14% in females at 1000 ppm, and by 11%-74% in males and 61%-78% in females at 2000 ppm. It is notable that brain cholinesterase activity in males at 2000 ppm remained above 80% of control activity during the first year of the study and not until the second year did it decline to 26% at 24 months. Brain cholinesterase activity in females at 2000 ppm, on the other hand, declined to 22%-39% of control activity at 3, 6, 12 and 24 months. At 2000 ppm, brain cholinesterase activity in females apparently was affected more severely earlier in the study than for males. Slight decreases in cholinesterase activity from plasma and brain were seen sporadically at 20 ppm, but were not statistically significant. The decreased activity compared to control levels in red blood cells was statistically significant ($p \leq 0.01$) at 20 ppm in males and females (21% and 19%, respectively) at 6 months, and was slightly, but consistently, less than the controls at the other time points.

The decreased plasma and brain cholinesterase activity observed at 2000 ppm and 1000 ppm in males and females is considered to be treatment-related. The decreased red blood cell cholinesterase activity observed at 2000 ppm, 1000 ppm and 20 ppm in males and females is considered to be treatment-related. The NOEL for plasma and brain cholinesterase inhibition for males and females is 20 ppm. No NOEL was determined for red blood cell cholinesterase inhibition in males and females (< 20 ppm).

TABLE 5A: Mean cholinesterase activity in plasma at different times during treatment with Malaoxon

| Time period | Cholinesterase activity (IU/ml) | | | |
|---|---|---|---|---|
| | Control | 20 ppm | 1000 ppm | 2000 ppm |
| | Males | | | |
| Month 3 | 0.531 ±0.050 | 0.525 ±0.050 | 0.132** ±0.022 | 0.092** ±0.021 |
| Month 6 | 0.615 ±0.042 | 0.588 ±0.046 | 0.124** ±0.023 | 0.071** ±0.015 |
| Month 12 | 0.740 ±0.107 | 0.787 ±0.093 | 0.193* ±0.030 | 0.087** ±0.015 |
| Month 24 | 1.603 ±0.280 | 1.619 ±0.715 | 0.306** ±0.055 | 0.151** ±0.108 |
| | Females | | | |
| Month 3 | 2.559 ±0.362 | 2.583 ±0.254 | 0.355* ±0.103 | 0.140** ±0.033 |
| Month 6 | 3.201 ±0.844 | 3.000 ±0.986 | 0.419** ±0.105 | 0.139** ±0.025 |
| Month 12 | 3.422 ±0.331 | 3.359 ±0.341 | 0.611* ±0.097 | 0.196** ±0.061 |
| Month 24 | 3.119 ±0.958 | 3.651 ±0.558 | 0.526* ±0.174 | 0.316** ±0.334 |

Data extracted from Appendix H, pp. 382-405, MRID 43975201.
*p ≤ 0.05, **p ≤ 0.01, significantly different from controls.

TABLE 5B: Mean cholinesterase activity in red blood cells at different times during treatment with Malaoxon

| Time period | Cholinesterase activity (IU/ml) | | | |
|---|---|---|---|---|
| | Control | 20 ppm | 1000 ppm | 2000 ppm |
| | Males | | | |
| Month 3 | 1.06 ±0.13 | 0.93 ±0.15 | 0.40** ±0.13 | 0.45** ±0.10 |
| Month 6 | 1.15 ±0.20 | 0.91** ±0.15 | 0.39** ±0.13 | 0.43** ±0.10 |
| Month 12 | 1.25 ±0.32 | 1.08 ±0.30 | 0.49** ±0.08 | 0.44** ±0.10 |
| Month 24 | 1.25 ±0.18 | 1.12 ±0.23 | 0.68** ±0.08 | 0.70** ±0.08 |
| | Females | | | |
| Month 3 | 1.25 ±0.38 | 1.00 ±0.23 | 0.47** ±0.21 | 0.53** ±0.10 |
| Month 6 | 1.29 ±0.21 | 1.04** ±0.24 | 0.56** ±0.09 | 0.52** ±0.12 |
| Month 12 | 1.43 ±0.33 | 1.18 ±0.23 | 0.58** ±0.08 | 0.49** ±0.10 |
| Month 24 | 1.32 ±0.50 | 1.10 ±0.10 | 0.72** ±0.07 | 0.71** ±0.07 |

Data extracted from Appendix H, pp. 382-405, MRID 43975201.
*p ≤ 0.05, **p ≤ 0.01, significantly different from controls.

TABLE 5C: Mean cholinesterase activity in brain at different times during treatment with Malaoxon

| Time period | Cholinesterase activity (IU/g) | | | |
|---|---|---|---|---|
| | Control | 20 ppm | 1000 ppm | 2000 ppm |
| | Males | | | |
| Month 3 | 10.45 ±0.30 | 10.25 ±0.45 | 9.51 ±2.60 | 8.53** ±0.68 |
| Month 6 | 10.22 ±0.39 | 10.49 ±0.41 | 10.03 ±0.28 | 9.05** ±0.58 |
| Month 12 | 11.45 ±0.41 | 11.24 ±0.30 | 10.58 ±1.12 | 9.46** ±0.35 |
| Month 24 | 10.73 ±0.32 | 10.61 ±0.61 | 7.52** ±3.04 | 2.82** ±1.45 |
| | Females | | | |
| Month 3 | 10.57 ±0.35 | 10.38 ±0.39 | 9.34** ±0.47 | 2.30** ±0.69 |
| Month 6 | 10.29 ±0.37 | 10.43 ±0.22 | 9.64** ±0.27 | 3.97** ±0.40 |
| Month 12 | 11.27 ±0.20 | 11.27 ±0.35 | 10.67* ±0.34 | 4.26** ±1.08 |
| Month 24 | 10.77 ±0.38 | 10.63 ±0.66 | 9.26 ±1.19 | 4.04** ±1.37 |

Data extracted from Appendix H, pp. 382-405, MRID 43975201.
*p ≤ 0.05, **p ≤ 0.01, significantly different from controls.

F. URINALYSIS

No statistically significant changes in urinalysis parameters were seen in the study with malaoxon. There was a trend toward decreased urine volume, increased specific gravity, and decreased urine pH at the high dose; however, wide variations and high standard deviations resulted in the values not reaching statistical significance. These trends are shown in Table 6. Decreased urine volume was seen in females at all time points.

TABLE 6: Trends in urinalysis parameters seen after treatment with Malaoxon

| Time period/ Urinalysis parameter | Urinalysis parameter value | | | |
|---|---|---|---|---|
| | Control | 20 ppm | 1000 ppm | 2000 ppm |
| | Males | | | |
| Month 12/pH | 8.3 ± 0.7 | 8.6 ± 0.5 | 7.9 ± 0.1 | 7.7 ± 0.5 |
| Month 12/Sp. Gr. | 1.038 ± 0.018 | 1.033 ± 0.011 | 1.051 ± 0.015 | 1.047 ± 0.016 |
| Month 12/Vol. (ml) | 6.6 ± 1.7 | 7.9 ±1.8 | 7.8 ± 1.4 | 8.8 ± 1.2 |
| Month 24/pH | 7.4 ± 0.7 | 7.1 ± 0.9 | 7.0 ± 0.7 | 6.4 ± 0.3 |
| Month 24/Sp. Gr. | 1.040 ± 0.008 | 1.040 ± 0.005 | 1.043 ± 0.005 | 1.069 ± 0.021 |
| Month 24/Volume | 8.9 ± 5.2 | 14.6 ± 7.1 | 8.8 ± 4.7 | 6.5 ± 4.3 |
| | Females | | | |
| Month 12/pH | 8.8 ± 0.3 | 8.6 ± 0.5 | 8.3 ± 0.7 | 7.8 ± 0.7 |
| Month 12/Sp. Gr. | 1.040 ± 0.009 | 1.038 ± 0.007 | 1.036 ± 0.009 | 1.052 ± 0.007 |
| Month 12/Volume | 11.9 ± 4.5 | 12.9 ± 6.8 | 8.3 ± 2.7 | 5.5 ± 2.4 |
| Month 24/pH | 7.1 ± 0.8 | 7.1 ± 0.4 | 6.7 ± 0.7 | 6.7 ± 0.6 |
| Month 24/Sp. Gr. | 1.047 ± 0.014 | 1.043 ± 0.015 | 1.052 ± 0.015 | 1.050 ± 0.010 |
| Month 24/Volume | 11.4 ± 4.7 | 7.9 ± 4.1 | 8.0 ± 4.2 | 5.8 ± 3.1 |

Data calculated from Appendix I, pp. 456-471, MRID 43975201.

## G. SACRIFICE AND PATHOLOGY

### 1. Organ weight

Statistically significant changes were seen at 2000 ppm in the absolute and relative (to brain) weights of liver, kidney, adrenals, and spleen either at 12 months or 24 months, in males or in females. None of the statistically significant changes in organ weights were seen in both sexes or at both time periods. The weight changes in these organs and their relative (to brain) weights are shown in Table 7. The relative organ weights to the terminal body weights reflect the significantly decreased terminal body weight at the high dose and, therefore, were not included in Table 7. The organ weights measured at 12 and 24 months in both sexes are given for comparison. In high dose males after 12 months, the mean absolute and relative (to brain) weights of liver were increased by about 22% and 24% ($p \leq 0.01$), respectively, compared to the controls; and the mean absolute and relative kidney weights were significantly ($p \leq 0.01$) increased in high dose males (about 10 and 11%, respectively). The mean absolute and relative adrenal weights were significantly increased in high dose males at 24 months (13% and 12%, respectively, $p \leq 0.05$). The mean liver and kidney weights were not significantly different from the controls in high dose females, and the mean adrenal weight was slightly decreased in high dose females at 24 months (11%,

N.S.). At 24 months, the mean spleen weight in high dose females was decreased by ~51% ($p \leq 0.01$) compared to the controls. The mean spleen weight in high dose males was increased by about 24% (N.S.) at 24 months compared to the control group. Since no corresponding clinical chemistry or histopathological changes in these same organs (liver, kidney, adrenals, spleen) were observed, the biological significance of these organ weight differences and their relationship to treatment with the test material is not clear.

TABLE 7: Mean absolute and relative (to brain) organ weights after 12 and 24 months treatment with Malaoxon

| Organ/ Treatment period | Mean absolute/relative organ weights (grams) | | | |
|---|---|---|---|---|
| | Control | 20 ppm | 1000 ppm | 2000 ppm |
| | Males | | | |
| Brain/12 mo. | 1.902 | 1.851 | 1.858 | 1.883 |
| Brain/24 mo. | 1.923 | 1.952 | 1.954 | 1.916 |
| Liver/12 mo. | 10.405/5.47 | 10.397/5.61 | 11.469/6.17 | 12.732**/6.76** |
| Liver/24 mo. | 13.568/7.05 | 12.450/6.39 | 14.037/7.19 | 11.372/5.93 |
| Kidney/12 mo. | 2.578/1.36 | 2.449/1.32 | 2.636/1.42 | 2.842**/1.51** |
| Kidney/24 mo. | 3.097/1.61 | 3.064/1.57 | 3.211/1.64 | 2.942/1.53 |
| Adrenals/12 mo. | 0.0532/2.80 | 0.0520/2.81 | 0.0534/2.88 | 0.0566/3.02 |
| Adrenals/24 mo. | 0.0693/3.65 | 0.0668/3.43 | 0.0797/4.11 | 0.0780*/4.07* |
| Spleen/12 mo. | 0.633/3.33 | 0.654/3.54 | 0.648/3.49 | 0.678/3.60 |
| Spleen/24 mo. | 1.424/7.45 | 2.460/12.82 | 1.846/9.48 | 1.769/9.34 |
| | Females | | | |
| Brain/12 mo. | 1.735 | 1.771 | 1.723 | 1.763 |
| Brain/24 mo. | 1.760 | 1.774 | 1.764 | 1.804 |
| Liver/12 mo. | 6.513/3.75 | 6.996/3.97 | 6.736/3.91 | 6.524/3.69 |
| Liver/24 mo. | 9.350/5.30 | 9.947/5.60 | 9.876/5.61 | 9.097/5.04 |
| Kidney/12 mo. | 1.682/9.70 | 1.739/9.84 | 1.624/9.45 | 1.725/9.80 |
| Kidney/24 mo. | 2.165/1.24 | 2.351/1.32 | 2.270/1.29 | 2.259/1.25 |
| Adrenals/12 mo. | 0.0635/3.66 | 0.0640/3.62 | 0.0597/3.48 | 0.0622/3.77 |
| Adrenals/24 mo. | 0.0789/4.67 | 0.0697/3.93 | 0.0694/3.97 | 0.0700/3.87 |
| Spleen/12 mo. | 0.505/2.91 | 0.519/2.95 | 0.499/2.90 | 0.444/2.51 |
| Spleen/24 mo. | 0.833/4.89 | 0.528/2.98 | 0.543/3.06 | 0.412**/2.29** |

Data extracted from Appendix J, pp. 3931-3946, MRID 43975201.
*$p \leq 0.05$, **$p \leq 0.01$, Statistically different from controls.

2. Gross pathology

The total incidences of rats from the 24 month main study with gross lesions that may be treatment-related and/or can be compared to the findings during the microscopic examinations are shown in Table 8. An increased incidence of emaciation was seen at the high dose in early decedents but not in survivors at the scheduled study termination at 24 months (totals in males: controls, 5.5%; high dose 14.5%, N.S.; in females: controls, 0; low dose, 11%, $p \leq 0.01$; middle dose, 23.6%, $p \leq 0.001$; high dose, 21.8%, $p \leq 0.001$). Discolored foci in the stomach was also only seen in early decedents. One high dose female had discolored stomach foci at 24 months, but 20.7% to 39.1% of early decedent males and 22.2% to 33.3% of early decedent females were found to have this lesion. Stomach foci were found in all dose groups, but the highest incidence in both sexes was seen at 1000 ppm malaoxon. The overall incidence of discolored stomach foci was significantly increased in females at 1000 ppm (control, 3.6%; mid dose, 14.5%, $p \leq 0.05$; high dose, 12.7%, N.S.). Enlarged spleens were also seen in all dose groups. The overall incidences were not dose related, but the incidence was significantly increased at 1000 ppm in females (control, 3.6%; mid dose, 14.5%, $p \leq 0.05$. In females, the incidences of mammary gland nodules and ovarian cysts decreased with malaoxon treatment, especially at 24 months. The decreases were dose dependent, and the decrease in the incidence of ovarian cysts in high dose females was statistically significant compared to the controls (control, 14.5%; high dose, 3.6%, $p \leq 0.05$).

TABLE 8: Incidences of animals in the main study with gross lesions during treatment with Malaoxon

| Organ/Lesion | Animal source | No. animals with lesion/no. animals/percent | | | |
|---|---|---|---|---|---|
| | | Control | 20 ppm | 1000 ppm | 2000 ppm |
| | | MALES | | | |
| Whole body/ emaciation | Early deaths | 2/16/12.5% | 3/19/15.8% | 2/23/8.7% | 8/29/27.6% |
| | 24-Months | 1/39/2.6% | 0/36 | 0/32 | 0/26 |
| | Total | 3/55/5.5% | 3/55/5.5% | 2/55/3.6% | 8/55/14.5% |
| Stomach/ Discolored foci | Early deaths | 4/16/25.0% | 5/19/26.3% | 9/23/39.1% | 6/29/20.7% |
| | 24 Months | 0/39 | 0/36 | 0/32 | 0/26 |
| | Total | 4/55/7.2% | 5/55/9.1% | 9/55/16.4% | 6/55/10.9% |
| Spleen/ Enlarged | Early deaths | 8/16/50% | 10/19/52.6% | 13/23/56.5% | 12/29/41.4% |
| | 24 Months | 5/39/12.8% | 4/36/11.1% | 4/32/12.5% | 4/26/15.4% |
| | Total | 13/55/23.6% | 14/55/25.5% | 17/55/30.9% | 16/55/29.1% |
| | | Females | | | |
| Whole body/ emaciation | Early deaths | 0/7 | 5/13/38.5% | 13/24/54.2% | 12/27/44.4% |
| | 24-Months | 0/48 | 1/42/2.4% | 0/31 | 0/28 |
| | Total | 0/55 | 6/55/10.9%** | 13/55/23.6%** | 12/55/21.8%*** |
| Stomach/ Discolored foci | Early deaths | 2/7/28.6% | 3/13/23.1% | 8/24/33.3% | 6/27/22.2% |
| | 24 Months | 0/48 | 0/42 | 0/31 | 1/28/3.6% |
| | Total | 2/55/3.6% | 3/55/5.5% | 8/55/14.5%* | 7/55/12.7% |

TABLE 8: Continued

| Organ/Lesion | Animal source | No. animals with lesion/no. animals/percent | | | |
|---|---|---|---|---|---|
| | | Control | 20 ppm | 1000 ppm | 2000 ppm |
| | | Females (Continued) | | | |
| Spleen/ Enlarged | Early deaths | 1/7/14.3% | 5/13/38.5% | 5/24/20.8% | 4/27/14.8% |
| | 24 Months | 1/48/2.1% | 1/42/2.4% | 3/31/9.7% | 0/28 |
| | Total | 2/55/3.6% | 6/55/10.9% | 8/55/14.5%* | 4/55/7.3% |
| Mammary gland/ nodules, masses | Early deaths | 1/7/14.3% | 0/13 | 3/24/12.5% | 2/27/7.4% |
| | 24 Months | 6/48/12.5% | 5/42/11.9% | 2/31/6.5% | 0/28 |
| | Total | 7/55/12.7% | 5/55/9.1% | 5/55/9.1% | 2/55/3.6% |
| Ovary/Cyst | Early deaths | 1/7/14.3% | 0/13 | 2/24/8.3% | 0/27 |
| | 24 Months | 7/48/14.6% | 4/42/9.5% | 4/31/12.9% | 2/28/7.1% |
| | Total | 8/55/14.5% | 4/55/7.3% | 6/55/10.9% | 2/55/3.6%* |

Data extracted from Appendix K, Table 1D, pp. 1917-1923 and Table 1E, pp. 1926-1931, MRID 43975201.
*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, Significantly different from controls. Fisher exact test performed by reviewer.

3. Microscopic pathology

a) Non-neoplastic - The total incidences of animals with selected non-neoplastic lesions found in the main study are included in Table 9. The incidences of foreign material found in the nasal lumen increased from 9.1% in control males and 1.8% in control females to 48.2% in high dose males and to 45.5% in high dose females (p ≤ 0.001). Inflammation of the nasal lumen was significantly increased in males at 2000 ppm (control, 23.6%; 2000 ppm, 55.6%, p ≤ 0.01) and in females at 1000 ppm (27.8%, p ≤ 0.05) and at 2000 ppm (45.5%, p ≤ 0.001) compared to the control (9.1%). Nasal epithelial hyperplasia was slightly increased in males and significantly increased in females at the middle and high doses (males: control, 20.0%; high dose, 35.2%, N.S.; females: control, 5.5%; mid dose, 46.3%, p ≤ 0.001; high dose, 36.4%, p ≤ 0.001). The nasal epithelial hyperplasia, observed in 2000 ppm males and females and in 1000 ppm females, was frequently associated with inflammation and increased severity of goblet cell hypertrophy and hyperplasia in the respiratory nasal mucosa and with epithelial degeneration in the olfactory nasal mucosa. In addition, in mid and high dose females, focal areas of olfactory epithelium were sometimes replaced with ciliated and non-ciliated columnar epithelial cells (most of which displayed hyperplasia). Squamous/ squamoid metaplasia of the nasal mucosa was also observed in a few mid and high dose males and females, presumably a sequela to continued injury.

The incidences of lung interstitium inflammation were significantly increased at the high dose in males and the mid and high doses in females (males: control, 21.8%; high dose, 41.8%, $p \leq 0.05$; females: control, 25.5%; mid dose, 52.7% $p \leq 0.01$; high dose, 58.2%, $p \leq 0.001$). Inflammation of the lungs was often accompanied by edema and foreign body granulomas.

The tympanic cavity epithelial inflammation was primarily seen in early decedent animals, and was significantly increased in females at 1000 ppm (85%, $p \leq 0.001$) and 2000 ppm (74%, $p \leq 0.001$) compared to the control (0%). The increased inflammation in the tympanic cavity of males (not significant) and females most likely occurred via the Eustachian tube from affected nasopharyngeal tissues.

Increased incidences of mineral deposits were seen in the muscularis of the stomachs of mid and high dose males and in mid and high dose females (males: control, 3.6%; mid dose, 23.6%, $p \leq 0.01$; high dose, 45.5%, $p \leq 0.001$; females: control, 0%; mid dose, 9.3%, $p \leq 0.05$; high dose, 41.8%, $p \leq 0.001$). The same trends were seen in most cases in animals that died before the end of the study and in those that were killed and examined at 24 months.

The statistically significant non-neoplastic microscopic lesions described above in 2000 ppm males and females and in 1000 ppm females are considered to be related to treatment with the test material. In addition, in 1000 ppm males, mineral deposits in the muscularis of the stomach was also considered to be treatment related.

The testing laboratory suggested that the rats in this study, particularly at the higher doses, tended to aspire food and other foreign particles (hair, inflammatory debris) into their nasal cavities and lungs. This phenomenon subsequently resulted in considerably increased inflammation and other changes in the epithelial cells of the nasal cavity, lungs, and tympanic cavity seen in high dose males and mid and high dose females. The microscopic findings in the nasal cavity, lungs and tympanic cavity in high dose males and females and mid dose females were considered by the testing laboratory to be secondary findings and to be the expected response to injury due to the presence o foreign material (food particles) in the nasal lumen. The testing laboratory further stated that "the pathogenetic mechanism whereby inhaled food particles are retained in the nasal cavity and its relationship to the test material is not known."

An alternative explanation of the histopathological findings in the nasopharyngeal tissues in this study is that malaoxon may have acted systemically through

the circulation to affect the nasal mucosa resulting in inflammation, degeneration, hypertrophy, hyperplasia and metaplasia as described in the study report. Due to this continued injury, food, hair, inflammatory debris and other foreign particles may have secondarily tended to collect in the nasal lumen.

TABLE 9: Incidences of animals in the main study with non-neoplastic microscopic lesions

| Organ/Lesion | | No. Animals with lesion/no. animals examined/% | | | |
|---|---|---|---|---|---|
| | | Control | 20 ppm | 1000 ppm | 2000 ppm |
| | | Males | | | |
| Nasal lumen/ foreign material | | 5/55/9.1% | 7/55/12.7% | 8/55/14.5% | 26/54/ 48.2%*** |
| Nasal lumen/ Inflammation | | 13/55/23.6% | 16/55/29.1% | 13/55/23.6% | 30/54/ 55.6%** |
| Nasal lumen/ Epithelial hyperplasia | | 11/55/20.0% | 17/55/30.9% | 12/55/21.8% | 19/54/35.2% |
| Lung interstitium/ Inflammation | | 12/55/21.8% | 9/55/16.4% | 12/55/21.8% | 23/55/ 41.8%* |
| Tympanic cavity/ Inflammation | Early deaths | 5/15/33.3% | 5/16/31.3% | 7/22/31.8% | 13/27/48.1% |
| | 24 Months | 3/39/7.7% | 0/0 | 0/0 | 2/24/8.3% |
| Stomach muscularis/ Mineral deposits | | 2/55/3.6% | 1/55/1.8% | 13/55/ 23.6%** | 25/55/ 45.5%*** |
| | | Females | | | |
| Nasal lumen/foreign material | | 1/55/1.8% | 5/55/9.1% | 17/54/ 31.5%*** | 25/55/ 45.5%*** |
| Nasal lumen/Inflammation | | 5/55/9.1% | 5/55/9.1% | 15/54/ 27.8%* | 25/55/ 45.5%*** |
| Nasal lumen/Epithelial hyperplasia | | 3/55/5.5% | 5/55/9.1% | 25/54/ 46.3%*** | 20/55/ 36.4%*** |
| Lung interstitium/ Inflammation | | 14/55/25.5% | 15/55/27.3% | 29/55/ 52.7%** | 32/55/ 58.2%*** |
| Tympanic cavity/ Inflammation | Early deaths | 0/7 | 3/8/37.5% | 17/20/ 85.0%*** | 17/23/ 73.9%*** |
| | 24 Months | 2/48/4.2% | 0/0 | 0/0 | 2/27/7.4% |
| Stomach muscularis/ Mineral deposits | | 0/54 | 0/55 | 5/54/ 9.3%* | 23/55/ 41.8%*** |

Data extracted from Appendix K, Table IIB, pp. 1988-2057 and Table IIC, pp. 2059-2127, MRID 43975201.
*p ≤ 0.05, **p ≤ 0.01, Significantly different from controls. Fisher exact test performed by reviewer.

b) Neoplastic - Testicular interstitial cell tumors and lymphoreticular system mononuclear cell leukemia were commonly seen in the animals in this study. The incidences of these neoplastic lesions are shown in Table 10. Neither of these tumor types, nor any other tumor type, however, was considered to be related to treatment with malaoxon.

Testicular interstitial cell tumors

For animals in the oncogenicity portion of the study (main study) that were permitted to live to the end of the study if possible (55/sex/group), the incidence of interstitial cell tumors was 53/55 (96%), 49/55 (89%), 53/55 (96%) and 47/55 (85%) for the control, low, mid and high dose male groups respectively. For these animals, there was no treatment related effect. Further, there was no indication that malaoxon treated animals had a decreased latency or time to tumor when compared to the control animals since incidences in treated animals and control animals were similar for animals which were sacrificed at 12 months and also for animals that died or were sacrificed moribund prior to termination of the study.

Only when animals from the 12-month interim sacrifice groups (available only for control and high dose groups) were included in the statistical calculations did statistically significant differences occur. When this was done, there was a statistically significant ($p < 0.01$) positive trend for interstitial cell tumors and a statistically significant increase ($p < 0.05$) for these tumors at the mid dose (Fishers exact test) and a decrease in these tumors (not statistically significant) at the high dose. The practice of including data from some interim sacrifice animals (control and high dose), but not from other interim sacrifice animals (low and mid dose) is questionable.

Regarding historical control data for testicular interstitial cell tumors from the same testing laboratory, data from 6 comparable 2 year studies using Fischer 344 rats were available (1987 - 1992). The mean incidence of interstitial cell tumors in these 6 studies was 87.5% (range of 82% to 94%). Further, the mean incidence of these tumors in the NTP historical control data base, published in 1990, was 87.8% (range of 64% to 98%). In this study, the incidences of interstitial cell tumors in the control and treated groups were clearly consistent with the historical control data.

The testicular interstitial cell tumors observed in the malaoxon treated male rats in this study are not considered to be treatment related.

Mononuclear cell leukemia

For animals in the oncogenicity portion of the study (55/sex/group), the incidence of mononuclear cell leukemia in males was 13/55 (24%), 12/55 (22%), 19/55 (35%) and 16/55 (29%) and in females was 8/55 (15%), 9/55 (16%), 10/55 (18%) and 5/55 (9%) for the control, low, mid and high dose groups respectively. For these animals, there was no treatment related effect.

When animals from the 12-month interim sacrifice groups (available only for control and high dose groups) were included in the statistical calculations, for male rats, there was a statistically significant ($p < 0.05$) positive

trend for mononuclear cell leukemia. There was no pairwise difference, however, between the male control group and any malaoxon treated male group. For female rats, neither a statistically significant trend nor pairwise difference was observed. As mentioned previously, the practice of including data from some interim sacrifice animals (control and high dose), but not from other interim sacrifice animals (low and mid dose) is questionable.

Historical control data for mononuclear cell leukemia from the same testing laboratory were available from 6 comparable 2-year studies using Fischer 344 rats (1987 - 1992). For males, the mean incidence of mononuclear cell leukemia in these 6 studies was 29.0% (range of 15% to 36%) and for females was 26.8% (range of 17% to 44%). Further, the mean incidence of mononuclear cell leukemia in the NTP historical control data base (1990) for males was 33.6% (range of 10% to 72%) and for females was 20.2% (range of 6% to 31%). In this study, the incidences of mononuclear cell leukemia in the control and treated groups, for both males and females, fell within or below the historical control range.

The mononuclear cell leukemia observed in the malaoxon treated male and female rats in this study are not considered to be treatment related.

Other types of tumors

No other types of tumors observed in the male and female rats in this study were of potential concern. They occurred with comparable incidence in control and malaoxon treated groups or occurred sporadically. In particular, no neoplasms considered to be related to treatment with malaoxon were observed in the nasoturbinal tissues or the liver.

In the liver, hepatocellular adenomas were observed in male rats at an incidence of 4/65, 3/55, 1/55 and 0/65 and in female rats at an incidence of 1/65, 1/55, 1/55 and 0/65 in the control, low, mid and high dose groups respectively. Hepatocellular carcinomas were observed only in female rats and at an incidence of 0/65, 1/55, 0/55 and 1/65 in the control, low, mid and high dose groups respectively. These hepatocellular neoplasms are not considered to be treatment related.

TABLE 10: Number of animals with neoplastic lesions in the 24-month rat study with Malaoxon

| Organ/Lesion/ Study period | No. Rats with lesion/no. rats examined/% with lesion | | | |
|---|---|---|---|---|
| | Control | 20 ppm | 1000 ppm | 2000 ppm |
| | Males | | | |
| Testes/Interstitial cell adenoma/total 12 mo. | 1/10/10% | Not examined | Not examined | 2/10/20% |
| Testes/Interstitial cell adenoma/Total early deaths | 15/16/94% | 15/19/79% | 22/23/96% | 22/29/76% |
| Testes/Interstitial cell adenoma/Total 24 months | 38/39/97% | 34/36/94% | 31/32/97% | 25/26/96% |
| Interstitial cell adenoma/ Total 24 month + total early deaths | 53/55/96% | 49/55/89% | 53/55/96% | 47/55/85% |
| Interstitial cell adenoma/ Total for study[1] | 54/65/83% | 49/55/89% | 53[F]/55/96% | 49*[T+]/65/75% |
| Mononuclear cell leukemia/Early deaths | 7/16/44% | 8/19/42% | 13/23/57% | 8/29/28% |
| Mononuclear cell leukemia/24 Months | 6/39/15% | 4/36/11% | 6/32/19% | 8/26/31% |
| Mononuclear cell Leukemia/24 month + early deaths | 13/55/24% | 12/55/22% | 19/55/35% | 16/55/29% |
| Mononuclear cell Leukemia/total for study[1] | 13/65/20% | 12/55/22% | 19/55/35% | 16[T]/65/25% |

| TABLE 10: Continued | | | | |
|---|---|---|---|---|
| Organ/Lesion/ Study period | No. Rats with lesion/no. rats examined/% with lesion | | | |
| | Control | 20 ppm | 1000 ppm | 2000 ppm |
| | Females | | | |
| Mononuclear cell leukemia/24 Months | 6/48/13% | 4/42/10% | 5/31/16% | 2/28/7% |
| Mononuclear cell leukemia/Early deaths | 2/7/29% | 5/13/38% | 5/24/21% | 3/27/11% |
| Mononuclear cell leukemia/Early deaths + 24 months | 8/55/15% | 9/55/16% | 10/55/18% | 5/55/9% |
| Mononuclear cell leukemia/Total for study[1] | 8/65/12% | 9/55/16% | 10/55/18% | 5/65/8% |

Data extracted from Appendix K, Table IIA, pp.1944-1986, Table IIB, pp. 198-2057, Table IIC, pp. 2059-2127, and pp, 76-77, MRID 43975201.
* ≤ 0.05, Significantly different from controls, Cox or Gehan-Breslow.
[T]p ≤ 0.05, [T+]p ≤ 0.01, statistically significant trend test.
F Statistically different from control by Fisher exact test at p ≤ 0.05.
[1]Includes early decedents, 24 month scheduled sacrifice rats as well as 12-month scheduled sacrifice rats at 0 and 2000 ppm.

## III. DISCUSSION

### A. DISCUSSION

Malaoxon (96.4% a.i. lot # 279-ABB-036) was administered to 85 Fischer 344 CDF® (F-344)/CrlBR rats/sex/dose in the diet at concentrations of 0, 20, 1000, or 2000 ppm for up to 24 months. The dietary concentrations resulted in calculated mean dose levels of 0, 1, 57, and 114 mg/kg/day for males and 0, 1, 68, and 141 mg/kg/day for females based on individual body weights and food consumption data. Ten animals/sex/group were sacrificed at 3 months, 6 months, and 12 months for blood and brain cholinesterase evaluations. All tissues from the 12-month control and high dose animals were retained and examined grossly and microscopically along with tissues from the main 24-month study.

The only clinical observation that was related to treatment with malaoxon was the appearance of yellow ano-genital staining seen especially from treatment week 90 through 105. This sign was seen in a maximum of 8% of control and 37% of high dose males (p ≤ 0.05), and in a maximum of about 10% of control and 43% of high dose females (p ≤ 0.01) during that time span.

The overall mortality was significantly increased in high dose males (control 29%; high dose, 53%, p ≤ 0.01) and in mid and high dose females (control, 13%; mid dose, 44%, p ≤ 0.01; high dose, 49%, p ≤ 0.01). The mortality in males was also

increased at the mid dose, but the increase was not statistically significant (42%).

The mean terminal body weight of males at the high dose was significantly decreased (about 14%, $p \leq 0.05$) compared to the control mean body weight. The mean female body weight was slightly decreased (about 11%, N.S.) at the high dose, and, although not significantly different from the control body weights, the decrease was dose related. With the exception of treatment week 1, food intake was significantly increased at all dose levels sporadically throughout the study. The increased food intake compared to the controls was seen most consistently at the high dose in both sexes. Although the food efficiency was not calculated for this study, the decreased body weight with increased food consumption indicates a decrease in food efficiency consistent with a toxic effect of malaoxon treatment.

Changes in hematology parameters were not biologically significant. Slight decreases in mean red cell volume (2%-5%, $p \leq 0.05$ and 0.01) and in mean cell hemoglobin (2%, $p \leq 0.01$) seen in high dose females were statistically significant, but the values were within the normal range seen in historic control animals.[1] Dose-related and statistically significant decreases in red blood cell, plasma and brain cholinesterase activities compared to control levels were seen in treated animals. The plasma cholinesterase activity was significantly decreased in males (74%-91%) and in females (82%-96%) after 3, 6, 12, and 24 months of Malaoxon treatment at the mid and high doses ($p \leq 0.05$ or 0.01). Red blood cell cholinesterase activity was also decreased (54%-66%) in males and females (45%-66%) at all time points at the mid and high doses. The red blood cell cholinesterase activity was also decreased by 21% in males and 19% in females after 6 months of malaoxon treatment at 20 ppm ($p \leq 0.01$). It was consistently, but not significantly, decreased at the other time points at 20 ppm in both sexes. Brain cholinesterase activity was significantly decreased (about 11%-74%, $p \leq 0.01$) at the high dose at all time points and at the mid dose by about 30% ($p \leq 0.01$) at 24 months in males. In females, it was decreased by 61%-78% ($p \leq 0.01$) compared to the controls at the high dose at all time points. At the mid dose, the brain cholinesterase activity was decreased in females by 5%-14% compared to control levels. The decreases in activity compared to the control seen at the mid dose in females were all statistically significant ($p \leq 0.05$ or 0.01) except at the 24 month time point.

Slight trends (not statistically significant) were seen toward decreased urine volume, increased specific gravity, and lower pH with increasing concentrations of malaoxon. The trends were more obvious in females than in males.

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[1]Charles River Breeding Laboratories. 1984. Baseline hematology and clinical chemistry values for Charles River Fischer-344 rats-CDF®(F-344)CrlBR as a function of sex and age. Charles River Breeding Laboratories, Inc., Wilmington, Mass.

The mean absolute liver and kidney weights of high dose males were increased by 22% and 10%, respectively, compared to the controls after 12 months of malaoxon treatment ($p \leq 0.01$), but they were not increased after 24 months of treatment. The mean adrenal weight was increased by about 13% in high dose males at 24 months ($p \leq 0.05$). The only mean organ weight that was affected by malaoxon treatment in females was the spleen, which was decreased in high dose females by 51% at 24 months ($p \leq 0.01$). The mean spleen weight in females was decreased by about 12% (N.S.) after 12 months of malaoxon treatment. There were no gross or microscopic findings that correlated with the organ weight changes.

No statistically significant differences were seen with treatment in the incidences of gross lesions seen in surviving animals at the 24 month scheduled necropsy. The greatest differences from the controls were seen in early decedents. Combining the animals examined at 24 months with the early decedents resulted in statistically significant increased incidences of emaciated female rats at the mid and high dose ($p \leq 0.001$), of discolored foci in the stomachs of mid dose females ($p \leq 0.05$), and of enlarged spleens in mid dose females ($p \leq 0.05$). Emaciation was increased in males at the high dose, and the incidence of discolored stomach foci was increased at the mid dose, but these findings were not statistically significant. No microscopic or additional data were available to explain the apparent inconsistency of decreased spleen weight and increased incidence of enlarged spleens seen in females. These observations are not likely to be toxicologically significant and may be spurious.

Treatment of the animals in this study with malaoxon may have tended to increase the aspiration of foreign particles (food, hair inflammatory debris) into the nasal cavities, and lungs resulting in most of the microscopic findings. This possibly could be indicative of a neurological effect secondary to the decrease in cholinesterase activity seen at the mid and high dose. Foreign material was found in the nasal cavities of 48% ($p \leq 0.001$) of high dose males and 46% ($p \leq 0.001$) of high dose females compared to 9% and 2% for male and female control groups, respectively. This may have resulted in a number of findings including nasal inflammation seen in 56% ($p \leq 0.01$) of high dose males compared to 24% of controls, and in 28% ($p \leq 0.05$) of mid dose females and 46% ($p \leq 0.001$) of high dose females compared to 9% of controls. Nasal lumen epithelial hyperplasia was increased in high dose males (control, 20%; high dose, 35%, N.S.) and in mid and high dose females (control, 6%; mid dose, 46%, $p \leq 0.001$; high dose, 36%, $p \leq 0.001$. An alternative explanation of the histopathological findings in the nasopharyngeal tissues in this study is that malaoxon may have acted systemically through the circulation to affect the nasal mucosa resulting in inflammation, degeneration, hypertrophy, hyperplasia and metaplasia as described in the study report. Due to this continued injury, food, hair, inflammatory debris and other foreign particles may have secondarily tended to collect in the nasal lumen. Lung interstitium inflammation was increased in high dose males (control, 22%; high dose, 42%, N.S.) and in mid and high dose females (control, 26%; mid dose, 53%, $p \leq 0.01$; high dose, 58%, $p \leq 0.001$), Tympanic

cavity inflammation was primarily seen in early decedents and was increased in high dose males (control, 33%; high dose, 48%, N.S.) and in mid and high dose females (control, 0; mid dose, 85%, $p \leq 0.001$; high dose, 74%, $p \leq 0.001$). The various areas of the respiratory system were apparently affected more in females than in males. An increased incidence of mineral deposits in the stomach muscularis was seen more in mid and high dose males (control, 4%; mid dose, 24%, $p \leq 0.01$; high dose, 46%, $p \leq 0.001$) than in females (control, 0; mid dose, 9%, $p \leq 0.05$; high dose, 42%, $p \leq 0.001$). The stomach lesions may correlate with the colored foci seen in the gross examination.

There were no treatment-related increases in neoplastic lesions in the 24-month study. A significant ($p \leq 0.05$) increase in the total incidence of testicular interstitial cell tumors seen in mid dose males was followed by a significant decreased incidence at the high dose compared to the total control incidence. A dose-related trend ($p \leq 0.05$) was seen in males for mononuclear cell leukemia. However, the increased incidence seen at the high dose was not significantly different from the control. Historic control data provided by the study authors showed the incidences to be within the expected range for F344 rats. The inclusion of the 12-month interim rat data obtained only for the control and high dose groups in the statistical calculations seems questionable, and there are no statistically significant increases or trends without its inclusion.

No-Observed-Effect-Levels (NOEL) were not determined for cholinesterase inhibition. The Lowest-Observed-Effect-Level (LOEL) of 20 ppm (1 mg/kg/day) for males and females was determined for cholinesterase inhibition based on a statistically significant 19-20% inhibition of red blood cell cholinesterase activity after 6 months of treatment with malaoxon. A NOEL of 20 ppm (1 mg/kg/day) and a LOEL of 1000 ppm (57 mg/kg/day for males and 68 mg/kg/day for females) for systemic toxicity were defined. The LOELs were based on increased mortality in females, microscopic changes in the nasoturbinal tissues in females, mineral deposits in the stomach muscularis especially in males, and microscopic changes in the lung interstitium and in the tympanic cavity in females.

B. STUDY DEFICIENCIES

The study authors grouped together all animals that were examined grossly and microscopically even though this included 10 control and 10 high dose animals killed at 12 months. It seems inappropriate to base statistics on this total grouping especially for tumors and other findings that occur mostly as the animals age. Summary tables combining the 24 month animals and the early decedents would have been helpful. No information was given on the basis for the dose selections.

These deficiencies do not detract significantly from the value of the chronic/oncogenicity study.

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

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OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Malathion Qualitative Risk Assessment Based On Fischer 344 Rat Dietary Study

P.C. Code 057701

TO: Brian A. Dementi, Toxicologist
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Health Effects Division (7509C)

AND: Edwin R. Budd, Toxicologist
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Health Effects Division (7509C)

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Health Effects Division (7509C)

THROUGH: Hugh M. Pettigrew, Secondary Reviewer
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Health Effects Division (7509C)
7/16/97

AND: Jesudoss Rowland, Branch Senior Scientist
Science Analysis Branch
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7/16/97

## Background

An oral toxicity/oncogenicity study with Malathion in Fischer 344 rats was conducted by Huntingdon Life Sciences, East Milestone, New Jersey, for Cheminova Agro A/S, Lemvig, Denmark, and completed February 27, 1996 (Study No. 90-3641; MRID No. 439429-01).

The study design allocated groups of 55 rats per sex to dose levels of 0, 100/50, 500, 6,000, or 12,000 ppm of Malathion for 105 weeks. An additional 15 rats per sex per dose were designated for interim sacrifice at week 54. Twenty additional rats per sex per dose were designated for interim sacrifice, 10 each at weeks 14 and 27. The animals of these first two sacrifice time periods (14 and 27 weeks) are not included in this analysis because only ocular tissues were microscopically examined. Following the 14 week interim sacrifice, the dosage level for the low dose group was reduced from 100 ppm to 50 ppm due to lack of an NOEL at that time.

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Survival Analyses

The statistical evaluation of mortality indicated significant increasing mortality with increasing doses of Malathion in male and female rats. Male rats of the high dose group had 100% mortality at week 97. See Tables 1 and 2 for rat mortality test results.

The statistical evaluation of mortality was based upon the Thomas, Breslow and Gart computer program.

Tumor Analyses

Male rats had a significant increasing trend, and a significant difference in the pair-wise comparison of the 12,000 ppm dose group with the controls, for testes interstitial cell tumors, both at $p < 0.01$. There were also significant differences in the pair-wise comparisons of the 500 and 6,000 ppm dose groups with the controls for testes interstitial cell tumors, both at $p < 0.05$. There was a significant trend in thyroid follicular cell adenomas and/or carcinomas combined at $p < 0.05$.

Female rats had significant increasing trends, and significant differences in the pair-wise comparisons of the 12,000 ppm dose groups with the controls, for liver adenomas, and adenomas and/or carcinomas combined, all at $p < 0.01$. There were also significant differences in the pair-wise comparisons of the 6,000 ppm dose group with the controls for liver adenomas, and adenomas and/or carcinomas combined, both at $p < 0.05$. In addition, there was a significant difference in the pair-wise comparison of the 100/50 ppm dose group with the controls for mononuclear cell leukemia, significant differences in the pair-wise comparisons of the 500 and 6,000 ppm dose groups with the controls for pituitary pars distalis carcinomas, and a significant difference in the pair-wise comparison of the 500 ppm dose group with the controls for pituitary pars distalis adenomas and/or carcinomas combined, all at $p < 0.05$.

The statistical analyses of the rats were based upon Peto's prevalence test since there were statistically significant positive trends for mortality in both male and female rats with increasing doses of Malathion. See Tables 3 through 7 for rat tumor analysis results.

Table 1. Malathion - Fischer 344 Rat Study

Male Mortality Rates[+] and Cox or Generalized K/W Test Results

| | Weeks | | | | | |
|---|---|---|---|---|---|---|
| Dose(ppm) | 1-26 | 27-53 | 54[i] | 54-78 | 79-106[f] | Total |
| 0 | 0/70 | 0/70 | 15/70 | 0/55 | 18/55 | 18/55 (33)** |
| 100/50 | 0/70 | 0/70 | 15/70 | 0/55 | 14/55 | 14/55 (25) |
| 500 | 0/70 | 0/70 | 15/70 | 3/55 | 23/52 | 26/55 (47) |
| 6000 | 0/70 | 0/70 | 15/70 | 1/55 | 38/52[a] | 39/53 (74)** |
| 12000 | 1/70 | 1/69 | 14/68 | 15/54 | 39/39 | 56/56 (100)** |

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[i]Interim sacrifices at week 14, 27 and 54. Only week 54 interim sacrifice animals are included in this analysis.

[f]Final sacrifice at week 105.

[a]Two accidental deaths at week 105, dose 6000 ppm.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 2. Malathion - Fischer 344 Rat Study

Female Mortality Rates[+] and Cox or Generalized K/W Test Results

| | Weeks | | | | | |
|---|---|---|---|---|---|---|
| Dose(ppm) | 1-26 | 27-53 | 54[i] | 54-78 | 79-106[f] | Total |
| 0 | 0/70 | 0/70 | 15/70 | 1/55 | 16/54 | 17/55 (31)** |
| 100/50 | 0/70 | 1/70 | 14/69 | 1/55 | 13/54 | 15/56 (27) |
| 500 | 0/70 | 0/70 | 15/70 | 2/55 | 12/53 | 14/55 (25) |
| 6000 | 0/70 | 1/70 | 15/69 | 1/54 | 19/53 | 21/55 (38) |
| 12000 | 0/70 | 1/70 | 15/70 | 4/55 | 30/51 | 35/55 (64)** |

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[i]Interim sacrifices at week 14, 27 and 54. Only week 54 interim sacrifice animals are included in this analysis.

[f]Final sacrifice at week 105.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 3. Malathion - Fischer 344 Rat Study

Male Testes Interstitial Cell Tumor Rates[+] and Peto's Prevalence Test Results (p values)

| | Dose (ppm) | | | | |
|---|---|---|---|---|---|
| | 0 | 100/50 | 500 | 6000 | 12000 |
| Tumors (%) | 52/55 (95) | 52/55 (95) | 53/55 (96) | 52/53 (98) | 53[a]/54 (98) |
| p = | 0.000** | - | 0.037* | 0.032* | 0.004** |

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

[a]First testes interstitial cell tumor observed at week 54, dose 0 ppm, in a 54-week interim sacrifice animal. First testes interstitial cell tumor not in an interim sacrifice or accidental death animal observed at week 64, dose 12,000 ppm.

Note: Interim sacrifice and accidental death animals are not included in this analysis. Two animals in the 0 ppm dose group and five animals in the 12,000 ppm dose group of the 54-week interim sacrifice group had testes interstitial cell tumors. Two accidental death animals in the 6,000 ppm dose group had testes interstitial cell tumors.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 4. Malathion - Fischer 344 Rat Study

Male Thyroid Tumor Rates[+] and
Peto's Prevalence Test Results (p values)

| | Dose (ppm) | | | | |
|---|---|---|---|---|---|
| | 0 | 100/50 | 500 | 6000 | 12000 |
| Adenomas (%) | 2/55 (4) | 1/54 (2) | 1/51 (2) | 4/51 (8) | 4[a]/43 (9) |
| p = | 0.063 | - | - | 0.150 | 0.378 |
| Carcinomas (%) | 0/42 (0) | 0/45 (0) | 2/41 (5) | 2[b]/26 (8) | 0/0 (0) |
| p = | 0.196 | - | 0.085 | 0.162 | - |
| Combined (%) | 2/55 (4) | 1/54 (2) | 3/51 (6) | 6/51 (12) | 4/43 (9) |
| p = | 0.035* | - | 0.321 | 0.077 | 0.160 |

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

[a]First thyroid follicular cell adenoma observed at week 76; dose 12,000 ppm.

[b]First thyroid follicular cell carcinoma observed at week 100, dose 6,000 ppm.

Note: Interim sacrifice and accidental death animals are not included in this analysis. There were no thyroid follicular cell tumors in any interim sacrifice or accidental death animals.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 5. Malathion - Fischer 344 Rat Study

Female Liver Tumor Rates[+] and
Peto's Prevalence Test Results (p values)

| | Dose (ppm) | | | | |
|---|---|---|---|---|---|
| | 0 | 100/50 | 500 | 6000 | 12000 |
| Adenomas (%) | 0/40 (0) | 1[a]/48 (2) | 1/43 (2) | 3/39 (8) | 3/29 (10) |
| p = | 0.007** | 0.240 | 0.168 | 0.032* | 0.008** |
| Carcinomas (%) | 0/41 (0) | 1/50 (2) | 1/44 (2) | 0/41 (0) | 3[b]/38 (8) |
| p = | 0.063 | 0.168 | 0.168 | - | 0.085 |
| Combined (%) | 0/41 (0) | 2/50 (4) | 2/44 (5) | 3/41 (7) | 6/38 (16) |
| p = | 0.002** | 0.134 | 0.085 | 0.032* | 0.003** |

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

[a]First liver adenoma observed at week 103, dose 100/50 ppm.

[b]First liver carcinoma observed at week 101, dose 12,000 ppm.

Note: Interim sacrifice animals are not included in this analysis. There were no liver tumors in any interim sacrifice animals.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 6. Malathion - Fischer 344 Rat Study

Female Mononuclear Cell Tumor Rates[+] and Peto's Prevalence Test Results (p values)

| | Dose (ppm) | | | | |
|---|---|---|---|---|---|
| | 0 | 100/50 | 500 | 6000 | 12000 |
| Leukemia (%) | 9/55 (16) | 18/55 (33) | 15/55 (27) | 13/54 (24) | 10[a]/55 (18) |
| p = | 0.917 | 0.025* | 0.059 | 0.181 | 0.670 |

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor. Also excludes week 54 interim sacrifice animals.

[a]First mononuclear cell leukemia observed at week 47, dose 12,000 ppm.

Note: Interim sacrifice animals are not included in this analysis. There were no mononuclear cell leukemias in any interim sacrifice animals.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 7. Malathion - Fischer 344 Rat Study

Female Pituitary Pars Distalis Tumor Rates[+] and Peto's Prevalence Test Results (p values)

| | Dose (ppm) | | | | |
|---|---|---|---|---|---|
| | 0 | 100/50 | 500 | 6000 | 12000 |
| Adenomas (%) | 25/51 (49) | 13/31 (42) | 20[a]/34 (59) | 17/33 (52) | 14/53 (26) |
| p = | 0.980 | - | 0.133 | 0.266 | - |
| Carcinomas (%) | 0/50 (0) | 1/30 (3) | 3[b]/32 (9) | 4/32 (12) | 1/49 (2) |
| p = | 0.778 | 0.319 | 0.029* | 0.027* | 0.369 |
| Combined (%) | 25/51 (49) | 14/31 (45) | 23/34 (68) | 21/33 (64) | 15/53 (28) |
| p = | 0.987 | - | 0.033* | 0.097 | - |

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

[a]First pituitary pars distalis adenoma observed at week 56, dose 500 ppm.

[b]First pituitary pars distalis carcinoma observed at week 79, dose 500 ppm.

Note: Interim sacrifice animals are not included in this analysis. There were no pituitary pars distalis tumors in any interim sacrifice animals.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

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WASHINGTON, D.C. 20460

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Malathion Qualitative Risk Assessment Based On $B_6C_3F_1$ Mouse Dietary Study

P.C. Code 057701

TO: Brian A. Dementi, Toxicologist
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FROM: Lori L. Brunsman, Statistician
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Health Effects Division (7509C)
Lori L. Brunsman 5/8/97

THROUGH: Hugh M. Pettigrew, Section Head
Statistics Section
Science Analysis Branch
Health Effects Division (7509C)
Hugh M Pettigrew 5/8/97

## Background

An oncogenicity study with Malathion in $B_6C_3F_1$ mice was conducted by International Research and Development Corporation, Mattawan, Michigan, for Cheminova Agro A/S, Lemvig, Denmark, and completed October 12, 1994 (Lab Project ID No. 668-001; MRID No. 434072-01).

The study design allocated groups of 55 mice per sex to dose levels of 0, 100, 800, 8,000, or 16,000 ppm of Malathion for 79 weeks. An additional 10 mice per sex per dose were designated for interim sacrifice at week 53.

## Survival Analyses

The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of Malathion in male or female mice. See Tables 1 and 2 for mouse mortality test results.

The statistical evaluation of mortality was based upon the Thomas, Breslow and Gart computer program.

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Tumor Analyses

Male mice had significant increasing trends, and significant differences in the pair-wise comparisons of the 8,000 and 16,000 ppm dose groups with the controls, for liver adenomas, and adenomas and/or carcinomas combined, all at $p < 0.01$. There were significant differences in the pair-wise comparisons of the 100 and 8,000 ppm dose groups with the controls for liver carcinomas, both at $p < 0.05$. There was also a significant difference in the pair-wise comparison of the 100 ppm dose group with the controls for liver adenomas and/or carcinomas combined at $p < 0.01$.

Female mice had significant increasing trends, and significant differences in the pair-wise comparisons of the 8,000 and 16,000 ppm dose groups with the controls, for liver adenomas, and adenomas and/or carcinomas combined, all at $p < 0.01$.

The statistical analyses of the mice were based upon the Exact trend test and the Fisher's Exact test for pair-wise comparisons. See Tables 3 and 4 for mouse tumor analysis results.

Table 1. Malathion - $B_6C_3F_1$ Mouse Study

Male Mortality Rates[+] and Cox or Generalized K/W Test Results

| | Weeks | | | | | |
|---|---|---|---|---|---|---|
| Dose(ppm) | 1-26 | 27-52 | 53[i] | 53-66 | 67-80[f] | Total |
| 0 | 0/65 | 3/65 | 8/62 | 3/54 | 1/51 | 7/57 (12) |
| 100 | 0/65 | 0/65 | 10/65 | 1/55 | 3/54 | 4/55 (7) |
| 800 | 0/65 | 0/65 | 10/65 | 4/55 | 3/51 | 7/55 (13) |
| 8000 | 0/65 | 0/65 | 10/65 | 0/55 | 0/54[a] | 0/54 (0) |
| 16000 | 2/65 | 2/63 | 10/61 | 1/51 | 0/49[b] | 5/54 (9) |

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[i]Interim sacrifice at week 53.

[f]Final sacrifice at week 79.

[a]One accidental death at week 78, dose 8000 ppm.

[b]One accidental death at week 79, dose 16000 ppm.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 2. Malathion - $B_6C_3F_1$ Mouse Study

Female Mortality Rates[+] and Cox or Generalized K/W Test Results

| | Weeks | | | | | |
|---|---|---|---|---|---|---|
| Dose(ppm) | 1-26 | 27-52 | 53[i] | 53-66 | 67-80[f] | Total |
| 0 | 0/65 | 0/65 | 10/65 | 0/55 | 0/55 | 0/55 (0) |
| 100 | 0/65 | 0/65 | 10/65 | 0/55 | 3/55 | 3/55 (5) |
| 800 | 0/65 | 0/64[a] | 10/64 | 1/54 | 0/52[a] | 1/53 (2) |
| 8000 | 1/64[b] | 0/63 | 10/63 | 0/53 | 0/52[b] | 1/53 (2) |
| 16000 | 2/65 | 0/62[c] | 10/62 | 0/52 | 0/51[c] | 2/53 (4) |

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[i]Interim sacrifice at week 53.

[f]Final sacrifice at week 79.

[a]Two accidental deaths, one each at weeks 36 and 73, dose 800 ppm.

[b]Two accidental deaths, one each at weeks 22 and 79, dose 8000 ppm.

[c]Two accidental deaths, one each at weeks 35 and 78, dose 16000 ppm.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 3. Malathion - $B_6C_3F_1$ Mouse Study

Male Liver Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

| | Dose (ppm) | | | | |
|---|---|---|---|---|---|
| | 0 | 100 | 800 | 8000 | 16000 |
| Adenomas (%) | 1/54 (2) | 6[a]/54 (11) | 2/55 (4) | 13/55 (24) | 49/51 (96) |
| p = | 0.000** | 0.056 | 0.507 | 0.001** | 0.000** |
| Carcinomas (%) | 0/54 (0) | 6/54 (11) | 3[b]/55 (5) | 6/55 (11) | 1/51 (2) |
| p = | 0.345 | 0.014* | 0.125 | 0.014* | 0.486 |
| Combined (%) | 1/54 (2) | 10[c]/54 (19) | 5/55 (9) | 18[d]/55 (33) | 49[d]/51 (96) |
| p = | 0.000** | 0.004** | 0.107 | 0.000** | 0.000** |

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 54. Also excludes week 53 interim sacrifice animals.

[a]First liver adenoma observed at week 53, dose 16000 ppm, in an interim sacrifice animal. Second liver adenoma observed at week 79, dose 100 ppm, in a terminal sacrifice animal.

[b]First liver carcinoma observed at week 65, dose 800 ppm.

[c]Two animals in the 100 ppm dose group had both an adenoma and a carcinoma.

[d]One animal in each of the 8000 and 16000 ppm dose groups had both an adenoma and a carcinoma.

Note: Interim sacrifice animals are not included in this analysis. One animal in the 16000 ppm dose group of the interim sacrifice group had a liver adenoma.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 4. Malathion - $B_6C_3F_1$ Mouse Study

Female Liver Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

| | Dose (ppm) | | | | |
|---|---|---|---|---|---|
| | 0 | 100 | 800 | 8000 | 16000 |
| Adenomas (%) | 0/55 (0) | 1/53 (2) | 0/53 (0) | 9/52 (17) | 42[a]/51 (82) |
| p = | 0.000** | 0.491 | 1.000 | 0.001** | 0.000** |
| Carcinomas (%) | 1[b]/55 (2) | 0/53 (0) | 2/53 (4) | 1/52 (2) | 2/51 (4) |
| p = | 0.183 | 0.509 | 0.486 | 0.738 | 0.471 |
| Combined (%) | 1/55 (2) | 1/53 (2) | 2/53 (4) | 10/52 (19) | 43[c]/51 (84) |
| p = | 0.000** | 0.743 | 0.486 | 0.003** | 0.000** |

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 54. Also excludes week 53 interim sacrifice animals.

[a]First liver adenoma observed at week 78, dose 16000 ppm.

[b]First liver carcinoma observed at week 79, dose 0 ppm.

[c]One animal in the 16000 ppm dose group had both an adenoma and a carcinoma.

Note: Interim sacrifice and accidental death animals are not included in this analysis. One animal in the 16000 ppm dose group which was killed accidentally had a liver adenoma.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

References

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May 13, 1998

Malathion Male Mouse

Hepatocellular Adenomas and/or Carcinomas Combined

Fisher's Exact Test/Cochran-Armitage trend test

Re-Read

H

| DOSE(ppm) | 0.0000 | 100.0000 | 800.0000 | 8000.0000 | 16000.0000 |
|---|---|---|---|---|---|
| | 4/54 (7) | 10/54 (19) | 9/55 (16) | 15/55 (27) | 49/51 (96) |
| | p= 0.0000** | p= 0.0751 | p= 0.1254 | p= 0.0058** | p= 0.0000** |

| | CHI-SQUARE | DF | P VALUE | |
|---|---|---|---|---|
| LINEAR TREND (Ho: no trend) | 106.7718 | 1 | 0.0000** | (one-sided) |
| DEPARTURE (Ho: linear) | 15.0302 | 3 | 0.0020** | (two-sided) |

STATS BASED on RE-READ By PWG of Male Mice Liver Tumors

- A Better memo will Be coming soon

WJB 2/12/99

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# MALATHION

## Summaries of the Cancer Assessment Review Committee Meetings of September 24 & October 8 1997

## FIRST MEETING - 9/24/97

1. The Carcinogenicity Study in B6C3F1 Mice, 1994. (MRID No. 43407201

Groups of B6C3F1 mice (55/sex/dose) were fed diets containing Malathion at 0, 100, 800 8000 or 16, 000 ppm for 18 months; 10/sex/dose received the treatment for 12 months (interim sacrifice)

A. Discussion of the Tumor Data

Hepatocellular tumors were increased in both sexes of mice and the incidence are as follows:

| Tumor Type | Sex | 0 ppm | 100 ppm | 800 ppm | 8000 ppm | 16000 ppm |
|---|---|---|---|---|---|---|
| Adenomas (%) | Males | 2** | 11 | 4 | 24** | 96** |
| | Females | 0** | 2 | 0 | 17** | 82** |
| Carcinomas (%) | Males | 0 | 11** | 5 | 11 | 2 |
| | Females | 2 | 0 | 4 | 2 | 4 |
| Combined (%) | Males | 2** | 19** | 9 | 33** | 96** |
| | Females | 2 | 2 | 4 | 19** | 84** |

Historical Control Incidences - Testing Laboratory (5 studies; 1985 - 90)

Male Mice at 18-months::

Adenomas: Range, 14 - 22%, Mean- NA
Carcinomas: Range, 0 -6%, Mean, - NA
[1 mouse /1 study (2.2%); 3 mice/1 study (6.4%)]

Liver tumors observed at 100 ppm were attributed to treatment because of the following factors:

-multiplicity of the component of the liver tumors in tumor bearing animals (i.e., the presence of adenomas and carcinomas in the different lobes of the liver in the same mice.
- Pair-wise significance for carcinomas and combined adenomas/carcinomas in males

- Incidence of carcinomas greater than historical controls

- in vitro studies positive at low doses in vivo positive at 120 mg/kg

B. Non-Neoplastic Lesions: Hypertrophy of the Liver was observed in both sexes of mice at 8( and 16000 ppm with incidence and severity of the lesion increasing with the doses.

C. Toxicity

- No increase in mortality

- No clinical signs

- Decrease in absolute body weight at 8000 and 16000 ppm; no effect on food consumption at these doses.

-Cholinesterase inhibition for all 3 compartments:

| % Inhibition | Plasma | | RBC | | Brain | |
|---|---|---|---|---|---|---|
| | Males | Females | Males | Females | Males | Females |
| 800 ppm | 24 | 36 | 44 | 58 | 7 | 3 |
| 8000 ppm | 90 | 92 | 90 | 92 | 23 | 37 |
| 16000 ppm | 95 | 96 | 92 | 92 | 37 | 43 |

D. Adequacy of the Dose Levels Tested: The Committee voted on the following:

**The two top dose levels tested** at higher than the Limit Dose of 1000 mg/kg/day(Males = 1476 & 2978 mg/kg/day; Females = 1707 & 3448 mg/kg/day) **were excessive** based on the severity of inhibition of cholinesterase activity in both sexes:

| | | |
|---|---|---|
| **Two top doses are Excessive:** | **Yes = 11** | **No = 2** |

**The 800 ppm dose is Adequate in both sexes:**

| | | |
|---|---|---|
| **800 ppm Adequate** | **Yes = 10** | **No = 2** |

E. Conclusions

Given the increase in hepatocellular tumors at the 100 ppm dose but not at the 800 ppm dose, the Committee concluded that the:

1) the liver slides from **male mice at all dose level should be re-evaluated** and;

2) this should be **referred to the Pathology Work Group**

The Committee voted: **Yes = 13** **No = 1**

2. The Carcinogenicity Study in Fischer 344 Rats 1996. (MRID No. 43942901

Groups of Fisher 344 rats (55/sex/dose) were fed diets containing Malathion at 0, 50, 500 6000 or
12, 000 ppm for 24 months; interim sacrifice (10/sex/dose) at 3, 6 and 12 months.

A. Adequacy of the Dose Levels Tested

Mortality: Increased in males at 6000 ppm & in both sexes at 12000 ppm (no survivors in males at 12000 ppm at termination)

Clinical signs: limited to anogenital staining in females

Anemia: observed in both sexes at 6000 and 12000 ppm

Body weight gain: decreases at 6000 (13% in males and 4% in females)
decreased at 12,000 ppm (4% in males and 15% in females)

% ChE inhibition at termination:

| | 6000 | | 12000 | |
|---|---|---|---|---|
| | M | F | M | F |
| Plasma | 64 | 61 | Dead | 89 |
| RBC | 43 | 44 | dead | 52 |
| Brain | 21 | 18 | dead | 67 |

**The Committee concluded that the:**

- **12000 ppm: excessive in both sexes**
- **6000 ppm: excessive in males** (due to mortality)
- **6000 ppm: adequate in females.**
- **500 ppm: adequate in males**

B. Discussion of Tumor Data

**I. LIVER TUMORS IN FEMALES**

| Tumor Type | 0 ppm | 50 ppm | 500 ppm | 6000 ppm | 12000 ppm |
|---|---|---|---|---|---|
| Adenomas (%) | 0** | 2 | 2 | 8** | 10** |
| Carcinomas (%) | 0 | 2 | 2 | 0 | 8 |
| Combined (%) | 0** | 4 | 5 | 7 | 16** |

Historical Control Incidences

| | Testing Laboratory | NTP |
|---|---|---|
| Adenomas: | Range, 0 - 5%; Mean= 1.6 | 0 .59% |
| Carcinomas: | Range, 0 - 2.4%, Mean = 1.1 | 0.07% |

**The Committee concluded that the:**

| | | |
|---|---|---|
| **- liver tumors at 6000 ppm are treatment-related** | **Yes = 9** | **No = 3** |
| **- liver tumors at 50 and 500 ppm are suggestive evidence and can not be discounted:** | **Yes = 8** | **No = 4** |

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## II. NASAL TUMORS:

The Committee determined that:

1) The nasal tissues from all animals/dose groups should be evaluated in its entirety.

2) Use the standard for location/trimming of nasal turbinates consult with the Pathologist, L. Brenneke for details and guidance.

**The Committee concluded that an assessment on the relevancy of this tumor to treatment can not be made at this time because of the need for a re-evaluation of the nasal tissues from all animals.**

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## SECOND MEETING - 10/8/97

At the First meeting on 9/24/97, the Committee determined that the 8000 ppm in the mouse study was an excessive dose and in the rat study, the 6000 ppm dose was adequate in females but excessive in males

At the Second meeting on 10/8/97, one member (who was not present at the first meeting) raised the concern that if the 8000 ppm in mice was determined to be an excessive dose should not the 6000 ppm in rats be also considered an excessive dose and not an adequate dose based on a comparable brain cholinesterase inhibition (ChEI) at these doses. Brain ChEI was 23% in male mice and 21% in female rats. In order to address this concern, the Committee discussed the brain ChEI in the mouse and rat studies. While it was true that the brain ChEI was comparable between these two species, ChEI in the other two compartments (plasm and RBC) were not comparable. In the mouse at 8000 ppm plasma ChEI was 90% & 92% in males and females, respectively,.whereas in the rat study plasma ChEI was only 64 & 61% in males and females, respectively. Similarly, in the mouse, the RBC ChEI was 90% & 92% in males and females, respectively, whereas in the rats RBC ChEI was 43% & 44% in males and females, respectively. Thus it was evident that the ChEI was more severe in mice involving all three compartments, whereas in the rats, only the brain ChEI was comparable to that of the mouse. In addition, at the 6000 ppm females, there was no increase in mortality, no cholinergic signs, and there was only a 4% decrease in body weight gain. The Committee further evaluated the acute and subchronic studies. Data from these studies showed that the ChEI seen at 6000 ppm in the chronic study was not supported by the ChEI seen at 5000 ppm in the 90-day study or at in the acute study. Based on thes data, the Commiitee reaffirmed its conclusion that the **6000 ppm** is an **adequate dose in females.**

## III. TESTICULAR TUMORS IN MALES

| Tumor Type | 0 ppm | 50 ppm | 500 ppm | 6000 ppm | 12000 ppm. |
|---|---|---|---|---|---|
| Interstitial cell tumor | 52/55 | 52/55 | 53/55 | 52/53 | 53/54 |
| (%) | 95 | 95 | 96 | 98 | 98 |
| p= | 0.000** | - | 0.037** | 0.032* | 0.004** |

- No historical control data are available from the testing laboratory

- The increase in tumor incidence at 6000 and 2000 ppm groups could be due to the high mortality at these doses ( NOTE: both doses were determined to excessive in males )

- Sufficient data are NOT available to determine if there was a decrese in the llatency period (i.e,.there was no serial sacrifice to determine latency (YES = 11 NO =1).

- This tumor type is not useful in overall evaluation.

**The Committee concluded that there is not adequate evidence to support a chemical-induced tumor response for this tumor type based on the incidence and lack of data to determine the onset of tumor (latency).** Members voted on the following options:

The significance of this tumor to treatement can not be determined: Yes = 6
The tumor response is non-positive: Yes = 5
Tumors are treatment-Related: Yes = 1

## IV. THYROID C-Cell TUMORS IN MALES

| Tumor Type | 0 ppm | 50 ppm | 500 ppm | 6000 ppm | 12000 ppm |
|---|---|---|---|---|---|
| Adenomas (%) | 13/54 24.1 | 14/54 25.9 | 10/53 18.9 | 6/54 11.1 | 4/55 7.4 |
| Carcinomas (%) | 1/54 1.8 | 2/54 3.7 | 6/53 11.3 | 2/54 3.7 | 0/55 0 |
| Combined (%) | 14/54 25.9 | 16/54 29.6 | 16/53 30.2 | 8/54 14.8 | 4/55 7.4 |

- No historical control data available from the testing laboratory

- No increase is seen either for the adenomas or the combined tumor incidences.

- There is a dose-related increase in carcinomas

- There is some evidence of increased incidence of C-Cell tumors at some doses (may be excessive), in three strains of rats ; the Osborne-Mendel (1978 study), Sprague-Dawley (1980; FDRL study), and F344 (1979, NCI-Malaoxon study) rats. However, no increase in C-Cell tumors were seen in the 1996 F344 rats with Malaoxon.

**The Committee concluded that there is suggestive evidence for this tumor type.** This conclusions was based on 1) the occurrence of a dose-related and statististically significant increase in carcinomas in the present study and 2) increases in C-Cell tumors in other strains of rats at some doses in other studies.

Vote: YES = 8; Can not be determined = 4.

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## V. THYROID FOLLICULAR CELL TUMORS IN MALES

| Tumor Type | 0 ppm | 50 ppm | 500 ppm | 6000 ppm | 12000 ppm |
|---|---|---|---|---|---|
| Adenomas (%) p= | 2/55 4 0.063 | 1/54 2 - | 1/51 2 - | 4/51 8 0.150 | 4/43 9 0.378 |
| Carcinomas (%) p= | 0/42 0 0.196 | 0/45 0 - | 2/41 5 0.085 | 2/26 8 0.162 | 0/0 0 - |

| | | | | | |
|---|---|---|---|---|---|
| Combined<br>(%)<br>p= | 2/55<br>4<br>0.035 | 1/54<br>2<br>- | 3/51<br>6<br>0.321 | 6/51<br>12<br>0.077 | 4/43<br>9<br>0.160 |

- No historical control data available from the testing laboratory

- When the two excessive toxic doses (6000 & 12000 ppm) are excluded, there is no increases in the incidences of adenomas, carcinomas or the combined tumors in treated animals when compared to controls.

**The Committee unanimously concluded that these tumors are NOT attributable to treatment.**

## VI. PITUITARY TUMORS IN MALES

| Tumor Type | 0 ppm | 50 ppm | 500 ppm | 6000 ppm | 12000 ppm |
|---|---|---|---|---|---|
| Adenomas<br>(%)<br>p= | 25/51<br>49<br>0.980 | 13/31<br>42<br>- | 20/34<br>59<br>0.133 | 17/3<br>52<br>0.266 | 14/53<br>26<br>- |
| Carcinomas<br>(%)<br>p= | 0/50<br>0<br>0.778 | 1/30<br>3<br>0.319 | 3/32<br>9<br>0.029 | 4/32<br>12<br>0.027 | 1/49<br>2<br>0.369 |
| Combined<br>(%)<br>p= | 25/51<br>49<br>0.987 | 14/31<br>45<br>- | 23/34<br>68<br>0.033 | 21/33<br>64<br>0.097 | 15/53<br>28<br>- |

- No historical control data available from the testing laboratory

- No increase in adenomas or the combined incidences

- Only carcinomas were increased (pair-wise) at 500 and 6000 ppm (exesssive dose)

**The Committee concluded that this tumor type can NOT be attributed to treatment YES = 11:**