UNITED ST TECTION AGENCY 013991

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Malathion

Date: February 10, 2000

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Memorandum

Subject: Malathion - Re t Review Committee

From: Jess Rowland, --
Cancer Assessment Review Committee
Health Effects Division

To: Paula Deschamp
Risk Assessor, Reregistration Branch 2
Health Effects Division

Attached please find the Final Cancer Assessment Document for Malathion

cc: K. Baetcke
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CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF
MALATHION

FINAL REPORT

February 2, 2000

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

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DATA PRESENTATION: Brian Dementi

Brian Dementi, Toxicologist

DOCUMENT PREPARATION: Jess Rowland

Jess Rowland., Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: AT ONE OR MORE OF THE MEETINGS

(Signature indicates concurrence with the assessment unless otherwise stated).

William Burnam, Chairman

Karl Baetcke

Vicki Dellarco

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Nancy McCarroll

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Yin Tak Woo — SEE ATTACHED FAX. (JR)

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report and statistical analysis of data, respectively)

Lucas Brennecke, Pathology Consultant — See ATTACHED FAX (JR)

Lori Brunsman, Statistical Analysis

DRAFT INTERNAL AND DELIBERATIVE MATERIAL

DATA PRESENTATION: ______________________
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<u>COMMITTEE MEMBERS IN ATTENDANCE:</u> AT THE **JUNE 23, 1999** MEETING

(Signature indicates concurrence with the assessment unless otherwise stated).

William Burnam, Chairman ______________________

Karl Baetcke ______________________

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<u>NON-COMMITTEE MEMBERS IN ATTENDANCE</u>

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Lucas Brennecke, Pathology Consultant ______________________

Lori Brunsman, Statistical Analysis ______________________

DATA PRESENTATION: Brian Dementi, Toxicologist

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Lucas Brennecke, Pathology Consultant

Lori Brunsman, Statistical Analysis

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EXECUTIVE SUMMARY

On September 24, October 8, October 15, 1997, June 10, 1998, February 24, 1999 and June 23, 1999, the Health Effects Division's Cancer Assessment Review Committee (CARC) evaluated the carcinogenic potential of malathion. The Committee reviewed the following studies: 1) Carcinogenicity study in B6C3F1 mice; 2) Combined chronic toxicity/carcinogenicity study in Fischer 344 rats with malathion; and 3) the Combined chronic toxicity/carcinogenicity study with malaoxon, the active cholinesterase inhibiting metabolite of malathion in F344 rats. Relevant subchronic, chronic and mutagenicity studies were also reviewed at these meetings, as well as the results of the studies conducted with malathion and/or malaoxon (during 1978-80) by the National Cancer Institute/National Toxicology Program (NC/NTP).

Dr. Brian Dementi, Toxicology Branch 1, presented the experimental designs including: survival data, body weight effects, cholinesterase data, treatment-related non-neoplastic and neoplastic lesions, statistical analysis of the tumor data, the adequacy of the dose levels tested, and the weight of the evidence for the carcinogenicity of malathion and malaoxon. Dr. Dementi's memoranda regarding the assessment of the carcinogenicity of malathion and malaoxon that were forwarded to the Chairman/Executive Secretary of CARC are presented in Attachment 1.

Groups of male and female **B6C3F1 mice** received **malathion** in their diet at at 0, 100, 800, 8000 or 16,000 ppm for 18 months. These dietary concentrations were equivalent to doses of 0, 17.4, 143, 1476 or 2978 mg/kg/day in males and 0, 20.8, 167, 1707 or 3448 mg/kg/day for females, respectively. The Committee concluded that in mice, the 800 ppm dose level was adequate to assess the carcinogenic potential of malathion, however, the 8000 and 16,000 ppm doses were excessive based on severe plasma (90 to 95%) and red blood cell (92 to 96%) and marked brain (20 to 43%) cholinesterase inhibition in both sexes.

Groups of male and female **Fischer 344 rats** received **malathion** in their diet at concentrations of 0, 50, 500, 6000 or 12,000 ppm for 24 months; the low dose 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 red blood cell cholinesterase inhibition among females at 100 ppm. These dietary concentrations were equivalent to doses of 0, 2, 29, 359 or 739 mg/kg/day in males and 0, 3, 35, 415 or 868 mg/kg/day for females, respectively. The Committee concluded that the dose level of 500 ppm in males and 6000 ppm in females were adequate to assess the carcinogenic potential of malathion; but 6000 ppm in males was excessive due to increased mortality (74%); and the 12,000 ppm was excessive in both sexes based on the severe inhibition of plasma (89%), red blood cell (52%) and brain (67%) cholinesterase activity in females and increased mortality in males (100%) and females (64%) at this dose.

Groups of **Fischer 344 rats** were fed diets containing **malaoxon** at 0, 20, 1000 or 2000 ppm for 24 months. These dietary concentrations were equivalent to doses of 0, 1, 57 or 114 mg/kg/day in males and 0, 1, 68 or 141 mg/kg/day for females, respectively. The Committee concluded that the dose level of 1000 ppm was adequate to assess the carcinogenic potential of malaoxon, but the 2000 ppm dose was excessive due to increased mortality (53% in males and 49% in females)and severe inhibition of plasma (83-96%), red blood cell (54-66%) and brain (11-78%) cholinesterase activity.

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When compared to the historical control data of the testing laboratory, the incidences of adenomas at 6000 ppm (8%) and 12,000 ppm (10%) exceeded the historical control range (0 to 5%) and mean (1.6%). The incidences of carcinomas at 12,000 ppm (8%) also exceeded the historical control range (0 to 2.4%) and mean (1.1%). In addition, the incidences of these two tumor types exceeded the historical control incidences (adenomas, 0.44% and carcinomas, 0%) of the NTP.

The increase in liver tumors in female rats when combined with the increase in liver tumors in both sexes of mice at 8,000 ppm and 16,000 ppm, somewhat increased the Committee's concern for the carcinogenic effects of malathion. However, this concern was lessened since these tumors occurred only at doses in mice which caused severe plasma and red blood cell cholinesterase inhibition (90 to 96%) and marked brain (20 to 43%) cholinesterase inhibition. No liver tumors occurred at the 800 ppm dose which was considered adequate based on less cholinesterase inhibition at this dose.

The most compelling evidence of carcinogenicity was shown in the response of liver tumors in female rats at doses which were not considered excessively toxic. Although the strongest evidence was seen at 12,000 ppm (which was determined to be excessively toxic in the female rat), this increase in liver tumors (mainly adenomas) was also significant at 6,000 ppm ($p=0.032$). The presence of two tumors per dose level at the lower doses of 50 ppm and 500 ppm was considered to be of biological significance and added to the overall concern for malathion's carcinogenic potential.

The Committee concluded that the incidence of liver tumors in female rats at 50 and 500 ppm provides suggestive evidence of carcinogenicity and cannot be discounted and that the liver tumor incidences at 6000 and 12,000 ppm (although considered to be excessive doses) provided positive evidence of carcinogenicity. There was no evidence of liver carcinogenicity in male rats at any dose level, but the potential for tumor induction may have been compromised by competing toxicity, particularly at 6000 ppm and 12000 ppm, where mortality was 74% and 100%, respectively.

Nasal Tumors - Rats. In **male rats**, there was an **adenoma of the olfactory epithelium** at 6000 ppm and an **adenoma of the respiratory epithelium** at 12,000 ppm compared to zero in the controls. In **female rats,** there was an **adenoma of the respiratory epithelium** at 6000 ppm and 12,000 ppm compared to zero in the controls.

When compared to the historical control data of the testing laboratory, the incidence of nasal adenomas in this study (1/90 at 6000 ppm and 1/90 at 12,000 ppm) in both sexes exceeded the historical control incidence (0/240 males and 0/240 females). In addition, the NTP reported respiratory tract tumors in the respiratory epithelium of 6/4000 male rats and in the olfactory epithelium of 0/4000 males. Furthermore, of the 6/4000 of the respiratory epithelium, four of the six were squamous cell tumors. Thus, the relevant incidence for the tumor type in question is 2/4000 control males.

The Committee concluded that there is evidence of carcinogenicity in both sexes of mice at the two highest dose levels of malathion tested which were considered excessive. There is no evidence of carcinogenicity in male or female mice at the lower doses. Evidence for carcinogenicity in mice was demonstrated by the presence of liver tumors in both sexes. **The Committee further concluded that there is evidence of carcinogenicity for malathion in female rats (but not males)** which manifested as liver tumors at all dose levels and tumors of the nasal mucosa at 6000 ppm, although nasal tumors were also seen at 12,000 ppm (a dose considered to be excessive).

Liver Tumors - Mice. In **male mice** (based on the Pathology Work Group Re-Read), there was a positive trend (p=0.000) for **liver** adenomas and the combined tumors (adenomas plus carcinomas). The incidence of adenomas was significantly increased at 8000 ppm (14/55, 25%, p = 0.0103) and 16,000 ppm (49/51, 96%, p = 0.000) when compared to controls (4/54, 7%). Similarly, the combined tumors (adenomas plus carcinomas) showed pair-wise significance at 8000 ppm (15/55, 27%, p=0.006) and 16000 ppm (49/51, 96%, p=0.000) when compared to controls (4/54, 7%). Although carcinomas were seen at 100 ppm, 800 ppm and 8000 ppm compared to zero in the controls, none of the incidences showed statistical significance nor there was a dose-related increase at any dose level.

When compared with the historical control ranges: the incidences of adenomas at the 8000 ppm (25%) and 16,000 ppm (96%) doses exceeded the historical control range (14 to 22%). The incidences of carcinomas at 800 ppm (5%) and 8000 ppm (4%) doses were within the historical control range (0 - 6.4%). No carcinomas were seen at 16,000 ppm. The incidence of carcinomas at 100 ppm (7%) was slightly outside the historical control range and well above the mean value in a small historical control data base.

In **female mice**, there was a positive trend (p=0.000) for **liver** adenomas and the combined tumors (adenomas plus carcinomas). The incidence of adenomas was significantly increased at 8000 ppm (9/52, 17%, p = 0.001) and 16,000 ppm (42/51, 82%, p = 0.000) when compared to controls (0/55). Similarly, the combined tumors (adenomas plus carcinomas) showed pair-wise significance at 8000 ppm (10/52, 19%, p=0.003) and 16,000 ppm (43/51, 84%, p=0.000) when compared to controls (1/55, 2%). No statistically significant increases in carcinomas alone were seen at any dose.

Liver Tumors - Rats. In **female rats**, increased incidence of **liver** adenomas and combined adenomas and carcinomas were seen at all dose levels of malathion tested. However, statistical significance was seen only at the two highest dose levels tested. There was a positive trend (p=0.007) for adenomas and the combined adenomas plus carcinomas (p=0.002). The incidence of adenomas was significantly increased at 6000 ppm (3/39, 8%, p = 0.032) and at 12,000 ppm (3/29, 10%, p = 0.008) when compared to controls (0/40). A dose-related increase in the incidences of combined tumor (adenomas plus carcinomas) were seen, reaching statistical significance only at the two highest dose levels. The combined tumors (adenomas plus carcinomas) were: 2/50 (4%) at 50 ppm; 2/44 (5%) at 500 ppm; 3/41 (7%, p=0.032) at 6000 ppm; 6/38 (16%, p=0.003) at 12,000 ppm compared 0/41 in the controls. No statistically significant increases in carcinomas alone were seen at any dose level.

Of the four nasal tumors, one in each sex at the two highest dose levels, only one tumor in the 6000 ppm dose in the female was at a dose that was not considered excessive. The Committee postulated that direct contact with malathion (by volatilization from the feed or by inhalation of the feed through the nose) was a plausible explanation for the nasal tumors. However, the Committee concluded that a systemic effect could not be unequivocally ruled out.

The Committee attributed the nasal tumors in female rats to treatment because: (1) spontaneous nasal tumors are very rare in rats and therefore the incidences in this study were considered to be biologically significant; (2) an adenoma of the respiratory epithelium was also seen in one male rat at 12,000 ppm (although an excessive dose); (3) an adenoma of the olfactory epithelium also was seen in one male rat at 6000 ppm; (4) there were no nasal tumors in the concurrent controls; and (5) the incidences exceeded the historical control incidence of the testing laboratory.

The Committee concluded that the tumors of the palate (squamous cell papilloma /carcinoma), thyroid gland (follicular cell and C-cell), pituitary gland (par distalis), testes (interstitial cell), and uterus (various types) as well as the mononuclear cell leukemia in rats are not treatment-related because: (1) the individual tumor incidences were low; (2) the tumor incidences and types in treated groups were comparable to those seen in the concurrent control group; (3) there was neither statistical nor biological significance; (4), there was no dose-response relationship; and/or (5) the incidences were within the historical control mean and/or range of the testing laboratory.

Results of the guideline genetic toxicology studies with malathion indicate that the test material did not cause gene mutations in bacteria or unscheduled DNA synthesis in cultured rat hepatocytes. Similarly, malathion was neither clastogenic nor aneugenic up to doses that showed clear cytotoxicity for the target tissue *in vivo*. By contrast, studies from the open literature indicated that malathion is a confirmed clastogen both *in vitro* and *in vivo*. However, there are uncertainties regarding the relevance of these findings to a mutagenic mode of action for malathion since the positive results from both *in vivo* and *in vitro* studies were seen at cytotoxic doses and/or the types of induced aberrations were asymmetric and, therefore, not consistent with cell survival. Nevertheless, malathion was shown to be weakly reactive with DNA and does contain a structure that suggests it is electrophilic. **Therefore, the Committee concluded that while the evidence for mutagenicity as an influence on the carcinogenicity of malathion is weak, at this time it can not be ruled out.**

Malaoxon, the active cholinesteraseinhibiting metabolite of malathion, was not carcinogenic in male or female rats when tested at doses that were judged to be adequate to assess its carcinogenic potential. Malaoxon was non-mutagenic in bacteria, was not clastogenic in cultured Chinese Hamster Ovary (CHO) cells, but did produce positive results without metabolic activation in the mouse lymphoma assay. Malaoxon caused sister chromatid exchanges in CHO cells in the absence of metabolic activation. Like Malathion, Malaoxon has the same electrophilic structure for DNA reactivity.

In accordance with the EPA *Proposed Guidelines for Carcinogen Risk Assessment* (April 10, 1996), the Committee classified malathion as a **"likely human carcinogen"**. This classification was based on the following factors:

(i) occurrence of liver tumors in male and female B6C3F1 mice and in female Fischer 344 rats; and
(ii) presence of a few rare tumors (nasal respiratory epithelium) in female Fischer 344 rats.

It should be noted however, that the classification of "likely human carcinogen" is primarily based on the presence of the liver tumors in the female rats at doses which were not considered excessive. With the exception of one nasal tumor in female rats, all other tumor types were determined to occur at excessive doses or were unrelated to treatment with malathion.

The Committee further observed that it is plausible that tumor occurrences in these studies are dose-limited (i.e., tumors are induced only at excessive doses), however, mode of action studies to demonstrate this hypothesis are not available. Consequently, some Committee members were of the opinion that malathion should be classified as a "suggestive human carcinogen" and that this classification best described the carcinogenic potential for malathion. The majority opinion, however, was that malathion should be classified as a "likely human carcinogen" based on animal data.

The Committee recommended a linear low-dose approach (Q_1^*) for human risk characterization and extrapolation based on the nasal and the liver tumors in rats at all dose levels tested. The linear dose extrapolation is supported by: (i) lack of mode of action and (ii) a possible involvement of a genetic component for malathion or malaoxon.

At the June 23, 1999 meeting, the Committee recommended that quantifications of risk be estimated for female rat liver tumors, female rat nasal tumors, and male rat nasal tumors and that the most potent unit risk, Q_1^*, of the these should be used for risk assessments. The following Q_1^*, were calculated:

Tumor Type	Q_1^*
Male nasal	1.06×10^{-3}
Female nasal	4.95×10^{-4}
Female liver	1.52×10^{-3}

In this case, the most potent unit risk, Q_1^*, is that for female rat liver adenoma and/or carcinoma combined tumor rates at 1.52×10^{-3} in human equivalents. Therefore, this slope factor will be used for human health risk assessments in the Reregistration Eligibility Document.

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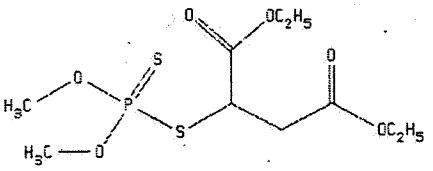
I. INTRODUCTION

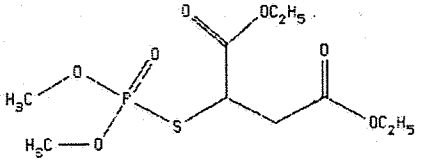
The Health Effects Division's Cancer Assessment Review Committee (CARC) met on September 24, October 8, October 15, 1997, June 10, 1998, February 24, 1999 and June 23, 1999 and evaluated the carcinogenic potential of Malathion. The CARC reviewed the following studies: (1) Carcinogenicity study in B6C3F1 mice; (2) Combined chronic toxicity/carcinogenicity study in Fischer 344 rats with Malathion; and (3) the Combined chronic toxicity/carcinogenicity study with Malaoxon (the active metabolite of malathion) in F344 rats. The Committee also discussed the results of the subchronic, chronic and mutagenicity studies as well as the results of the studies conducted with Malathion/Malaoxon by the National Cancer Institute (NCI)/National Toxicology Program (NTP).

Dr. Brian Dementi of Toxicology Branch presented the experimental design including: survival data, body weight effects, cholinesterase data, treatment-related non-neoplastic and neoplastic lesions, statistical analysis of the tumor data; the adequacy of the dose levels tested, and the weight of the evidence for the carcinogenicity of malathion.

II. BACKGROUND INFORMATION



Malathion



Malaoxon

PC Code: 057701
CAS No. 121-75-5

In 1990, the malathion carcinogenicity data base was considered by the HED Cancer Peer Review Committee (CPRC). At that time five NCI carcinogenicity studies plus a contract laboratory 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 in F344 rats and B6C3F1 mice. The contract laboratory study was a 2-year malathion study in Sprague-Dawley rats performed by Food and Drug Laboratories, Waverly, New York.

In 1990, the CPRC review of these six studies took into consideration the registrant's (Cheminova Inc) assessment of the studies as well as 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 suggested carcinogenic response of these studies included the following:

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Species	Strain	Tumor Type/Sex
		Malathion
Mouse:	B6C3F1	Neoplastic nodules/hepatocellular carcinomas, males
Rat:	Sprague-Dawley	C-cell neoplasms of thyroid glands, female Mammary tumors and uterine polyps, female
Rat:	Fischer 344	Pheochromocytoma of adrenal gland, male Leukemia, male
Rat:	Osborne-Mendel	C-cell neoplasms of thyroid glands, male Follicular cell neoplasms of thyroid glands, both sexes Pheochromocytoma of adrenal gland, male
		Malaoxon
Mouse:	B6C3F1	No evidence of carcinogenicity
Rat:	Fischer 344	C-Cell neoplasms of thyroid glands, male and female Pheochrocytoma of the adrenal gland, male Mammary gland adenomas, female Lymphoma of hematopoietic, male

In 1990, the CPRC classified malathion as a Group D chemical, (not classifiable as to human carcinogenicity) based on the inadequacy of the available studies to make definitive determinations on the carcinogenicity of malathion or malaoxon. The CPRC agreed with the NTP re-analysis that there was no clear evidence of carcinogenicity due to malathion or malaoxon administration in most of these studies. However, the Committee felt that there were many issues regarding the adequacy of each study which needed to be addressed before a firm conclusion regarding the carcinogenic potential of malathion could 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 tumors of thyroid gland and pheochromocytoma of adrenal gland) and of multiple tumors occurring in more than one study. There was also some evidence from mutagenicity studies that suggested a genetic component for malathion and malaoxon was possible. These factors provided weight to the evidence of possible carcinogenic effects that could not be totally dismissed (Cancer Peer Review for Malathion dated April 12, 1990; HED Document No. 008386).

In 1990, the CPRC reaffirmed the recommendation of the 1988 Registration Standard for the Registrant to perform additional carcinogenicity studies with malathion and malaoxon. The 1988 Registration Standard had required a new malathion carcinogenicity study in B6C3F1 mice, a malaoxon chronic toxicity/carcinogenicityin Fischer 344 rats, and a malathion chronic toxicity study in Fischer 344 rats (Cancer Peer Review for Malathion dated 4/12/90; HED Document No. 008386).

These studies have now been completed and reviewed by the Agency and they constitute the principal body of information in the current evaluation of the carcinogenic potential of malathion and malaoxon.

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III. EVALUATION OF CARCINOGENICITY STUDIES

1. Carcinogenicity Study with Malathion in B6C3F1 Mice

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

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 16,000 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.

B. Discussion of Tumor Data

(i) Liver Tumors

The incidences of hepatocellular tumors were increased in both sexes of mice as shown in **Table 1**.

Male mice had significant increasing trends, and significant differences in pair-wise comparisons of the 8000 and 16000 ppm dose groups with the controls, for liver adenomas, and adenomas/carcinomas combined, all at $p < 0.01$. There were significant differences in the pair-wise comparisons of the 100 and 8000 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 combined adenomas/carcinomas ($p < 0.01$)

Female mice had significant increasing trends, and significant differences in the pair-wise comparisons of the 8000 and 16,000 ppm dose groups with the controls, for liver adenomas, and adenomas/carcinomas combined, all at $p < 0.01$.

The Committee concluded that the liver tissues/slides from male mice from all dose levels should be re-evaluated and submitted to a pathology working group (PWG) for peer review. This conclusion was based on: 1) the statistically significant increases in hepatocellular tumors in male mice at the low-(100 ppm), mid-high (8000 ppm) and high-(16000 ppm) doses but not at the mid dose (800 ppm) and 2) the apparent low tumor incidences in the concurrent control (male) mice.

As requested by the Committee a re-read of the male mouse liver pathology slides was conducted by a PWG and the results were submitted to the Agency. **The Committee accepted the results of the re-read of the male mouse liver tumors by the PWG.** The qualitative analysis of the re-read of the liver tumors is presented in **Table 2.**

Table 1: Mice: Original Pathology Report, 1997 - Liver Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results.

Tumor Type	Sex	0 ppm	100 ppm	800 ppm	8000 ppm	16000 ppm
Adenomas	**Males**	1/54	6[a]/54	2/55	**13/55**	**49/51**
%		2	11	4	**24**	**96**
p=		**0.000****	0.056	0.507	**0.001****	**0.000****
%	**Females**	**0/55**	1/53	0/53	**9/52**	**42[b]/51**
p=		**0**	2	0	**17**	**82**
		0.000**	0.491	1.000	**0.001****	**0.000****
Carcinomas	**Males**	0/54	**6/54**	3[c]/55	**6/55**	1/51
%		0	**11**	5	**11**	2
p=		0.345	**0.014***	0.125	**0.014***	0.486
%	**Females**	1[d]/55	0/53	2/53	1/52	2/51
p=		2	0	4	2	4
		0.183	0.509	0.486	0.738	0.471
Combined	**Males**	1/54	**10[e]/54**	5/55	**18[f]/55**	**49[f]/51**
%		2	**19**	9	**33**	**96**
p=		**0.000****	**0.004****	0.107	**0.000****	**0.000****
%	**Females**	1/55	1/53	2/53	**10/52**	**43[g]/51**
p=		2	2	4	**19**	**84**
		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 (Statistical Analysis - Brunsman, May 2, 1997)

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

[b] First liver adenoma in female observed at week 78, dose 16000 ppm.

[c] First liver carcinoma in male observed at week 65, dose 800 ppm.

[d] First liver carcinoma in female observed at week 79, dose 0 ppm.

[e] Two males at 100 ppm had both an adenoma and a carcinoma.

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

[g] One female at 16000 ppm dose group had both an adenoma and a carcinoma.

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

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Table 2. Male Mice: PWG Re-read, 1998 - Liver Tumor Rates[+] and Exact Trend and Fisher's Exact Test Results

Tumor Type	0 ppm	100 ppm	800 ppm	8000 ppm	16000 ppm
Adenomas	4/54	8[a] /54	7/55	**14[a] /55**	**49[a] /51**
%	7	15	13	**25**	**96**
p=	**0.000****	0.180	0.274	**0.0103***	**0.000****
Carcinomas	0/54	4/54	2[b] /55	2/55	0/51
%	0	7	5	4	0
p=	0.128	0.059	0.252	0.252	1.0
Combined	4/54	10[c] /54	9/55	**15[d] /55**	**49/51**
%	7	19	16	**27**	**96**
p=	**0.000****	0.075	0.125	**0.006****	**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 (Statistical Analysis, Brunsman, February, 16, 1999).

[a] First liver adenoma observed at week 53, dose 16000 ppm, in an interim sacrifice animals. Subsequent liver adenomas observed at week 79, simultaneously in the 100, 8000 and 16000 ppm dose groups, in terminal sacrifice animals.

[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 the 8000 ppm dose group had both an adenoma and a carcinoma

Note: Interim sacrifice animals are not included in this analysis. One male 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 comparisons with control denoted at dose level.
If *, then $p < 0.05$; If **, then $p < 0.01$

There were significant differences in the pair-wise comparisons of the 16,000 ppm dose group with the controls, for liver adenomas, and combined adenomas/carcinomas, all at $p < 0.01$. There were significant differences in the pair-wise comparisons of the 8000 ppm dose group with the controls for liver adenomas at $p < 0.05$, and combined liver adenomas /carcinomas at $p < 0.01$. There also were significant increasing trends for adenomas and combined adenomas/carcinomas ($p < 0.01$). Increased incidences of adenomas, carcinomas and combined adenomas/carcinomas were seen at 100 ppm and 800 ppm, but none of the increases showed either statistical significance or a dose-response relationship.

The Committee discussed the differences between the original diagnosis of the tumor incidences and those following the re-read by the PWG (**Table 3a**). The Committee also discussed the "multiplicity" 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 mouse (**Table 3b**).

Table 3a. Male Mice: Summary of the changes in tumor incidences between the original diagnosis (i.e., the original pathology report, Table 1) and the re-evaluation (PWG re-read, Table 2)

	ORIGINAL DIAGNOSIS			REEVALUATION- PWG		
Dose(ppm)	**Adenoma**	**Carcinoma**	**Combined**	**Adenoma**	**Carcinoma**	**Combined**
Control	1	0	1	4	0	4
100	6	6	10	8	4	10
800	2	3	5	7	2	9
8,000	13	6	18	14	2	15
16,000	49	1	49	49	0	49

Table 3b. Male Mice: Incidences of "single" and "multiple" tumors after re-evaluation (PWG re-read).

		0 ppm	100 ppm	800 ppm	8000 ppm	16,000 ppm
Adenomas-	Single	4	8	6	14	13
	Multiple	0	0	1	0	36
Carcinomas	Single	0	2	2	2	0
	Multiple	0	2	0	0	0
Adenoma/Carcinoma		0	2	0	1	0

Dr. Brennenke, the consulting pathologist, commented that in the evaluation of carcinogenicity, "tumor bearing animal" counts as one regardless of the number or multiplicity of any tumor type. Although carcinomas were observed in both sexes at all dose levels (except in males at 16,000 ppm and females at 100 ppm) the incidences showed neither a dose-response relationship nor statistical significance at any dose level. In addition, tumor incidences at the two high doses should be considered carefully since these dose levels were determined to be excessive for assessing carcinogenicity (Section D on Pages 9-10).

The incidences of liver tumors in male mice in this study (censored data[1]) were compared to the historical control data (non-censored) for male mice from five studies conducted at the testing laboratory (International Research and Development Corporation, Mattawan, MI). Based on the results of the PWG re-read, when compared with the historical control ranges: the incidences of adenomas at 8000 ppm (25%) and 16,000 ppm (96%) exceeded the historical control range (14 to 22%); and the incidences at 100 ppm (15%) and 800 ppm (13%) were within the historical control range (data for mean incidences are not available). When compared to the historical control data, the incidence of carcinomas at 100 ppm (7%) was slightly outside the range (0 to 6%), and the incidences of carcinomas at 800 ppm (5%) and 8000 ppm (4%) were within the historical control range. In the 5 historical control studies, the incidences of liver carcinomas were: 0 in 3 studies; 1 mouse in one study (2.2%); and 3 mice in an another study (6.4%).

The Committee concluded that there is evidence of carcinogenicity in both sexes of mice at the 8,000 and 16,000 ppm dose groups and there is no evidence of carcinogenicity in male or female mice at 100 or 800 ppm.

In the 1978 NCI study with B6C3F1 mice, liver tumors (11 carcinomas and 6 adenomas) were seen in 17 of 55 male mice at the highest dose tested (16,000 ppm); there was no carcinogenic response in female mice. Also in the NCI study, among females, the combined adenomas/carcinomas incidences were 0% at 8000 ppm and 4% at 16,000 ppm in contrast to the present study where the tumor incidences in females were 19% at 8000 ppm and 84% at 16,000 ppm. The Committee noted that the tumor responses in the present study at the same dose levels were more pronounced than those seen in the NCI study.

(ii) <u>Nasal Tumors (Mice)</u>

At the October 8, 1997 meeting, the Committee elected to require histopathologic examination and peer review of microscopic slides of the nasal tissues from all animals in all dose groups in this carcinogenicity study in mice because of the concern for nasal tumors seen in the chronic toxicity/carcinogenicity study in rats (discussed later).

The tissue sections taken from five nasal regions in all mice were microscopically examined. This examination identified four neoplasms: a periodontal hemagiosarcoma in one control male; an odontoma in another control male; and an odontoma in each of two male mice in the low dose group.

The CARC concluded that the nasal neoplasms are not attributable to treatment since there is neither a statistical or biological significance nor a dose-response relationship. Additionally, there is no evidence of a carcinogenic response in the nasal turbinate at any dose level.

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[1] Number of animals examined excluding those that died prior to the observance of the first tumor.

C. Non-Neoplastic Lesions

Treatment-related non-neoplastic lesions of the liver manifested as hepatocellular hypertrophy in both sexes of mice at the 8000 and 16,000 ppm dose levels. Incidence and severity of these lesions increased with dose. The incidences are summarized in **Table 4.**

Table 4. Non-Neoplastic Lesions of the Liver in Mice Fed Malathion for 18 Months

Type of Lesion	0 ppm	100 ppm	800 ppm	8000 ppm	16000 ppm
MALES					
No. Examined	54	55	55	55	51
Hepatocellular Hypertrophy	0	0	0	55[a] (2.1)[b]	55 (3.1)
Mononuclear Cell Infiltration, Portal	0	2 (1.0)	0	4 (1.0)	4 (1.5)
Mononuclear Cell Foci, Parenchyma	5 (1.0)	4 (1.0)	7 (1.0)	9 (1.2)	4 (1.3)
Necrosis	2 (1.5)	2 (2.0)	1 (2.0)	3 (2.0)	5 (2.0)
FEMALES					
No. Examined	55	55	54	53	52
Hepatocellular Hypertrophy	0	0	0	53 (1.7)	52 (3.1)
Mononuclear Cell Infiltration, Portal	8 (1.0)	7 (1.3)	4 (1.0)	5 (1.0)	7 (1.0)
Mononuclear Cell Foci, Parenchyma	19 (1.0)	21 (1.0)	12 (1.1)	18 (1.1)	24 (1.0)
Necrosis	1 (1.0)	0	0	0	0

a = Incidences include mice that died, sacrificed in extremis, sacrificed at 18 month terminal sacrifice.

b = Indicate average severity score as follows: trace = 1.0; mild = 2.0, moderate = 3.0; severe = 4.0

Treatment-related non-neoplastic lesions of the nasal tissues were characterized as exudate, suppurative, increased glandular secretion, olfactory degeneration, olfactory atrophy and olfactory respiratory metaplasia in females at 800 ppm and in both sexes at 8000 and 16,000 ppm. The incidences are presented in **Table 5.**

Table 5. Non-Neoplastic Lesions of the Nasal Tissue in Mice [a]

Type of Lesion	No. Nasal Sections/ Animal	0 ppm	100 ppm	800 ppm	8000 ppm	16000 ppm
MALES						
No. of Animals Examined		54	55	55	55	51
Exudate, Suppurative	5	3	7	5	31	35
Increased Glandular Secretion	5	0	5	4	116	108
Olfactory Degeneration	4	0	0	0	183	159
Olfactory Atrophy	3	0	0	0	58	46
Olfactory Respiratory Metaplasia	3	1	0	0	24	28
Hyperplasia of Bowman's Gland	4	0	0	0	59	54
FEMALES						
No. of Animals Examined		55	55	54	53	52
Exudate, Suppurative	5	0	2	12	30	43
Increased Glandular Secretion	4	7	6	89	149	133
Olfactory Degeneration	4	0	0	10	187	191
Olfactory Atrophy	3	1	3	48	71	67
Olfactory Respiratory Metaplasia	4	0	0	1	126	86
Hyperplasia of Bowman's Gland	3	0	0	0	0	32

a = Incidences presented are the <u>total of the lesions</u> observed in all sections of the nasal tissue.

D. <u>Adequacy of the Dosing for Assessment of Carcinogenicity</u>

There were no effects on survival rate in mice at any dose level for either sex. There were decreased absolute body weights at 8000 ppm and 16,000 ppm in both sexes, ranging from 14.3 to 20.0% in males and 9.7 to 16.1% in females, throughout the study. There were no treatment-related clinical signs of toxicity at any dose level. The percent cholinesterase inhibition data are summarized in **Table 6.**

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Statistically significant inhibition of plasma and RBC cholinesterase activity was observed in males at 8000 and 16000 ppm and in females at 800, 8000, and 16,000 ppm; inhibition of brain cholinesterase activity was seen in males and females only at 16,000 ppm. At necropsy, liver "masses" were seen at all dose levels. Treatment-related non-neoplastic lesion, hypertrophy of the liver, was observed in both sexes of mice at 8000 and 16,000 ppm with incidence and severity of the lesion increasing with dose.

Table 6. Cholinesterase Activity in Mice Fed Malathion For 18 Months.

Percent Inhibition of Cholinesterase Activity At 18- Months						
Dose/Sex	Plasma		Red blood cell		Brain	
	Males	Females	Males	Females	Males	Females
800 ppm	24	**36***	44	**58***	7	3
8000 ppm	**90****	**92****	**90****	**92****	23	20
16,000 ppm	**95****	**96****	**92****	**92****	**37****	**43****

If *, = $p \leq 0.05$; If, ** = $p \leq 0.01$

The Committee concluded that, based on the severity of cholinesterase inhibition in both sexes, the two top dose levels (8000 and 16,000 ppm) were excessive and that the 800 ppm dose was adequate to assess the carcinogenic potential of malathion in this strain of mice. The 800 ppm dose was determined to be adequate based on the statistically significant decrease in plasma and RBC cholinesterase activity (36% and 58%, respectively) in females and biologically significant decrease (24% and 44%) in males. The Committee noted that the degree of cholinesterase inhibition was less severe when compared to 8000 ppm and 16,000 ppm dose levels. The Committee further noted that the 8000 ppm (1476 mg/kg/day dose in males and 1707 mg/kg/day in females) dose was higher than the Limit Dose (1000 mg/kg/day) and the 16,000 ppm (2978 mg/kg/day in males and 3448 mg/kg/day in females) dose was more than twice the Limit Dose for carcinogenicity studies.

The two highest dose levels tested in this study (8000 and 16,000 ppm) were required by the Agency (Data Call-In, 6/15/92) since they duplicated the levels tested in the 1978 NCI study in this strain of mice. In the 1978 NCI study, increased incidences of liver tumors in male mice were reported at 16,000 ppm, however, due to the equivocal nature of the findings, a clear association between liver tumors and malathion administration 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 analysis of the data further compromised the usefulness of the NCI study. Therefore, the Agency required a new study to be performed under similar conditions in order to resolve the question of possible carcinogenicity of malathion in B6C3F1 mice.

2. Combined Chronic Toxicity/Carcinogenicity Study with Malathion in Fischer 344 Rats

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

A. Experimental Design

Malathion Technical (97% a.i.) was administered in the diet to groups of (90/sex/dose) F344 rats at 0, 100/50, 500, 6000 or 12,000 ppm [equivalent to respective mean values of 0, 2, 29, 359 and 739 mg/kg/day (males) and 0, 3, 35, 415 and 868 mg/kg/day (females)] for two years. Ten rats/sex/group were sacrificed at 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 RBC cholinesterase inhibition in females.

B. Discussion of Tumor Data

(i) Liver Tumors

There were no statistically significant increases in hepatocellular carcinomas at any dose level in male rats. As shown in **Table 7,** there were pair-wise significant increases relative to the control group for adenomas and combined liver adenomas/ carcinomas at 6000 ppm ($p < 0.05$) and 12,000 ppm ($p < 0.01$) in female rats. There were also significant increasing trends for adenomas and combined adenoma/carcinoma ($p < 0.01$).

The incidences of liver tumors in this study (censored data) were compared to the historical control data (non-censored) from studies conducted at testing laboratory. The incidences of adenomas at 6000 ppm (8%) and 12,000 ppm (10%) exceeded the historical control range (0 to 5%) and mean (1.6%). The incidences of carcinomas at 12,000 ppm (8%) also exceeded the historical control range (0 to 2.4%) and mean (1.1%). In addition, the incidences of these two tumor types exceeded the historical control incidences of the National Toxicology Program [adenomas, 4/901 (0.44%) and carcinomas, 0/901 (0%)].

The Committee concluded that the incidence of liver tumors at the 50 and 500 ppm dose levels provide suggestive evidence of carcinogenicity and cannot be discounted. The Committee also concluded that the liver tumor incidences at 6000 ppm and at 12,000 ppm (although considered to be excessive doses) provide positive evidence of carcinogenicity.

This conclusion was based on: (1) absence of liver tumors in the concurrent controls; (2) the positive increasing trends for adenomas as well as combined adenomas/ carcinomas; (3) pair-wise significance (6000 ppm and 12,000 ppm); and (4) the incidences of adenomas exceeded the mean incidences (at 50 and 500 ppm) and range (at 6000 ppm and 12,000 ppm) of the historical control data from both the testing laboratory and the NTP.

Table 7. Female Rat: Liver Tumor Rates[+] and Peto's Prevalence Test Results.

Tumor Type	0 ppm	100/50 ppm	500 ppm	6000 ppm	12000 ppm
Adenomas	0/40	1[a] /48	1/43	**3/39**	**3/29**
%	0	2	2	**8**	**10**
p=	**0.007****	0.240	0.168	**0.032***	**0.008****
Carcinomas	0/41	1/50	1/44	0/41	3[b] /38
%	0	2	2	0	8
p=	0.063	0.168	0.168	-	0.085
Combined	0/41	2/50	2/44	**3/41**	**6/38**
%	0	4	5	**7**	**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 (Statistical Analysis, Brunsman, July 16, 1997).

[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 comparisons with control denoted at dose level.
If *, then p <0.05; If **, then p <0.01

(ii). Nasal / Palate Tumors (Rat)

At the September 24, 1997 meeting, the Committee determined that nasal tissues had not been fully evaluated histopathologically in the original submission. Therefore, the Committee elected to require the histopathologic examination and peer review of microscopic slides of nasal tissues among rats of both sexes. The nasal/oral tissue sections taken from five nasal regions from all rats underwent microscopic examination. This was a nasal tissue reevaluation, and oral tissue findings (tumors of the palate and tooth) were incidental in the nasal tissue assessment, which reflects only a partial histopathologic assessment of oral cavity tissues. The nasal/oral tumor incidences are presented in **Table 8**.

Table 8. Neoplastic Findings of the Nasal/Oral Tissues in Rats

TUMOR TYPE	Dose (ppm)				
	0	100/50	500	6000	12000
MALES (No. Examined: 90/dose)					
Nasal Olfactory Epithelium **Adenoma**	0	0	0	**1**	0
Nasal Respiratory Epithelium **Adenoma**	0	0	0	0	**1**
Palate, Squamous Cell papilloma	0	1	0	0	0
FEMALES (No. Examined: 90/dose)					
Nasal Respiratory Epithelium **Adenoma**	0	0	0	**1**	**1**
Tooth, Alveolus, Squamous Cell Carcinoma	0	1	0	0	0
Palate, Squamous Cell Papilloma	0	0	0	1	0
Palate, Squamous Cell Carcinoma	0	0	0	0	1

The Committee compared these nasal tumors to the historical control data from the testing laboratory as well as to the tumors of the "respiratory tract" seen in studies conducted at NTP. When compared to the historical controls of the testing laboratory, the incidences of nasal adenomas in this study (1/90 at 6000 ppm and 1/90 at 12,000 ppm) in both sexes exceeded the historical control incidence (0/240 males and 0/240 females). The NTP reported "respiratory tract" tumors in the respiratory epithelium of 6 of 4000 male rats and in the olfactory epithelium of 0 of 4000 males. Of the 6/4000 of the respiratory epithelium, four of the six were squamous cell tumors. Therefore, the relevant incidence for the tumor type in question is 2/4000 control males.

The Committee concluded that the nasal tumors are treatment related. This conclusion was based on the following factors: (1) spontaneous nasal tumors are very rare and are therefore, biologically significant; (2) there were no nasal tumors in the concurrent controls; (3) the incidences in this study exceeded the zero historical control incidence of the testing laboratory; and (4) incidences exceeded the NTP historical control database. Additionally, the two nasal tumors in females were found in the nasal turbinate section 5, a region in which minimal non-neoplastic lesions were seen.

The Committee postulated that direct contact with malathion (by volatilization from the feed or by inhalation of the feed through the nose) was a plausible explanation for the nasal tumors; however, it was concluded that a systemic effect could not be unequivocally ruled out. The Committee noted that the Hazard Identification Assessment Review Committee (HIARC) determined that a new subchronic inhalation toxicity study in rats is required based on the results of the two-week range-finding study (MRID No. 44554301) and the lack of a NOAEL for cholinesterase inhibition and non-neoplastic lesions in the 90-day study (MRID No.43266601) (HIARC Report dated 12/22/98; HED Document No. 013032).

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Palate tumors were observed at 100/50 ppm (a squamous cell papilloma in 1/90 males), at 6000 ppm (a squamous cell papilloma in 1/90 females) and at 12,000 ppm (a squamous cell carcinoma in 1/90 females). These tumors were not attributed to malathion treatment due to lack of statistical significance, and absence of a dose-response in either sex.

(iii). Thyroid Follicular Cell Tumors (Rat)

Thyroid gland follicular cell tumors in male rats are presented in **Table 9.**

Table 9. Male Rat: Thyroid Follicular Cell Tumor Rates[+] and Peto's Prevalence Test Results.

Tumor Type	0 ppm	100/50 ppm	500 ppm	6000 ppm	12000 ppm
Adenomas	2/55	1/54	1/51	4/51	4[a]/43
(%)	4	2	2	8	9
p=	0.063	-	-	0.150	0.378
Carcinomas	0/42	0/45	2/41	2[b] /26	0/0
(%)	0	0	5	8	0
p=	0.196	-	0.085	0.162	-
Combined	2/55	1/54	3/51	6/51	4/43
(%)	4	2	6	12	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 (Statistical Analysis, Brunsman, July 16, 1997).

[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 of the interim sacrifice or accidental death animals.
Significance of trend denoted at control.
Significance of pair-wise comparisons with control denoted at dose level.
If *, then $p < 0.05$; If **, then $p < 0.01$

The Committee concluded that the thyroid follicular cell tumors are NOT treatment-related since there is neither a pair-wise significance nor a dose-response relationship for any tumor type (i.e., adenomas, carcinomas or combined adenomas/ carcinomas); only a trend was seen for the combined tumors.

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(iv). Thyroid C-Cell Tumors (Rat)

At the October 8, 1997 meeting, the Committee requested additional statistical analysis (Peto' Prevalence Test) of the thyroid C-cell tumor incidences in male rats as well as historical control data from the testing laboratory. Tumor incidences and results of the statistical analysis are presented in **Table 10a** (for all dose groups) and in **Table 10b** (excluding the top two doses).

Table 10a. Male Rat: Thyroid C-Cell Tumor Rates[+] and Peto's Prevalence Test Results Including All Dose Groups

Tumor Type	0 ppm	100/50 ppm	500 ppm	6000 ppm	12000 ppm
Adenomas p=	13/53 (25%) 0.326	14/54 (26%) 0.461	10/50 (20%) -	6/50 (12%) -	4[a]/35 (11%) 0.242
Carcinomas p=	1/51 (2%) 0.556	2/50 (4%) 0.310	6[b]/45(13%) **0.012***	2/43 (5%) 0.178	0/9 (0%) -
Combined p=	14/53 (25%) 0.430	16/54 (30%) 0.389	14[c]/50(28%) 0.403	8/50 (16%) -	4/35 (11%) 0.242

[+] Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor (Statistical Analysis, Brunsman, May 03, 1999).

[a] First thyroid c-cell adenoma observed at week 81, dose 12,000 ppm. [b] First thyroid c-cell carcinoma observed at week 90, dose 500 ppm. [c] Two animals in the 500 ppm had both an adenoma and a carcinoma.

Table 10b. Male Rat: Thyroid C-Cell Tumor Rates[+] and Peto's Prevalence Test Results Excluding Top Two Dose (6000 & 12,000 ppm) Groups

Tumor Type	0 ppm	100/50 ppm	500 ppm
Adenomas p=	13[a]/46 (28%) 0.737	14/47 (30%) 0.461	10/44 (23%) -
Carcinomas p=	1/51 (2%) **0.006****	2/50 (4%) 0.310	6[b]/45 (13%) **0.013***
Combined p=	14/51 (27%) 0.356	16/50 (32%) 0.394	14[c]/50 (31%) 0.332

[+] Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor (Statistical Analysis, Brunsman, May 03, 1999).
[a] First thyroid c-cell adenoma observed at week 97, dose 0 ppm. [b] First thyroid c-cell carcinoma observed at week 90, dose 500 ppm. [c] Two animals in the 500 ppm had both an adenoma and a carcinoma.
Note: Significance of trend denoted at control.
Significance of pair-wise comparisons with control denoted at dose level.
If *, then $p < 0.05$; If **, then $p < 0.01$

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The Committee noted when all dose groups were included (**Table 10a**), male rats had a statistically significant (p=0.012) difference in the pair-wise comparison of the 500 ppm dose group with the controls for thyroid C-cell carcinomas. There were no other statistically significant differences in the pair-wise comparisons of the dosed groups with the controls, or any significant trends for C-cell adenomas, carcinomas or combined adenomas/carcinomas. Additionally, there was no dose-related increase for any tumor type.

The Committee also observed that when the top two doses (6000 ppm and 12,000 ppm) were excluded (**Table 10b**) from the analysis there was a dose-related increase (2%, 4% and 13% at 0 ppm, 50 ppm and 500 ppm, respectively), a pair-wise significance (p=0.013) at 500 ppm, and the incidences at both doses exceeded the mean historical control incidence (6/239; 2.5%) for carcinomas in male rats. However, the pathologist stated that thyroid C-cell adenomas and carcinomas are difficult to differentiate. In addition, there were no other statistically significant differences in the pair-wise comparisons of the dosed groups with the controls nor any significant trends for C-cell adenomas or combined adenomas/carcinomas. The reviewers postulated that the excessive mortality in males at the top doses (74% at 6000 ppm and 100% at 12,000 ppm) may have compromised the expression of this tumor at these (higher) doses. However, the Committee noted that although excessive mortality was also seen in female rats at the top doses (64% at 12,000 ppm) liver tumors were seen at this dose.

The Committee concluded that the thyroid C-cell tumors are NOT attributable to treatment based on the combined tumor (adenomas/carcinomas) incidences. The combined tumors were determined to be the most appropriate tumor type for evaluation due to the difficulty in distinguishing the individual tumor types (i.e., adenomas and carcinomas). For the combined tumors, there was no statistically significant trend, pair-wise significance, or dose-response at any dose level when all dose groups were included or when the top two doses were excluded from the analyses. Additionally, there was no evidence of malathion induced thyroid toxicity in the database and there were no supportive pre (non) neoplastic lesions in the thyroid glands of male or female rats.

(v). <u>Pituitary Tumors</u> (Rat)

At the October 15, 1997 meeting, the Committee noted that not all female pituitary glands had been examined microscopically, therefore, histopathologic examination and peer review of microscopic slides of pituitary glands from all female rats was required. The pituitary gland tumors (original results) observed in female rats are presented in **Table 11.**

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Table 11. Female Rat: Pituitary Pars Distalis Tumor Rates[+] and Peto's Prevalence Test Results (Original Study Report).

Tumor Type	0 ppm	100/50 ppm	500 ppm	6000 ppm	12000 ppm
Adenomas	25/51	13/31	20[a]/34	17/33	14/53
(%)	49	42	59	52	26
p=	0.980	-	0.133	0.266	-
Carcinomas	0/50	1/30	3[b]/32	4/32	1/49
(%)	0	3	9	12	2
p=	0.778	0.319	**0.029***	**0.027***	0.369
Combined	25/51	14/31	23/34	21/33	15/53
(%)	49	45	68	64	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 (Statistical Analysis, Brunsman, July 16, 1997).

[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 and accidental death animals are not included in this analysis. There were no pituitary pars distalis tumors in any of the interim sacrifice animals.
Significance of trend denoted at control.
Significance of pair-wise comparisons with control denoted at dose level.
If *, then $p < 0.05$; If **, then $p < 0.01$

The histopathology examination of pituitary glands from all females (re-read) were completed and the results are presented in **Table 12.**

Table 12. Female Rat: Histopathology of the Pituitary Glands from ALL Animals (Re-Read).

Tumor Type	0 ppm		100/50 ppm		500 ppm		6000 ppm		12000 ppm	
No. Examined	88	87	90	90	87	89	87	88	88	89
Sex	M	F	M	F	M	F	M	F	M	F
Adenomas	20	25	23	23	16	27	17	18	5	17
Carcinomas	0	0	0	1	0	2	0	1	0	1

The Committee concluded that the pituitary tumors are NOT attributable to treatment since the incidences and types of tumors (adenoma and carcinoma) observed in the treated groups were comparable to those seen in the control group and since there was neither statistical significance nor dose-response for either pituitary tumor type.

(vi). Uterine Tumors

At the October 15, 1997 meeting, the Committee noted the presence of some rare/unusual uterine tumors which are shown in **Table 13.** Individually, the incidences of the uterine tumors were low. However, collectively the incidences of the uterine tumors were of a concern to the Committee. It was also noted that not all animals at the low, mid and mid-high doses were examined. Therefore, the Committee requested histopathologic examination and peer review of microscopic slides of the uteri from all females. The re-read histopathology of the uterine tumors from all animals is presented in **Table 14.**

Table 13. Female Rats Incidence of Uterine Tumors (Original Pathology Report)

Tumor Type	0 ppm	100/50 ppm	500 ppm	6000 ppm	12000 ppm
No. Examined	70	26	24	31	70
Deciduoma	0	1	0	0	0
Hemangioma	0	0	0	0	1
Endometrial Carcinoma	1	2	0	0	2
Endometrial, Carcinosarcoma	0	0	0	0	1
Stromal Sarcoma	0	1	0	0	0
Fibrosarcoma	0	1	0	0	0
Leiomyosarcoma	0	0	0	1	0

Table 14. Female Rat: Incidence of Uterine Tumors (Re-Read of ALL Animals)

Tumor Type	0 ppm	100/50 ppm	500 ppm	6000 ppm	12000 ppm
No. Examined	90	90	90	90	90
Deciduoma	0	0	0	0	0
Hemangioma	0	0	0	0	1
Endometrial Adenoma	0	0	1	0	0
Endometrial Carcinoma	1	2	1	0	2
Endometrial Stromal Polyp	20	15	17	11	11
Fibrosarcoma	0	1	0	0	0
Fibroma	0	0	0	1	0
Leiomyoma	0	0	0	1	0
Leiomyosarcoma	0	0	0	1	0
Stromal Sarcoma	0	1	0	0	0

The Committee concluded that the uterine tumors are NOT treatment related since the incidences and types of tumors observed in the treated groups were comparable to those seen in the control animals and since there was neither statistical significance nor dose-response for any tumor type.

(vii). Testicular Tumors (Rat)

At the October 8, 1997 meeting the Committee evaluated the interstitial cell tumors of the testes presented in **Table 15.**

Table 15. Male Rat: Testes Interstitial Cell Tumor Rates[+] and Peto's Prevalence Test Results (p values)

Tumor Type	0 ppm	100/ 50 ppm	500 ppm	6000 ppm	12000 ppm
Interstitial cell tumor	52/55	52/55	53/55	52/53	53[a]/54
(%)	95	95	96	98	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 (Statistical Analysis, Brunsman, July, 16, 1997).

[a] First testicular tumor observed at week 54, dose 0 ppm, in a 54-week interim sacrifice animal. First testicular tumor not in an interim sacrifice or accidental death animal observed at wee 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 this tumor. Two accidental death animals in the 6000 ppm dose group had this tumor.

Significance of trend denoted at control.
Significance of pair-wise comparisons with control denoted at dose level.
If *, then $p < 0.05$; If **, then $p < 0.01$

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 the interstitial cell tumor, both at $p < 0.01$. There were also significant differences in the pair-wise comparisons of the 500 ppm and 6000 ppm dose groups with the controls for this tumor type, both at $p < 0.05$. Statistical analyses of this tumor in the study report concluded that increases in testicular tumors were statistically significant at all dose levels. Statistical analysis by HED obtained essentially the same results, except for the low dose group which did not show pair-wise significance.

The Committee concluded that the testicular tumors are NOT treatment related since: 1) this non-lethal tumor was observed in nearly 100% of male rats including controls; 2) the apparent statistical significance of the tumor incidence at 6000 and 12000 ppm groups is probably due to the high mortality at these doses [*Note:* both doses were determined to be excessive in males] and was considered to be an artifact of the Peto's Prevalence Analyses protocol; (3) sufficient data are not available to determine if there was a decrease in the latency period [i.e.,there was no serial sacrifice to determine latency]; and (4) this tumor type is not useful in overall evaluation since its occurrence is similar at all dose levels.

(viii). Mononuclear Cell Leukemia (Rat)

At the October 15, 1997 meeting the Committee evaluated the mononuclear cell leukemia and concluded that the occurrence of this tumor type in female rats is not attributable to treatment due to lack of statistical significance at any dose level and the incidences were within in the historical control range of the testing laboratory (15 to 36%).

At the February 24, 1999 meeting, the Committee, determined that additional statistical analysis using Peto's prevalence test will be needed for this tumor type in male rats. Results of this analysis presented below in **Table 16** were evaluated at the June 23, 1999 Committee meeting.

Table 16. Mononuclear Cell Tumor Rates[+] and Peto's Prevalence Test Results.

Tumor Type	0 ppm	100/ 50 ppm	500 ppm	6000 ppm	12000 ppm
Male	23/55	16/55	24/55	17/53	1[a]/52
(%)	42	29	44	32	2
p=	-	-	0.463	-	-
Female	9/55	**18/55**	15/55	13/54	10[b]/55
(%)	16	**33**	27	24	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 (Statistical Analysis, Brunsman, July 16, 1997 & May 03, 1999).

[a] First mononuclear cell leukemia observed in a males at week 64, dose 12,000 ppm.
[b] First mononuclear cell leukemia observed in a female at week 47, dose 12,000 ppm

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

The Committee concluded that the mononuclear cell tumors in male and female rats are NOT treatment related based on the lack of statistical significance at any dose level, absence of a dose-response relationship, and the incidences were within the historical control range of the testing laboratory (15 to 36%). Additionally, mononuclear cell tumors were not seen in three strains of rats: the Osborne-Mendel (1978 NCI-malathion); Sprague-Dawley (1980-FDRL-malathion); and F344 (1979, NCI-malaoxon and the 1996 malaoxon studies).

C. Non-Neoplastic Lesions

The nasal/oral tissue sections taken from five nasal regions from all rats were histopathologically examined. **Table 17** on Page 22 presents the treatment-related non-neoplastic lesions of the nasal mucosa were seen in both sexes at all dose levels including the controls. In both sexes, lesions of the olfactory/respiratory mucosa were more severe at 500, 6000 and 12,000 ppm dose groups. Most of the non-neoplastic lesions did not occur in section 5, the section where the nasal tumors in females occurred.

D. Adequacy of the Dosing for Assessment of Carcinogenicity

Mortality was increased in males at 6000 ppm and in both sexes at 12,000 ppm. Statistical analyses of mortality data are presented in **Tables 18 and 19** for males and females, respectively on Page 23. There was a significant increasing trend with increasing doses of malathion in males and females. Males at 12,000 ppm had 100% mortality at week 97. Decrements in body weight gain were 13% in males and 4% in females at 6000 ppm and 32% in males and 15% in females at 12,000 ppm. Both sexes of rats at 6000 and 12,000 ppm exhibited anemia. The statistically significant ($p < 0.01$) cholinesterase inhibition in plasma, red blood cell and brain observed at 6000 and 12,000 ppm are summarized below in **Table 20:**

Table 20 Cholinesterase Activity in Rats Fed Malathion for 24 Months

Percent Cholinesterase Inhibition in Rats At 24 months						
Dose	**500 ppm**		**6000 ppm**		**12000 ppm**	
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Plasma	29**	18	64**	61**	Dead	89**
RBC	17	27**	43**	44**	Dead	52**
Brain	3	1	21**	18**	Dead	67**

The Committee further evaluated the acute and subchronic studies. Data from these studies showed that the cholinesterase inhibition seen at 6000 ppm in the chronic study was not supported by the cholinesterase inhibition observed at a comparable dose (5000 ppm) in the 90-day study or at 2000 ppm in the acute study. The Committee concluded that the 12,000 ppm dose in both sexes (due to severe cholinesterase inhibition) and the 6000 ppm dose in males (due to mortality) were excessive. Thus, it was determined that the 500 ppm dose in males and 6000 ppm dose in females were adequate to assess the carcinogenic potential of malathion.

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Table 17. Non-neoplastic Lesions of the Nasal Mucosa in Male and Female Rats [a]

Type of Lesion	No. of Sections Examined/ Animal	0 ppm	100/50 ppm	500 ppm	6000 ppm	12,000 ppm
MALES						
Olfactory/Respiratory Mucosa: subacute (chronic active)/ chronic inflammation	3	3/8	2/14	32/23	113/63	55/27
Olfactory Epithelium, Degeneration/Atrophy	3	4	3	14	197	150
FEMALES						
Olfactory Mucosa Congestion Respiratory Mucosa Congestion	5	10 25	11 27	16 46	25 40	50 70
Olfactory Edema Respiratory Edema	4	0 0	1 4	3 9	67 28	45 38
Squamous/Squamoid Metaplasia, Focal Multi-Focal	 3 2	 0 1	 4 4	 0 7	 3 8	 0 0
Olfactory/Respiratory Mucosa: subacute (chronic active) /chronic inflammation), Multi-Focal	4	0/5	0/28	2/7	2/11	4/6
Nasal Mucosa: Olfactory Epithelium, Degeneration/Atrophy	3	0	6	5	239	150
Nasal Mucosa: Olfactory Epithelium, Degeneration / Atrophy, Multi-Focal	3	2	1	1	26	103
Paranasal Sinus(es): Maxillary Gland-Atrophy	2	1	0	19	45	13
Nasal Mucosa (Vestibular), Congestion	1	8	12	18	16	33
Nasal Mucosa (Vestibular), Squamous Cell Hyperplasia	1	1	0	1	7	2
Nasal Mucosa (Vestibular), Squamous Cell Hyperplasia, Focal	1	1	2	1	1	1
Nasal Mucosa (Vestibular), Squamous Cell Hyperplasia, Multi-Focal	1	1	8	5	10	0

a =Incidences are the total of the lesions seen in all sections of the nasal mucosa. Essentially 90 animals/group were examined for all tissues except for section 5 where 78-81/group (males) and 78-85/group (females) were examined. Most of the non-neoplastic lesions did not occur in section 5, the section where the nasal tumors in females occurred.

Table 18. Male Rat Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Study Weeks						
	1-26	27-53	54 [i]	54-78	79-106[f]	Total	Percentage
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)****
12,000	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 included in this analysis.
[f] Final sacrifice at week 105
[a] Two accidental deaths at weeks 105, dose 6000 ppm

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

Dose (ppm)	Study Weeks						
	1-26	27-53	54[i]	54-78	79-106[f]	Total	Percentage
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)
12,000	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 included in this analysis.
[f] Final sacrifice at week 105
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 $p < 0.01$.

3. Combined Chronic Toxicity/Carcinogenicity Study with Malaoxon in Fischer 344 Rats

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

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

As shown in **Table 21,** there was a statistically significant ($p < 0.05$) increase in mononuclear cell leukemia in male rats at the highest dose tested (2000 ppm). There was also a statistically significant trend ($p < 0.05$) for these tumors.

Table 21. Mononuclear Cell Leukemia in Rats Fed Malaoxon for 24 Months.

Sex	0 ppm	20 ppm	1000 ppm	2000 ppm
Males	13/55 (24%) **p=0.03***	12/55 (22%)	19/55 (35% p =0.07	16/55 (29%) **p =0.05***
Females	8/55 (15%)	9/55 (16%)	10/55 (18%)	5/55 (9%)

The Committee concluded that the mononuclear cell leukemia is NOT treatment related since the increase was seen only in males at a dose that was determined to be an excessive dose, there was no dose-response, and the incidences were within the historical control range (15 to 36%) of the testing laboratory. Additionally, mononuclear cell tumors were not seen in three strains of rats: the Osborne-Mendel (1978, NCI-malathion); Sprague-Dawley (198, FDRL-malathion); and F344 (1979, NCI-malaoxon) studies.

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.

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D. Adequacy of Dosing for Assessment of Carcinogenicity

Mortality was significantly ($p<0.01$) increased at the high dose (2000 ppm) in males (53%) and females (49%) compared to controls (males, 29% and females, 13%). There was severe inhibition of cholinesterase activity for all three compartments (plasma, RBC and brain) in both sexes at 1000 and 2000 ppm at various time points during treatment compared to controls. At 1000 ppm, cholinesterase inhibition was: plasma, 74 to 81% in males and 82 to 87% in females; RBC, 54 to 66% in males and 45 to 62% in females; and brain, 2 to 30% in males and 5 to 14% in females. At 2000 ppm cholinesterase inhibition was: plasma 83 to 91% in males and 90 to 96% in females; RBC, 56 to 65% in males and 54 to 66% in females; and brain, 11 to 74% in males and 61 to 78% in females. Based on the increased mortality and severe cholinesterase inhibition in all three compartments, the Committee concluded that 2000 ppm was excessive and 1000 ppm was an adequate dose.

IV. MUTAGENICITY

Three acceptable studies [*Salmonella typhimurium/Escherchia coli* reverse gene mutation assay, *in vivo* bone marrow cytogenetic assay in rats, and an unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes were available for review. Findings from the submitted guideline studies indicate that malathion did not cause gene mutations in bacteria or UDS in cultured rat hepatocytes. Similarly, malathion was neither clastogenic nor aneugenic up to doses that showed clear cytotoxicity for the target tissue *in vivo*. By contrast, studies from the open literature indicated that malathion is a confirmed clastogen both *in vitro* and *in vivo*. However, there are uncertainties regarding the relevance of these findings to a mutagenic mode of action for malathion since the positive results both *in vivo* and *in vitro* were seen only at cytotoxic doses and the types of induced aberrations were asymmetric (i.e., chromatid and chromosome breaks and exchanges) which were not stable. Nevertheless, malathion was shown to be weakly reactive with DNA and does contain a structure that suggests electrophilicity. The Committee concluded, therefore , that while the evidence for mutagenicity as an influence on the carcinogenicity is weak, it can not be ruled out. Summarized findings supporting the above conclusions are presented below:

A. Gene Mutation

In a *Salmonella typhimurium/Escherichia coli* reverse gene mutation assay, Malathion (94.5%) was non-mutagenic when tested at concentrations up to 5000 µg/plate (highest dose) with or without metabolic (S9) activation (MRID No. 40939302).

B. Chromosome Aberrations

In an *in vivo* bone marrow cytogenetic assay, malathion (94%) was negative following oral doses at 500-2000 mg/kg to male and female Sprague-Dawley rats. A dose-related reduction in mitotic indices (MIs) was seen in the females of all treatment levels at 24 hours. Reduced MIs were also recorded for high-dose males and females at 48 hours (MRID No. 41451201).

3

C. Other Mutagenic Effects

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

D. Other Information

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.

The overall assessment from this review indicated that there is overwhelming confirmation from the published literature demonstrating 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 (Peer Review of Malathion, April, 12, 1990; HED Document No. 008386).

It should be noted however, that while 5 of the 7 *in vivo* bone marrow studies were reported positive, evidence of structural chromosome damage was either accompanied by cytotoxic effects (i.e., significantly reduced mitotic indices or increased cell cycle delay) or asymmetrical structural aberrations (i.e., chromatid and chromosome breaks and exchanges). A similar observation regarding cytotoxicity and the induction of unstable aberrations, which generally lead to death and hence do not directly contribute to carcinogenesis, can also be made for the 5/6 positive *in vitro* cytogenetic assays. Nevertheless, the review prepared by Flessel *et al.*, also indicated evidence of malathion's *in vitro* interaction with DNA. Weak but positive results were shown for sister chromatid exchange (SCE) induction and methylation and denaturation of DNA. Moreover, the methyl ester moiety of malathion is listed by Ashby and Tennant (1991) as a structural alert to DNA reactivity. No assays with germinal cells have been submitted to the Agency. However, malathion was negative in *Drosophila melanogaster* sex linked recessive lethal assay, mouse dominant lethal assays and spermatogonia/orspermatocyte cytogenetic assays. A questionable clastogenic response was reported in mouse spermatocytes following subacute exposure to commercial grade malathion (Salvadori *et al.*, 1988). Nevertheless, the data from developmental and reproduction studies, as well as epidemiological surveys of pregnant women exposed to malathion (Arevalo *et al.*, 1987; Spielman, 1986; Grether *et al.*, 1987), do not suggest an adverse heritable effect.

No mutagenicity studies have been submitted to OPP on malaoxon. The consensus opinion from the above cited reviews of the open literature is that malaoxon is not mutagenic in bacteria but is a confirmed positive without S9 activation in the mouse lymphoma forward gene mutation assay. 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. Non-activated malaoxon also caused SCEs in independently performed investigations with CHO cells. Malaoxon has the same structural alert that was identified by Ashby and Tennant for malathion.

E. Conclusions:

Results of the guideline genetic toxicology studies with malathion indicate that the test material did not cause gene mutations in bacteria or UDS in cultured rat hepatocytes. Similarly, malathion was neither clastogenic nor aneugenic up to doses that showed clear cytotoxicity for the target tissue *in vivo*. By contrast, studies from the open literature indicated that malathion is a confirmed clastogen both *in vitro* and *in vivo*. However, there are uncertainties regarding the relevance of these findings to a mutagenic mode of action for malathion since positive results from both *in vivo* and *in vitro* studies were seen at cytotoxic doses and/or the types of induced aberrations were asymmetric and therefore, not consistent with cell survival. Nevertheless, malathion was shown to be weakly reactive with DNA and does contain a structure that suggests electrophilicity. The Committee concluded therefore, that while the evidence for mutagenicity as an influence on the carcinogenicity of malathion is weak, at this time it can not be ruled out.

V. STRUCTURE-ACTIVITY RELATIONSHIP

Both malathion and malaoxon should be considered structural analogs of each other.

VI. TOXICOLOGY

A. Acute Toxicity

In acute toxicity studies conducted in rats, malathion exhibits low acute toxicity potential via the oral (LD50 = 5500 mg/kg), dermal (LD50 >2,000 mg/kg) and the inhalation (LC50 of >5.2 mg/L) routes.

In an acute neurotoxicity study, groups of Sprague-Dawley rats (27/sex/dose) received a single oral administration of malathion (96.4%) in corn oil at doses of 0, 500, 1000, or 2000 mg/kg. For neurotoxicity, the NOAEL was 1000 mg/kg and the LOAEL was 2000 mg/kg/day based on decreased motor activity at peak effect time (day 1) and clinical signs (salivation, body staining, tremors in one animal, labored breathing, stained fur, decreased defecation and urination). Plasma and RBC cholinesterase were inhibited in both sexes at 2000 mg/kg. Also, there was an equivocal inhibition of plasma cholinesterase for females at 500 and 1000 mg/kg, characterized by a poor dose response. No inhibition of brain cholinesterase activity was seen in either sex at any dose level. Equivocal neuropathological findings at 2000 mg/kg included axonal degeneration in the lumbar root and bilateral retinal rosette in one male, digestion chambers in the lumbar dorsal root fibers in one male and in the sciatic and tibial nerve in another male rat. Digestion chambers and axonal degeneration of the sciatic nerve were also seen in one male control rat (MRID No. 43146701).

B. Subchronic Toxicity

In a subchronic neurotoxicity study, groups of Sprague-Dawley rats (25/sex/dose) were fed diets containing Malathion (96.4%) at 0, 50, 5000 or 20,000 ppm (0, 4, 352, or 1486 mg/kg/day in males and 0, 4, 395, or 1575 mg/kg/day in females, respectively). For systemic toxicity, the NOAEL was 5000 ppm (352/395 mg/kg/day for M/F) and the LOAEL was 20,000 ppm (1486/1575 mg/kg/day in M/F) based on decreased body weight and food consumption and on increased clinical signs (anogenital staining, and dried red material around the nose). For cholinesterase inhibition, the overall NOAEL was 50 ppm (4 mg/kg/day) and the LOAEL was 5000 ppm (352/395 mg/kg/day in M/F) based on inhibition of plasma and red blood cell cholinesterase in males and females and on brain cholinesterase in females. There were no treatment-related effects on brain weight or neuropathology (MRID No. 43269501).

In a subchronic inhalation study, groups of Sprague-Dawley rats (15/sex/concentration) were exposed in whole body inhalation chambers to malathion (96.4%) at aerosol (analytical) concentrations of 0.1, 0.45, or 2.01 mg/L for 6 hours/day, 5 days/week for 13 weeks. Treatment had no effects on survival, body weights or food consumption. Cholinergic signs observed at 2.01 mg/L and sporadically in a few animals at the lower doses included red staining of the urogenital areas, excess salivation and ungroomed oily fur. Treatment-related histopathological lesions were seen in the nasal cavity and the larynx of both sexes of rats at all concentrations tested. The lesions in the nasal cavity were characterized as slight to moderate degeneration and/or hyperplasia of the olfactory epithelium which was locally extensive. The lesions of the larynx were characterized as epithelial hyperplasia, with squamous keratinization occurring in some rats. In addition, the olfactory/respiratory epithelial junction was severely affected in most animals. For systemic toxicity, a NOAEL was not established and the LOAEL was 0.1 mg/kg/day based on histopathologic lesions of the nasal cavity and larynx. Inhibition of plasma and red blood cell cholinesterase activity was seen in females rats at all concentrations. In male rats, inhibition of cholinesterase activity was observed in plasma at 2.01 mg/L and in red blood cells at $\geq$ 0.45 mg/L. Inhibition of brain cholinesterase activity was seen only at the highest concentration. A NOAEL was not established for plasma and RBC cholinesterase inhibition; the LOAEL was 0.1 mg/L. For inhibition of brain cholinesterase, the NOAEL was 0.45 mg/L and the LOAEL was 2.01 mg/L (MRID No. 43266601). The HIARC has requested for another subchronic inhalation toxicity study due to the lack of a NOAEL for cholinesterase inhibition as well as non-neoplastic lesions in this study (See HIARC Report dated 12/28/98). The HIARC has requested another subchronic inhalation toxicity study.

C. Chronic Toxicity

In a combined chronic toxicity/carcinogenicity study in rats (discussed earlier), mortality was increased in males at 6000 ppm and in both sexes at 12,000 ppm. There was a significant increasing trend with increasing doses of malathion in males and females. Male

rats at 12,000 ppm had 100% mortality at week 97. Decrements in body weight gain were 13% in males and 4% in females at 6000 ppm and 4% in males and 15% in females at 12,000 ppm. Both sexes of rats at 6000 and 12,000 ppm exhibited anemia. Significant inhibition of plasma, RBC and brain cholinesterase activity was seen in both sexes at 6000 and 12,000 ppm. Based on the re-assessment of the nasal tissues, for males, the NOAEL was 100/50 ppm and the LOAEL was 500 ppm based on non-neoplastic lesions of the nasal mucosa; a NOAEL was not identified for females (MRID No. 43942901; 44782301).

In a carcinogenicity study in B6C3F1 mice (discussed earlier), mortality rates, clinical signs of toxicity and hematological parameters were not affected by treatment with malathion at any dose. 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 NOAEL for plasma and RBC cholinesterase inhibition was 100 ppm, and that for brain cholinesterase inhibition was 800 ppm for both sexes (MRID No. 43407201).

D. Metabolism

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 RBCs 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 (MRID No. 41367701).

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 (MRID No. 41367701).

VII. COMMITTEE ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

The Committee's assessment of the weight-of-the-evidence is presented below:

1. Carcinogenicity

Evidence for carcinogenicity was demonstrated by the presence of liver tumors in male and female B6C3F1 mice as well as the liver and nasal tumors in female Fischer 344 rats.

A. Liver Tumors

In **male mice** (based on the PWG re-read), there was a positive trend (p=0.000) for **liver** adenomas and the combined tumors (adenomas/carcinomas). The incidence of adenomas was significantly increased at 8000 ppm (14/55, 25%, p = 0.0103) and 16,000 ppm (49/51, 96%, p = 0.000) when compared to controls (4/54, 7%). Similarly, the combined tumors (adenomas/carcinomas) showed pair-wise significance at 8000 ppm (15/55, 27%, p=0.006) and 16000 ppm (49/51, 96%, p=0.000) when compared to controls (4/54, 7%). Although carcinomas were seen at 100 ppm, 800 ppm and 8000 ppm compared to none in the controls, none of the incidences exhibited statistical significance nor was there a dose-related increase at any dose level.

When compared with the historical control ranges: the incidences of adenomas at the 8000 ppm (25%) and 16,000 ppm (96%) doses exceeded the historical control range (14 to 22%). The incidences of carcinomas at 800 ppm (5%) and 8000 ppm (4%) were within the historical control range (0 to 6.4%). No carcinomas were seen at 16,000 ppm while the incidence of carcinomas at 100 ppm (7%) was slightly outside the historical control range.

In **female mice**, there was a positive trend (p=0.000) for **liver** adenomas and the combined tumors (adenomas/carcinomas). The incidence of adenomas was significantly increased at 8000 ppm (9/52, 17%, p = 0.001) and 16,000 ppm (42/51, 82%, p = 0.000) when compared to controls (0/55). Similarly, the combined tumors (adenomas/carcinomas) showed pair-wise significance at 8000 ppm (10/52, 19%, p=0.003) and 16000 ppm (43/51, 84%, p=0.000) when compared to controls (1/55, 2%). No statistically significant increases in carcinomas alone were seen at any dose level.

The Committee concluded that there is evidence of carcinogenicity in both sexes of mice at the two highest dose levels tested. There is no evidence of carcinogenicity in male or female mice at the lower doses.

In **female rats**, increased incidences of **liver** adenomas and combined adenomas/ carcinomas were seen at all dose levels tested. However, statistical significance was seen only at the two highest dose levels tested. There was a positive trend (p=0.007) for adenomas and the combined adenomas/carcinomas (p=0.002).

The incidence of adenomas was significantly increased at 6000 ppm (3/39, 8%, p = 0.032) and 12,000 ppm (3/29, 10%, p = 0.008) when compared to controls (0/40). A dose-related increase in the incidences of combined tumor (adenomas/carcinomas) were seen, reaching statistical significance only at the two highest dose levels. The combined tumors (adenomas/carcinomas) were: 2/50 (4%) at 50 ppm; 2/44 (5%) at 500 ppm; 3/41, 7%, p=0.032 at 6000 ppm; 6/38, 16%, p=0.003 at 12,000 ppm compared 0/41 in the controls. No statistically significant increases in carcinomas alone were seen at any dose level.

When compared to the historical control data of the testing laboratory, the incidences of adenomas at 6000 ppm (8%) and 12,000 ppm (10%) doses exceeded the historical control range (0 to 5%) and mean (1.6%). The incidences of carcinomas at 12,000 ppm (8%) also exceeded the historical control range (0 to 2.4%) and mean (1.1%). In addition, the incidences of these two tumor types exceeded the historical control incidences of the National Toxicology Program (adenomas, 0.44% and carcinomas, 0%).

The increase in liver tumors in female rats, when considered with the increase in liver tumors in both sexes of mice at 8000 and 16,000, somewhat increased the Committee's concern for the carcinogenic effects of malathion. However, this concern was lessened since these tumors occurred in mice only at doses which caused severe plasma (90 to 95%) and red blood cell (92 to 96%) cholinesterase inhibition and marked brain (20 to 43%) cholinesterase inhibition. No statistically significant increases in liver tumors were seen at 800 ppm, which was considered adequate based on the less severe plasma (24 to 36%), RBC (44 to 58%) and brain (3 to 7%) cholinesterase inhibition seen at this dose.

The most compelling evidence of carcinogenicity was shown in the response of liver tumors in female rats at doses which were not considered excessively toxic. Although the strongest response was seen at 12,000 ppm (which was determined to be excessively toxic in the female rat), the increase in liver tumors (mainly adenomas) was also significant at 6,000 ppm (p=0.032). The occurrence of two tumors per dose level in the lower doses of 50 ppm and 500 ppm was considered to be of biological significance and increased the level of the Committee's concern.

The Committee concluded that the incidence of liver tumors in female rats at 50 and 500 ppm provides suggestive evidence of carcinogenicity and cannot be discounted but the liver tumor incidences at 6000 and 12,000 ppm provided positive evidence of carcinogenicity. There was no evidence of liver carcinogenicity in male rats at any dose level but the potential for tumor induction may have been compromised by competing toxicity, particularly at 6000 ppm and 12,000 ppm, where mortality was 74% and 100%, respectively.

B. Nasal Tumors

In **male rats**, there was an **adenoma of the olfactory epithelium** at 6000 ppm and an **adenoma of the respiratory epithelium** at 12,000 ppm compared to none in the controls. In **female rats,** there was an **adenoma of the respiratory epithelium** at 6000 and 12,000 ppm compared to none in the controls.

When compared to the historical control data of the testing laboratory, the incidence of adenomas in this study (1/90 at 6000 ppm and 1/90 at 12,000 ppm) in both sexes exceeded the historical control incidence (0/240 males and 0/240 females). In addition, the NTP reported respiratory tract tumors in the respiratory epithelium of 6 of 4000 male rats and in the olfactory epithelium of 0 of 4000 males. Of the 6/4000 of the respiratory epithelium, four of the six were squamous cell tumors. Therefore, the relevant incidence for the tumor type (adenomas) in question is 2/4000 control males.

Of the four nasal tumors, one in each sex at the two highest doses (6000 and 12,000 ppm), only one tumor at 6000 ppm in the females was at a dose that was not considered excessive. The Committee postulated that direct contact with malathion (by volatilization from the feed or by inhalation of the feed through the nose) was a plausible explanation for the nasal tumors. However, the Committee concluded that a systemic effect could not be unequivocally ruled out.

The Committee attributed the nasal tumors in female rats to treatment because: (1) spontaneous nasal tumors are very rare in rats and therefore the incidences in this study were considered to be biologically significant; (2) an adenoma of the respiratory epithelium was also seen in one male rat at 12,000 ppm; (3) an adenoma of the olfactory epithelium was seen in one male rat at 6000 ppm; (4) there were no nasal tumors in the concurrent controls; and (5) the incidences exceeded the historical control incidence of the testing laboratory.

C. Other Tumors

The Committee concluded that the tumors of the palate (squamous cell papilloma/carcinoma), thyroid gland (follicular cell and C-cell), pituitary gland (par distalis), testes (interstitial cell), and uterus (various types) as well as the mononuclear cell leukemia are not treatment-related because: (1) the individual tumor incidences were low; (2) the tumor incidences and types in treated groups were comparable to those seen in the concurrent control group; (3) there was neither statistical nor biological significance; (4) there was no dose-response relationship; and/or (5) the incidences were within the historical control mean and/or range of the testing laboratory.

2. **Mutagenicity**

Results of the guideline genetic toxicology studies with malathion indicate that the test material did not cause gene mutations in bacteria or UDS in cultured rat hepatocytes. Similarly, malathion was neither clastogenic nor aneugenic up to doses that showed clear cytotoxicity for the target tissue *in vivo*. By contrast, studies from the open literature indicated that malathion is a confirmed clastogen both *in vitro* and *in vivo*. However, there are uncertainties regarding the relevance of these findings to a mutagenic mode of action for malathion since positive results from both *in vivo* and *in vitro* studies were seen at cytotoxic doses and/or the types of induced aberrations were asymmetric and, therefore, not consistent with cell survival. Nevertheless, malathion was shown to be weakly reactive with DNA and does contain a structure that suggests electrophilicity. The Committee concluded, therefore, that while the evidence for mutagenicity as an influence on the carcinogenicity of malathion is weak, at this time, it can not be ruled out.

3. **Structure Activity Relationship**

Malaoxon, the active cholinesterase inhibiting metabolite of malathion, was not carcinogenic in male or female rats when tested at doses that were judged to be adequate to assess its carcinogenic potential. Mutagenicity studies published in the open literature indicate that malaoxon was non-mutagenic in bacteria, was not clastogenic in cultured CHO cells, but did produce positive results without metabolic activation in the mouse lymphoma and caused sister chromatid exchanges in CHO cells in the absence of metabolic activation.

VIII. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA *Proposed Guidelines for Carcinogen Risk Assessment* (April 10, 1996), the Committee classified malathion as a **"likely human carcinogen"** by all routes of exposure. The classification was based on:

(i) the occurrence of liver tumors in male and female B6C3F1 mice and in female Fischer 344 rats; and

(ii) the presence of a few rare tumors of the nasal mucosa in female Fischer 344 rats.

It should be noted that the classification of "likely human carcinogen" is primarily based on the presence of the liver tumors in the female rats at doses which were not considered excessive. However, with the exception of one nasal tumor in female rats, all other tumor types were determined to occur at excessive doses or were unrelated to malathion. The Committee further observed that it is plausible that tumor occurrences in these studies are dose-limited (i.e., tumors are induced only at excessive doses), however, mode of action studies to demonstrate this hypothesis are not available. Consequently, some committee members were of the opinion that malathion should be classified as a "suggestive human carcinogen" and that this classification best described the carcinogenic potential for malathion. The majority opinion, however, was that malathion should be classified as a "likely human carcinogen".

IX. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The Committee recommended a linear low-dose approach (Q_1*) for human risk characterization and extrapolation based on the nasal and the liver tumors in rats at all dose levels tested. The linear dose extrapolation is supported by: (i) the lack of mode of action data and (ii) a plausible genetic component for malathion or malaoxon, the major metabolite formed by oxidation.

The Committee recommended that quantifications of risk be estimated for female rat liver tumors, female rat nasal tumors, and male rat nasal tumors and that the most potent unit risk, Q_1* of these should be used for risk assessments. The following Q_1* s were calculated (Brunsman, July 13, 1999):

Tumor Type	**Q_1*,**
Male nasal	1.06×10^{-3}
Female nasal	4.95×10^{-4}
Female liver	**1.52×10^{-3}**

In this case, the most potent unit risk, Q_1^*, is that for female rat liver adenoma and/or carcinoma combined tumor rates at 1.52×10^{-3} in human equivalents. Therefore, this slope factor will be used for human health risk assessments in the Reregistration Eligibility Document.

X. BIBLIOGRAPHY

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44554301 Beattie (1993). A 2-Week Toxicity Study of Aerosolized Malathion Administered by Whole-body Inhalation Exposure to the Albino Rat. Bio-Research Laboratories, Senneville, Canada. Unpublished Study.

44554901 Review of Pathology Working Group (PWG) Peer Review of Proliferative Lesions of the Liver in Male B6C3F! Mice in An 18-Month Oral (Dietary) Oncogenicity Study in Mice of Malathion *Memorandum:* B. Dementi, HED to Dana Latulere, SRRD. Dated December 1, 1998.

44733501 Review of the Histopathology Assessment of Nasal Tissues for the Malathion 18-Month Oral (Dietary) Carcinogenicity Study in Mice. *Memorandum*: B. Dementi, HED to Phillip Poli, SRRD. Dated May 5, 1999.

44782301 Review of the Histopathology Re-Assessment of Nasal Tissues for the Malathion 24-Month Oral (Dietary) Combined Toxicity/Crcinogenicity Study in the F344 Rat. *Memorandum*: B. Dementi, HED to Phillip Poli, SRRD. Dated May 27, 1999.

44744201 Review of the Histopathology Assessment of Pituitary and Uterus Tissue for the Malathion 24-Month Oral (Dietary) Combined Toxicity/Carcinogenicity Study i n the F344 Rat. *Memorandum:* B. Dementi, HED to Phillip Poli, SRRD. Dated June 9, 1999.

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---------Brunsman, L (1999). Malathion REVISED Qualitative Risk Assessment Based on Re-Read of B6C3F1 Mouse Dietary Study Liver Slides. February, 16, 1999.

---------Brunsman, L (1999). ADDENDUM to Malathion Qualitative Risk Assessment Based on Fischer 344 Rat Dietary Study. May 03,, 1999.

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

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Members In Attendance At the Previous Cancer Assessment Review Committee Meetings

September 24, 1997:

Karl Baetcke, Luke Brennecke (Pathology Consultant), William Burnam (Chairman), Marion Copley, Virginia Dobozy, Pam Hurley, Mike Ioannou, Nancy McCarroll, Hugh Pettigrew, Esther Rinde, Jess Rowland (Executive Secretary), Joycelyn Stewart, Linda Taylor, and Yin-Tak Woo. Also present were Alberto Protzel, Branch Senior Scientist, Toxicology Branch 1 and Mr. Richard Brown, of Institute for Individual and Organizational Excellence. Data were presented by Brian Dementi, Toxicology Branch 1.

October 8, 1997:

Karl Baetcke, William Burnam (Chairman), Marion Copley, Vicki Dellarco, Richard Hill, Pam Hurley, Mike Ioannou, Nancy McCarroll, Jess Rowland (Executive Secretary), Joycelyn Stewart, Linda Taylor, and Yin-Tak Woo. Also present were Alberto Protzel, Branch Senior Scientist, Toxicology Branch 1 and Mr.Richard Brown, Institute for Individual and OrganizationalExcellence. Data were presented by Brian Dementi, Toxicology Branch 1.

October 15, 1997:

Karl Baetcke, Luke Brennecke (Pathology Consultant), William Burnam (Chairman), Marion Copley, Pam Hurley, Mike Ioannou, Nancy McCarroll, Hugh Pettigrew, Jess Rowland (Executive Secretary), Joycelyn Stewart, Linda Taylor, and Yin-Tak Woo. Also present were Alberto Protzel, Branch Senior Scientist, Toxicology Branch 1. Data was presented by Brian Dementi, Toxicology Branch 1.

June 10, 1998:

Karl Baetcke, Luke Brennecke (Pathology Consultant), William Burnam (Chairman), Marion Copley, Virginia Dobozy, Mike Ioannou, Hugh Pettigrew, Esther Rinde, Jess Rowland (Executive Secretary), Joycelyn Stewart, and Linda Taylor. Also present were Edward Budd, Toxicologist, Registration Action Branch 2; Lori Brunsman, Science Analysis Branch, Diana Locke Risk Assessor, Reregistration Branch 2; and Alberto Protzel, Branch Senior Scientist, Toxicology Branch 1. Data were presented by Brian Dementi, Toxicology Branch 1.

February 24, 1999:

Luke Brennecke (Pathology Consultant), William Burnam (Chairman), Marion Copley, Sanju Diwan (Executive Secretary), Virginia Dobozy, Mike Ioannou, Esther Rinde, Jess Rowland, Joycelyn Stewart, Clark Swentzel, and Linda Taylor. Also present were Lori Brunsman and Brenda Tarplee, Science Analysis Branch; Paula Deschamp, Risk Assessor, Reregistration Branch 2; Randolph Perfetti, Associate Director. Data were presented by Brian Dementi, Toxicology Branch 1.

June 23, 1999:

Karl Baetcke, Luke Brennecke (Pathology Consultant), William Burnam (Chairman), Vicki Dellarco, Sanju Diwan, (Executive Secretary) Virginia Dobozy, Mike Ioannou, Nancy McCarroll, Esther Rinde, Jess Rowland, Joycelyn Stewart, Clark Swentzel, and Linda Taylor. Also present were Lori Brunsman, Science Analysis Branch and Brenda Tarplee, Science Analysis Branch. Data were presented by Brian Dementi, Toxicology Branch 1.

September 20, 1999:

At this meeting, the following members reviwed the Draft Report dated September 20, 199. *Karl Baetcke, William Burnam (Chairman), Marion Copley, Sanju Diwan (Executive Secretary,) Virginia Dobozy, Mike Ioannou, Nancy McCarroll, Jess Rowland, Clark Swentzel, and Linda Taylor.* Non-members who participated in the review were Lori Brunsman, Science Analysis Branch and Brian Dementi, Toxicology Branch 1. Also present was Paula Deschamp (observer), Risk Assessor of Reregistration Branch 2.

October 4, 1999:

This meeting continued the review of the Draft Report dated September 20, 199. *Karl Baetcke, William Burnam (Chairman), Marion Copley, Virginia Dobozy, Mike Ioannou, Jess Rowland, Clark Swentzel, and Linda Taylor.* Non-members who participated in the review were Lori Brunsman, Science Analysis Branch and Brian Dementi, Toxicology Branch 1. Also present was Paula Deschamp (observer), Risk Assessor of Reregistration Branch 2.

Chronology of the Cancer Assessment Review Committee Meetings

A chronology of the six meetings held and a summary of the conclusions reached at each meeting are presented below.

September 24, 1997

The Cancer Assessment Review Committee (CARC) reviewed and evaluated the non-neoplastic and neoplastic lesions of the liver as well as the adequacy of the dose levels tested in the carcinogenicity study in B6C3F1 mice. The CARC also reviewed and evaluated the liver and the nasal tumors, and the adequacy of the dose levels tested in the combined chronic toxicity/carcinogenicity study in rats.

The Committee concluded that the liver pathology slides from male mice at all dose levels should be re-evaluated and referred to a pathology work group (PWG) and the PWG evaluation should be done in compliance with the August 24, 1994 Pesticide Regulation Notice 94-5. The Committee also concluded that an assessment on the relevancy of the nasal tumors to treatment could not be completed at this meeting because of the need for a re-evaluation of the nasal tissues from all animals.

October 8, 1997

The Committee re-assessed the adequacy of the dose levels tested in the combined chronic toxicity/carcinogenicity study in Fischer 344 rats and evaluated the testicular (males), thyroid (males), and the pituitary (females) tumors observed in this study.

Due to lengthy discussions and lack of time, the Committee decided to continue the discussion the following week on October 15, 1997.

October 15, 1997

The Committee re-evaluated the pituitary tumors and continued the review and evaluation of the uterine tumors and mononuclear cell leukemia in female rats. The Committee reviewed and evaluated the non-neoplastic and neoplastic lesions as well as the adequacy of the dose levels tested, in the carcinogenicity study with malaoxon in Fischer 344 rats.

The Committee concluded that a re-evaluation of the following tissues/slides was required in order to ascertain the relevance of the tumors seen in these organs/tissues to treatment.

Species	*Tissue/Slides*	*Dose Levels*
Mouse	*Nasal Turbinate*	*All Animals / all Doses*
Mouse	*Liver*	*Males - All Doses*
Rat	*Nasal Turbinate*	*All Animals / All Doses*
Rat	*Pituitary glands*	*All Females / All Doses*
Rat	*Uterus*	*All Females / All Doses*

The Committee also concluded that a definitive classification on the carcinogenic potential of malathion could not be made at that time due to the need for the re-evaluation of the tissues/slides listed above, but the available data indicate suggestive evidence of carcinogenicity. The Committee also concluded that there are no compelling reasons to deviate from the current linear low-dose approach (Q_1) for human risk characterization (i.e., status quo). However, the method for quantification would be re-assessed after review and evaluation of the requested pathology data.*

June 10, 1998

The Committee evaluated the conclusions reached by the pathology working group (PWG) in their review of liver pathology slides from the carcinogenicity study in male mice (as requested at the 9/24/97 meeting) and discussed whether HED should continue to use the existing linear low dose approach (Q_1*) for risk assessments based on *all* liver tumors seen at *all* dose levels in female mice.

The Committee accepted the PWG report on the re-read and concluded that the use of the existing Q_1 for risk assessments should continue since the re-read of the male mouse liver tumors did not provide any compelling reasons to change from the use of the linear low dose extrapolation for human risk characterization.*

February 24, 1999

The Committee continued with the review and evaluation of the re-examination of the following tissues/slides: liver, male mice based on the PWG re-read; nasal tumors, all mice; nasal, thyroid, pituitary and uterine tumors of rats (as requested at the October 15, 1997 meeting).

The Committee concluded that the following additional information and/or data analyses were required:

- ***Nasal tumors - Rat:*** *Independent review of re read data submitted to the Agency (and associated analyses by the EPA reviewer) is required. Only a letter (from Dr. James Swenberg) was made available to the Committee members. There was also a discrepancy between the Study Pathologist in the Original Report (listed as Dr. William Wooding) and in the letter from Dr. Swenberg (listed as Dr. Henry Bolte).*

Tooth Tumor - Rat: *Re-evaluation of the "diagnosis" of the tumor morphology.*

- ***Thyroid C-cell Tumor - Male Rat:*** *Statistical (Peto's prevalence test) analyses of tumor incidences by SAB/HED and historical control data from the testing laboratory.*
- ***Mononuclear Cell Leukemia - Male Rat:*** *Statistical (Peto's prevalence test) analyses of tumor incidences and historical control data from the testing laboratory.*

June 23, 1999

The Committee evaluated the additional information and/or data requested at the February 24, 1999 meeting.

> *The conclusions are presented in this report. In summary, the Committee classified malathion as a "likely human carcinogen" and recommended a linear low-dose approach for human risk characterization.*

September 20, 1999

The Committee reviewed the Draft -Cancer Assessment Document, dated September 20, 1999

October 4, 1999

The Committee continued the review of the Draft -Cancer Assessment Document, dated September 20, 1999.

XI. ATTACHMENTS

Dementi, B.(1997). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **November 26, 1997.**

Dementi, B.(1998). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **February 23, 1998.**

Dementi, B.(1998). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **April 9, 1998.**

Dementi, B.(1998). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **May 4, 1998.**

Dementi, B.(1998). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **May 29, 1998.**

Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to Sanju Diwan, Executive Secretary, Cancer Assessment Review Committee, dated **February 11, 1999.**

Dementi, B.(1999). *Recommendation to CARC Members* from Brian Dementi, Toxicology Branch 1, dated **February 24, 1999.**

Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1to Jess Rowland, Executive Secretary, Cancer Assessment Review Committee, dated **April 1, 1999.**

Dementi, B.(1999). *Memorandum:* Brian.Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **April 27, 1999.**

Dementi, B.(1999). Addendum to Malathion Qualitative Risk Assessment Based on Fischer 344 Rat Dietary Study. *Memorandum*: Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **May 18, 1999.**

Dementi, B.(1999). Malathion Combined Chronic Toxicity/Carcinogenicity Study in the F344 Rat (MRID No. 43942901). *Memorandum*: Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **June 7, 1999.**

--------- Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **June 21, 1999.**

--------- Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **July 13, 1999.**

--------- Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **July 22, 1999**.

--------- Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **September 21, 1999.**

--------- Dementi, B.(1999). *Memorandum:* Comments on September 20, 199 Draft CARC Report on Malathion. Brain Dementi, Toxicology Branch 1 to Jess. Rowland, Executive Secretary, Cancer Assessment Review Committee, dated **October 6, 1999.**

--------- Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to Jess Rowland, Executive Secretary, Cancer Assessment Review Committee, dated **October 28, 1999.**

--------- Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **November 12, 1999**.

--------- Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **December 7, 1999.**

--------- Dementi, B.(2000). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **January 12, 2000.**

--------- Dementi, B.(2000). *Memorandum:* Brian Dementi, Toxicology Branch 1 to Jess Rowland, Executive Secretary, Cancer Assessment Review Committee, dated **February 7, 2000**.

--------- Dementi, B.(2000). *Memorandum:* Brian Dementi, Toxicology Branch 1 to Jess Rowland, Executive Secretary, Cancer Assessment Review Committee, dated **February 9, 2000.**