Background Document for the Session:

A Consultation on the Proposed Health Effects Division Classification of the Human Carcinogenic Potential of Malathion

presented by:

Dr. Marion Copley
Health Effects Division, Office of Pesticide Programs,
United States Environmental Protection Agency, Washington, D.C.

To the:

FIFRA Scientific Advisory Panel,
Arlington, Virginia
August 16 and 17, 2000

Background

On 24-September, 8-October, 15-October-1997, 10-June-1998, 24-February-1999 and 23-June-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) 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 (NCI/NTP).

On 12-April-2000, the CARC met to evaluate: (1) a new Pathology Working Group (PWG) report submitted by the pesticide registrant on the female Fischer 344 rat liver tumors; (2) two issues raised by Dr. Brian Dementi regarding the evaluation of malathion (items #4–mononuclear cell leukemia in Fischer 344 male rats and #7–oral tumors in Fischer 344 female rats from Attachment 25); (3) the 29-March-2000 letter from Jellinek, Schwartz & Connally, Inc. to Patricia Moe, "Re: Comments on EPA's Risk Assessments for Malathion;" (4) and, discuss the weight of evidence and cancer classification for malathion based on the previously listed information.

Groups of **B6C3F1 mice** were fed diets containing **malathion** at 0, 100, 800, 8000 or 16,000 ppm for 18 months. The Committee concluded that in mice, the 800 ppm dose level was adequate to assess the carcinogenic potential of malathion 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. 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 as well as decreased absolute body weight compared to controls (9.7 to 20%) in both sexes. Groups of **Fischer 344 rats** were fed diets containing **malathion** at concentrations of 0, 100/50, 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. The Committee concluded that the 500 ppm dose in males was adequate to assess the carcinogenic potential of malathion based on a non-statistical, but biologically significant increase in mortality at this concentration (47% as compared to 33% in controls); and a decrease in plasma cholinesterase (29%, $p \leq 0.01$). In females, the 6000 ppm dose was considered adequate based on a decrease in plasma, RBC and brain cholinesterase (61, 44 and 18 %, respectively). This dose was one-half the next dose where mortality was increased. Toxicity at 6000 ppm in males was considered 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. The Committee concluded that the dose level of 1000 ppm was adequate to assess the carcinogenic potential of malaoxon because it was one-half the dose (2000 ppm) causing excessive toxicity. 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.

The Committee concluded that there was evidence of carcinogenicity in both sexes of mice at the two highest dose levels of malathion tested which were considered excessive. Evidence for carcinogenicity in mice was demonstrated by the presence of liver tumors in both sexes. There was no evidence of carcinogenicity in male or female mice at the lower doses. **The Committee further concluded that there was evidence of carcinogenicity for malathion in female rats** at the highest dose, although this dose was considered excessive. **The Committee determined that the oral (females at 6000 and 12,000 ppm) and nasal tumors (females at 6000 and 12,000 ppm and males at 12,000 ppm) could not be distinguished as either treatment-related or of random occurrence.**

Liver Tumors - Mice. In **male 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 (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 zero in the controls, none of the incidences showed 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%) 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/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 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. There was no treatment-related increase in liver tumors in **male rats**. In **female rats**, there was a positive trend (p = 0.005) for adenomas. The incidence of adenomas was significantly increased by pair-wise comparison at 12,000 ppm (5/38, 13%, p = 0.009) when compared to controls (0/41). There were no carcinomas in any group. These incidences are based on the April 2000 PWG reread of the female rat livers.

When compared to the historical control data of the testing laboratory, the incidences of adenomas at 12,000 ppm (13%) exceeded the historical control range (0 to 5%) and mean (1.6%). In addition, the incidence of adenomas exceeded the historical control incidence (adenomas, 0.44%) of the NTP (1998 report).

The Committee concluded that, although the incidence of liver tumors in female rats was observed only at an excessively toxic dose (12,000 ppm), it provided evidence of carcinogenicity because: (1) the incidence was statistically significant by pair-wise comparison; (2) there was a statistical trend; (3) the incidence was outside the range of both the testing facility and NTP historical control data bases.

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. The incidence of nasal adenomas of the respiratory epithelium in this study (1 in the 6000 ppm females and 1 at 12,000 ppm in both sexes) exceeded the historical control incidence (0/240 males and 0/240 females). In addition, the NTP (1990 report, combined dietary and inhalation studies) reported respiratory tract tumors in the respiratory epithelium of 6/4000 male rats, in the olfactory epithelium of 0/4000 males and none of either type in females. Furthermore, four of these 6/4000 respiratory epithelial tumors were squamous cell tumors, not adenomas of the respiratory epithelium. Therefore, the relevant historical control incidence for respiratory epithelial adenoma is only 2/4000 in males.

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 biological significance of the adenoma of the olfactory epithelium (6000 ppm male) is unknown since it is from a different cell of origin and this type of tumor (esthesioneural epithelial neoplasm) should not be combined with other tumors of the respiratory nasal cavity. The Committee postulated that direct contact with malathion (by volatilization from the feed or by inhalation of the feed through the nose) was a possible explanation for the nasal tumors. However, there was no evidence to support or refute that the tumorigenicity was due to exposure by the inhalation or systemic route. To the contrary, the tumors occurred in section five, a section where there was little to no evidence of increased inflammation. Therefore, the Committee concluded that a systemic effect could not be unequivocally ruled out.

The Committee concluded that it could not determine whether nasal tumors were either treatment-related or due to random occurrence. 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 6000 ppm (not an excessive dose); (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. The CARC also concluded that for males, the biological significance of the single olfactory epithelial tumor at 6000 ppm is unknown, since it is from a different cell of origin (esthesioneural epithelial neoplasm) and this type of tumor should not be combined with nasal respiratory epithelial neoplasms.

Oral Cavity Tumors - Rats. In **male rats**, there was one **squamous cell papilloma** of the palate at 100/50 ppm compared to zero in all other groups, including controls. In **female rats,** there was a **squamous cell carcinoma** of the alveolus of the tooth at 100/50 ppm, a **squamous cell papilloma** of the palate at 6000 ppm and a **squamous cell carcinoma** of the palate at 12,000 ppm compared to zero of all three tumor types in the controls. There is considerable uncertainty however, as to the actual incidence of these tumors and how many animals had this tissue examined since the oral mucosa was not considered a routine tissue for histologic examination.

The single occurrence of a low dose tumor in males was considered to be incidental background since there were no tumors at the higher doses, even with the large dose spread from 100/50 to 12,000 ppm. For females however, the incidence of oral squamous cell tumors in this study (1 at 6000 ppm and 1 at 12,000 ppm) exceeded the historical control incidence from inhalation studies at the testing facility (0/240 males and 0/240 females). In addition, the NTP historical control summary (1998 report) reported: squamous cell papilloma - females 2/901 (0.22%), squamous cell carcinoma - females 0/901 (0%).

It was difficult to judge the significance of the low dose alveolar tumor since the oral cavity was not routinely examined in this study and the tumor was only seen in one low dose female. Of the two oral palate tumors, one at each of the two high doses, only the one adenoma in the 6000 ppm female was at a dose that was not considered excessive.

The Committee concluded that it could not determine whether the oral cavity tumors in females were treatment-related or due to random occurrence. 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) 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 are 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; (3) due to the lack of systematic pathologic evaluation of the oral mucosa, there is uncertainty as to the actual incidence of oral tumors. However, the CARC determined that a recut would not alter their conclusion.

The Committee concluded that the following tumors are NOT treatment related:

Male rats - (1) **thyroid gland (follicular cell)** - there was neither statistical (other than a positive trend for combined adenomas and carcinomas) nor biological significance for any tumor type. Although there was no evidence that the above tumors are treatment related in rats at any dose level, 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. There is, however, no evidence to either support or refute this supposition.
(2) **thyroid gland (C-cell)** - there was neither statistical (other that carcinomas in the 500 ppm group) nor biological significance, there was no dose-response relationship, and the combined tumor incidences in treated groups were comparable to those seen in the concurrent control group.
(3) **testes (interstitial cell)** - tumor incidences of this nonfatal tumor were approaching 100% in all groups including controls, and positive statistical significance was considered to be an artifact in the Peto's Prevalence Analyses due to high mortality rather than biological significance.
(4) **liver** - there was neither statistical nor biological significance and there was no dose-response relationship. Although there was no evidence that the above tumors were treatment related in rats at any dose level, 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. There is, however, no evidence to either support or refute this supposition.
(5) **mononuclear cell leukemia** (MCL) - there was no indication of increased incidence or early onset, this tumor occurs commonly in Fischer 344 rats, the incidences were within historical control ranges, there was no statistical significance at any dose, and there was no dose response. Further more: (a) the CARC considered attributing the cause of death to MCL as subjective and not a reliable indicator of increased severity of this tumor; (b) using the incidence of deaths in leukemic animals caused by MCL as a measure of severity is not reliable because establishing a cause of death is subjective in older rats with possible multiple aging processes.

Female rats - (6) **pituitary gland (par distalis)** - the tumor incidences and types in treated groups were comparable to those seen in the concurrent control group; there was neither statistical nor biological significance; and there was no dose-response relationship.
(7) **uterus (various types)** - the individual tumor incidences were low, the tumor incidences and types in treated groups were comparable to those seen in the concurrent control group; there was neither statistical nor biological significance; and there was no dose-response relationship.

Results of the guideline genetic toxicology studies with malathion indicated 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*. The CARC included that *in vitro* and *in vivo* findings from the open literature should be interpreted with caution since positive results were seen at cytotoxic doses and/or the types of induced aberrations were asymmetric and, therefore, not consistent with cell survival. The question of test material purity in several of these studies was also an issue. Although the structure of malathion suggests electrophilicity, **the Committee concluded that the weight of the evidence supports neither a mutagenic hazard nor a role for mutagenicity in the carcinogenicity associated with malathion.**

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. 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. Malaoxon has a structure similar to malathion; hence, the possibility of electrophilicity may also apply, despite the evidence of no carcinogenicity.

In accordance with the EPA *Guidelines for Carcinogen Risk Assessment* (Draft, July 1999), the Committee 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: (1) occurrence of liver tumors in male and female B6C3F1 mice and in female Fischer 344 rats only at excessive doses; (2) 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; (3) the evidence for mutagenicity is not supportive of a mutagenic concern in carcinogenicity; and (4) 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 "suggestive" classification was supported by the majority of the CARC members present at the meeting. Several of the members of the CARC present at the meeting thought that the evidence for malathion's cancer potential was weaker than a "suggestive" classification. There were two votes for, "data are inadequate for an assessment of human carcinogenic potential" and two votes for "not likely to be carcinogenic to humans." These opinions were based, in part, on the consideration that: (1) the increase in liver tumors was due to hepatocellular adenomas (benign tumors); (2) there was no statistical significance at non-excessive doses (significance only in the presence of excessive toxicity); (3) the oral and nasal tumors were not considered treatment-related. In addition, they believed that the dose range for malathion's cancer effects was well defined and limited to excessive or near excessive doses. One member abstained.

ISSUES FOR THE SCIENTIFIC ADVISORY PANEL

NOTE: The actual questions are identified by a question mark "?" to the left of the question.

Issue 1. The HED 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 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?

? Question 2.2: Does the SAP consider the statistically significant trend and pair-wise increases in liver tumors in female B6C3F1 mice at 8000 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 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 6000 ppm (not an excessive dose); (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 5 nasal sections per rat. Historical control studies usually have only 1 or 2 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 6000 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 6000 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; (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; (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 5 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?

? 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?
