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

013721

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

December 1, 1998

SUBJECT: Review of Pathology Working Group Report (PWG) Peer Review of Proliferative Lesions of the Liver in Male B6C3F1 Mice in an 18-Month Oral (Dietary) Oncogenicity Study in Mice of Malathion (MRID 44554901)

FROM: Brian A Dementi, Ph.D., D.A.B.T.
Toxicology Branch I
Health Effects Division (7509C)

THRU: Alberto Protzel, Ph.D.
Senior Branch scientist
Toxicology Branch I
Health Effects Division (7509C)

TO: Dana Lateulere
PM Team 53
Special Review and Reregistration Division (7508W)

TO: Diana Locke
Toxicologist
Reregistration Branch 2
Health Effects Division (7509C)

Registrant: Cheminova Agro A/S
Chemical: Malathion
Case No.: 818961 Submission No.: S544067
DP Barcode: D246737 PC Code: 057701
MRID No.: 44554901

Action: Review the subject Pathology Working Group Peer Review of the carcinogenicity study of malathion in male mice.

Conclusion: Presented below are the citation and executive summary of the reviewed study;

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the Review follows.

Citation: Pathology Working Group (PWG) Peer Review of Proliferative Lesions of the Liver in Male B6C3F1 Mice in an 18-Month Oral (Dietary) Oncogenicity Study in Mice of Malathion. Environmental Pathology Laboratories, Inc., Research Triangle Park, NC. EPL Project No. 297-003. May 8, 1998 (MRID No. 44554901).

Executive Summary: Toward fulfilling a requirement of HED's Carcinogen Assessment Review Committee for the re-evaluation of microscopic slides for liver tumor response among male mice in the mouse carcinogenicity study (MRID 43407201), the Sponsor has submitted results of a Pathology Working Group (PWG) convened in April 28-29, 1998. The PWG defined the nomenclature and diagnostic criteria used to classify the proliferative hepatocellular lesions. Through invoking this definition, the PWG assessment resulted in a number of revised interpretations as to the identity of lesions, as discussed in this review. The combined incidences of adenomas and carcinomas in the original evaluation were 2%, 19%, 9%, 33% and 96% as contrasted with the results of the re-evaluation of 7%, 19%, 16%, 27% and 96%, respectively for the 0, 100, 800, 8000 and 16000 ppm dose groups.

In consideration of these findings taken in concert with the finding of hepatocellular hypertrophy and differing tumor morphology in the 8000 ppm and 16000 ppm groups, the PWG concluded that malathion was tumorigenic in male mice at these two dose levels, but not at the lower doses. The PWG identified a LOEL of 8000 ppm for this effect. The PWG also noted that 8000 ppm exceeded the limit dose. This conclusion was based on the finding of adenomas. It should be noted that according to this re-evaluation, the NOEL would be 800 ppm, with no further assessment between this dose and the limit dose.

This review of the PWG report recommends that the re-evaluations of the very microscopic slides examined by the PWG be classified as **Acceptable.** However, this review documents a number of issues that would question the PWG's interpretation of the study as to the carcinogenicity of malathion. Thus, this reviewer recommends against accepting the PWG's conclusions beyond those of the interpretation of slides, and refers the question of interpretation of carcinogenicity and any additional work that may be needed to HED's Carcinogen Assessment Review Committee.

REVIEW OF PATHOLOGY WORKING GROUP REPORT

I. BACKGROUND

The HED Carcinogen Assessment Review Committee (CARC) convened during September and October 1997 to consider the malathion cancer asessment data base elected to require a Pathology Working Group (PWG) re-evaluation of the pathology readings of the male mouse liver slides from the recent malathion study in B6C3F1 mice, MRID 43407201. Principal matters of concern in this study were the high incidences of hepatocellular tumors, particularly at the high dose, and

the statistically significant finding of such tumors among male mice at the lowest dose level. The incidences of hepatocellular adenomas and carcinomas as presented in the May 8, 1997 report of HED's Lori Brunsman, which were revised somewhat with respect to those incidences reported in the February 10, 1995 DER, are presented as follows:

Dietary Concentration of Malathion (ppm):	0	100	800	8000	16000
Hepatocellular Adenomas (%):	2	11	4	24	96
Hepatocellular Carcinomas (%):	0	11	5	11	2
Combined Adenomas and Carcinomas (%):	2	19	9	33	96

These above tabulated incidences do not incorporate multiple tumor findings, e.g., a liver having both an adenoma and a carcinoma is simply counted as an incidence of carcinoma.

On April 27-28, 1998, Environmental Pathology Laboratory, Inc (EPL) conducted the PWG assessment, as required, on the male liver tumor data. The report of that assessment, dated May 8, 1998, entitled Pathology Working Group (PWG) Peer Review of Proliferative Lesions of the Liver in Male B6C3F1 Mice in an 18-Month Oral (Dietary) Oncogenicity Study in Mice of Malathion, has been submitted to the Agency (MRID 44554901), and is the subject of this review. The PWG was chaired by Dr. Jerry Hardisty (EPL), who organized and presented the material to a panel of five pathologists: Dr. Robert Geil (study pathologist), Dr. Robert Mann (reviewing pathologist) and Drs. Ray Brown, James Swenberg and Jerrold Ward, PWG consultant pathologists. Also present at the meeting as observers were Ms. Meena Sonawanee and Dr. Judy Hauswirth, both of Jellinek, Schwartz and Connolly, Inc. and Dr. Mike Ioannou and Dr. Brian Dementi of EPA.

II. The PWG Report

i) Review Procedure

According to the study report, the procedure was as follows: the reviewing pathologist first examined all slides from the study. All hepatocellular lesions identified by either the *study pathologist during the initial examination* or the *reviewing pathologist during the peer review* were referred to the entire PWG for purposes of re-evaluation. "The PWG examined coded slides without knowledge of treatment group. The PWG examined all slides containing sections of liver with a previous diagnosis of either altered foci of hepatocytes or an hepatic neoplasm reported either by the study pathologist or the reviewing pathologist. Each participant recorded his diagnoses and comments on worksheets which were prepared by the PWG chairperson. The PWG examined the slides only for proliferative hepatocellular lesions and did not consider other systemic neoplasms (such as lymphoma or histiocytic sarcoma) or nonproliferative lesions which may have also been present. Each lesion was discussed by the group, reexamined if necessary, and the final opinions were recorded on the chairperson's worksheets. The consensus diagnoses of the PWG were reached when at least three of the five PWG participants were in agreement.

"After the PWG completed the slide review and the diagnoses were recorded by the PWG chairperson, the slides were decoded and the microscopic findings were tabulated by treatment group. No changes were made to the consensus diagnoses after the slides were decoded by

treatment group. PWG consensus diagnoses for individual animals reviewed in each group are presented in Appendix A." (pp. 13-14 of the study report)

The study report contains a signed Statement of No Data Confidentiality Claims, a signed Certificate of Good Laboratory Practices and a signed EPA Flagging Criteria Statement.

ii) Results of PWG Review

As the result of the re-evaluation of original slides from the study, there were in the consensus of the peer review members, revisions in interpretation of certain slides with respect to those in the original study report. The revised incidences of adenomas, carcinomas and the combinations of the two are tabulated as follows, presenting both the original tumor data at termination in the original study and revised tumor incidences, for purposes of direct comparison:

Dietary Concentration of Malathion (ppm):	0	100	800	8000	16000
Hepatocellular Adenomas (%)					
Original:	2	11	4	24	96
Re-evaluation:	7	15	13	25	96
Hepatocellular Carcinoma (%)					
Original:	0	11	5	11	2
Re-evaluation:	0	7	4	4	0
Combined Adenomas and Carcinomas (%)					
Original:	2	19	9	33	96
Re-evaluation:*	7	19	16	27	96

(Re-evaluation % calculated by reviewer from incidence data in PWG report disclosed in Attachment 3; *data not combined in PWG study report)

The HED statistical treatment of the combined incidence data, and incidence data from the PWG report (p. 16) are appended (Attachment 3).

iii) Discussion of the PWG Report

In comparison with the original interpretations, changes of diagnoses are reviewed as follows: In the control group, the study pathologist had identified 1 adenoma and 3 basophilic foci. However, PWG consensus was to call all three foci, adenomas. Thus by re-evaluation there are 4 adenomas in the control group. *None of the pathologists identified carcinoma in the control group*. It should be noted that the reviewing pathologist had offered the interpretation of 2 adenomas and 2 basophilic foci. This is mentioned by way of indicating there was not unanimity among the five pathologists, as was also true to varying degrees in other dose groups. In the 100 ppm group, the study pathologist had identified 4 adenomas and 6 carcinomas, while the PWG interpreted 2 of the 6 carcinomas to be adenomas, yielding 6 adenomas and 4 carcinomas. In the 800 ppm group, the study pathologist had identified 4 basophilic foci, 2 adenomas and 3 carcinomas, whereas the PWG consensus opinion was to upgrade all basophilic foci to adenomas and to down grade one carcinoma to adenoma yielding 7 adenomas and 2 carcinomas. In the 8000 ppm group, the study pathologist identified 2 eosinophilic foci, 12 adenomas and 6 carcinomas, while the PWG, in finding an additional eosionphilic focus, downgrading certain

adenomas to eosinophilic foci and certain carcinomas to adenomas, yielded revised incidences of 6 eosinophilic foci, 13 adenomas and 2 carcinomas. In the 16000 ppm group, there was little difference between the study pathologist's interpretations and those of the PWG. Adenomas (often multiple) were found in essentially all animals. The study pathologist had identified one carcinoma that the PWG called adenoma.

The PWG report makes note of the fact that in the original final report for the study, treatment-related increases in liver weight were observed in male mice at 8000 and 16000 ppm and that treatment-related hepatocellular hypertrophy was characteristic of mice in the 8000 ppm and 16000 ppm dose groups, being more severe in the 16000 ppm group. Hypertrophy was not evident in the control or lower dose groups. The PWG also makes note of the fact that morphologic appearance of most of the adenomas in the 16000 ppm group and the majority of the 8000 ppm group was different from that of the adenomas in the control and lower dose groups. All adenomas in the control, 100 ppm, 800 ppm and a few in the 8000 ppm groups were more typical in appearance to those of spontaneous hepatocellular adenomas in B6C3F1 mice. The PWG report makes note of the fact that a few carcinomas were identified in the 100, 800 and 8000 ppm groups, while none were observed in the control or 16000 ppm groups. Most of these were single solitary masses at gross necropsy and were diagnosed as single hepatocellular carcinomas. Mention is made of the fact that multiple carcinomas were diagnosed by the PWG in two of the 100 ppm dose group mice. The PWG report advises that the historical control data base for carcinoma from the performing laboratory is limited, but the range was from 0% to 6.38%. This is to be compared with incidences of carcinoma of 7%, 4% and 4% in 100, 800, and 8000 ppm groups, respectively. As to the relevance of carcinoma findings in this study, the PWG concluded these were not the result of treatment, as there was no dose response and no evidence of progression of the adenomas to carcinomas in the high dose group.

iv) PWG Conclusions

The PWG re-evaluated liver histopathology for male mice in the recent 18-month carcinogenicity study of malathion in the B6C3F1 mouse (MRID 43407201). This re-evaluation resulted in a number of revised interpretations as to the identity of lesions, as discussed in this report. The combined incidences of adenomas and carcinomas in the original evaluation were 2%, 19%, 9%, 33% and 96% as contrasted with the results of the re-evaluation of 7%, 19%, 16%, 27% and 96%, respectively for the 0, 100, 800, 8000 and 16000 ppm dose groups.

In consideration of these findings taken in concert with the finding of hepatocellular hypertrophy and differing tumor morphology in the 8000 ppm and 16000 ppm groups, the PWG concluded that malathion was tumorigenic in male mice at these two dose levels, but not at the lower doses. The PWG identified a LOEL of 8000 ppm for this effect. The PWG also noted that 8000 ppm exceeded the limit dose. This conclusion was based on the finding of adenomas. It should be noted that according to this re-evaluation, the NOEL would be 800 ppm, with no further assessment between this dose and the limit dose.

III. Reviewer's Discussion and Comment

i) Procedural Aspects

According to the study report, the purpose of the PWG was to determine the incidences of hepatic neoplasms in male mice following currently accepted nomenclature and diagnostic criteria and to discuss the relevance, for purposes of risk assessment of the hepatic neoplasms which occurred in the study (p. 10 of the study report). The PWG asserted that its evaluation was conducted in accordance with EPA Pesticide Registration Notice 94-5 (EPS, August 24, 1994). Actually, according to the January 7, 1998 letter of Walter Waldrop of SRRD to Blane Dahl of Jellinek, Schwartz & Connolly, Inc., (Attachment 1) the PWG was being asked to provide re-evaluations of pathology readings of slides according to PR 94-5 (Attachment 2). This notice provides a mechanism for registrants to submit revised pathology diagnoses following a designated peer review process, similar to the one used by NTP. *The question of relevance, for purposes of risk assessment should be viewed as a somewhat more complex matter that is the responsibility of the Agency.*

It is not certain that it was the purpose of the PWG, as requested by the Agency and under PR Notice 94-5, to do anything other than re-evaluate the previously generated histopathology slides, and to supply the results of the re-evaluations to the Agency for its own unique interpretation. Nevertheless, in addition to the results of the re-reevaluations, an interpretation has been rendered by the PWG that must be addressed. That particular interpretation is that malathion yielded a positive tumorigenic response at the top two doses, but not at the lower two doses. This reviewer is of the opinion that this is a much more complex study, interpretatively, than is portrayed in the PWG report, and that many concepts of carcinogenesis have gone unacknowledged. It is the duty of the Agency to consider important relevant information before merely approving or accepting the conclusions of this PWG.

One of the purposes for the presence of the observers, including this reviewer, at the PWG meeting was "to insure that all important questions are resolved." (July 24, 1997 letter of R. R. Maronpot to B. Dementi, Attachment 4; March 6, 1998 letter of W. Burnam to J. Hauswirth, Attachment 5). Indeed, several questions were asked and good answers provided. This was an effective process that should be proposed as standard procedure. Nevertheless, at the termination of the meeting, the reviewer raised a question concerning *macro*scopic pathology, namely, why so many liver lesions listed as "masses" in the low dose group while there were *none* so described in the control group. Response to my question was put off with the suggestion of Jellineck's representatives that we pursue the matter after the meeting. I had no complaint with that, as the needed data was not in hand, and everyone was preparing to leave. Upon returning to my office, I immediately set about examining the individual histopathology sheets from the study, and also had opportunity for the first time to read and study the 1980 publication on mouse liver tumors by Dr. Jerrold Ward, appearing in *Cancer Letters.* This publication by one of the PWG members, distributed by the chairman at the April PWG meeting, evidently serves as a source of guidance in such workshops. The publication claims, among other concepts, that with increasing size of hepatocellular adenomas, there is increasing likelihood that trabecular formations, viewed as evidence of carcinoma, will be found within the adenoma, i.e. "a tumor within a tumor". According to Dr. Ward's paper, a large fraction of adenomas having diameters in the 10 mm range are said to exhibit regions of trabecular formations, yet it is not clear in this publication how many histopathology sections are examined from large adenomas in order to establish whether carcinoma is present or not. Now my observations were that many of the "masses" in the low dose group of the malathion study were in this size range. So being curious as to whether

more than one section through large lesions would be necessary to rule out the real possibility of trabecular formations; and not knowing how many slides were taken in the malathion study nor whether the PWG was aware of the largeness of the tumors, I drafted a letter to Dr. Hardisty (Attachment 6). This letter was intended to be an informal letter written almost as if I were still at the meeting, continuing with questions. Furthermore, as stated previously, we left the PWG meeting with the understanding there would be a follow-up to my question regarding liver masses. I had intended that Dr. Hardisty be the recipient of my questions before the PWG was finalized. Accordingly, the letter was ready for faxing on May 4. However, for reasons beyond my control, it was not possible for me to send the letter until May 11. Unfortunately, by that time the PWG report was finalized. This having been said and done is now history, the letter is appended and Dr. Hardisty's June 4 letter of response addressed to M. Sonawane is also appended (Attachment 7).

ii) Scientific Aspects

As a result of the PWG assessment, the tumor incidence in the control group (Group 1) rose from 1 adenoma and 3 basophilic foci, identified in the original study, to 4 adenomas, **as tabulated above**. For two of these four adenomas, the study pathologist and reviewing pathologist (the only pathologists that read all slides in the study) identified them as basophilic foci, while the other three pathologists identified them as adenomas. So, the consensus was 3 to 2 in favor of the these two adenoma designations. One must pose the question, in the interest of the public health, should this level of certitude be considered acceptable where the assessment is so critical to the statistical significance of findings in dose groups? Similarly, a 3 to 2 split vote occurred in Group 3 for a number of adenoma vs basophilic foci interpretations. It should be emphasized that no carcinomas were identified in Group 1.

All 4 of the lesions in question in Group 1 were identified *macro*scopically as "nodules", having dimensions at the upper end of the "small" size for liver adenomas as described in Dr. Ward's 1980 paper. By contrast, in Group 2, of 13 *macro*scopic lesions, two were described as "nodules" having dimensions similar to those in Group 1, while 11 were described as "masses" of greater proportions, satisfying the "larger" and, in greater number, the "largest" tumor sizes as discussed in Dr. Ward's paper. A significant question is whether such large liver tumors identified after 90-104 weeks in Dr. Ward's publication would be expected after 78 weeks, as in the malathion study. **(Note, see Addendum at the end of this review, p. 18, for comments on the historical control data now received)**

In Group 2, the original assessment identified 10 mice with hepatocellular adenomas and/or carcinomas. One of these mice had a basophilic focus, not reported macroscopically, in addition to a carcinoma. The PWG confirmed the basophilic focus while revising the carcinoma to an adenoma in that mouse. According to the original study report, among the 10 mice involved, two mice had an adenoma and a carcinoma on differing liver lobes, a third had two carcinomas, one on each of two liver lobes and a fourth had a large carcinoma attached to two lobes, possibly, according to the PWG report, the result of two carcinomas that arose independently with subsequent fusion. In any event, whether one large carcinoma, or two that fused, this suggests an advanced stage for such a lesion for but an 18-month study. The net finding for Group 2 in the original study report was that of 10 mice with liver adenomas/carcinomas, where the number of

such tumors was 13 (possibly 14), due to the presence of 3 (possibly 4) instances of multiplicity. Lest there be any uncertainty, the one basophilic focus reported microscopically is not included in this tally.

The PWG agreed with the study report on all but two carcinomas in Group 2, which were concluded to be adenomas instead. So while the study report had identified 10 mice harboring 6 adenomas and 7 carcinomas (possibly 8 carcinomas), the PWG concluded that 10 mice were affected, having 8 adenomas and 5 carcinomas (possibly 6 carcinomas). The differences of opinion among pathologists for this dose group were over the question of whether the identified lesions are adenomas as opposed to carcinomas. One basophilic focus did not enter the picture for Group 2 as explained above. As contrasted with Group 1, where two of the four adenoma calls were on a consensus 3 to 2 split vote, in Group 2, involving 10 mice (13 and possibly 14 tumors), the pathologists agreed 100% as to diagnosis of 12 tumors and split 4 to 1 on two diagnoses. Hence, the consensus was much enhanced for this group over that of Group 1.

In order to facilitate interpretation of this study, discussion is offered here under headings that set forth recognized principles of carcinogenesis assessment that were not apparent in the PWG assessment, namely: ***1)*** *Definition of a carcinogen;* ***2)*** *Tumor progression;* ***3)*** *Variable mechanism of carcinogenesis.*

1) *Definition of a carcinogen.* The Office of Science and Technology Policy (OSTP) (1985) defines a carcinogen as follows: "*A chemical carcinogen may be a substance which either significantly increases the incidence of cancer in animals or humans or significantly decreases the time it takes a naturally occurring (spontaneous) tumor to develop relative to an appropriate background or control group.* ***Either*** *(emphasis added) phenomenon is said to represent the effects of a carcinogen.*" (pp. 10414-10415)

There is evidence of a tumorigenic response in the malathion study *both* in terms of increased tumor incidence and decreased time of tumor development, i.e. decreased latency.

Determination of incidence must include reliable evaluation of tissues. In response to my question concerning the number of slides taken from a "mass", Dr. Hardisty says in his June 4 letter a single such slide is usually prepared, and presumably was so in this case. He also indicates that morphologic criteria other than trabecular formations are considered when rendering a diagnosis of adenoma versus carcinoma. While I am certain this is true, Dr. Ward's paper seems to say trabeculation alone will suffice. It appears it would be questionable to dismiss it as diagnostic. I have examined others of his publications, e.g. Frith and Ward (1980), Ward (1984) and Jang et al (1992), and believe this is a correct rendering of his views. Furthermore, the same view is expressed in Maronpot et al (1987), a publication also distributed at the PWG meeting. Frith and Ward (1980) says "Few small liver neoplasms (< 5 mm) have this trabecular pattern, and it is more common in large tumors" (p. 338). Ward (1984) says: " In B6C3F1, C3H and other strains of mice, perhaps 40% of the large adenomas have foci of prominent trabecular formations (nodule in nodule, focal atypia, focal carcinoma) within the adenoma.", and "The trabecular foci or areas of atypia are almost never seen in situ or in small nodules in control mouse liver." (p. 8) Further along, "Grossly, carcinomas are large and have prominent blood vessels. *In control mice with a low incidence of liver tumors they are usually single, but are frequently multiple in mice*

exposed to a carcinogen." (emphasis added) (p. 9) These statements suggest that larger tumor **size** constitutes evidence of a more advanced stage of tumorigenesis and that largeness together with multiplicity is evidence of effects of a carcinogen. I am concerned that in cases where large "masses" are being characterized and where trabecular formations have been identified in certain of these in a particular dose group, based upon inspection of the one slide prepared, additional slides would be necessary for satisfactory diagnosis of remaining tumors in that group. As to this particular question, one expert I spoke with, Dr. Gary Williams of the Naylor Dana Institute for Disease Prevention, Valhalla, NY, says that one slide is good enough, deriving from his belief that all such liver tumors will eventually progress to carcinomas, such that the diagnosis of adenoma versus carcinoma is not that critical. In his view, its a neoplasm, and all neoplasms count in the assessment of carcinogenicity. However, when I raised the question of using adenoma versus carcinoma diagnosis to help establish progression (a topic discussed further in item 2 below) or stage of tumor development and, hence, latency, Dr. Williams acknowledged it becomes important. As relevant background, I should note that the National Cancer Institute Carcinogenesis Technical Report Series No. 1, "Guidelines for Carcinogen Bioassay in Small Rodents" (1976) says that multiple portions of tumors or masses shall be submitted if these are large or variable in appearance. (p. 54) In Reznik and Ward (1979), step sections were made of the livers from all mice with liver tumors in the carcinogenicity study of a hair-dye chemical. I was unable to find any other statements in Dr. Ward's work as to how many slides are prepared on a given liver or lesion for adequate diagnostic distinctions between adenoma and carcinoma.

Tumor size is recognized as a factor to be considered in the assessment or determination of benign versus malignant hepatocellular tumor incidence. In his June 4 letter, Dr. Hardisty makes it clear the PWG members were not aware of the gross description of tumors, only microscopic morphology, yet it is questionable whether conclusions as to a compound's carcinogenicity can be rendered on the basis of microscopic assessment alone. Reference is made here to Ward et al (1995) which defines the principles of " Peer Review in Toxicologic Pathology" that presumably guided this PWG for malathion, where among many other views the following quotation may be found: "Equally important, missing information of potential value in interpretation of effects should be identified during the review and included in the appropriate report(s) to include better characterization of the lesions (size, multiplicity, presence of related lesions, etc.)." (pp. 227-228). Indeed, the question of tumor size was raised by me at the meeting. OSTP (1985) says "The pathological examination, macroscopic as well as microscopic, is the cornerstone of the carcinogenicity study" (p. 10414); "In addition to tumor incidence at specific sites, the stage in the development of neoplasia should be evaluated. For example, the finding that the *majority* (emphasis added) of neoplastic lesions at a specific site is more advanced in a treated group compared to its control may provide additional evidence of a treatment-related effect." (p. 10377); and "Accurate interpretation of tumor data is contingent upon careful attention to gross observation ....." (p. 10377) The Interagency Regulatory Liaison Group (IRLG) (1979) says "General evaluation of neoplastic pathology for carcinogenesis bioassays includes consideration of the total number of animals with tumors in each group, the total number of individual tumors, and the index of tumor multiplicity in tumor-bearing animals. The tumor response can be further characterized by detailed observation of the tumor morphology and related preneoplastic changes. The extent of tumor growth and spread and special morphologic characteristics may give useful indications of the time of development of the neoplastic response.

The quality of the pathologic response is determined by a comprehensive evaluation of all of the pathologic changes observed in both treated and control animals." (pp. 254-255). Maronpot et al (1987) says under the topic of Hepatocellular Carcinoma: "The diagnosis of hepatocellular carcinoma is made when there is distinct trabecular or adenoid pattern, when the cells are poorly differentiated or anaplastic, and/or when there is histologic evidence of local invasiveness or metastasis. The distinction between hepatocellular adenoma and well differentiated hepatocellular carcinoma is relative and depends upon the perceived degree of cytologic differentiation, the internal and peripheral growth pattern, and the *size of the neoplasm* (emphasis added)." (p. 12)

One of my reasons in writing to Dr. Hardisty was to let him know of the macroscopic pathology in time for the PWG to consider it before submitting their report. Given the principles enunciated here, many parameters are important with respect to identifying a carcinogen on the basis of increased progression, i.e. decreased latency. Dr. Hardisty also speaks of differential tumor growth rates as opposed to time of onset in explaining tumor size. Actually, under the OSTP (1985) characterization of a carcinogen based on decreased (lag) time of spontaneous tumor development, either compound-induced earlier initiation or increased growth rate would be sufficient to establish a finding. Dr. Hardisty has no answer to my question concerning how common large tumors are in 18-month studies, so it would appear not to have been considered.

One question I posed concerned the *incidence of multi-lobed tumors (a weighing factor in evaluating incidence of benign versus malignant tumorigenic response)* in the 100 ppm dose group, where three such cases and probably a fourth were observed, compared to none in the control. Dr. Hardisty responded to my question by saying the evidence of the finding was infrequent and never involved more than two lobes. My view is that these may be relatively rare or uncommon events in control mice, particularly in 18-month studies, so their finding in the low dose group is of added concern, both in terms of incidence as rare, and as evidence of a more advanced stage. The PWG identified *none* in the control group that involved even two lobes.

Another question which I posed concerned *historical control incidences* of hepatocellular tumors. Dr. Hardisty says little about the adequacy of the performing laboratory's 18-month data base used to support interpretation in this study. Also, he feels no real need to distinguish adenomas from carcinomas in the data base, as in his words it will not likely change things much. This may be true in assessing tumor incidence alone (though PWG should have combined the tumors in the malathion study), but the nature of the historical findings, if adequate, would help evaluate the question of progression or latency in the malathion study. However, the data base must be viewed as inadequate at this critical juncture. Reasons for this conclusion are its smallness and age. Study completion dates for the five historical studies range 1/19/87 to 10/12/90 as compared to the malathion study completion date of 10/12/94. EPA's 1996 Proposed Guidelines for Carcinogen Risk Assessment says: "The most relevant historical data come from the same laboratory and same supplier, gathered within 2 or 3 years one way or the other of the study under review, other data should be used only with extreme caution." (p. 53) So the historical data base in question, ranging from 4 to nearly 8 years prior to the study is certainly soft in terms of age by EPA's standard. In the historical data base for the performing laboratory as cited in this study, the five control groups incorporate a total of 4 carcinomas among 205 male mice examined, or 1.95%. If one added to that the contemporaneous control of zero incidence for carcinoma, the incidence of carcinoma in the entire control data base would be 1.5%, while

respective incidences in the malathion 100, 800 and 8000 ppm groups were 11%, 5% and 11% by original diagnosis and 7%, 4% and 4% according to the PWG revised diagnoses. Now as stated in the Executive Summary of this review, the PWG defined the nomenclature and diagnostic criteria to classify proliferative hepatocellular tissues in this pathology re-evaluation. To the extent these criteria differ from those employed in the five historical control studies of the performing laboratory, the historical controls are irrelevant to the evaluation of responses in this malathion study. Had the current PWG re-evaluated the historical controls by the same criteria set forth in the PWG, and this resulted in a down grading of carcinomas to adenomas in numbers proportionate to those down graded in the malathion study, the contrast in the malathion study would be enhanced to that degree. *This in fact should be done. In the mean time, given the weakness in the historical data base, the* ***contemporaneous control*** *must be viewed as the defining control in the assessment of this study.* For that group, there were no carcinomas.

2) *Tumor progression.* A characteristic of hepatocellular tumorigenesis in the B6C3F1 mouse is that of progression through the following "natural history of neoplasia": foci of cellular alteration > adenoma > carcinoma (Jang et al, 1992; Maronpot et al, 1987; Ward, 1985). In consideration of this principle, adenomas and carcinomas in the malathion study must be combined in rendering tumor incidence, and for statistical treatment of the data.

The following views I would offer in support of my concern that a compound-related tumorigenic response may be evident in Group 2 *in terms of the second aspect of OSTP's definition of a carcinogen, namely decreased time to tumor development.* Dr. Ward's 1980 publication, provided at the PWG meeting, appears to instruct that in the case of the B6C3F1 mouse, there is a morphologic progression for spontaneous liver tumors, focus > adenoma > carcinoma over a 24-month (90-104 week) period, and that this progression is manifested in terms of increasing tumor size and concomitant increased likelihood and size of regions of carcinoma within the tumor. In his publication, Dr. Ward groups the size of lesions as "small" (1-5 mm diameter), "larger" (5-10 mm diameter) and "largest" (> 10 mm diameter). In the malathion study, the Group 2 tumorigenic incidence is elevated relative to Group 1. Group 2 tumor expression appears more advanced, with several adenomas and carcinomas being identified, i.e. there appears to be a frame shift, qualitatively, in the tumorigenic expression between the two groups, Group 1 being in the "focus of cellular alteration" > adenoma stage, with Group 2 being in the adenoma > carcinoma stage of the "natural history of neoplasia". This is also supported by the small size of the adenomas in Group 1. The evidence of a more advanced stage, and hence decreased latency, in Group 2 rests with larger tumor size (where 11 of 13 lesions are described as "masses" as opposed to none being so described in Group 1, multiplicity and the absence of carcinomas in Group 1. [See discussion on latency in IRLG (1979) under the topic "Evaluation of Pathologic Results"]

Interpretatively, it should be noted that any effort to separate adenomas from carcinomas, as if these were not part of a continuum in the tumorigenic response, and to treat these as independent and fundamentally different phenomena flies in the face of both the concepts expressed in Dr. Ward's paper and reason. Such a segregation or segmentation of tumor incidences which diminishes the impact of the concerted findings of both adenomas and carcinomas in a particular group, should not be considered acceptable. This is probably why HED's Cancer Peer Review Committee combines adenomas and carcinomas for statistical purposes. But it really goes beyond

statistics, for in the strict numerical sense, statistics for combined incidences do not quantitate the added evidence of a tumorigenic response (i.e. advanced stage, decreased latency) inherent in an increased proportion of carcinomas to adenomas, tumor size and multiplicity.

3) *Variable mechanism of carcinogenesis.* The mechanism of carcinogenicity for a given chemical may not necessarily be uniform across all doses. In the malathion study, the dose range is so wide, 100 ppm to 16000 ppm, that a substantially different profile of parent compound and metabolites may be expected at the extreme ends of the dose range. Any assumption that but one mechanism operates at all doses for a given chemical, particularly across a wide dose range, must be questioned. EPA (1996) says: "The possibility that an agent may act differently in different tissues or have more than one mode of action in a single tissue must also be kept in mind." (p. 66) I have no other reference readily at hand in support of this assertion, but I have read it and heard it said at cancer assessment symposia. Furthermore, it is fundamentally self-evident.

The dose levels in this study were 0, 100, 800, 8000 and 16000 ppm. There is clearly an hepatocellular response in male mice at 8000 and 16000 ppm. The PWG appears to conclude that a treatment-related increase of adenomas (exclusive of carcinomas) occurred in Groups 4 and 5, but that a tumorigenic effect was not observed in Groups 2 and 3. Since correlates of an hepatocellular tumorigenic response were seen only in Groups 4 and 5 (e.g. enlarged liver, hypertrophy), where adenomas were abundant, the absence of this pathology at lower doses is used to conclude the compound was not exerting a biological effect at those lower doses, and, hence, the liver tumors identified at those doses ***must*** be spontaneous in nature. This kind of reasoning imposes an interpretation that but one biological effect and one mechanism of carcinogenesis is possible for the chemical, regardless of the evidence of a tumorigenic effect at the lower doses, particularly the lowest dose in this case. Also, according to the PWG, the adenomas in Groups 4 and 5 differ qualitatively from those in Groups 1-3, thus further suggesting those in the latter group are to be regarded as spontaneous in nature. To the extent that carcinomas are not considered, this may be more defensible. However, the philosophy employed here assumes *a priori* that but one carcinogenic mechanism operates in this study, despite the elevated incidence of combined adenomas and carcinomas, the presence of carcinomas, large tumors, multiplicity and decreased latency, especially in Group 2 relative to the control group. *Alternative proposals are possible*. For the sake of discussion, it could be posed that at 100 ppm, the *in vivo* concentration of malathion is not great enough to appreciably induce hepatic metabolic enzymes, or at least not to an extent necessary to meaningfully metabolize the malathion molecule, while at appreciably higher doses (800, 8000 and 16000 ppm) such induction progressively increases to the point where some protective metabolic effect seen somewhere between 100 and 800 ppm becomes progressively overwhelmed at 8000 and 16000 ppm. The liver may be so turned on and malathion so modified metabolically that a different profile of malathion derived chemical entities operate to induce tumors differently at these doses. There is nothing in the PWG assessment that would address or refute this alternative interpretation.

Indeed, as to the relevance of carcinoma findings in this study, the PWG concluded these were not the result of treatment, as there was no dose response and no evidence of progression of the adenomas to carcinomas in the high dose group, i.e. the absence of carcinoma at the high dose is used to discount those observed at the low dose. Given the PWG endorses the concept of progression, i.e. focus > adenoma > carcinoma, it is a curiosity the PWG expressed no surprise

over the absence of findings of carcinoma in the high dose group, despite a 96% incidence of adenoma. This suggests to this reviewer a fundamentally different tumorigenic response or mechanism at the higher doses that has its purest expression at the highest dose. Perhaps due to altered liver metabolic response with increasing dose (at these high doses), there is a progressive modification of the tumorigenic response that tends to preclude transformation of adenomas to carcinomas, since surely given a 96% adenoma response, if progression as usual and expected, particularly in response to a xenobiotic, were to occur, the probability is very high among so many tumors (multiplicity was high in the high dose group) for carcinoma to have been seen. The fact that progression did not occur in the 16000 ppm group, therefore, should not be used to discount the considerable finding of carcinoma in the 100 ppm group, particularly in view of the absence of carcinoma in the control and the enormous spread in dose that could elicit differing mechanisms of neoplasia.

IV RECOMMENDATIONS

A) *PWG's Re-evaluation of Slides*

This reviewer recommends acceptance of the PWG re-evaluations of the individual slides examined and discussed at the PWG meeting in April. However, there is the concern over the lack of unaniminity of interpretation for the four "nodules" identified macroscopically in the control group. Two of the only four confirmed adenomas in this group rest on a 3 to 2 vote of the PWG. To the extent that the interpretation of these particular two lesions are allowed to drive the interpretation of the study, statistically, is problematical. *Public health considerations demand greater certainty than this.* In addition, to the extent that the tumorigenic response is considered positive only at doses exceeding the limit dose, and to the extent such findings might be discounted for that reason, there is no assessment of malathion in this study between 800 ppm and the limit dose, estimated to be 7000 ppm. In essence additional testing at low doses up to and including the limit dose would be indicated for proper assessment of the tumorigenic potential of malathion, given the nature of the findings in this study.

B) *PWG's Assessment of Relevance for Purposes of Risk Assessment*

The PWG concluded that a tumorigenic response occurred at the 8000 and 16000 ppm dose levels, but not at the lower doses. This reviewer recommends acceptance of the conclusion for the high dose groups, but against acceptance of the conclusion at lower doses, particularly at 100 ppm. As discussed in this review, testing at 100 ppm may constitute a fundamentally different study from that at 800, 8000 and 16000 ppm. Reasons for recommending a departure from the PWG rests with evidence at the lower doses (particularly the lowest dose) of a positive finding for carcinogenicity in accordance with the OSTP (1985) definition, and deficiencies in the body of information to rule out such an effect.

Rationale based on increased tumor *incidence* include: a) at the low dose, combined incidence of adenomas and carcinomas was increased, though not significantly so by the $p = 0.05$ criterion, yet this hinges on the close vote among pathologists of the PWG mentioned above for the four adenomas in the control; b) carcinomas were present in the low dose group but absent in the control (the PWG did not combine adenomas and carcinomas in its interpretation in spite of its

endorsement of the concept of progression); and c) carcinoma incidence exceeded the historical control incidence. Rationale supporting a tumorigenic effect in this study at the lowest dose based on OSTP's (1985) concept of *decreased time to tumor development* as identifying a carcinogen include: a) large tumors, macroscopically; b) substantial fraction of tumors as carcinomas; c) multiplicity; d) absence of carcinomas in the control; e) in control group, PWG weighed foci versus adenoma designations, while in low dose it was adenomas versus carcinomas, a more advanced stage in the "natural history of neoplasia".

Deficiencies include a) assessment of the full potential for development of the neoplastic response was compromised in this study in that it was conducted but for 18-months. On the other hand, OSTP (1985) claims the sensitivity of bioassays decreases with time, because of the natural appearance of age-related tumors in the control animals (p. 10414); b) the historical data base is too small (total of 205 male mice) and perhaps too old (4 to nearly 8 years) to use without considerable concern; c) diagnostic criteria and nomenclature defined by PWG may not apply to historical control findings, while PWG did not examine any of the few historical controls for confirmatory diagnosis; d) inadequate information in general as to hepatocellular tumor incidence and adenoma/carcinoma proportions in 18-month studies in B6C3F1 mice; and e) evidently only one slide was prepared for each tumor, though a substantial number of tumors were large, macroscopically, in the low dose group compared to control.

C) *Additional Comments*

1) If malathion is positive for carcinogenicity at 100 ppm, there is no NOEL for carcinogenicity in this study.

2) An 18-month study may be inadequate to assess the potential carcinogenicity *of malathion*, owing to the peculiar nature of the findings. On the other hand, a longer term study may have washed out distinctions observed after only 18 months of dosing. The National Toxicology Program (NTP) advises that mouse carcinogenicity studies be conducted for two years. The entire NTP mouse historical control data base is for two year studies, and, hence, is not helpful as background for the malathion study.

3) A low incidence of carcinomas in the control group, zero in fact, of the malathion study is not particularly surprising, for 18-month studies, which adds to the concern for the low dose group findings, where six lesions among four mice were diagnosed as carcinomas in the PWG re-evaluation and eight lesions among six mice were originally so diagnosed by the study pathologist. None of the pathologists identified carcinoma in the control group. It is significant that OSTP (1985) says: "These pathologists believe that truly benign tumors in rodents are rare and that most tumors diagnosed as benign really represent a stage in the progression to malignancy. For some tissue sites, this view is widely accepted. Examples of this are adenomas versus adenocarcinomas in the pituitary, thyroid, lung, kidney tubules, and according to some experts, in mouse liver. *In each of these cases, it is argued that the judgement of the pathologist as to whether the lesion is an adenoma or an adenocarcinoma is so subjective that it is essential they be combined for statistical purposes* (emphasis added). It is also argued, in these specific cases, that the adenoma is a precursor of the adenocarcinoma. Indeed, the Subcommittee on Environmental Carcinogenesis of the National Cancer Advisory Board recommended in 1976 that these lesions

be combined for statistical purposes." (p. 10416)

4) The fact that this finding of concern occurred in males is consistent with the more remarkable effects in males as opposed to females at the higher doses, males only evidenced a tumorigenic response in the 1979 National Cancer Institute study, the results of which prompted requirement of the new study. In other words, the liver is a target organ.

5) This study was required by the Agency in order to address equivocal hepatocellular tumorigenic findings (adenomas and carcinomas) in male B6C3F1 mice in the 1978 National Cancer Institute study, where doses employed were 0, 8000 and 16000 ppm. The Agency required that the same high doses be employed, disregarding the fact that these doses exceeded the limit dose. It is not clear how positive findings in males at 16000 ppm was perceived to be interpreted should they be confirmed in a repeat study. Presumably, it was hoped the effect would not be there. In the 1978 study, there was no significantly increased incidence of hepatocellular tumors in females (combined incidence of adenomas and carcinomas: 2%, 0% and 4%, respectively, for the control, 8000 and 16000 ppm groups), while in males (respective combined incidences: 16%, 15% and 35%) an increase was seen only at 16000 ppm, where $p = 0.031$ by pairwise comparison. In the current study, the incidence of adenomas at 16000 ppm among males was 96% and among females was 84%, versus control incidences of 2% for both males and females. No explanation has been rendered for the more remarkable effects in males nor the positive finding for females in the new study. The fact that in the recent study only adenomas were seen in males at the 16000 ppm dose is also puzzling. It may have its explanation in the fact that it was conducted for but 18 months (78 weeks) while the NCI study was for 95 weeks (80 weeks dosing plus 15 added weeks in-life). It is recognized that progression of hepatocellular tumors from adenomas to carcinomas accelerates post week 80 in such studies in B6C3F1 mice (Maronpot et al, 1987). The existence of the contrast in response between the NCI and the recent study enhances the concern as to the reliability of the historical control data base across time for use with the present study, reinforcing the notion that primary reliance should reside with the contemporaneous control. The different responses between the two studies may serve to underscore a fundamental problem associated with B6C3F1 mouse carcinogenicity studies having to do with their interpretability. OSTP (1985) also says: "Despite its long history, the continued use of the B6C3F1 hybrid mouse by the NTP is currently under review because of the difficulty in interpreting the significance of proliferative liver lesions." (p. 10412). In spite of this statement, the B6C3F1 mouse remains the strain for current mouse NTP bioassays.

D) *Issues Referrable to HED's Carcinogen Assessment Review Committee*

1) Whether the tumorigenic response in Group 2 (100 ppm) is a compound-related effect by the OSTP (1985) definition of a carcinogen, based on increased incidence *and/or* decreased latency.

2) Whether there was adequate sampling of liver tissue, specifically adequate sections of large tumors, particularly of the low dose (100 ppm) group, for proper diagnosis.

3) The need to confirm carcinoma diagnoses of historical control carcinomas by current PWG standards, should any reliance be placed on these controls.

4) The concept of a different mechanism of carcinogenesis at 100 ppm, as distinct from that at the higher doses.

5) Requiring another carcinogenicity study in the low dose range, but to include the limit dose..

6) PWG's expressing no concern over the absence of carcinomas at the highest dose in spite of a 96% incidence of adenomas, in view of concepts of progression.

7) PWG's treating the carcinoma response separate from the adenoma response, even though progression is a well accepted principle as cited by PWG in the form of supporting references

8) PWG's discounting carcinomas at the lower doses as allegedly spontaneous in nature, rather than as evidence of progression, because progression to carcinoma was not observed at the highest dose, this despite the fact that none were observed in the control.

9) PWG's not addressing the evidence of a tumorigenic response at the lowest dose, in spite of the fact that in contrast to the control group, the lowest dose group exhibited increased incidence, large tumors (indeed the PWG was not aware of macroscopic pathology), high proportion of carcinomas, multiplicity, evidence that the group was in the adenoma > carcinoma phase of progression, while the control group remained in the foci > adenoma phase.

10) PWG's expressing no concern over the fact that examining but one slide from large adenomas in the low dose group may not be adequate to rule out regions of carcinoma, while not combining adenomas and carcinomas.

11) PWG's acknowledging the weakness of the historical control data base, while expressing no real concern over its usefulness, nor any need to evaluate the few carcinomas (four) in that entire data base by the same standards employed in the malathion re-reads.

12) PWG's not discussing the influence an 18-month study versus a 24-month study poses interpretively. NTP scientists advise that studies in the mouse should be 24-month studies for adequate carcinogenicity potential.

This whole issue is of more than academic interest, for it is both surprising and of considerable concern where protection of the public health is concerned should malathion be a carcinogen at doses as low as 100 ppm. By this I mean that with respect to the public health, the stakes are too to high to dismiss positive findings, or to accommodate much that is uncertain in the face of evidence of positive findings.

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ADDENDUM

This reviewer being concerned about the substantial number of tumors described macroscopically as "mass" among Group 2 male mice versus none so described in the control group, and also by the much larger size of most of the tumors in Group 2 versus control, advised the PWG of this in his letter of May 4, 1998 to Dr. Hardisty (Attachment 6). He also requested by internal memorandum dated July 8, 1998, descriptors of macroscopic pathology for the five historical

control groups from MPI Research. The information (MRID 44662600) was submitted by the registrant's representative, Jellinek, Schwartz & Connolly, Inc. September 29, 1998 under the Study Title: "18-Month Oral (Dietary) Carcinogenicity Study in Mice Supplementary Information for MRID 43407291", MPI Study No. 668-001dated September 25, 1998; the author was Dr. C. Fred Morris. This supplemental report is further identified as MRID 44662600. Comments on the macroscopic findings for those historical controls are presented below.

First, be it acknowledged that there are a substantial number of hepatocellular tumors among the historical controls described as "mass", and several of these are of the same or similar order of magnitude of size as those appearing in Group 2 of the malathion study. Second, the characterization of hepatocellular tumors in the five historical control groups (Groups A thru E) including macroscopic (i.e. "focus", "nodule", "mass") and microscopic (adenoma, carcinoma) descriptors, including dimensions are tabulated below:

Group A (study termination: 1/19/87; number rats examined: 47)

Animal ID #	Adenoma (incidence: 19.2%)	Carcinoma (incidence: 6.4%)
8416		"mass" 1.4 x 1.2 x 0.6 cm
8422	"mass" 0.8 x 0.8 x 0.5 cm	
8427		"mass" 1.8 x 1.5 x 1.2 cm
8429		"mass" 2.1 x 1.8 x 0.9 cm
8431	"nodule" 2 mm	
8434	"mass" 0.8 x 0.6 x 0.4 cm	
8436	"focus" 4 mm	
8441	"mass" 0.7 x 0.4 x 0.3 cm	
8442	"focus" 2 mm	
8449	"focus", multilobular, 1-2 mm	
8451	"mass" 1.0 x 1.0 x 0.3 cm	
8453	"mass", multilobular, largest 2.0 x 1.7 x 0.6 cm	

Group B (study termination: 3/23/87; number rats examined: 47)

Animal ID #	Adenoma (incidence: 19.2%)	Carcinoma (incidence: 0%)
11259	"mass" 1.0 x 1.9 x 0.6 cm	
11263	"mass" 3.0 x 1.5 x 1.5 cm, multilobular	
11271	"mass" 0.8 x 0.7 x 0.6 cm	
11279	"focus" 4 mm	
11286	"nodule" 4 mm	
11289	"mass" 2.0 x 2.0 x 2.0 cm, multilobular	
11290	"mass" 0.8 x 0.7 x 0.7 cm	
11292	"nodule" 5 mm	
11295	"focus" 2 mm	

Group C (study termination: 8/22/89; number rats examined: 21)

Animal ID #	Adenoma (incidence: 14.3%)	Carcinoma (incidence: 0%)
27707	"mass" 0.5 cm diameter	
27728	"mass" 1.5 x 0.5 x 0.3 cm	

27735	"mass" 2.0 x 1.0 x 0.5 cm

Group D (study termination: 4/13/90; number rats examined: 46)

Animal ID #	Adenoma (incidence: 21.7%)	Carcinoma (incidence: 2.2%)
31692		"mass" 2.0 x 1.5 x 1.0 cm
31701	"mass" 2.0 x 1.0 x 0.5 cm	
31716	"mass" 1.5 x 1.0 x 0.5 cm	
31722	"nodule" 1.0 mm diameter	
31727	"mass" 3.0 cm diameter, multiple	
31731	"mass" 3.0 x 2.0 x 1.5 cm	
31732	"focus" minute	
31735	"focus" or "foci" multiple minute	
31739	"nodule" 4.0 mm diameter	
31740	"mass" 1.5 x 1.0 x 0.5 cm	
31742	"nodule" 1.0 mm diameter	
31743		"foci" 1.0 mm diameter, multilobular

Group E (study termination: 10/12/90; number animals examined: 44)

Animal ID #	Adenoma (incidence: 15.9%)	Carcinoma (incidence: 0 %)
34853	"nodule" 0.5 cm diameter	
34863	"mass" 1.0 x 0.6 x 0.5 cm	
34869	"mass" 0.7 cm diameter	
34875	"nodule" 3.0 mm diameter	
34876	"mass" 1.5 x 1.0 x 0.5 cm	
34886	"nodule" 2.0 mm diameter	
34887	"nodule" 2.0 mm diameter	

The following are summary statements for each of the five historical control groups, given by study date:

1) *Study A* (termination date 1/19/87) from among 47 male mice there were 9 adenomas and 3 carcinomas diagnosed. Of these 12 tumors, 8 are described as "mass", 1 as "nodule" and 3 as "focus". The nodule and foci are small. Among the 8 masses, this reviewer would estimate that 3 to 4 are of the "largest" size (estimated equivalent $\geq$ 10 mm diameter, per Ward 1980). Among these, 3 are carcinomas, 1 an adenoma.

2) *Study B* (termination 3/23/87), from among 47 male mice there were 9 adenomas and no carcinomas diagnosed. Of the 9 tumors, 5 are described as "mass", 2 as "nodule" and 2 as "focus". Nodules and foci are small. Among the 5 masses, this reviewer would estimate 2 as "largest".

3) *Study C* (termination 8/22/89), from among 21 male mice there were 3 adenomas and no carcinomas diagnosed. All 3 tumors were described as "mass" in which case this reviewer would estimate none as "largest".

4) *Study D* (termination 4/13/90), from among 46 male mice there were 10 adenomas and 1 carcinoma (the individual animal data sheets show one additional small foci, 1 mm, multilobular, diagnosed as carcinoma, that evidently in someone's judgement was not rendered on the summary sheet for historical control data appearing in the mouse study report, p. 1404.) Of the 11 tumors, 6 were described as "mass", 3 as "nodule" and 2 as "focus". The nodules and foci are small. Among the 6 masses this reviewer would estimate 3 to 6 as "largest".

5) *Study E* (termination 10/12/90), from among 44 male mice there were 7 adenomas and no carcinomas diagnosed. Of the 7 tumors, 3 were described as "mass" and 4 as "nodule". The nodules were small, and among the 3 masses this reviewer would estimate 1 as "largest".

For this performing laboratory, evidently the term "mass" is reserved for lesions that are of larger proportions than about 5 mm, the approximate upper limit of size for the term "nodule". Foci are generally smaller still. Unlike the control group of the malathion study, where but the four tumors identified were described as nodules, the historical controls do record a number of tumors (25 total among 205 control mice) as masses. An estimated 50-75% of which are here estimated as "largest". So the contemporaneous control differs substantially from the historical controls in having not only a lower tumor incidence, but all four are relatively small and described as nodules.

Had the historical data base been similarly absent (or harbored few) masses, the finding of the large number of masses in Group 2 in the malathion study would be more persuasive of a unique effect. It was thus needful to examine the macroscopic pathology for the historical controls, given the contrast that existed in the malathion study.

A few additional comments are necessary. 1) Though masses do appear in the historical data base they are fewer, with respect to the malathion Group 2, in proportion to the number of mice examined. 2) There are no examples in any of the controls of distinct masses on two lobes, of which there are 3 (possibly 4, in fact PWG diagnosed the latter as multiple carcinoma) examples in Group 2. We should qualify this by saying a few lesions in the historical controls are said to be multilobular, however they may be interpreted. This observation supports the uniqueness of Group 2 in terms of showing multiplicity and a more advanced stage of tumor progression. 3) The data base is very small. 4) The contemporaneous control should be regarded as the defining control for reasons stated in this review.

MALATHION - PATHOLOGY WORKING GROUP (PWG), MOUSE LIVER TUMORS

The Following Attachments Are Not Available Electronically

Attachment 1: January 7, 1998 Letter Walter Waldrop, Chief, Reregistration Branch III, SRRD to Blane Dahl (Jellinek, Schwartz and Connolly, Inc.) (JSC), setting forth the Agency's requirements for additional information on malathion carcinogenicity studies.

Attachment 2: EPA's Pesticide Regulation (PR) Notice 94-5.

Attachment 3: a) HED's May 13, 1998 Statistical Analysis of Tumor Incidence Re-Read Data.
b) Tumor Incidence (Re-Read) Data From The PWG Report.

Attachment 4: July 24, 1997 Letter of R. R. Maronpot (NIEHS) to Dr. Brian Dementi (USEPA).

Attachment 5: March 6, 1998 Fax of William Burnam (HED) to Judy Hauswirth (JSC).

Attachment 6: May 4, 1998 Letter of Brian Dementi (OPP) to Jerry Hardisty, Environmental Pathology Laboratories (EPL).

Attachment 7: June 4, 1998 Letter of Jerry Hardisty (EPL) to Meena Sonawane (JSC).

SignOff Date:	1/27/99
DP Barcode:	D246737
HED DOC Number:	013721
Toxicology Branch:	TOX1