SAP Report No. 2000-04
December 14, 2000

REPORT

FIFRA Scientific Advisory Panel Meeting, August 17-18, 2000, held at the Holiday Inn-Ballston Hotel, Arlington, Virginia

Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding:

A Consultation on the EPA Health Effect Division's Proposed Classification of the Human Carcinogenic Potential of Malathion

NOTICE

This report has been written as part of the activities of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP). This report has not been reviewed for approval by the United States Environmental Protection Agency (Agency) and, hence, the contents of this report do not necessarily represent the views and policies of the Agency, nor of other agencies in the Executive Branch of the Federal government, nor does mention of trade names or commercial products constitute a recommendation for use.

The FIFRA SAP was established under the provisions of FIFRA, as amended by the Food Quality Protection Act (FQPA) of 1996, to provide advice, information, and recommendations to the Agency Administrator on pesticides and pesticide-related issues regarding the impact of regulatory actions on health and the environment. The Panel serves as the primary scientific peer review mechanism of the EPA, Office of Pesticide Programs (OPP) and is structured to provide balanced expert assessment of pesticide and pesticide-related matters facing the Agency. Food Quality Protection Act Science Review Board members serve the FIFRA SAP on an ad-hoc basis to assist in reviews conducted by the FIFRA SAP. Further information about FIFRA SAP reports and activities can be obtained from its website at http://www.epa.gov/scipoly/sap/ or the OPP Docket at (703) 305-5805. Interested persons are invited to contact Larry Dorsey, SAP Executive Secretary, via e-mail at dorsey.larry@.epa.gov.

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Mr. Paul Lewis
Designated Federal Official
FIFRA Scientific Advisory Panel
Date:_____

Mary Anna Thrall, D.V.M.
FIFRA SAP Session Chair
FIFRA Scientific Advisory Panel
Date:_____

Federal Insecticide, Fungicide, and Rodenticide Act
Scientific Advisory Panel Meeting
August 17-18, 2000

A Consultation on the EPA Health Effect Division's Proposed Classification of the Human Carcinogenic Potential of Malathion

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PUBLIC COMMENTERS

Oral statements were made by:
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Jerry Hardisty, D.V.M., on behalf of Experimental Pathology Laboratories, Inc.
Judith Hauswirth, Ph.D., on behalf of Jellinek, Swartz, and Connolly,
J. William Hirzy, Ph.D., on behalf of the National Treasury Employees Union, Chapter 280
Mr. Greg Kidd, on behalf of the National Coalition Against the Misuse of Pesticides
Don O'Shaughnessy, Ph.D. D.A.B.T., on behalf of Cheminova, Inc.
Ms. Joyce Shepard, on behalf of the Citizens Action Network for Change

Written statements were received from:
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James Swenberg, Ph.D., on behalf of the University North Carolina

INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP) has completed its review of the set of scientific issues being considered by the Agency issues pertaining to a consultation on the EPA Health Effect Division's proposed classification of the human carcinogenic potential of malathion. Advance notice of the meeting was published in the *Federal Register* on July 20, 2000. The review was conducted in an open Panel meeting held in Arlington, Virginia, on August 17-18, 2000. The meeting was chaired by Mary Anna Thrall, D.V.M. Mr. Paul Lewis served as the Designated Federal Official.

This 2-day meeting concerned the evaluation of the human carcinogenic potential of malathion. In accordance with the EPA *Guidelines for Carcinogen Risk Assessment* (Preliminary Draft, July 1999), the EPA, Office of Pesticide Programs, has proposed to classify malathion as having "**suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential"** by all routes of exposure. 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 only at excessive doses; (ii) the presence of a few rare tumors, oral palate mucosa in females and nasal respiratory epithelium in male and female Fischer 344 rats. These tumors cannot be distinguished as either treatment related or due to random occurrence; (iii) the evidence for mutagenicity is not supportive of a mutagenic concern in carcinogenicity; and (iv) malaoxon, a structurally related chemical, is not carcinogenic in male or female Fischer 344 rats. With the exception of 1 nasal and 1 oral tumor in female rats, all other tumor types were determined to occur at excessive doses or were unrelated to treatment with malathion. The toxicology data considered included chronic toxicity, carcinogenicity, subchronic toxicity and mutagenicity studies on malathion and as well as carcinogenicity and mutagenicity studies on malaoxon.

Marion Copley, D.V.M., D.A.B.T. (EPA, Office of Pesticide Programs) presented the

adequacy of studies, positive tumor findings results, weight of evidence/cancer classification and negative tumor findings results. Brian Dementi, Ph.D., D.A.B.T. (EPA, Office of Pesticide Programs) presented an alternative approach to the Agency's analysis.

In preparing this report, the Panel carefully considered all information provided and presented by the Agency presenters, as well as information presented by public commenters. This information included evaluations of the significance of liver tumors in B6C3F1 mice; liver tumors in male and female F344 rats; thyroid tumors in male F344 rats; nasal tumors in F344 rats; oral squamous cell tumors in F344 rats; interstitial cell testicular tumors in F344 rats; leukemia in male F344 rats; mutagenicity of malathion; cancer classification of malathion; and the incidence of other tumorigenic responses in rats and mice. This report addresses all these issues within the structure of the charge presented by the Agency.

CHARGE

Issue 1. The HED Cancer Assessment Review Committee (CARC) determined that all three new studies, a rat and mouse study with malathion and a rat study with malaoxon, were adequate to evaluate the carcinogenic potential of the test substance. Although excessive toxicity was present at the high dose or two high doses in all of the studies, the next lower dose was either adequate based on marginal evidence of toxicity or was less than one half of the excessively toxic dose.

Question 1: Does the SAP agree that each of the three above-mentioned cancer studies were adequate to assess potential carcinogenicity? If yes, why. If no, why not.

Issue 2. The HED CARC classified malathion as “suggestive.” This is based on the occurrence of liver tumors in male and female B6C3F1 mice, and in female Fischer 344 rats at excessive doses and the presence of a few rare nasal respiratory epithelial tumors in male and female Fischer 344 rats. The nasal and oral tumors could not be distinguished as either treatment related or due to random occurrence.

Question 2.1: Does the SAP consider the statistically significant trend and pair-wise increases in liver tumors in male B6C3F1 mice at 8,000 and 16,000 ppm (adenomas - 14/55 and 49/51 compared to 4/54 in controls; and adenomas/carcinomas - 15/55 and 49/51 compared to 4/54 in controls) to be related to malathion exposure? Why or why not? What weight should be placed on these data since there is evidence of excessive toxicity based on marked (brain) to severe (RBC and plasma) cholinesterase inhibition in all three compartments and decreased body weight at both doses?

Question 2.2: Does the SAP consider the statistically significant trend and pair-wise increases in liver tumors in female B6C3F1 mice at 8,000 and 16,000 ppm (adenomas - 9/52 and 42/51 compared to 0/55 in controls; and adenomas/carcinomas - 10/52 and 43/51 compared to 1/55 in controls) to be related to malathion exposure? If not, why not? What weight should be placed on these data since there is evidence of excessive toxicity based on severe cholinesterase inhibition in

all three compartments (RBC, plasma and brain) and decreased body weight at both doses?

Question 2.3: The CARC considered the April 2000 Pathology Working Group (PWG) report for female Fischer 344 rat liver tumors to be valid and used these values in the cancer hazard assessment.

2.3.1. Does the SAP agree that the female rat liver tumor PWG report (from April 2000) should be considered valid and that these values should be used in this hazard assessment for malathion? If yes, why? If not, why not?

2.3.2. Does the SAP consider the statistically significant trend and pair-wise increases in liver tumors in female Fischer 344 rats at 12,000 ppm (adenomas - 5/38 compared to 0/41 in controls) to be related to malathion exposure? If not, why not? What weight should be placed on this data since there is evidence of excessive toxicity based on severe cholinesterase inhibition in all three compartments (RBC, plasma and brain) and mortality?

Question 2.4: The Committee could not determine whether the nasal tumors in rats were due to treatment or random occurrence because: on the one hand: (1) there was no dose response over a wide range of doses (100/50 to 12,000 ppm); (2) there was no statistical significance; (3) there were only adenomas, one in each of two doses for females and only one at the high dose in males; (4) the high dose in both male and females were considered excessively toxic; and (5) these tumors occurred in section 5 where there was little to no evidence of non-neoplastic lesions in the nasal mucosa. On the other hand: (1) an adenoma of the respiratory epithelium was seen in one female at 6,000 ppm (not an excessive dose) and; (2) spontaneous nasal tumors are very rare in rats, there were no nasal tumors in the concurrent controls and the incidences exceeded the historical control incidence of the testing laboratory and NTP. It should be noted that the biological significance of the olfactory epithelial tumor is unknown since it is from a different cell of origin and these types of tumor (esthesioneural epithelial neoplasms) should not be combined with other tumors of the respiratory nasal cavity. The biological significance of this in relation to tumors of the respiratory epithelium is unknown. It should be pointed out that there were five nasal sections per rat. Historical control studies usually have only one or two sections.

2.4.1 Does the SAP agree that nasal respiratory epithelial tumors in Fischer 344 rats are rare tumors in light of the number of sections in the current study as compared to the historical control data base? Why or why not?

2.4.2 Does the SAP agree that the increase in these nasal respiratory epithelial tumors in Fischer 344 rats cannot be conclusively attributed to either treatment with malathion or random occurrence? If the SAP feels this increase can be attributed to treatment, why?

2.4.3 What, if any, is the significance of the adenoma of the olfactory epithelium in one male Fischer 344 rat at 6,000 ppm?

Question 2.5: The CARC could not determine whether the oral cavity squamous cell tumors in Fischer 344 female rats (palate—one papilloma at 6,000 and one carcinoma at 12,000 ppm; carcinoma of tooth alveolus at 100/50 ppm) were due to treatment or random occurrence because on the one hand, (1) there was no dose response over a wide range of doses (100/50 to 12,000 ppm); (2) there was no statistical significance and; (3) the high dose in the females was considered excessively toxic. On the other hand, (1) a squamous cell papilloma of the palate was seen in one female at 6000 ppm (not an excessive dose); (2) spontaneous oral tumors may be very rare in rats, there were no oral tumors in the concurrent controls and the incidences exceeded the historical control incidence of the testing laboratory and NTP and; (3) due to the lack of systematic pathologic evaluation of the oral mucosa, there is uncertainty as to the actual incidence of oral tumors. The one papilloma in males at 100/50 ppm was considered incidental. It should be pointed out that oral epithelium (often palate) is usually present on nasal sections and that there were five nasal sections per rat. Historical control studies usually have only one or two sections. However, the CARC determined that a recut would not alter their conclusion.

2.5.1: Does the SAP agree that the oral squamous cell tumors in Fischer 344 rats are rare tumors in light of the number of sections in the current study as compared to the historical control data base? Why or why not?

2.5.2: Does the SAP agree that a recut of tissue would not significantly alter the conclusions of this study? If yes, why? If not, why not?

2.5.3: Does the SAP agree that the increase in the oral tumors in Fischer 344 rats cannot be conclusively attributed to either treatment with malathion or random occurrence? If the SAP feels this increase can be attributed to treatment, why?

2.5.4: What, if any, is the significance of the squamous cell carcinoma of the alveolus of the tooth in one female Fischer 344 rat at 100/50 ppm?

Question 2.5: Does the SAP agree with the proposed CARC classification of malathion as "suggestive?" Why or why not?

Issue 3. There were several other neoplastic lesions that the CARC considered and determined **not** to be indicative of a carcinogenic potential of malathion. These included: mouse liver tumors at low doses in males, male rat nasal tumors, male rat thyroid follicular cell tumors, male rat thyroid C-cell tumors, rat interstitial cell testicular tumors, male rat liver tumors, male rat leukemia, female rat pituitary gland tumors, and female rat uterine tumors (various types). The CARC considered and determined that leukemia in the male rat was not related to malaoxon treatment.

Question 3. Does the SAP agree that these tumors are not related to malathion treatment and as such do not contribute to the weight of the evidence?

DETAILED RESPONSE TO THE CHARGE

The specific issues to be addressed by the Panel are keyed to the Agency's background document "A Consultation on the EPA Health Effect Division's Proposed Classification of the Human Carcinogenic Potential of Malathion ", dated July 20, 2000, and are presented as follows:

Issue 1. The HED Cancer Assessment Review Committee (CARC) determined that all three new studies, a rat and mouse study with malathion and a rat study with malaoxon, were adequate to evaluate the carcinogenic potential of the test substance. Although excessive toxicity was present at the high dose or two high doses in all of the studies, the next lower dose was either adequate based on marginal evidence of toxicity or was less than one half of the excessively toxic dose.

Question 1: Does the SAP agree that each of the three above-mentioned cancer studies were adequate to assess potential carcinogenicity? If yes, why. If no, why not.

The SAP agrees that the three new studies reviewed by HED's CARC (i.e., the 1994 IRDC Malathion mouse bioassay, the 1996 Huntington rat Malathion bioassay and the 1996 Huntington rat Malaoxon bioassay) are adequate to assess the potential carcinogenicity of technical grade malathion and malaoxon. A detailed review of the three studies is provided below.

Mouse malathion study - This was an 18 month dietary (feed) study of technical grade malathion in male and female B6C3F1 mice at doses of 0, 100, 800, 8,000 and 16,000 ppm continuously, per EPA guidelines. The number of mice per dose group was adequate, as was the conduct of the study and survival.

The pathology was reviewed independently by both the study pathologist and a reviewing pathologist. The only major areas of concern in the study were the length of the study at 18 months (the ideal length for this strain of mouse would be 24 months) and the spacing of the dose levels (the gap between 800 ppm and 8000 ppm does not provide for a good dose response relationship). Overall, this was an adequate study.

Toxicity was severe at the two top doses (as described later in this report). There was little, if any, signs of toxicity and pathology at 800 ppm, although some nasal irritation was reported at 800 ppm in both sexes. There was evidence of carcinogenic activity (liver tumors) in both sexes at the 8,000 and 16,000 ppm dose groups.

Rat malathion study - This was a 24 month feed study of technical grade malathion in male and female F344 rats at doses of 0, 50, 100, 500, 6,000 and 12,000 ppm. The spacing of these doses was not ideal. The number of rats per group was adequate, as was the conduct of the study, including the pathology evaluation, which was subjected to a Pathology Working Group (PWG) review. Overall, this was an adequate study.

Toxicity was severe at both 6,000 and 12,000 ppm as evidenced by a marked increase in mortality and decrease in body weight gain. Poor survival, in principle, could reduce the ability to detect tumors at the two highest doses, but the mortality did not appear until after 18 months, which suggests that the animals survived at these high doses for a sufficient time to exhibit neoplastic responses.

The only evidence of carcinogenic activity reported was a small, but significant, increase in benign liver tumors (hepatocellular adenomas) in females at the high dose. The lowered body weights at the higher doses may have reduced the incidence of some tumor types, making their detection as a result of exposure to malathion more difficult. Nevertheless, no neoplastic response was observed at 6,000 ppm in either sex even at these excessive (toxic) doses, and therefore, it can be assumed that none would have been found at lower doses between 6,000 and 500 ppm.

Rat malaoxon study - This was a 24 month feed study of technical grade malaoxon in male and female F344 rats at doses of 0, 20, 1000, and 2000 ppm. The number of rats per group was adequate, as was the conduct of the study and the pathology evaluation. There was significant mortality at 2,000 ppm, but the study was adequate to determine the potential carcinogenic activity of the compound. Overall, this was an adequate study. No evidence of carcinogenic activity was found in either sex, even in the presence of excessive toxicity.

Issue 2. The HED CARC classified malathion as "suggestive." This is based on the occurrence of liver tumors in male and female B6C3F1 mice, and female Fischer 344 rats at excessive doses and the presence of a few rare nasal respiratory epithelial tumors in male and female Fischer 344 rats. The nasal and oral tumors could not be distinguished as either treatment related of due to random occurrence.

Question 2.1: Does the SAP consider the statistically significant trend and pair-wise increases in liver tumors in male B6C3F1 mice at 8000 and 16,000 ppm (adenomas - 14/55 and 49/51 compared to 4/54 in controls; and adenomas/carcinomas - 15/55 and 49/51 compared to 4/54 in controls) to be related to malathion exposure? Why or why not? What weight should be placed on these data since there is evidence of excessive toxicity based on marked (brain) to severe (RBC and plasma) cholinesterase inhibition in all three compartments and decreased body weight at both doses?

The SAP concluded that the liver tumor increases in male mice at the doses of 8,000 ppm and 16,000 ppm are related to malathion exposure. There were significant increasing trends in adenomas ($p < - 0.0000$) and adenomas/carcinomas combined ($p < - 0.0000$). The incidences of adenomas at 8,000 ppm was 25% and at 16,000 ppm was 96%; both were higher than the concurrent control of 7% and the historical control range of 14% to 22%. Thus, only benign tumors were increased.

The survival rates of the five groups were about equal with the numbers alive in week 78 at 48 to 54 per group. Thus, treatment had no apparent effect on the rate of mortality. However, body weights were reduced in high dose groups; the percentage decreases were 14% and 20% for 8,000 ppm and 16,000 ppm, respectively. The MTD was exceeded at the two highest doses, as evidenced by the lower body weights and toxicity, including increased liver weight and hypertrophy, both of which were found early in the study.

The SAP concluded that tumors were increased in the presence of severe toxicity. Most members agreed that these findings should be given no weight in the cancer classification because the tumors produced at these doses may be the result of indirect effects of malathion that may not be present at lower doses. Other members opined that they should be given a non-zero weight. The tumor incidence rates of the two low dose groups (100 ppm and 800 ppm) were higher than the tumor rates observed in the concurrent control group in both adenomas (7%) and carcinomas (0%), but there was no statistical significance in the pair-wise comparisons.

The Panel also decided that using AchE levels to define an excessive dose has no biological basis. Since AchE has not been shown to be associated with cancer risk, it is not a confounder.

Question 2.2: Does the SAP consider the statistically significant trend and pair-wise increases in liver tumors in female B6C3F1 mice at 8,000 and 16,000 ppm (adenomas - 9/52 and 42/51 compared to 0/55 in controls; and adenomas/carcinomas - 10/52 and 43/51 compared to 1/55 in controls) to be related to malathion exposure? If not, why not? What weight should be placed on these data since there is evidence of excessive toxicity based on severe cholinesterase inhibition in all three compartments (RBC, plasma and brain) and decreased body weight at both doses?

The SAP concluded that liver tumor increases in female mice at the doses of 8,000 ppm and 16,000 ppm are also related to malathion exposure. The tumor incidences of adenomas at the 8,000 ppm and 16,000 ppm were 17% and 92%, respectively. In addition, both were higher than the concurrent control (0%) and the historical control range of 0% to 10.6%. Both groups showed statistically significant decreases in mean body weights, percentage decreases of 9.7% and 16% for 8000 ppm and 16000 ppm, respectively. The survival rates of these two groups were more than adequate. However, the numbers of alive animals in week 78 were 55, 52, 53, and 51 for the control, 100, 800, 8000, and 16000 ppm, respectively.

The Panel did not consider the cholinesterase inhibition to be related to tumorigenesis. The decreased body weights at the two high doses did indicate some toxicity. The SAP concluded that the liver tumors were increased in the presence of toxicity. The reduced body weights may actually have been associated with a lowering of the incidence of tumors observed in the two high doses. Most members agreed that these findings should not be given any weight in the cancer classification because the tumors produced at these doses may be the result of indirect effects of malathion that may not be present at lower doses. However, a few members of the SAP considered the liver tumors to be relevant to cancer risk in humans.

Question 2.3: The CARC considered the April 2000 Pathology Working Group (PWG) report for female Fischer 344 rat liver tumors to be valid and used these values in the cancer hazard assessment.

2.3.1. Does the SAP agree that the female rat liver tumor PWG report (from April 2000) should be considered valid and that these values should be used in this hazard assessment for malathion? If yes, why? If not, why not?

The majority of the Panel concluded that the PWG report should be accepted and used for malathion hazard assessment even though a few Panel members did express reservations with the report. The histologic diagnoses of some tumors in rodents were reviewed by the PWG and were altered to reflect the consensus of the PWG as noted below. No information was given as to the background information or charge given to the PWG. The PWG consisted of the original pathologist, the reviewing pathologist, and three new consulting pathologists. The PWG was conducted according to EPA guidelines and the pathologists involved were highly qualified to perform the task. The reviewers were given slides selected by the project and reviewing pathologists and evaluated blindly. The diagnoses were made on the basis of a consensus tally (three of five required to agree). After revision of the pathologic diagnoses by the PWG, significant differences for carcinomas was achieved for no exposure group; the combined incidence of adenomas and carcinomas was significantly elevated for the rodents exposed at 8,000 and 16,000 ppm.

As a result of this second evaluation, the incidence of tumors in the control group, which had been 1 in 108 males in the original report, was revised to 4 in 108. The number of carcinomas in the exposed mice, which had been 16 was reduced to 8. The increase in the number of adenomas in the control group served to bias the conclusions towards the null. Also, regression to the mean may be at work; in any study, high estimates will tend to be lower on a second repetition, and low estimates higher.

Concerns were expressed by a few members of the Panel about the changes in diagnosis that arose during the course of the PWG review of lesions. Specifically, one Panel member argued that the *post hoc* revision of diagnoses, examining only the positive or conflicting diagnoses, and with knowledge of the final conclusions, cannot be rectified by blinding the reviewers and is not scientifically justified. Other Panel members commented that the finding that certain hepatic proliferative lesions were downgraded from adenoma to focus of cellular alteration is easily explainable and would not be surprising to experienced toxicologic pathologists who are involved with rodent lesions of questionable classification. In the evaluation of rodent hepatic lesions, there is a morphologic continuum that entails some overlap with respect to diagnostic criteria. Unfortunately, the pathology assessment of rodent hepatic proliferative lesions involves a degree of subjectivity. Most toxicologic pathologists would be of the opinion that the consensus diagnosis of a properly conducted PWG (as was done in this study), should be given more credence than the individual opinion of a single pathologist.

The Panel also expressed concern regarding whether the PWG properly selected lesions for review because not all foci of cellular alteration were subjected to PWG examination. The majority of Panel members believed that there was no reason to be concerned that only the largest foci were selected for PWG review by the reviewing pathologist since these lesions would be the only ones that would pose diagnostic dilemmas to the pathologist for the determination of what would constitute the neoplastic hepatic proliferative lesions. Studies have demonstrated (Harada et al., 1989) that numerous small foci are present in the livers of aging rats although very few progress to neoplasms. The majority of foci of cellular alteration in the liver pose little diagnostic difficulty for the diagnosis of hepatocellular adenoma and thus have no need for being brought forward to the PWG.

2.3.2. Does the SAP consider the statistically significant trend and pair-wise increases in liver tumors in female Fischer 344 rats at 12,000 ppm (adenomas - 5/38 compared to 0/41 in controls) to be related to malathion exposure? If not, why not? What weight should be placed on this data since there is evidence of excessive toxicity based on severe cholinesterase inhibition in all three compartments (RBC, plasma, and brain) and mortality?

The Panel agreed that the statistically significant pair-wise increase in liver tumors at the highest dose in female F344 rats was related to administration of the test material. Most members of the Panel were of the opinion that the tumors produced at this dose were the result of indirect effects of malathion that would not be present at lower doses and warranted no weight in assessment of potential human cancer hazard. However, it needs to be stressed that the Panel was not certain whether the response was directly related to malathion or to the minor contaminants in the material. In addition, it needs to be noted that the response was restricted to the high dose in one sex (females), that the tumors (adenomas) were all benign, and that they only occurred in animals showing severe toxicity, as evidenced by increased mortality, decreased body weight gain, markedly increased liver weights and hepatocellular hypertrophy. The rats were obviously suffering from cholinergic stress but this observation is not necessary to establish that the animals were severely intoxicated.

Hepatic tumors in female Fischer rats are not a common spontaneous finding (<1%). Most of the Panel believed that little significance should be attributed to this finding due to the fact that the increase in liver tumors was limited to a concentration that had exceeded the MTD, as judged by evidence of systemic toxicity. It was felt that little significance should be accorded to rodent liver tumors when these findings were limited to exposure concentrations that exceeded MTD levels and that the liver is an important site of metabolism for the chemical in question.

Important information that contributed to the finding of the Panel that little weight should be given to this high dose finding was the fact that there were no neoplastic proliferative lesions at lower concentrations and no carcinomas were found in the study. The lack of findings at concentrations that have not exceeded MTD and the complete lack of hepatic carcinomas in the study diminish the significance of the finding of the five adenomas. Although somewhat

speculative at this point with respect to mode of action, the weight of the evidence suggests that extremely high exposures of malathion are associated with excessive hepatic toxicity (hypertrophy and increased hepatic weight) that were possibly associated with overloading of hepatic metabolism.

Question 2.4: The Committee could not determine whether the nasal tumors in rats were due to treatment or random occurrence because: on the one hand: (1) there was no dose response over a wide range of doses (100/50 to 12,000 ppm); (2) there was no statistical significance; (3) there were only adenomas, one in each of two doses for females and only one at the high dose in males; (4) the high dose in both male and females were considered excessively toxic; and (5) these tumors occurred in section 5 where there was little to no evidence of non-neoplastic lesions in the nasal mucosa. On the other hand: (1) an adenoma of the respiratory epithelium was seen in one female at 6,000 ppm (not an excessive dose) and (2) spontaneous nasal tumors are very rare in rats, there were no nasal tumors in the concurrent controls and the incidences exceeded the historical control incidence of the testing laboratory and NTP. It should be noted that the biological significance of the olfactory epithelial tumor is unknown since it is from a different cell of origin and these types of tumor (esthesioneural epithelial neoplasms) should not be combined with other tumors of the respiratory nasal cavity. The biological significance of this in relation to tumors of the respiratory epithelium is unknown. It should be pointed out that there were five nasal sections per rat. Historical control studies usually have only one or two sections.

2.4.1 Does the SAP agree that nasal respiratory epithelial tumors in Fischer 344 rats are rare tumors in light of the number of sections in the current study as compared to the historical control data base? Why or why not?

The usual definition of "rare" is an incidence of less than 1%. Thus, these tumors appear to be rare, but occasionally are found as spontaneous lesions in the anterior nose. The Panel was in agreement with the conclusion that there is an extremely limited database upon which to evaluate the true incidence of spontaneous nasal tumors in aged Fischer 344 rats due to the five section trimming scheme used in this study (typically three sections are taken). None of the pathologists present were aware of any long-term dosed feed studies in F344 rats in which the nasal cavities were subjected to the trim scheme utilized in the malathion studies.

Tumors in the nasal sections in the F344 rat are uncommon. In a recent publication by Haseman, Hailey, and Morris (1998), seven tumors were reported in the nasal cavity of male F344/N rats from the NTP control groups (7/1341) from two-year feeding studies, six of the seven tumors were of epithelial origin (one squamous cell papilloma and five squamous cell carcinomas). In that same publication, four tumors were reported in the nasal cavity of female control F344/N rats from feeding studies (4/1351), three tumors were of epithelial origin (1 adenoma and 2 squamous cell carcinomas). Since that publication, other nasal tumors have been found. A review of the NTP Technical Reports appearing in 1999 (excluding pentachlorophenol and furfuryl alcohol which had nasal tumor responses) and observing all dose groups, six tumors

were found in male rats and none in 1295 female rats. In the male rats, no more than one tumor was found in any group (no tumors were found in any high dose groups). There was one adenoma, two squamous cell carcinomas, two chrondromas, and one tumor of the olfactory epithelium (6/1295).

In a Chemical Industry Institute of Toxicology (CIIT) sponsored bioassay conducted in the early 1980's using a similar tissue trimming scheme as the present malathion study, there was a single polypoid adenoma reported in the anterior nose of a control male F344 rat out of a group of 118 animals. No nasal tumors were reported in 114 female controls (Kerns et al., 1983). This publication did not discuss the exact section that the control tumor was found in but did note that the neoplasm was found in the anterior nose in the Sections I-III region. Section I corresponds to Section V in the malathion bioassay. In a follow-up bioassay a decade later conducted at CIIT (Monticello et al., 996) using a similar tissue trimming method, there were no nasal neoplasms reported in a control group of 90 male animals.

2.4.2 Does the SAP agree that the increase in these nasal respiratory epithelial tumors in Fischer 344 rats cannot be conclusively attributed to either treatment with malathion or random occurrence? If the SAP feels this increase can be attributed to treatment, why?

Considering jointly the nasal respiratory epithelium adenoma observed in male rats and the two adenomas observed in female rats, and using only the concurrent controls without reliance on historical data, the exact permutation test showed a statistical significance of $P<0.025$. This association was not strong enough to attribute to treatment.

In the male rats, there were two tumors of the nasal cavity, one of epithelial origin and one of olfactory origin (Bowman's Gland). The Panel was of the opinion that normally these two tumor types are not combined. Thus, there is one tumor of each type that has arisen and most Panel members were of the opinion that solitary tumor findings cannot be definitively attributed to treatment. In the female rats, there are two respiratory tract adenomas, one at 6000 ppm and one at 12,000 ppm. As pointed out, these tumors are uncommon in the F344 rat but have been occasionally reported in control animals. While it is unlikely that these two tumors were related to malathion treatment, it cannot be unequivocally ruled out.

2.4.3 What, if any, is the significance of the adenoma of the olfactory epithelium in one male Fischer 344 rat at 6,000 ppm?

The Panel was informed that a re-evaluation of this lesion indicated it to be a Bowman's gland tumor rather than an olfactory epithelial tumor. This uncommon tumor is considered to be a spontaneous lesion and not related to malathion exposure. This conclusion is based on the circumstances that it is a single observation in a single animal, in a single sex, in a single dose and, most importantly, there is no evidence of precursor lesions, e.g., hyperplasia or dysplasia in the same tissue. Additionally, it is not appropriate to combine this tumor with the respiratory

adenomas noted in a few other animals because the respiratory epithelium and Bowman's gland have a different histogenesis and thus cannot be assumed to exhibit a common response.

Question 2.5: The CARC could not determine whether the oral cavity squamous cell tumors in Fischer 344 female rats (palate—one papilloma at 6,000 and one carcinoma at 12,000 ppm; carcinoma of tooth alveolus at 100/50 ppm) were due to treatment or random occurrence because on the one hand, (1) there was no dose response over a wide range of doses (100/50 to 12,000 ppm); (2) there was no statistical significance and; (3) the high dose in the females was considered excessively toxic. On the other hand, (1) a squamous cell papilloma of the palate was seen in one female at 6,000 ppm (not an excessive dose); (2) spontaneous oral tumors may be very rare in rats, there were no oral tumors in the concurrent controls, and the incidences exceeded the historical control incidence of the testing laboratory and NTP and; (3) due to the lack of systematic pathologic evaluation of the oral mucosa, there is uncertainty as to the actual incidence of oral tumors. The one papilloma in males at 100/50 ppm was considered incidental. It should be pointed out that oral epithelium (often palate) is usually present on nasal sections and that there were five nasal sections per rat. Historical control studies usually have only 1 or 2 sections. However, the CARC determined that a recut would not alter their conclusion.

2.5.1: Does the SAP agree that the oral squamous cell tumors in Fischer 344 rats are rare tumors in light of the number of sections in the current study as compared to the historical control data base? Why or why not?

Tumors in the oral cavity of the F344 rat are uncommon but not rare as judged by recent NTP historical controls. In a publication by Haseman et al., (1998), 10 squamous tumors were reported in the oral cavity of male F344/N rats in dosed feed studies (1,354 animals at risk) and 15 squamous tumors in male F344/N rats in inhalation studies (905 animals at risk). In the female rat studies with similar numbers of animals at risk, the results were 18 squamous tumors in dosed feed groups and 14 tumors in inhalation study controls. Thus, tumor incidence runs approximately 1% in control animals. The higher incidence in inhalation study animals probably is reflective of the emphasis placed on this site in the examination process. It should be noted that in rats, squamous tumors of the oral cavity have been noted in association with oral cavity inflammatory processes and have been linked to diet related factors (Madsen, 1989). The fact that additional sections were taken in the present study (five versus three in NTP studies) makes it very difficult to compare the malathion incidences with historical databases.

2.5.2: Does the SAP agree that a recut of tissue would not significantly alter the conclusions of this study? If yes, why? If not, why not?

Recut of additional tissue always has the possibility to alter the findings, although in the case of either oral or nasal tumors, it is likely that the lack of a dose response will remain. Moreover, it would be impractical, if not impossible, to sample the oral cavity in a way that would meaningfully add to the knowledge of the potential carcinogenicity of this chemical. The method

used in this study was to examine the oral cavity at necropsy and then conduct histopathology on any suspect lesion. This is the standard practice and is the only one that is practical or even reasonably possible. Neoplasms in the oral cavity are typically exophytic and easily recognized at necropsy. If other areas of the oral cavity were to be sectioned, criteria would need to be formulated for what tissues were to be sampled and how many sections of each tissue would be needed.

2.5.3: Does the SAP agree that the increase in the oral tumors in Fischer 344 rats cannot be conclusively attributed to either treatment with malathion or random occurrence? If the SAP feels this increase can be attributed to treatment, why?

Oral tumors are uncommon neoplasms, but the absence of a dose response and a lack of statistical significance precludes a conclusion of a carcinogenic effect. Additionally, there was no evidence of other treatment-related effects in the oral cavity tissue available. Thus, the Panel concluded that the increase in oral tumor could not be attributed to treatment.

2.5.4: What, if any, is the significance of the squamous cell carcinoma of the alveolus of the tooth in one female Fischer 344 rat at 100/50 ppm?

The occurrence of a solitary tumor at the low dose cannot be attributed to the exposure without violating the basic concepts of toxicology. Squamous cell tumors of the oral cavity are not uncommon according to Haseman et al., 1998. In NTP two-year feeding studies, 10/1354 male and 12/1351 female rats had squamous cell papillomas of the oral cavity. In these same control groups, 0 males and 6 females had squamous cell carcinomas of the oral cavity. Similar rates are seen for inhalation studies with 13/905 squamous cell papillomas of the oral cavity for males and 8/903 squamous cell papillomas for females. In these same control groups, two males and six females had squamous cell carcinomas of the oral cavity. When the squamous cell carcinomas become larger, they often involve teeth and it is very difficult to determine whether the site of origin is the alveolus of the tooth or other areas of oral epithelium. Thus, this tumor is highly unlikely to be related to the administration of the compound.

Question 2.5: Does the SAP agree with the proposed CARC classification of malathion as "suggestive?" Why or why not?

The Panel was almost equally divided on its recommendation of the proposed CARC classification of malathion. The Panel was almost equally divided between "suggestive" and "not likely to be carcinogenic to humans" classification. One Panel member indicated that the classification should be "likely".

About half of the Panel members agreed with the HED CARC classification of malathion as ``suggestive" based on the occurrence of liver tumors in male and female B6C3F1 mice and female Fischer 344 rats at excessive doses and the presence of a few rare nasal respiratory epithelial adenomas in male and female rats ($P<0.025$). The nasal and oral tumors could not be

distinguished as either treatment related or due to random occurrence. In addition, there was a statistically significant increasing dose-response trend in female mice and the tumor incidence rates of the two low dose groups (100 ppm and 800 ppm) were higher than the tumor rates observed in the concurrent control group. Also, there was a statistically significant trend ($P<0.04$) for thyroid follicular cell tumors in male rats and testicular interstitial cell tumors occurred earlier in rats ($P<0.05$) exposed to as low as 500 ppm of malathion. There was no mechanistic data to support an existence of threshold effect. There were evidences that malathion and/or malaoxon caused sister chromatid exchanges and/or other mutagenic effects.

An almost equal number of Panel members concluded that "suggestive" was not supportable because the tumor increases occurred only under conditions of toxicity that are not relevant to humans. In particular, at the exposures at which benign liver tumors were increased, the animals exhibited reduced body weight gain, hepatomegaly and liver cell hypertrophy. These latter findings are indicative of metabolic overload. In the opinion of some Panel members, a weight-of-the-evidence evaluation of the animal carcinogenicity data on malathion and malaoxon best fits the category of "not likely to be carcinogenic to humans". There is neither a positive nor biologically significant tumor response for any organ site after discounting dose-groups in which there was marked toxicity. The tumor responses noted in these studies were unequivocally a result of excessive toxicity and have no relevance to any possible exposure scenario that could be encountered by humans. The exposures at which no tumors were increased and the much lower environmental exposures are unlikely to constitute a human cancer hazard. Tumor outcomes in the bioassays were limited to rodent liver tumors at excessive exposure concentrations and the liver is an important site of malathion metabolism. The rare nasal and oral palate tumors that were found cannot be attributed to malathion exposure. Liver tumors in male and female B6C3F1 mice occurred only at exposure concentrations that exceed MTD levels. These tumors were all benign with a lack of carcinomas noted, an unusual finding in mouse bioassays with hepatocarcinogens. The occurrence of liver tumors in female F344 rats was similarly limited to adenomas at concentrations that exceeded the MTD level. There was no evidence for mutagenic concern. Malaoxon was not carcinogenic in male or female F344 rats.

Some Panel members expressed the opinion that if the highest doses are given no weight because of excessive toxicity, then the study did not include a MTD. Without including a MTD, testing would be inadequate to conclude that malathion is “not likely to be carcinogenic to humans”.

One Panel member differed with the recommendations of either “suggestive” or “not likely to be carcinogenic to humans”. Instead, the Panel member proposed that the appropriate classification should be "likely". The basis for this decision was that the downgrading of the estimation of the carcinogenic properties of malathion from "likely" to "suggestive" with no new data was to be expected given the data analytic techniques employed by CARC 2. The Panel member suggested that the exclusion of selected groups and the changing of selected diagnoses impacted the association. That is, the *post hoc* exclusion of the two highest exposure groups, with knowledge of their tumor rates was a seriously flawed procedure. The highest exposure

groups are needed to achieve statistical power. The power to find an effect is fixed by three variables: the alpha level selected, the size of the effect under study, and the number of subjects. Reducing the dose level can be expected to reduce the number of tumors seen. With a sample size of 54 subjects in each group, the power to find a relatively small effect is between 0.4 and 0.6. This means that without the highest exposure groups, there is only a 40 to 60% chance of finding a true carcinogenic effect. This Panel member also believed that the carcinogenicity animal studies were confirmatory studies with well spelled out hypotheses and methods and, as such, they do not permit exclusion of subject groups.

Second, this Panel member noted that CARC 2 dismissed the positive studies of sister chromatid exchange, stating that the doses were excessive without mentioning two papers indicating that malathion is genotoxic, which may be due to oxidating properties on DNA (Blasiak et al. "*In vitro* studies on the genotoxicity of the organophosphorus insecticide malathion and its two analogues". *Mutation Research* 1999; Blasiak and Kowalik. "Protective action of sodium ascorbate against the DNA -damaging effect of malaoxon". *Pesticide Biochem and Phyisol*. 65: 110-118. 1999). These papers were made available at the Panel meeting and should be a part of the CARC database and decision-making.

However, other Panel members suggested that "likely" to be an inappropriate classification because there is neither strong experimental evidence of carcinogenicity nor does the evidence implicate the tumor increases (mouse and rat liver adenomas) to be due to a mode of action relevant to humans. Notably there was no evidence for a DNA-reactive mechanism.

Issue 3. There were several other neoplastic lesions that the CARC considered and determined not to be indicative of a carcinogenic potential of malathion. These included: mouse liver tumors at low doses in males, male rat nasal tumors, male rat thyroid follicular cell tumors, male rat thyroid C-cell tumors, rat interstitial cell testicular tumors, male rat liver tumors, male rat leukemia, female rat pituitary gland tumors, and female rat uterine tumors (various types). The CARC considered and determined that leukemia in the male rat was not related to malaoxon treatment.

Question 3. Does the SAP agree that these tumors are not related to malathion treatment and as such do not contribute to the weight of the evidence?

In addressing this question, the SAP called attention to the complexity of animal chronic toxicity/carcinogenicity studies. There are approximately 40 different tissues routinely examined histopathologically from each animal. By chance alone, a few of these endpoints will show an increase or decrease in a given tumor type. To account for this, the endpoint showing a difference from the controls needs to be examined for its biological importance as well as its statistical significance. This is accomplished in many ways. Foremost is consideration of whether the tumor in question is of a type that would be expected to be due to exposure to the chemical. Added weight is given to the finding if: (1) it occurs in multiple exposure groups, (2) occurs in both sexes, (3) is supported by precursor lesions, e.g. hyperplasia/dysplasia, weight changes in the

target organ, (4) is found in a dose-related fashion and, (5) is supported by findings in other studies of the chemical and in other species. The endpoint must also be examined as to whether the most ideal representation of the data is employed, e.g,. for a given type of tumor, is it more appropriate to examine benign and malignant neoplasms individually, or is it better to evaluate them combined? Thus, the Agency needs to consider each of these issues for each tumor type noted in the question.

The Panel concluded that none of the neoplasms (i.e. male rat liver tumors, male rat leukemia, female rat pituitary tumors, and rat uterine tumors) were related to exposure of technical grade malathion. The Panel provided a detailed analysis of each neoplasm below. Finally, the Panel agreed that there was no evidence of a treatment related increased incidence of leukemia in male rats after life-time exposure to malaoxon. Several Panel members expressed the opinion that the Agency should examine the results of additional statistical analysis or await confirmation that appropriate statistical methods were employed in the examination of the mononuclear cell leukemia response in the male rats exposed to technical grade malathion. However, these suggestions should not limit the Agency's ability to conduct a risk assessment. Even though this additional information would be helpful, the Panel did not conclude this tumor in Fischer 344 rats can be attributed to malathion exposure due to the incidence and pattern of dose response.

An animal carcinogenicity study is conducted to determine if the test chemical can significantly increase tumor incidences or significantly reduce time of tumor onset in animals. One factor that affects the analysis of tumorgenicity data is the animal survival time. A high degree of animal mortality, due to either treatment toxicity or tumor lethality, will cause a significant censoring of the tumor response. Comparisons should be adjusted for the survival time because the crude tumor incidence rate can be biased by the differential mortality across groups.

A survival-adjusted method, that has been widely accepted, e.g., US Food and Drug Administration and International Agency for Research on Cancer, is based on the ``context of observation" (cause-of-death) developed by Peto et al. (1980). In the ``context of observation" approach, tumors are classified as either ``incidental" or ``fatal". Tumors that do not alter an animal's risk of death and are observed only as the result of a death from an unrelated cause are classified as an incidental context. Tumors that affect mortality by either directly causing death or indirectly increasing the risk of death are classified as a fatal context.

An alternative survival-adjusted method is the so-called Poly-3 test (Bailer and Portier, 1988; Bieler and Williams, 1993). The Poly-3 test modifies the Cochran-Armitage test to account for the survival times of those animals that die prior to study termination without tumor presence. The Poly-3 test recently has been adopted by the NTP as a standard test for the analysis of tumorgenicity data.

Statistical methods used in the analyses were generally appropriate with an exception of the mononuclear cell leukemia in male rats (below). Additionally, the interpretation of statistical

significance for the testicular tumors in male rate is incorrect, regardless of biological relevance of this particular tumor type.

A description of each tumor type is described below.

Mouse liver tumors at lower doses - there is no evidence that the tumors observed at lower doses are treatment related. The primary reason for this conclusion is that they are not statistically different from the controls and are in the range that would be expected in this strain of mouse at 18 months of age.

Male nasal tumors - as the Panel noted in response to questions 2.4.1 and 2.4.2, it concluded that the tumors appear to be rare. However they have occasionally been found as spontaneous lesions in the anterior nose. In addition, the Panel concluded that the increase in nasal respiratory epithelial tumors was unlikely due to malathion treatment, however it can not be unequivocally dismissed.

Male rat thyroid follicular cell tumors - the most appropriate assessment of this tumor is to examine the adenomas/carcinomas combined. The reason for this is that this tumor is a continuum in its natural history, i.e., progresses from hyperplasia, to adenoma to carcinoma. The criteria used for differentiating these lesions, particularly from adenoma to carcinoma, are quite arbitrary and probably do not have a great deal of meaning as to the potential of the lesion. This is true for most tumors involving endocrine organs. The thyroid tumors were statistically significant by Peto's Prevalence Test, which considers the age of the animal at the time of tumor recognition and gives more weight to tumors found at an earlier age. It seems reasonable to conclude that this statistical event is not related to malathion exposure because the *p*-value was so weak ($P<0.04$) and is not supported by any pairwise comparison.

Male rat thyroid C-cell tumors - the most appropriate assessment of this tumor is to examine the adenomas/carcinomas combined for the reasons given above for assessment of follicular cell tumors. While there was a pair-wise statistical difference for carcinomas at the high dose, this was not apparent when adenomas and carcinomas were combined, nor was there any dose trend.

Rat interstitial tumors of the testes - this is the most common neoplasm found in the F344 rat, occurring in close to 100% male rats surviving to 2-years. It is invariably benign, typically bilateral, and is never the cause of death. In addition, it can be found in a high proportion of rats as early as 12 months. Since there was excellent survival until 18 months (a time at which almost all of the rats would have this tumor), but a precipitous increase in mortality after this time, the Panel concluded that the statistical significance (only by Peto's Prevalence Test) is not biologically supportable.

The Peto's prevalence analysis showed statistical significant trend and significant differences in the pair-wise comparisons of the 500 ppm, 6,000 ppm, and 12,000 ppm dose groups. In the 500 ppm group, the interstitial cell tumors occurred earlier ($P<0.05$), which is not considered

particularly significant for a common tumor. The test compares the development of a tumor among control and dosed groups during the time course of the experiment. There are sufficient numbers of animals that died during the experiment (in all five groups). The numbers of deaths before the terminal sacrifice were 18, 14, 26, 39, and 55 for 0, 100/50, 500, 6,000, and 12,000 ppm, respectively. If there was no treatment effect, then the proportion of the animals with the testicular tumor observed in the control and the dosed groups should be no different at any time point in the experiment. That is, approximately the same proportion of animals with this tumor will be observed in all groups. Since this is a non-lethal tumor, there is an equal chance of death from toxicity for the animals in the dosed groups, regardless of the presence or absence of this tumor.

Male rat liver tumors - a review of the pathology data in the PWG report does not show any statistical increase in liver tumors in males.

Male rat leukemia - this is a common neoplasm in the F344 strain of rat and is readily influenced by diet and body weight during the course of the study. The only statistical event was in the low dose group, which had an incidence of twice the controls (18/55 vs 9/55). The Panel concluded this was a spurious observation because it was not supported by an increase at the next highest dose and or by Peto's Prevalence Test. In addition, it did not meet a level of significance ($p<0.01$) required for a commonly occurring tumor.

Female rat pituitary tumors - a review of the pathology data in the PWG report does not show any statistical increase in pituitary tumors.

Rat uterine tumors - a review of the pathology data in the PWG does not show any statistical increase in uterine tumors.

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