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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

February 11, 1999

SUBJECT: Supplemental Information for the Cancer Assessment Review Committee Meeting Scheduled for February 24, 1999 to Resume Evaluation of the Malathion Carcinogenicity Data Base.

FROM: Brian Dementi, Ph.D., DABT
Toxicologist
Toxicology Branch
Health Effects Division

Brian Dementi 2/11/99

TO: Sanju Diwan, Ph.D.
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division

THRU: Alberto Protzel, Ph.D.
Branch Senior Scientist
Toxicology Branch
Health Effects Division

2/11/99

The Cancer Assessment Review Committee (CARC) met September 24 and October 8 and 15, 1997 to consider the malathion carcinogenicity data base. The purpose of this memorandum is to comment on the status of the work which has been pursued since the 1997 CARC meeting, and to convey all relevant documents to the CARC that have been generated since that meeting. The complete background package of DERs and other information in support of the September/October 1997 meeting remain in the hands of the committee and will not be re-submitted under this memorandum.

No complete report of the results of the 1997 CARC meeting deliberations and conclusions was

ever produced by the committee. However, certain additional testing requirements were imposed as set forth in the November 3, 1997 memorandum of Jess Rowland, CARC Executive Secretary, a copy of which is appended. (Attachment 1) In summary, the CARC requirements included: 1) a Pathology Working Group (PWG) assessment of the male mouse liver tumor response in the recently submitted mouse carcinogenicity study (MRID 43407201); 2) full pathology assessment of nasal tissues from the same mouse carcinogenicity study, a tissue site not examined in the original pathology evaluation; and 3) pathology re-evaluation of nasal, pituitary and uterine tissues in the recently submitted malathion combined chronic toxicity/carcinogenicity study in the F344 rat (MRID 43942901).

As to the status of fulfillment of these particular requirements, the PWG assessment of male mouse liver tumors, performed by Experimental Pathology Laboratories, Inc., Research Triangle Park, NC, has been completed. The PWG's May 8, 1998 report (MRID 44554901) and HED's January 27, 1999 review of the same are here forwarded to the CARC. (Attachments 2 and 3, respectively)

The pathology report (MRID 44733501) of evaluations of nasal tissues from the mouse carcinogenicity study dated January 8, 1999, received in HED February 1, has not been reviewed. HED awaits the registrant's submission of a missing summary table of the findings, expected soon, before drafting a final review of the submission. The pathology assessments of the pituitary and uterus from the rat study, received in HED January 27, have been submitted to HED's pathologist for comment, following which a brief statement of the findings for both tissues will be rendered within HED. This is not expected to be a time consuming matter. The pathology re-evaluations of the nasal tissues from the rat study have not been received by the Agency as of this date. The reviewing pathologist has advised HED the report is imminent. (Attachments 4, 5 and 6 reserved, respectively, for these outstanding submissions)

Following the Agency's receipt of the male mouse liver PWG report, the CARC re-convened June 10, 1998 to consider those particular findings. This meeting was an expedite that occurred prior to full HED review of the PWG report. While no official report of this meeting of the CARC was produced, as best remembered the committee's conclusions were that the PWG report should result in no immediate change in the regulatory status of malathion, and that final decision on interpretation of the study be deferred until such time as all other outstanding work has been received and final review of the malathion carcinogenicity data base is undertaken by the CARC.

Not long after the September/October, 1997 CARC, the HED reviewer/presentor of the data base to the CARC, Dr. Dementi, submitted a memorandum dated November 26, 1997 to the CARC Chairman, taking issue with certain decisions rendered at the meeting. A copy of that memorandum is here being conveyed to the CARC for consideration. (Attachment 7) Furthermore, in connection with his concern over the lack of a full report of the results of the 1997 CARC meeting, i.e. complete minutes of the meeting, Dr. Dementi expressed his views in a January 19, 1998 memorandum to Mr. Steve Johnson, then Acting Director of OPP. (Attachment 8)

Also, since the September/October 1997 CARC meeting, leukemia and interstitial cell testicular tumor incidence data from the malaoxon combined chronic toxicity/carcinogenicity study (MRID 43975201) have received statistical re-evaluations by the registrant at HED's request, the results of which are here being communicated to the CARC. (Attachment 9)

An issue previously before the CARC was that of the response of nasal tissues in the combined chronic toxicity/carcinogenicity studies in the rat. It may be of value for the CARC to have in hand the results of the subchronic inhalation study on malathion, which received particular attention by the HIARC in its December 22, 1998 report. (Attachment 10, selected pages from the 12/22/98 HIARC report) The HIARC is requiring another subchronic inhalation study, most particularly to identify the NOEL for nasal tissue hyperplasia/degeneration and cholinesterase inhibition. The HIARC had been made aware at its last meeting of a 2-week range-finding inhalation study in the rat, not previously submitted to the Agency, performed by the registrant for purposes of dose selection in the subchronic inhalation study. The range-finding study demonstrated nasal hyperplasia/degeneration and cholinesterase inhibition, at all doses, after only two weeks of treatment. So when the CARC considers nasal tissue effects in the chronic oral studies, findings in the inhalation studies may be instructive in the interpretation. The relevant subchronic inhalation DER was present in the background package provided for the September/October 1997 meeting of the CARC. The question of carcinogenicity as it may relate to the microscopic lesions of nasal passages was raised in the DER (p. 2). Nasal tissue findings in the 2-week range-finding inhalation study are summarized in a March 10, 1998 memorandum of Brian Dementi to Jess Rowland, HIARC Secretary. (Attachment 11)

ATTACHMENT 1

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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: **MALATHION**: Request for Re-Evaluation of Tissues/Slides by the Cancer Assessment Review Committee

FROM Jess Rowland, Executive Secretary 11/3/97
Cancer Assessment Review Committee
Health Effects Division (7509C)

THROUGH William Burnam, Chairman
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Mike Ioannou, Chief
Toxicology Branch 1
Health Effects Division (7509C)

The Health Effects Division's Cancer Assessment Review Committee (CARC) met on September 24, October 8 and October 15, 1997 to evaluate the carcinogenic potential of Malathion. The CARC reviewed the following studies: 1) Carcinogenicity study in B6C3F1 mice; 2) Combined chronic toxicity/carcinogenicity study in Fischer 344 rats with Malathion; and 3) the Combined chronic toxicity/carcinogenicity study with Malaoxon in F344 rats. The CARC recommended re-evaluation of certain tissues/slides from these studies since an assessment on the relevancy of the tumors to treatment could not be made at this time due to the absence of critical histopathology data. Details for CARC's request are attached:

cc: P. Wagner
D. Locke
B. Dementii
R. Whiting

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REQUEST FOR REEVALUATION OF TISSUES/SLIDES FROM CARCINOGENICITY STUDIES WITH MALATHION

The Health Effects Division's Cancer Assessment Review Committee (CARC) met on September 24, October 8 and October 15, 1997 to evaluate the carcinogenic potential of Malathion. CARC reviewed the following studies: 1) Carcinogenicity study in B6C3F1 mice with Malathion; 2) Combined chronic toxicity/carcinogenicity study in Fischer 344 rats with Malathion; and 3) the Combined chronic toxicity/carcinogenicity study with Malaoxon in F344 rats. The CARC recommended re-evaluation of certain tissues/slides from these studies since an assessment on the relevancy of the tumors to treatment could not be made due to the lack of critical histopathology data.

II. DATA REVIEW

1. Carcinogenicity Study in B6C3F1 Mice with Malathion (MRID No. 43407201)

(I) Liver

In this study, the incidences of hepatocellular tumors were increased in both sexes of mice as shown below:

Liver Tumor Rates and Exact Trend Test and Fisher's Exact Test Results

Tumor Type	Sex	0 ppm	100 ppm	800 ppm	8000 ppm	16000 ppm
Adenomas	Males	1/54	6/54	2/55	**13/55**	**49/51**
%		2	11	4	**24**	**96**
p=		0.000**	0.056	0.057	**0.001****	**0.000****
%	Females	0/55	1/53	0/53	**9/52**	**42/51**
p=		0	2	0	**17**	**82**
		0.000**	0.491	1.000	**0.001****	**0.000****
Carcinomas	Males	0/54	**6/54**	3/55	**6/55**	1/51
%		0	**11**	5	**11**	2
p=		0.345	**0.014****	0.125	**0.014***	0.486
%	Females	1/55	0/53	2/53	1/52	2/51
p=		2	0	4	2	4
		0.183	0.509	0.486	0.738	0.471
Combined	Males	1/54	**10/54**	5/55	**18/55**	**49/51**
%		2	**19**	9	**33**	**96**
p=		0.000**	**0.004****	0.107	**0.000****	**0.000****
%	Females	1/55	1/53	2/53	**10/52**	**43/51**
p=		2	2	4	**19**	**84**
		0.000**	0.743	0.486	**0.003****	**0.000****

Given the statistically significant (If *, then p <0.05; If **, then p <0.01) increases in hepatocellular tumors in male mice at the low-(100 ppm), mid-high (8000 ppm) and high-(16000 ppm) doses but not at the mid dose (800 ppm), CARC concluded that: 1) the **liver tissues/slides from male mice from all dose levels should be re-evaluated and 2) should be referred to a Pathology Work Group.**

(ii) Nasal Tissue

Because of the concern for nasal tumors in the rat study (to be discussed later), CARC recommended that nasal tissues from **all animals from all dose groups should be re-evaluated.**

2. <u>Carcinogenicity Study in B6C3F1 Mice with Malathion</u> (MRID No. 43942901).

(I) Nasal Tumors

The following tumors were observed in the nasal turbinate tissue:

<u>Dose</u>	<u>Sex</u>	<u>Tumor Type</u>
6,000 ppm	Male	Adenoma
12,000 ppm	Male	Carcinoma
50 ppm	Female	Squamous cell carcinoma of the alveolus of the root of a tooth was seen
12,000 ppm	Female	Same as above

In addition, hyperplasia of the olfactory epithelium as well as other nasal tissue lesions were relevant in both sexes of rats at 6,000 and 12,000 ppm dose groups. Of added concern is the fact that nasoturbinate slides were not examined for all animals at the low -dose group despite the facts that nasal tumors are rare in rats and hyperplasia was seen at the top two doses.

The 1984 Subdivision F Guidelines (Page 124) requires examination of animals at lower doses: :

*"if significant difference is observed in hyperplasia, pre-neoplastic or neoplastic lesions between the high dose and control groups, microscopic examination should be made on that particular organ or tissue **of all animals** in the study excessive"*

"if excessive early death or other problem occur in the high dose group compromising the significance of the data, the next lower dose level shall be examined for complete histopathology"

The factors listed below fulfill both the criteria specified above:

Hyperplasia of the nasal turbinate were seen in rats at the 6,000 and 12,000 ppm; therefore, histopathological examinations of the nasal tissues from all animals should have been conducted.

In addition, there was excessive mortality in males at 6,000 ppm (74%) and 12,000 ppm (100%) and in females at 12,000 ppm (64%); therefore, the nasal tissues from lower levels should have been examined.

Based on these factors, CARC determined that in order to conduct an accurate assessment on the relevancy of "nasal tumors" to Malathion exposure, **the nasal tissue from all animals from all dose groups should be evaluated.**

(iii) Pituitary Glands

Pituitary Pars Distalis Tumor Rates and Peto's Prevalence Test Results

Tumor Type	0 ppm	50 ppm	500 ppm	6000 ppm	12000 ppm
Adenomas	25/51	13/31	20/34	17/33	14/53
%	49	42	59	52	26
p=	0.98	-	0.133	0.266	-
Carcinomas	0/50	1/30	3/32	4/32	1/49
%	0	3	9%	12	2
p=	0.778	0.319	**0.029***	**0.027***	0.369
Combined	25/51	14/**31**	23/**34**	21/**33**	15/53
%	49	45	68	64	28
p=	0.987	-	**0.033***	0.097	-

The CARC noted that the incidences of carcinomas were significantly (pair-wise) increased in female rats at 500 and 6,000 ppm dose levels and the high mortality in this sex (64%) at 12,000 ppm could have compromised expression of this tumor at that dose level.

The CARC also noted that not all animals were examined at 50, 500 and 6,000 ppm dose groups; only 31, 34 and 33 animals, respectively, (out of 60/sex/dose) were examined

The 1984 Subdivision F Guidelines (Page 124) requires examination of animals at lower doses: :

> *"if significant difference is observed in hyperplasia, pre-neoplastic or neoplastic lesions between the high dose and control groups, microscopic examination should be made on that particular organ or tissue* ***of all animals*** *in the study excessive*
>
> *if excessive early death or other problem occur in the high dose group compromising the significance of the data, the next lower dose level shall be examined for complete histopathology"*

The factors listed below fulfill the criteria specified above:

In this study, there was a significant difference in the occurrence of carcinomas (neoplastic lesions) at 6,000 ppm (4/32, 12%, p =0.027) and the controls (0/50,, 0%).

Although the highest dose tested was 12,000 ppm, due to excessive morality (100% in males and 64% in females), the 6,000 ppm should considered the high dose .

Therefore, CARC concluded that the pituitary glands **from all animals at the 50, 500 and 6,000 ppm** dose groups should be evaluated.

(iv) Uterus

The CARC noted the presence of some rare/unusual uterine tumors as shown below. The CARC was concerned about the number, collectively, of low individual incidence of these rare tumor types in conjunction with the fact that all animals at the low, mid and mid-high doses were not examined.

UTERINE TUMORS IN FEMALE RATS

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

Therefore, CARC concluded that the uterus **from all females at the 50, 500 and 6,000 ppm** dose groups should be evaluated.

III CONCLUSIONS

The CARC determined the need for re-evaluation of the following tissues/slides from studies conducted in Malathion:

Species	Tissue/Slides	Dose Levels
Mouse	Nasal Turbinate	ALL ANIMALS/ALL DOSES
	Liver	MALES - ALL DOSES
Rat	Nasal Turbinate	ALL ANIMALS/ALL DOSES
	Pituitary glands	ALL ANIMALS/ ALL DOSES
	Uterus	ALL FEMALES/ALL DOSES

For the re-evaluation of the nasal tissue, the Agency recommends examination of five levels of nasal passage, numbered I through V from rostral to caudal for each animal. Details of this procedure can be found in the publication by *Eldrige et al.*, 1995. Fundamental and Applied Toxicology. 27: 25-32.(see Attached).

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FUNDAMENTAL AND APPLIED TOXICOLOGY 27, 25–32 (1995)

Effects of Propylene Oxide on Nasal Epithelial Cell Proliferation in F344 Rats[1]

SANDRA R. ELDRIDGE,*,[2] MATTHEW S. BOGDANFFY,† MICHEAL P. JOKINEN,* AND LARRY S. ANDREWS‡

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Effects of Propylene Oxide on Nasal Epithelial Cell Proliferation in F344 Rats. ELDRIDGE, S. R., BOGDANFFY, M. S., JOKINEN, M. P., AND ANDREWS, L. S. (1995). *Fundam. Appl. Toxicol.* 27, 25–32.

In chronic inhalation studies, propylene oxide (PO), widely used in the chemical and food industries, induced nasal tumors in F344 rats. Nonneoplastic findings of the chronic studies suggest a strong cytotoxic and proliferative component in the mechanism of PO carcinogenicity. A 4-week cell proliferation study was conducted to establish a no-observed-adverse-effect level (NOAEL) for nonneoplastic changes in the nasal epithelium of rats. Male F344 rats were exposed to 0, 10, 20, 50, 150, or 525 ppm PO vapor for up to 4 weeks with up to 4 weeks of recovery. Histopathology showed that the incidence and severity of respiratory epithelial hyperplasia increased with exposure time and regressed after termination of exposure with complete recovery after 4 weeks. Similarly, cell proliferation, as determined by bromodeoxyuridine incorporation into replicating cells, was elevated following 1 and 4 weeks of exposure, but decreased to control values after 1 week of recovery. Degeneration of the olfactory epithelium was found after 4 weeks of exposure with a decrease in incidence and severity after termination of exposure. Cell proliferation at this site was elevated during the 4-week exposure period and 1 week postexposure with return to control values after 4 weeks of recovery. Based on the cytotoxic and proliferative findings, the NOAEL for PO in nasal epithelium is 50 ppm. © 1995 Society of Toxicology

Propylene oxide (PO) is widely used in the chemical- and food-manufacturing industries in the production of polyurethane foams. *In vitro* studies show that PO is genotoxic, whereas *in vivo* genotoxicity studies have been negative (Health and Safety Commission, 1992), with the exception of micronuclei formation following ip exposure (Booman *et al.*, 1979). However, the ip route of exposure has no relevance to potential workplace exposures. Results from the *in vitro* studies suggest that direct contact of cells with PO has potential mutagenic consequences. This conclusion is supported by *in vitro* studies with mammalian cells placed in direct contact with PO which show that PO is mutagenic (Health and Safety Commission, 1992). Thus, genotoxicity data are suggestive of a carcinogenic effect at the point of contact of the chemical with tissues and not at remote tissues. Results of a long-term gavage study in which PO was shown to be carcinogenic only in forestomach support the premise (Dunkelberg, 1982).

Previous chronic inhalation studies have demonstrated a carcinogenic effect in the nasal cavity of F344 rats and B6C3F1 mice at 300 and 400 ppm (Renne *et al.*, 1986; Lynch *et al.*, 1984). Lynch *et al.* (1984) reported adenomas in the nasal passages of F344 rats at 300 ppm and epithelial hyperplasia in the nasal cavity of rats exposed to 100 and 300 ppm. At 400 ppm, Renne *et al.* (1986) observed an increase in the incidence of hemangiomas or hemangiosarcomas of the nasal submucosa in B6C3F1 mice and papillary adenomas involving the nasal respiratory epithelium and underlying submucosal glands in F344 rats. Nasal epithelial hyperplasia was seen in rats at 200 and 400 ppm. The epithelial tumors observed in the nasal cavity of rats were thought to have developed from the areas of epithelial hyperplasia (Renne *et al.*, 1986). PO did not induce nasal epithelial tumors in Wistar rats exposed by inhalation for 28 months at concentrations of up to 300 ppm (Kuper *et al.*, 1988). However, hyperplasia of nasal respiratory and olfactory epithelium was demonstrated after 28 months of exposure at PO concentrations between 30 and 400 ppm.

Differences in tumorigenic response between strains of rats (Kuper *et al.*, 1988; Lynch *et al.*, 1984) and between rats and mice (Renne *et al.*, 1986) raise uncertainty regarding the relevance of these tumors for human health risk assessment. In contrast to the oncogenic response, nonneoplastic effects observed in all three chronic inhalation studies (Kuper *et al.*, 1988; Lynch *et al.*, 1984; Renne *et al.*, 1986) were fairly consistent in that all demonstrated cytotoxic and

[1] Presented in part at the 33rd Annual Meeting of the Society of Toxicology, Dallas, TX, March 13–17, 1994.

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hyperplastic changes in the nasal cavity in both strains of rats and in mice. Therefore, these data can be used for risk assessment purposes with a higher degree of certainty regarding relevance to human health risk.

The nasal cavity is the site of the most notable morphologic effects of chronic exposure to PO. Nonneoplastic effects on the nasal mucosa have been seen after chronic exposure to 30 ppm (Kuper *et al.*, 1988), and epithelial tumors have been observed in the nasal cavity of animals exposed to 300 and 400 ppm. A no-observed-adverse-effect level (NOAEL) has not been found for PO. Given that the nonneoplastic effects on the nasal mucosa appear to be the most sensitive indicator of PO toxicity, a 4-week cell proliferation study was undertaken to (1) examine the nature of the concentration response relationship of cell proliferation to PO exposure, (2) determine whether lesions and/or cell proliferation are reversible after cessation of PO exposure, (3) compare lesions at 28 days to those described after long-term PO exposures, and (4) determine the NOAEL for PO in nasal tissues of rats after 1 and 4 weeks of whole-body exposure and at 1 and 4 weeks postexposure. The parameters examined were histopathology findings and a quantitative measure of cell proliferation in the target tissues. The exposure concentrations (0, 10, 20, 50, 150, and 525 ppm) were chosen in an attempt to identify a NOAEL and compare lesions after short-term exposure to higher concentrations (i.e., 525 ppm) to those described after chronic exposure to lower concentrations (i.e., 300 or 400 ppm).

MATERIALS AND METHODS

Animals. This study was conducted in compliance with Good Laboratory Practice regulations as promulgated by the U.S. Environmental Protection Agency and the Organization for Economic Cooperation and Development. This study was also conducted under NIH guidelines for the care and use of laboratory animals and was approved by ManTech Environmental Technology's Institutional Animal Care and Use Committee. Five-week-old F344 male rats were purchased from Charles River Breeding Laboratories (Raleigh, NC) with certification ensuring the animal colony to be free of disease or parasites. Following a 3-week quarantine period, animals were randomized by body weight into treatment groups (body weight range: 174 to 218 g; mean body weight for all groups was 196 g at the start of the study) and housed individually in suspended stainless steel (6 × 8 × 6-inch) cages. The animal rooms were maintained at 72°F and 51% humidity with a 12-hr light/dark cycle. Control and treated animals were provided food (Purina Certified Rat Chow, St. Louis, MO) and water *ad libitum* except during the actual inhalation exposure periods. Animals were observed for clinical signs of toxicity immediately before and after each exposure. Individual animal body weights were recorded weekly and at necropsy.

Experimental design. Five groups of 10 male F344 rats each were exposed to 0, 10, 20, 50, 150, or 525 ppm PO vapor 6 hr per day, 5 days per week. Chamber control rats were exposed to filtered room air. After 1 and 4 weeks of exposure, and after 1 and 4 weeks postexposure, 10 rats from each group were sacrificed for gross evaluation of all organs and histopathologic evaluation of the nasal cavity. Sixteen hours after the last exposure for each time point, 5 of the 10 rats were injected intraperitoneally with 5-bromo-2'-deoxyuridine (BrdU; 100 mg/kg, 5 ml/kg, Sigma Chemical Co., St. Louis, MO) in saline for cell proliferation evaluation. This protocol was shown to be effective at capturing peak proliferative responses of nasal epithelium to formaldehyde exposure (Swenberg *et al.*, 1986). BrdU is a thymidine analog that is incorporated into DNA during S phase of the cell cycle. Two hours later, animals were euthanized by aortic exsanguination under sodium pentobarbital anesthesia and necropsied.

Propylene oxide exposures. PO (CAS 75-56-9) was obtained from ARCO Chemical Co. (Channelview, PA). A sample was analyzed by gas chromatography prior to the first exposure and found to be 99.99% pure. The exposures were conducted in Hazelton 1300 liter chambers (Lab Products, Maywood, NJ) constructed of stainless steel and glass. The exposure chamber atmospheres were generated by metering liquid PO into a 1-in-diameter glass U-tube with 0.5-inch stainless steel Kovar ends using a Harvard Syringe Drive (Harvard Apparatus, South Natick, MA) with a glass syringe connected to the U-tube. PO entered the U-tube, dripped onto glass beads, and was vaporized. Conditioned compressed air carried the vapors to the exposure chamber at a rate of 300 liters per minute. Chamber concentrations were controlled by adjusting the flow rate of liquid PO into the U-tube. Chamber concentrations were monitored every 60 min during each 6-hr exposure with an infrared analyzer (Foxboro Analytical, South Norwalk, CT). Mean chamber concentrations were 10 ± 0.5, 22 ± 1.9, 53 ± 2.6, 151 ± 8.2, and 529 ± 11.3 ppm, representing target concentrations of 10, 20, 50, 150, and 525 ppm. Uniformity of vapor concentration in the exposure chambers was evaluated before animal exposures began to ensure spatial homogeneity of PO exposure. In addition, animals were rotated daily from the bottom shelf to the upper shelf of the chambers in a cyclic manner.

Tissue preparation. At necropsy, the head (with the skin, lower jaw, and soft tissues removed), larynx, trachea, and duodenum were immersed in 10% neutral-buffered formalin (NBF). Tissues were fixed in NBF for 24 hr and then transferred to 70% ethanol for 3 to 5 days. Nasal cavities were wrapped in gauze and decalcified in a formic acid–sodium citrate solution for 7 to 10 days. Five sections from each nasal cavity (Fig. 1) were taken and placed into fresh decalcification solution overnight. Nasal cavities were rinsed in running tap water for approximately 2 hr before processing. The nasal tissue sections were oriented to expose crucial landmarks and were embedded in paraffin. After facing the blocks, tissues were surface decalcified for 5 to 10 min. The blocks were then rinsed in warm tap water and placed on ice for 20 to 30 min prior to sectioning. Five -micrometer sections of nasal cavity were taken and stained with hematoxylin and eosin (H&E) for histopathology. Serial sections were taken for immunohistochemistry and placed on 3-aminopropyltriethoxysilane-coated slides (Fisher Scientific, Norcross, GA) to ensure adhesion during the immunohistostaining procedure.

Previously described methods (Eldridge *et al.*, 1990) were used to stain tissues for BrdU using a MicroProbe Manual Staining System (Fisher Scientific). Briefly, tissue sections were stained using a monoclonal antibody to BrdU (Becton–Dickinson, Mountain View, CA) and the avidin–biotin peroxidase (Vectastain ABC peroxidase kit, Burlingame, CA) method for detection of the antigen–antibody complex. BrdU incorporation was localized by the chromagen 3,3'-diaminobenzidine tetrahydrochloride (DAB; Sigma Chemical Co.).

Histopathology. Nasal tissues were examined from animals, beginning at 8 weeks of age, at each of the four time points: after 1 week of exposure, after 4 weeks of exposure, after 1 week postexposure, and after 4 weeks postexposure. Five levels of nasal passage, numbered I through V from rostral to caudal, were examined from each animal (Fig. 1). Level I was across the tip of the nose caudal to the external nares and included the stratified squamous epithelium lining the nasal vestibule. Level II was just rostral to the incisor teeth through the tips of the naso- and maxilloturbinates and included stratified squamous and respiratory epithelium. Level III was caudal to the incisor teeth at the level of the incisive papilla through the

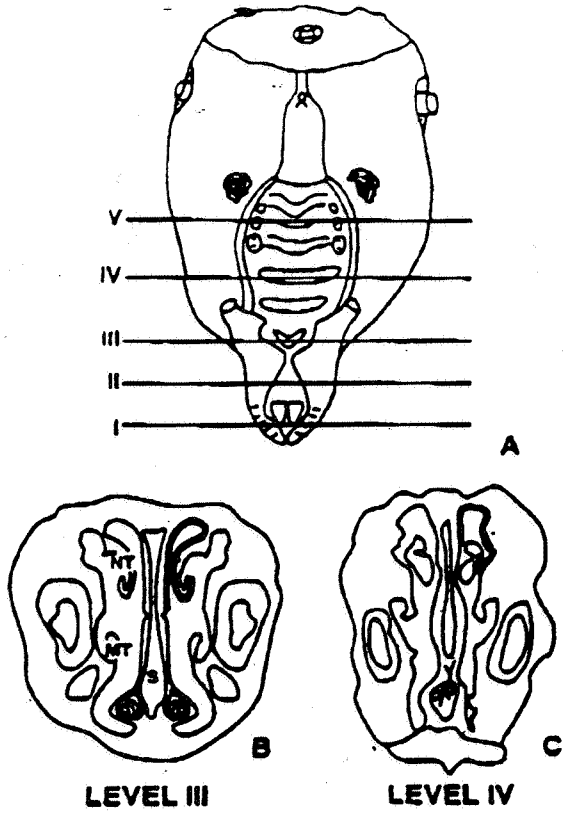

FIG. 1. (A) Nasal passages of F344 rat opened adjacent to the midline with the septum removed. Lines indicate the five section levels taken for histopathology of nasal tissues. (B) Level III (respiratory) and (C) level IV (olfactory) depict sites selected for histopathology and cell proliferation evaluations. NT, nasoturbinate; MT maxilloturbinate; S, nasal septum. The heavy lines depict the areas evaluated for cell proliferation. Modified from Monticello *et al.* (1991a).

naso- and maxilloturbinates and included respiratory and frequently some olfactory epithelium. Level IV was at the level of the first palatal ridge through the nasoturbinate and included olfactory and respiratory epithelium; sometimes the caudal tip of the maxilloturbinate was visible in level IV. Level V was at the level of the second palatal ridge through the ethmoturbinates and included olfactory and respiratory epithelium. In addition, larynx and trachea from animals in the control and high-concentration (525 ppm) groups at the Week 4 time point were examined histopathologically. All tissue sections were cut 5 μm thick. For histopathologic evaluation, severity grades were given for the lesions observed: 0, none; 1, minimal; 2, mild; 3, moderate; and 4, marked. For the respiratory epithelium, lesion severity grades were based on a combination of the degree of cellular change within the affected epithelium and the amount of epithelial area involved. Minimal lesions were focal to multifocal in the epithelium on the surface of the ventral ends of the nasoturbinates as well as on the adjacent nasal septum. As lesion severity increased, the respiratory epithelium of the dorsal meatus was also affected; moderate hyperplasia was characterized by diffuse involvement of the respiratory epithelium of the dorsal meatus. Some of the more severe lesions also diffusely involved the epithelium on the dorsal half of the nasal septum. For olfactory epithelium, severity was graded according to the number and size of cysts present.

Immunohistochemistry and cell proliferation measurements. Cells that had incorporated BrdU were identified by brown to black pigment over their nuclei. A section of duodenum, a tissue with a high cell proliferative rate, was included on each side to confirm the delivery of BrdU to the animal.

For evaluation of cell proliferation, five animals per group received BrdU by ip injection 2 hr prior to necropsy. In addition to these animals, five more animals per group were evaluated for histopathology. After evaluating the BrdU and H&E slides from the fourth week of exposure, it was determined that cell proliferation would be measured in the respiratory epithelium in level II or III lining the medial aspect of the dorsal meatus and in the olfactory epithelium in level IV or V extending from the dorsal meatus down the ossified portion of the nasal septum. These sites correlated with the sites observed to have lesions based on histopathologic evaluation. The unit length labeling index (ULLI) was used to quantify the degree of cell proliferation (Monticello *et al.*, 1991b). The number of BrdU-labeled epithelial cells was counted in 16 and 8 fields for the respiratory and olfactory epithelium, respectively. The same sites/levels evaluated for cell proliferation were also examined histopathologically.

Statistical analysis. The multivariate analysis of variance (MANOVA) technique was used to analyze the cell proliferation data for dose and time effects. Statistical significance was further confirmed using the Student's *t* test to compare ULLI between treatment groups and controls at each time point. The 5% significance level was used as the criterion for statistical significance. No attempt was made to statistically analyze the histopathology data due to the discrete nature of the severity grades.

RESULTS

Body weights are shown in Fig. 2. Group summaries of histopathology and cell proliferation findings are listed in Table 1.

There were no treatment-related clinical observations of toxicity. Body weight gain was significantly decreased in the 525-ppm group starting after 1 week of exposure. By 4 weeks postexposure, the body weight of the remaining 525-ppm rats (289 ± 23 g) did not differ from controls (302 ± 11 g). Body weight gains reflected the significant differences in body weight between the 525-ppm and control groups.

Histopathology Findings

Treatment-related effects included hyperplasia of the respiratory epithelium and degeneration of the olfactory epithelium. Respiratory epithelial hyperplasia was most common in animals from the 525-ppm group (5/10, 9/9, 2/10, and 1/10) and to a lesser extent in the 150-ppm (3/10, 7/10, 2/10, and 1/10) concentration group at Week 1, Week 4, Postexposure Week 1, and Postexposure Week 4, respectively. Minimal hyperplasia of the respiratory epithelium also occurred in a few animals in other concentration groups, including two control animals. Degeneration of the olfactory epithelium occurred in 1/10, 8/9, 7/10, and 3/10 animals in the 525-ppm group at Week 1, Week 4, Postexposure Week

HED Info- Cancer Assessment Review Committee file – Malathion

Page _____ is not included in this copy.

Pages 13 through 84 are not included in this copy.

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ATTACHMENT 3

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

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)

Brian Dementi 12/10/98

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

1/27/99

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

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

Pgs 85-105 already cleared

Registrant: Cheminova Agro A/S
Chemical: Malathion
Case No.: 818961
DP Barcode: D246737
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:

85

85

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 13 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

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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.

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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	

3488" "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 ≥ 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.

ATTACHMENT 1

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

JAN 7 1998

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Recvd 1-9-98 pw

CERTIFIED MAIL - MA008

Blane Dahl
Jellinek, Schwartz & Connolly, Inc.
1525 Wilson Boulevard, Suite 600
Arlington, VA 22209

Re: Malathion Cancer Assessment

Dear Mr. Dahl,

The Agency's Cancer Assessment Review Committee (CARC) met to evaluate the carcinogenic potential of malathion. The CARC reviewed the following studies: 1) Carcinogenicity study in B6C3F1 mice; 2) Combined chronic toxicity/carcinogenicity study in Fischer 344 rats with malathion; and 3) the Combined chronic toxicity carcinogenicity study with Malaoxon in F344 rats. The CARC has recommended the evaluation of certain tissues/slides from these studies since an assessment on the relevancy of the tumors could not be made due to the absence of critical histopathology data. (See the attached November 3, 1997 memo from Jess Rowland to Mike Ioannou for details).

As a result of the CARC's recommendation, the Agency is requesting that Cheminova have the following tissues/slides evaluated, as described in PR Notice 94-5:

1) MRID No. 43407201, Carcinogenicity in B6C3F1 Mice with Malathion
- the liver tissue/slides from male mice from all dose levels should be re-evaluated;
- the nasal tissues from all animals from all dose groups should be evaluated.

2) MRID No. 43942901, Carcinogenicity in Fischer 344 Rats with Malathion
- the nasal tissues from all animals from all dose groups should be evaluated (or where appropriate, *re*-evaluated);
- the pituitary glands from all animals at the 50, 500 and 6000 ppm dose groups should be evaluated (or where appropriate, *re*-evaluated);
- the uterus from all females at the 50, 500 and 6000 ppm dose groups should be evaluated.

For the evaluation of the nasal tissues, the Agency recommends examination of five levels of nasal passage, numbered I through V from rostral to caudal for each animals. Details of this

procedure can be found in the publication by *Eldgridge et al.*, 1995, Fundamental and Applied Toxicology; 27: 25-32. (See attached).

The Ageney has also reviewed your submission "Overview of the Subchronic and Chronic Toxicity of Malathion", MRID No. 44279701. The Agency concluded that examination of the document did not add any new information regarding the cancer assessment of malathion, the review is attached.

The Agency is requiring that you respond within two weeks of receipt of this letter with your commitment to have the evaluations of the above mentioned slides/tissues performed within six months. The evaluations should be done in compliance with the August 24, 1994 PR Notice 94-5, which outlines the procedure for submission of pathology re-reads to the Agency. (See attached). If you have any questions regarding this matter, please contact Dana Lateulere of my staff at (703) 308-8044.

Sincerely,

Walter I. Waldrop, Chief
Reregistration Branch III
Special Review and
Reregistration Division

cc: Diana Locke, HED - 7509C

Enclosures:
PR Notice 94-5;
November 3, 1997 memo from J. Rowland to M. Ioannou;
Fundamental and Applied Toxicology; 27: 25-32: "Effects of Propylene Oxide on Nasal Epithelial Cell Proliferation in F344 Rats";
Review of MRID No. 44279701; November 19, 1997 memo from B. Dementi to D. Lateulere.

EPA Office of Pesticide Programs

Pesticide Regulation (PR) Notice 94-5

August 24, 1994

PESTICIDE REGULATION (PR) NOTICE 94-5 NOTICE TO REGISTRANTS OF PESTICIDE PRODUCTS

ATTENTION: Persons Responsible For Registration of

Pesticide Products

SUBJECT: Requests for Re-considerations of Carcinogenicity Peer Review Decisions Based on Changes in Pathology Diagnoses.

This notice sets forth a procedure to be followed for submission of pathology re-reads to the Agency.

I. BACKGROUND

From time to time the Office of Pesticide Programs receives requests for re-consideration of Peer Review decisions based on re-evaluations of the pathology readings. These re-evaluations reflect voluntary activity on the part of the registrants, and are not the result of a requirement imposed by the Agency. The Agency is then asked to disregard the original readings and base its evaluation on the most recent ones. As a result the Agency may have two (or at times even more) pathological diagnoses for the same study.

Since this situation is occurring more and more frequently, the Agency is instituting a procedural requirement for any voluntary submissions of revised pathology diagnoses. This procedure will require a comprehensive peer review process, similar to the one used by the National Toxicology Program (NTP).

The National Toxicology Program (NTP) has a protocol for quality assurance in pathology, involving a quality assessment (peer review) pathologist and a Pathology Working Group (PWG) which is used to resolve differences in diagnoses between the laboratory (study) pathologist and the peer review pathologist. The PWG consists of a chair, the peer review pathologist and other pathologists (to include the study pathologist), all of whom are experienced in rodent toxicologic pathology. This group examines the tissues without knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differs from the opinion of the study pathologist, the diagnosis is changed. Thus, the final diagnoses represent a consensus of study, peer review, and consultant pathologists on the PWG. This procedure is described in the NTP Technical Reports under the section: "Clinical Examinations and Pathology." EPA believes that the use of a PWG, similar to one used by NTP, should be part of every pathology re-evaluation.

II. POLICY AND RATIONALE

The Agency believes that a procedure for obtaining consensus in pathology re-reads will improve the quality of decision-making in classifying pesticide chemicals having carcinogenic potential. The Agency has determined that unless the re-reads have been conducted using a Peer Review procedure, the Agency will base its evaluations upon the original readings.

The following will be required:

For any target tissue which is being re-evaluated, all slides containing that tissue in all dose groups, as well as the controls, must be re-read by the peer review pathologist. This is to include slides previously classified by the study pathologist as within normal limits, in addition to those having tumors.

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hyperplasia, or other type of cellular alteration or other non-neoplastic lesions.

The pathology reports from both the study and peer review pathologist and the original slides are to be submitted to a Pathology Working Group (PWG) similar to that described in the NTP Technical Reports under the section: "Clinical Examinations and Pathology." The PWG will review, as a minimum, all slides about which there were significantly differing diagnoses between the study and peer review pathologists.

Finally, the Agency should be provided with a detailed pathology report, which presents the PWG findings and includes the original diagnosis and the new diagnosis for each slide read, and a comment column to note any discrepancies, missing slides, etc.

The Agency also is considering including the requirement for review by a PWG for all original submissions in the future. This present Notice deals only with re-reads.

III. EFFECTIVE DATE

This policy notice is effective immediately. If you have questions, contact Esther Rinde at (703) 305-7492.

Penelope A. Fenner-Crisp,
Deputy Director (Acting)
Office of Pesticide Programs

May 13, 1998

Malathion Male Mouse

Hepatocellular Adenomas and/or Carcinomas Combined

Fisher's Exact Test/Cochran-Armitage trend test Re-Read

DOSE(ppm)	0.0000	100.0000	800.0000	8000.0000	16000.0000
	4/54	10/54	9/55	15/55	49/51
	(7)	(19)	(16)	(27)	(96)
	p= 0.0000**	p= 0.0751	p= 0.1254	p= 0.0058**	p= 0.0000**

	CHI-SQUARE	DF	P VALUE	
LINEAR TREND (Ho: no trend)	106.7718	1	0.0000**	(one-sided)
DEPARTURE (Ho: linear)	15.0302	3	0.0020**	(two-sided)

EPL PROJECT NO. 297-003

Table 2: 18-Month Oncogenicity Study in B6C3F1 Mice with Malathion (MPI Study No: 668-001)

Incidence of Altered Foci and Hepatocellular Neoplasms in Male Mice (PWG Consensus)

	Interim Sacrifice[1]					Terminal Sacrifice[2]				
Dose (ppm)	0	100	800	8000	16000	0	100	800	8000	16000
No. of Animals	11	10	10	10	14	54	54*	55	55	51
Altered Foci, Basophilic	0	0	0	0	0	0	1	0	1	3
Altered Foci, Eosinophilic	0	0	0	0	5	0	0	0	7	33
Hepatocellular Adenoma, Single	0	0	0	0	1	4	8	6	14	13
Hepatocellular Adenoma, Multiple	0	0	0	0	0	0	0	1	0	36
Hepatocellular Carcinoma, Single	0	0	0	0	0	0	2	2	2	0
Hepatocellular Carcinoma, Multiple	0	0	0	0	0	0	2	0	0	0
Total Tumor Bearing Animals	0	0	0	0	1	4	10**	9	15**	49

[1] Includes deaths from 0-12 months and 12-month Interim Sacrifice

[2] Includes deaths from 12-18 months and 18-month Terminal Sacrifice.

* Animal No. 49014 (Group 2) was too autolyzed for diagnosis.

** Animal No. 49019 and 49052 (Group 2) and Animal No. 49297 (Group 4) had both Hepatocellular Adenoma and Hepatocellular Carcinoma.

ATTACHMENT 4

DEPARTMENT OF HEALTH & HUMAN SERVICES — Public Health Service

National Institutes of Health
National Institute of
Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, NC 27709

July 24, 1997

Dr. Brian Dementi
U.S. Environmental Protection Agency
Mail Code 7509C
401 M Street SW
Washington, DC 29460

Dear Dr. Dementi:

I have reviewed the material you provided to Dr. Haseman and his letter to you dated July 17, 1997. I concur with all of Dr. Haseman's suggestions and opinions. Since the liver tumor response across the various dose groups is unusual, a formal peer review of the histopathological diagnoses is warranted. In the absence of such a peer review, the present findings would indicate a clear liver tumor response at the 100, 8000, and 16000 ppm levels. Should all of the original diagnoses be confirmed, you could proceed with more certainty in arriving at a judgment regarding the outcome of this study. Because of the unusual tumor incidence findings, it is imperative that any peer review be carried out without knowledge of the treatment status for each mouse. I recommend that all liver slides from all mice be subjected to peer review and that particular attention be paid to insuring that all grossly observed liver nodules have been appropriately made into histologic slides. Furthermore, it is imperative that the peer review insure that equivalent amounts of liver tissue have been made into histologic sections from all mice. Thus, all gross liver lesions should have a corresponding histologic diagnosis and equivalent amounts of grossly normal liver should have been processed into histologic slides.

A number of private pathology organizations have experience in conducting pathology peer reviews and I can provide names and addresses

should you desire. I am willing to offer the participation of our National Toxicology Program senior pathologists in the peer review exercise. It is also wise to include the original study pathologist in the process and a qualified pathologist from academia. It would also be beneficial to have yourself or another EPA toxicologist participate in the peer review process as an observer to insure that all important questions you might have are resolved. Again, I stress the importance of reviewing all liver tissues, even from animals without diagnosed liver tumors, since additional neoplasms may be found and preneoplastic lesions of the liver may be documented in some mice without overt liver tumors.

Sincerely yours,

R R Maronpot

R. R. Maronpot, DVM
Chief, Laboratory of Experimental Pathology

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ATTACHMENT 5

US ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDE PROGRAMS
HEALTH EFFECTS DIVISION (7509C)
FAX NUMBER 703-305-5147

FACSIMILE COVER SHEET

DATE: 3/6/98

TO: Judy Hauswirth

OFFICE: JSC

PHONE #: _____ FAX #: 703-527-5477

FROM: William L Burnam

PHONE #: 305 7491

NUMBER OF PAGES (INCLUDE THIS COVER SHEET) 2

COMMENTS:

NOTE: Judy Hauswirth
JSC
1525 Wilson Blvd., Suite 600
Arlington, VA 22209

This is in response to your questions in your February 12, 1998, fax to me. Brian Dementi, Mike Ioannou, Jess Rowland, and myself met on February 18, 1998 along with Luke Brennecke (via the telephone) to discuss your questions about Malathion and pathology issues.

Our answers and comments are in the same order as those in your fax of 2-12-98.

1. The Path Working Group (PWG) needs to be convened only for a reevaluation of male mouse liver slides. There should be a determination that an adequate sampling of liver tissue was examined.

2. The other tissues/slides for the nasal turbinate in rats and mice and pituitary glands and uterus in rats do not need the PWG. Your proposal for having the original pathologist look at the unread slides and a reviewing pathologist look at all tissues from all doses is a good idea. We are advising that inspection of nasal tissue slides should include careful examination of the squamous epithelium lining the alveoli of roots of teeth, where two rare tumors (squamous cell carcinoma) have been identified so far in dosed groups of this study. Concerning the uterus, we recommend that three sections be examined, one from each uterine horn plus one from the cervix of each rat. Concerning the pituitary, we agreed that the critical section for examination is one through the widest region of the pituitary such that both lobes are represented.

3. For the nasal turbinates in the rats, we want the best effort made to examine all five sections, even if this means a recut of the nasal area.

In addition, as previously discussed by Brian Dementi, please submit additional statistical analyses of tumor data from the malaoxon chronic toxicity/carcinogenicity study in rats (MRID 43975201). Specifically, the additional work should be directed to mononuclear cell leukemia, both sexes, and interstitial cell testicular tumors in males.

We think that it may be beneficial to have an EPA toxicologist participate in the peer review process as an observer to insure that all important questions are resolved. We are interested in your views about this.

William L. Burnam

Jerry Hardisty, D.V.M.
Experimental Pathology Laboratories, Inc
P.O. Box 12766
Research Triangle Park, NC 27709

May 4, 1998

Dear Dr. Hardisty,

As a follow-up to the Pathology Working Group (PWG) convened April 28-29, I have a few comments.

Having now had the opportunity to read the 1980 publication you provided by Dr. Ward on "Morphology of Hepatocellular Neoplasms in B6C3F1 Mice", I find it significant that the article indicates that the size of a liver tumor appears to bear a positive relationship with the likelihood that trabecular formations are present. Accordingly, the paper says the following: "Small tumors, usually 1-5 mm in diameter, were most commonly composed of a uniform population of basophilic hepatocytes growing in a solid pattern, with a cell size smaller than normal hepatocytes (Fig. 1). Other small nodules contained predominantly eosinophilic or vacuolated hepatocytes, or a mixture of all 3 cytoplasmic types. The eosinophilic and vacuolated cells were generally larger than normal hepatocytes. The uniform population of hepatocytes and the general difficulty in transplanting these tumors [5,8,19] led to their diagnoses as hepatocellular adenomas." (p. 321) Further along the paper says: " The large liver tumors [5-10 mm] frequently resemble the small tumors histologically, but also frequently had foci of vacuolated (glycogen or fat) cells, intracytoplasmic inclusions and areas of prominent trabecular formations (Fig. 2), the inclusions were of the Type 2 previously reported [6]. The morphology of hepatocytes in trabecular areas found in 53-55% of the mouse liver tumors were identical to those found in trabecular carcinomas. These trabecular foci in adenomas have been previously reported in mice [3,4,11,15] and may represent the early stages of trabecular carcinoma. The presence of these foci should lead to diagnosis as carcinomas(emphasis added). The largest tumors (greater than 1 cm in diameter) were generally composed of a variety of areas; some resembling adenomas and other larger areas of prominent trabecular formations (Fig. 3)." (pp. 321-323) "The small tumors were composed primarily of basophilic hepatocytes which grew in a solid adenomatous pattern. Large solid tumors had foci of prominent trabecular formations." (p. 319)

The reason for citing this information from Dr. Ward's publication rests with the fact that with respect to hepatocellular adenomas and carcinomas, eleven of the thirteen tumors identified macroscopically in Group 2 (100 ppm) appear to be much larger than the four identified in Group 1 (0 ppm) in the study being considered by the PWG. Given my uncertainty as to just what information was available to the committee, I have decided you should be advised of the size disparity. Accordingly, tumor sizes for Groups 1 and 2 as provided on individual animal pathology sheets are reproduced as follows by animal identification number (note in certain instances dimensions were given in cm which I converted to mm):

Group 1

#48885: "nodule", 6 mm diameter
#48897: "nodule" 2 to 6 mm diameter
#48916: "nodule", 5 mm diameter
#48919: "nodule", 4 mm diameter

Group 2

#48994: "nodule", 5 mm diameter
#48995: "nodule", 6 mm diameter
#49006: "mass", 12 x 8 x 6 mm
#49012: "mass", 15 x 10 x 10 mm
#49018: "mass", 8 x 8 x 6 mm
#49019: "mass"A, 7 x 9 x 4 mm
"mass"B, 6 x 7 x 5 mm
#49020: "mass"A, 25 x 20 x 20 mm
"mass"B, 15 x 10 x 10 mm
#49025: "mass", 28 x 16 x 12 mm
#49026: "mass", 20 x 12 x 5 mm
#49052: "mass"A, 15 mm diameter
"mass"B, 10 mm diameter

As you can see, lesions from all four Group 1 mice are described as "nodules" having sizes approximating those of the upper end of the range for small tumors (1-5 mm) as characterized in Dr. Ward's paper. In Group 2 mice, there are ten animals with lesions. Three of these have lesions on two lobes of the liver yielding a total of 13 macroscopic lesions. Two of the lesions are described as nodules having sizes similar to those in Group 1, however eleven of the lesions are described as "masses" rather than nodules, and depending upon the formula one uses to compute relative volumes of these lesions, those described as masses exceed the volume of a sphere of 5 mm diameter and six to eight of these exceed the volume of a sphere having a 10 mm diameter. Hence, all of the masses appear to qualify as large lesions with six to eight falling into the largest category as defined in Dr. Ward's paper.

According to Dr. Ward's paper, given that large tumors are likely to harbor foci characteristic of that of the carcinoma classification and given that four carcinomas have already been identified in Group 2 mouse livers, it would appear appropriate to examine several slides from each of the "masses" in Group 2 in order to be satisfied with the diagnosis as to tumor type. So a principal question I would have is that of whether a sufficient number of sections through these larger lesions were available to the PWG in order to rule out the presence of localized regions of carcinoma within them, in all cases?

Interpretatively, as to the question of whether Group 2 lesions are spontaneous in character, how much significance should be ascribed to the fact that all four macroscopic lesions in Group 1 are described as "nodules", whereas in Group 2, two are said to be "nodules" while eleven are

described as "masses"? Historically, are such masses common among control mice in 18-month, or even 24-month B6C3F1 mouse studies? Does the largeness of these masses suggest earlier onset, i.e. decreased tumor latency? Should any significance be ascribed to the presence of three cases in Group 2 of lesions on two lobes of the liver (note also the lesion in #49025 is described as attached to two lobes), particularly in an 18 month study?

Another point I would mention is that, given the weak historical control data base for 18-month studies, as was discussed at the meeting, how much confidence can be placed in that data base. I should note that among the five studies recorded for the performing laboratory, the incidence of carcinoma was 0 in three of the studies, with the present study contributing yet a forth 0 incidence of carcinoma. Among the remaining two historical studies, one carcinoma occurred in one and three carcinomas occurred in the other from among 50 animals in each group. Suppose these four historical carcinoma slides were on the table at the PWG, do we have any sense as to whether the classifications would have survived the re-examination? Since this historical data base is so small and yet so important, should these historical controls also be examined by the PWG members for purposes of uniformity of interpretation?

Permit me to reiterate that I am not aware of just what information was available to the committee, but having now read Dr. Ward's paper and in view of my uncertainty, I felt it appropriate to advise you concerning the macroscopic findings, which you might consider in addressing the question of whether the tumorigenic findings in Group 2 should be characterized as spontaneous in nature. At your request, the macroscopic pathology for all groups would be available.

I felt that your committee made a very conscientious effort to interpret this study, and having worked more closely with you, personally, at the meeting, I was impressed by your resolve to find consensus on the interpretations of slides. You were also very helpful in explaining things to the observers. All members of the PWG group were sources of insight and enjoyment.

Best Wishes,

Brian Dementi

Brian Dementi, Ph.D.
Office of Pesticide Programs
Environmental Protection Agency
Washington DC 20460
Mail Code 7509C

ATTACHMENT -

EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.
P.O. BOX 474 HERNDON, VIRGINIA 20172-0474 (703) 471-7060 Fax: (703) 471-8447

June 4, 1998

Ms. Meena Sonawane
Jellinek, Schwartz & Connolly, Inc.
1525 Wilson Blvd., Suite 600
Arlington, VA 22209-2411

Dear Meena:

Following the Pathology Working Group (PWG) conducted on April 27-28, 1998 to review proliferative lesions in the liver of male mice from a Carcinogenicity Study in B6C3F1 Mice with Malathion, I received a letter including several comments from Dr. Brian Dementi from the Office of Pesticide Programs at the Environmental Protection Agency. In his letter Dr. Dementi presented several comments and concerns about the macroscopic observations of liver nodules and masses described at necropsy in the control (Group 1) and low dose (Group 2) male mice. Dr. Dementi's presence at the PWG was very helpful and his comments and concerns are understandable. I appreciate the opportunity to address them. I have also shared Dr. Dementi's letter and my comments, which are included in this letter, with Dr. Ward and his opinion is consistent with mine.

A principal question in Dr. Dementi's letter is whether a sufficient number of sections through the larger lesions were available to the PWG in order to rule out the presence of localized regions of carcinoma. Most testing laboratories follow Standard Operating Procedures when preparing sections of nodules or masses regardless of the organ involved. For studies conducted for regulatory purposes, this usually requires that a single representative section of each nodule or mass be trimmed for histopathologic examination. This is generally adequate to properly classify the nodule or mass as benign or malignant. Although areas of obvious trabecular formation are indicative of malignancy in hepatocellular neoplasms, other criteria such as the pattern of growth (compressive or infiltrate), thickness of hepatic cords, cellular morphology, mitotic activity, and secondary pathologic features such as necrosis and hemorrhage are also considered when rendering a morphologic diagnosis of benign or malignant neoplasia. It would be unusual for a laboratory to risk introducing bias in a study by making additional sections of some nodules or masses and not all nodules or masses. The PWG examined all sections prepared from each of the nodules or masses that were prepared by the testing laboratory in a coded manner without knowledge of treatment group. Although they were not informed on the gross description of each mass, their morphologic diagnosis was based on the histologic features present in the sections examined. This is the standard method used in the histopathologic examination of tissues from

carcinogenicity studies conducted under regulatory guidelines. Another question presented by Dr. Dementi was how much significance should be ascribed to the fact that all four macroscopic lesions in Group 1 are described as "nodules," whereas in Group 2, two are said to be "nodules" while 11 are described as "masses." The PWG made their diagnoses based only on the morphology of the microscopic sections of the nodules or masses. Since they were examining the sections in a coded manner, they did not know the gross description of the lesions being examined. However, it is unlikely that their morphologic diagnosis would have been influenced knowing the gross description of the lesion being examined. The distinction between a "nodule" and a "mass" is not always clear. Depending on the individual making the gross description, the two terms may be used interchangeably. Often the distinction is based on size, "nodule" used to describe smaller well demarcated lesions and "mass" for larger lesions. The testing laboratory may have specific size criteria to indicate when a lesion will be called a "nodule" or a "mass."

Regarding Dr. Dementi's question about tumor latency, the biology of cellular proliferation of hepatic proliferative lesions is not well understood. Assuming that the rate of cellular prolif- eration is constant within a "nodule" or "mass," the size of the nodule or mass may correlate with the onset of the tumor. However, the rate of cellular proliferation within liver tumors is not constant, and the difference in size of the mass may only reflect differences in the rate of growth. Cellular proliferation studies of each neoplasm would be necessary to measure these differences. These types of investigations are not routinely performed on studies of this type and are often inconclusive since there are differences over time in the rate of cellular proliferation within tumors. Although a relative measure of the rate of proliferation at the time of death could be done using immunohistochemical staining for Proliferating Cell Nuclear Antigen (PCNA), cellular proliferation at other time intervals cannot be measured with the material available.

Another question concerns whether hepatic masses are common among control mice in 18-month, or even 24-month B6C3F1 mouse studies. Historical control data from 18-month studies with B6C3F1 mice are limited to those submitted by the testing laboratory. In 24-month studies conducted by the National Toxicology Program, hepatocellular tumors are one of the most frequent neoplasms observed in control male mice. The high incidence of hepatocellular neoplasms in concurrent control groups and historical data for male B6C3F1 mice is well known to be one of the most confounding and often highly criticized problems in the interpretation of test results from rodent carcinogenicity bioassays performed by the NTP. In a recent report (Haseman, et al, 1998), the range of hepatocellular adenoma was (4-60%) and the range of hepato-cellular carcinoma was (6-29%) in control male B6C3F1 Hybrid mice used

in 24-month carcinogenicity bioassays conducted by the NTP.[1] Dr. Dementi also asked if any significance should be ascribed to the presence of three cases in Group 2 of lesions on two lobes of the liver, particularly in an 18-month study. With neoplasms in solid parenchymal organs it is difficult to determine the significance of neoplasms involving two lobes of the liver. It may indicate that there are two separate tumors or, if the hepatic lobes are adjacent, may have resulted from a single neoplasm extending from one lobe to the adjacent lobe. Since this was very infrequent and never involved more than two hepatic lobes, this does not seem to be unusual and of little overall significance with respect to the biology of the tumors observed in Group 2.

Dr. Dementi's last point concerned how much confidence can be placed on the limited historical control data base available for 18-month studies conducted in male B6C3F1 mice. Historical control data should be used to evaluate not only the unusually high incidence of tumors in treated groups but should also be used to determine the validity of unusually low incidences of tumors in control groups. In the 18-month study of Malathion, the incidence of tumors in the control group is slightly low as compared to the available historical control data while the incidence in the low dose group is slightly elevated as compared to the available historical control data. Given the study-to-study variability in the incidence of tumors in the historical control data, there is considerable difficulty in using the available historical control data to interpret the difference in incidence of hepatic neoplasms in Group 1 and Group 2. When only these types of data are available, then interpretation of the study results must rely on the overall weight of the evidence including differences in organ weights, evidence of hepatic toxicity, and the morphologic appearance of the hepatic neoplasms in each group.

Dr. Dementi's suggestion that the historical controls also be examined by the PWG for purposes of uniformity of interpretation would be costly and time consuming. Although I feel that pathology peer review should be conducted on all critical studies being submitted for regulatory purposes, it is not always practical. However, if the historical control groups were to undergo such a review, then it would require that all liver sections from all historical control groups be examined during this review and not only the tumors. This is to assure that all histologic neoplasms have been identified during examination by the study pathologist. Following the initial peer review, then the PWG panel would have to be convened to examine all neoplasms diagnosed either during the study pathologist's initial evaluation or by the reviewing pathologist during the reexamination of all liver sections. It would be expected that there may be some differences in tumor classification as the result of such a review. However, it would be expected that the overall incidence of tumor bearing animals would not

be significantly different and; therefore, the current historical Ms. control data is adequate for the relative comparison of tumor incidences. As concluded in the final PWG report, the increased incidence of hepatocellular adenoma in the 8,000 ppm and 16,000 ppm dose groups was considered to be associated with the dietary administration of Malathion. All of the neoplasms in the 16,000 ppm dose group and most of the neoplasms in the 8,000 ppm dose group had a distinct morphology and were accompanied by nonneoplastic changes in the hepatic parenchyma (hepatocellular hypertrophy) which were also considered to be associated with treatment. Statistically significant increases were also reported in relative liver weights in these two dose groups.

The hepatocellular adenomas and carcinomas in the control (0 ppm), 100 ppm, and 800 ppm dose groups had a different morphology than those present in the higher dose groups and were not associated with relative increases in liver weight or other hepatocellular changes indicative of a treatment-related effect. The hepatocellular neoplasms present in these dose groups were not considered to be related to the dietary administration of Malathion.

In closing, I would like to thank Dr. Dementi and Dr. Ioannou for attending the PWG as observers. Their presence provided the panel of experts additional insight into the problem at hand and allowed in depth discussion concerning of the diagnostic criteria and differences in the histologic appearance of induced tumors in the 8,000 and 16,000 ppm dose groups and those observed in the lower dose groups, which were considered to be unrelated to the dietary administration of Malathion. The presence of representatives from the EPA at future Pathology Working Groups convened to address critical issues concerning pivotal studies submitted for regulatory review should be encouraged.

Sincerely,

Jerry F. Hardisty

JERRY F. HARDISTY, D.V.M.
Chairman, Pathology Working Group

JFH/wk

Reference

1. Haseman, J.K., Hailey, J.R., and Morris, R.W., 1998. Spontaneous Neoplasm Incidences in Fischer 344 Rats and B6C3F1 Mice in Two-Year Carcinogenicity Studies: A National Toxicology Program Update, Toxicol. Pathol., Vol. 26, No. 3, pp. 428-441.

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ATTACHMENTS 4, 5 AND 6

OUTSTANDING

ATTACHMENT 7

Mr. William Burnam, Chairman
Cancer Assessment Review Committee
Health Effects Division

November 26, 1997

Permit me to compliment you and the Cancer Assessment Review Committee (CARC) on the fine work and general decision rendering process pursued at the September/October assessment of the malathion carcinogenicity data base. I was particularly pleased over the invitation extended to Richard Brown to participitate at the meetings in the capacity of facilitator. Richard was very helpful to me in re-ordering some of the information for presentation to the committee as well as in rendering advice on how to be a presentor/commentor.

The Committee's decisions to require additional histopathologic assessments of various tissues, as enunciated in the November 3 memorandum of Jess Rowland was in all cases entirely appropriate. Also it was very encouraging to me to find in Jess' memorandum the publication by Eldridge, et al (1995) depicting the appropriate techniques for histopathologic assessment of nasal tissues.

Having expressed these views, and not knowing what the future holds with respect to my continued involvement with malathion, particularly at such time as when those final deliberations are held on the carcinogenicity of malathion, I consider it imperative to introduce into the record (as if I were to no longer be involved) certain specific follow-up views on the recent CARC.

I find unacceptable the notion that cholinesterase inhibition in a chronic carcinogenicity study should be used to conclude that dosing was excessive in the absence of clinical signs, increased mortality, substantial deficits of body weight gain or other evidence an MTD was exceeded. In all of my extensive involvement with the cholinesterase project I have witnessed nothing that reveals a relationship between cholinesterase inhibition and carcinogenicity when animals were not exhibiting clinical signs or increased mortality. Cholinesterase is assayed in the case of organophosphates because these are cholinesterase inhibitors and the LOEL/NOEL is needed to address cholinergic toxicity. Other enzymes, possibly more related to carcinogenicity such as DNA-repair enzymes, adenyl cyclase, glycolytic enzymes, plus a host of others are not assayed. If any of these were remarkably inhibited, would we conclude as in the case of cholinesterase inhibition that dosing is excessive, and discount tumor findings at those doses? Mechanisms of carcinogenesis are not understood, and due in part to this deficiency of understanding, high dose testing is pursued. It is well recognized that such doses likely far exceed those levels people would be exposed to for any length of time, and would be anticipated to alter many enzyme systems,

cholinesterase inhibition not withstanding. It could be argued that there is a selective testing advantage for cholinesterase inhibitors over other classes of chemicals (pesticides included) that don't suffer this compromise in reaching high doses because of interfering cholinesterase inhibition. Further, it could be argued that to properly test the more potent cholinesterase inhibitors, cholinesterase inhibition needs to be circumvented to get to those higher doses as is done in the case of testing for delayed neuropathy (OPIDN) through the use of atropine. In the case of the recent malathion mouse carcinogenicity study (MRID 43407201) this was in effect achieved as the animals survived high doses, without evidence an MTD was exceeded. In essence, my point is that cholinesterase inhibition should not have been used in the case of the malathion mouse carcinogenicity study to discount the very remarkable tumorigenic responses in mice of both sexes at the two high dose levels. In support of this I would quote from EPA's 1996 Proposed Guidelines for Carcinogen Risk Assessment:

> "Animal studies are conducted at high doses in order to provide statistical power, the highest dose being one that is minimally toxic (maximum tolerated dose). Consequently, the question often arises whether a carcinogenic effect at the highest dose may be a consequence of cell killing with compensatory cell replication or of general physiological disruption, rather than inherent carcinogenicity of the tested agent. There is little doubt that this may happen in some cases, but skepticism exists among some scientists that it is a pervasive problem (Ames and Gold, 1990; Melnick et al, 1993a; Melnick et al, 1993b; Barrett, 1993). In light of this question, the default assumption is that effects seen at the highest dose tested are appropriate for assessment, but it is necessary that the experimental conditions be scrutinized. If adequate data demonstrate that the effects are solely (emphasis added) the result of excessive toxicity rather than carcinogenicity of the tested agent per se, then the effects may be regarded as not appropriate to include in assessment of the potential for human carcinogenicity of the agent." (p. 27)

Now in view of all of these considerations, I am not aware that anyone demonstrated at the CARC, either on the rationale of cholinesterase inhibition or any other parameter of toxicity, that the tumorigenic findings in the mouse study were "solely the result of excessive toxicity rather than carcinogenicity of the tested agent per se". Indeed the remarkable tumorigenic findings in male mice at the lowest dose (100 ppm) would dispute such a conclusion barring a change of mechanism.

As to the question of the limit dose being exceeded in the mouse study (dosage levels: 0, 100, 800, 8000 or 16000 ppm), this is

clearly marginal at 8000 ppm and not sufficiently exceeded at 16000 ppm to merit discounting the findings at these doses. I say this in light of the fact that under the FIFRA Subdivision F Guidlines for carcinogenicity testing the limit dose is 5% of the diet, or 50,000 ppm. Only in more recent times has it been revised by internal memorandum to 7000 ppm for mice. If one were designing a study today perhaps the highest concentration of malathion in the food to be tested would be 7000 ppm, but now that the study has been conducted at 8000 and 16000 ppm as required of the registrant specifically to address so called questionable findings in the earlier National Cancer Institute study, I find it incredible that people would elect to discount positive findings at these doses in the current study if they were seriously interested in providing public assurance of the minimal risk. Furthermore, in my judgement, cholinesterase inhibition alone does not satisfy as sufficient reason to discount these findings. From the perspective of public health considerations, a much more compelling argument must be presented before the liver tumor findings are to be discounted. But if the Committee insists upon discounting findings at these two dose levels, there is an encumbency to test at 7000 ppm, viewed as the limit dose for mice. To permit the next lower dose below 8000 ppm, namely 800 ppm, to serve as an adequate high dose for the current study is to deny proper testing in the mouse for carcinogenicity of malathion.

I find it unfortunate to have to express these views after the CARC meetings, but quite frankly I was surprised at the invoking of cholinesterase inhibition as a way of discounting tumorigenic findings and needed additional time to reflect on the issue. At such time as this matter is revisited after the Pathology Working Group has rendered an opinion on the mouse liver tumors, I must put the challenge to CARC members to produce reasonable evidence to substantiate that cholinesterase inhibition, in the absence of other evidence of excessive toxicity, was somehow responsible solely, or even primarily (walking that extra mile), for the tumorigenic findings in the malathion mouse study. **I recommend this question to the Science Advisory Panel, if not previously addressed by that body, the question being the appropriateness of using cholinesterase inhibition in the absence of any other evidence an MTD was exceeded, to discount tumor findings.**

Another matter of considerable importance is that of nasal tissue lesions identified in the new malathion chronic toxicity/carcinogenicity study in the F344 rat (MRID 43942901). As I recall, when the Committee engaged this topic, there was little, or inadequate, discussion of the tumorigenic findings. Rather, the Committee quickly acknowledged that all nasal tissues had either not been examined or not fully examined, and elected to call in the additional histopathology assessments, deferring until such data is received a decision on this tumorigenic endpoint. To the extent that malathion may continue to be used during this interim period, I consider it my responsibility to

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advise the Committee of certain findings that exist in the data base. Firstly, the nasal tumors identified in this study, an adenoma among males in the 6000 ppm group and a carcinoma among males of the 12000 ppm group were described in the MRID study report itself as rare compound related tumorigenic findings. However, as explained in the DER of the study, while the tumors were characterized in the MRID study report as rare: "Spontaneous neoplasms of the nasoturbinal tissues are rare in F344 rats. In untreated dietary and corn oil control animals from eight recent NTP studies only six were identified from nearly 4000 control males and none occurred in a similar number of control females (citing Boorman et al, 1990). None have been observed in this laboratory in six previous studies (238 control males and 241 control females." (P. 93 of MRID study report) As explained in the DER (p. 62), both nasal tumors identified in the study were of the olfactory region of the nasal mucosa. An independent reading of Boorman et al (1990) confirms nasal tumors as rare among NTP historical controls, but just how rare was understated in the MRID study report. As written in the DER, "However, the claim of some six tumors among nearly 4000 control males is with reference to the respiratory epithelium (confirmed by personal communication with the principal author and inspection of Haseman et al (1990). Boorman et al (1990) and Haseman et al (1990) claim/identify **zero** incidence of tumors of the olfactory epithelium from among nearly 4000 control male rats, and none among a similar number of control females. In fact, Boorman et al (1990) says 'Neoplasms of the olfactory epithelium have occurred in F344 rats exposed to certain carcinogens, but have not been observed in controls.' (P. 332) So the finding in this study of two such tumors of the olfactory epithelium is exceedingly rare indeed, and heretofore unique to carcinogens." (p. 62 of DER)

Given the above, it is important to recognize that in addition to the rare tumors, hyperplasia and other non-neoplastic lesions of the olfactory epithelium were observed with high incidence in rats of both sexes at both 6000 and 12000 ppm. However, these lesions were not increased at or below the 500 ppm dosing level in the chronic toxicity/carcinogenicity study after a full two years. To the extent that hyperplasia was a precursor event to tumorgenic findings of the olfactory epithelium in this study (which we cannot actually say in this case), the fact that hyperplasia of the olfactory epithelium was not observed at or below 500 ppm is of some encouragement that such tumors may not be expected at those doses.

The added concern I have and wish to make note of is that the subchronic inhalation study on malathion (MRID 43266601) revealed hyperplasia of the olfactory epithelium in nearly all animals, both sexes and all dose levels. There was no NOEL, where concentrations employed were 0.1, 0.45 and 2.01 mg/liter. These concentrations when expressed in dosages delivered to the animal as calculated from inhalation concentrations using the best in-house procedure (mathematical formula) available for obtaining

such estimates, were 4.7, 21.2 and 94.5 mg/kg/day, or in terms of ppm in the diet, 75, 340 and 1508 ppm, respectively. These are very gross estimates with several qualifiers, as related to me by one of HED's inhalation experts who provided the formula. However, in terms of comparative inhibitions of erythrocyte cholinesterase, inhibitions among female rats in the chronic toxicity/carcinogenicity study after three months were 24% and 30% at 100 and 500 ppm, as compared to 11% and 27%, respectively, at the estimated doses of 75 and 340 ppm in the inhalation study at the same time point, three months. There is fair agreement in these findings in the two studies, suggesting that the estimated dosages delivered in the inhalation study were not far removed from those by the oral route. Thus after only 90 days of treatment, hyperplasia in the inhalation study extended to a much lower dose, estimated to be equivalent to that of 75 ppm in the diet (without a NOEL), than in the oral feeding study where the NOEL/LOEL was 500/6000 ppm. This may not be surprising given the direct application of the agent to vulnerable nasal tissues via the inhalational route. We have no idea how much earlier than 90 days of treatment hyperplasia might have occurred.

Since there was no NOEL in the inhalation study, exposures to low and possibly unknown concentrations by this route are problematic in terms of affirming public safety via the inhalational route of exposure. In my opinion, there is the need for additional review of this topic during the interim period that malathion continues to be used while the nasal tissue effects are being evaluated.

Also, it is my recommendation that when the CARC convenes again to consider the carcinogenicity of malathion, that you re-visit the mononuclear cell leukemia and interstitial cell testicular tumor data. I am concerned that these findings were dismissed too quickly by the Committee at the September/October meetings. In my judgement, in both cases competing toxicity, excessive early mortality and causes of death as matters pertaining to the proper selection of statistical methods of analysis and interpretation were not given adequate attention.

In closing, I would again compliment you and the Cancer Assessment Review Committee for your fine work in evaluating the malathion carcinogenicity data base.

Brian Dementi

Brian Dementi, Ph.D.
Toxicologist

cc Jess Rowland
Richard Brown

ATTACHMENT 8

MEMORANDUM

January 19, 1998

SUBJECT: Status of minutes of Cancer Assessment Review Committee (CARC), convened September 24, October 8 and October 15, 1997 to evaluate the carcinogenic potential of malathion.

FROM: Brian Dementi, Ph.D., DABT Brian Dementi
Toxicologist
Health Effects Division/OPP

TO: Steve Johnson
Acting Director
Office of Pesticide Programs

In my memo to you of January 13, 1998, I advised that even though it was agreed at our two meetings in December (11 and 18) that I would be included as a participant in the report of the Hazard ID Committee meeting on malathion held on November 6, 1997, that promise had not yet been implemented. My principal reason for pursuing that matter was to correct the lack of adequate opportunity for me to express my views as a long time expert on the malathion toxicology data base. Another problem now exists which I also believe stands to preclude the expression of my views in a timely fashion on the subject of the malathion data base. This particular concern rests with the Cancer Assessment Review Committee report, discussed as follows.

The Hazard ID committee meeting on malathion was held November 6, and the report of that meeting has now been generated. Yet the report of the CARC meeting on malathion which was held during September and October has not been produced. Now no one has explained to me, as presenter at that meeting, why the report of the Hazard ID Committee was given priority over the CARC report which preceeded the former in time. Nevertheless, it is certainly needful that the CARC report be produced at this time given the importance of the issue of the carcinogenicity of malathion. I say this not only because the entire community deserves to see the report, but because as time passes the remembrance of committee members as to what took place at the meeting diminishes, particularly since so much was said on this topic as it extended across three sessions. Furthermore, I am being requested at this time to finalize the toxicology RED chapter on malathion without the benefit of the CARC report. On earlier occasions it was decided to hold the draft RED document, pending receipt of the Hazard ID and CARC Committee reports. Without the CARC report in hand, I would find it unacceptable for me to sign the RED chapter, as I cannot state with certainty what the final decisions were, either in general or in particular, for various tumorigenic findings at the CARC meeting. There were numerous views expressed, and until a draft has been circulated for committee member and presenter comments, and these addressed for final sign off, one would not have in hand full authorative information to cite with respect to the conclusions reached by that committee.

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I should advise that having received no report of the CARC meetings on malathion, I drafted a memorandum on November 26, 1997 to William Burnam, Chairman, offering comments on that meeting, a copy of which is attached. I would anticipate that when the CARC report issues, this memo would be appended to that report, perhaps as a minority report. However, until the CARC report issues, there is evidently no benefit in holding these comments from consideration by those persons within the agency who may be making important decisions, such as whether to grant a Section 18 for malathion.

It was my understanding at the December 18 meeting in your office that issues I have raised pertaining to conclusions reached by the Hazard ID Committee on malathion will be submitted to external peer review. Little more was said with respect to the specifics of what will be submitted for external review. I would be pleased to be made aware of what is being sent in order to confirm that what I have said is adequately presented before the reviewing individuals. In addition, I am here requesting that my concerns expressed in the November 26 memo to William Burnam regarding carcinogenicity issues be included in the package for such review, in the event the memo is not already in the offing.

I remain concerned as I have expressed on previous occasions, that as toxicologist for malathion for some eleven years, with a wealth of information on the subject, I am rarely invited to be present when important management meetings are held to discuss malathion risk assessment issues. I believe this represents uncharacteristic treatment of expert toxicologists within the Agency.

I hereby request your attention to these important matters, as you were so generous to do in the case of the Hazard ID Committee concerns.

Attachment

cc: Margaret Stasikowski, HED
Dwight Welch, NFFE

Mr. William Burnam, Chairman
Cancer Assessment Review Committee
Health Effects Division

November 26, 1997

Permit me to compliment you and the Cancer Assessment Review Committee (CARC) on the fine work and general decision rendering process pursued at the September/October assessment of the malathion carcinogenicity data base. I was particularly pleased over the invitation extended to Richard Brown to participitate at the meetings in the capacity of facilitator. Richard was very helpful to me in re-ordering some of the information for presentation to the committee as well as in rendering advice on how to be a presentor/commentor.

The Committee's decisions to require additional histopathologic assessments of various tissues, as enunciated in the November 3 memorandum of Jess Rowland was in all cases entirely appropriate. Also it was very encouraging to me to find in Jess' memorandum the publication by Eldridge, et al (1995) depicting the appropriate techniques for histopathologic assessment of nasal tissues.

Having expressed these views, and not knowing what the future holds with respect to my continued involvement with malathion, particularly at such time as when those final deliberations are held on the carcinogenicity of malathion, I consider it imperative to introduce into the record (as if I were to no longer be involved) certain specific follow-up views on the recent CARC.

I find unacceptable the notion that cholinesterase inhibition in a chronic carcinogenicity study should be used to conclude that dosing was excessive in the absence of clinical signs, increased mortality, substantial deficits of body weight gain or other evidence an MTD was exceeded. In all of my extensive involvement with the cholinesterase project I have witnessed nothing that reveals a relationship between cholinesterase inhibition and carcinogenicity when animals were not exhibiting clinical signs or increased mortality. Cholinesterase is assayed in the case of organophosphates because these are cholinesterase inhibitors and the LOEL/NOEL is needed to address cholinergic toxicity. Other enzymes, possibly more related to carcinogenicity such as DNA-repair enzymes, adenyl cyclase, glycolytic enzymes, plus a host of others are not assayed. If any of these were remarkably inhibited, would we conclude as in the case of cholinesterase inhibition that dosing is excessive, and discount tumor findings at those doses? Mechanisms of carcinogenesis are not understood, and due in part to this deficiency of understanding, high dose testing is pursued. It is well recognized that such doses likely far exceed those levels people would be exposed to for any length of time, and would be anticipated to alter many enzyme systems,

cholinesterase inhibition not withstanding. It could be argued that there is a selective testing advantage for cholinesterase inhibitors over other classes of chemicals (pesticides included) that don't suffer this compromise in reaching high doses because of interfering cholinesterase inhibition. Further, it could be argued that to properly test the more potent cholinesterase inhibitors, cholinesterase inhibition needs to be circumvented to get to those higher doses as is done in the case of testing for delayed neuropathy (OPIDN) through the use of atropine. In the case of the recent malathion mouse carcinogenicity study (MRID 43407201) this was in effect achieved as the animals survived high doses, without evidence an MTD was exceeded. In essence, my point is that cholinesterase inhibition should not have been used in the case of the malathion mouse carcinogenicity study to discount the very remarkable tumorigenic responses in mice of both sexes at the two high dose levels. In support of this I would quote from EPA's 1996 Proposed Guidelines for Carcinogen Risk Assessment:

> "Animal studies are conducted at high doses in order to provide statistical power, the highest dose being one that is minimally toxic (maximum tolerated dose). Consequently, the question often arises whether a carcinogenic effect at the highest dose may be a consequence of cell killing with compensatory cell replication or of general physiological disruption, rather than inherent carcinogenicity of the tested agent. There is little doubt that this may happen in some cases, but skepticism exists among some scientists that it is a pervasive problem (Ames and Gold, 1990; Melnick et al, 1993a; Melnick et al, 1993b; Barrett, 1993). In light of this question, the default assumption is that effects seen at the highest dose tested are appropriate for assessment, but it is necessary that the experimental conditions be scrutinized. If adequate data demonstrate that the effects are <u>solely</u> (emphasis added) the result of excessive toxicity rather than carcinogenicity of the tested agent per se, then the effects may be regarded as not appropriate to include in assessment of the potential for human carcinogenicity of the agent." (p. 27)

Now in view of all of these considerations, I am not aware that anyone demonstrated at the CARC, either on the rationale of cholinesterase inhibition or any other parameter of toxicity, that the tumorigenic findings in the mouse study were "solely the result of excessive toxicity rather than carcinogenicity of the tested agent per se". Indeed the remarkable tumorigenic findings in male mice at the lowest dose (100 ppm) would dispute such a conclusion, barring a change of mechanism.

As to the question of the limit dose being exceeded in the mouse study (dosage levels: 0, 100, 800, 8000 or 16000 ppm), this is

advise the Committee of certain findings that exist in the data base. Firstly, the nasal tumors identified in this study, an adenoma among males in the 6000 ppm group and a carcinoma among males of the 12000 ppm group were described in the MRID study report itself as rare compound related tumorigenic findings. However, as explained in the DER of the study, while the tumors were characterized in the MRID study report as rare:
"Spontaneous neoplasms of the nasoturbinal tissues are rare in F344 rats. In untreated dietary and corn oil control animals from eight recent NTP studies only six were identified from nearly 4000 control males and none occurred in a similar number of control females (citing Boorman et al, 1990). None have been observed in this laboratory in six previous studies (238 control males and 241 control females." (P. 93 of MRID study report) As explained in the DER (p. 62), both nasal tumors identified in the study were of the olfactory region of the nasal mucosa. An independent reading of Boorman et al (1990) confirms nasal tumors as rare among NTP historical controls, but just how rare was understated in the MRID study report. As written in the DER, "However, the claim of some six tumors among nearly 4000 control males is with reference to the respiratory epithelium (confirmed by personal communication with the principal author and inspection of Haseman et al (1990). Boorman et al (1990) and Haseman et al (1990) claim/identify **zero** incidence of tumors of the olfactory epithelium from among nearly 4000 control male rats, and none among a similar number of control females. In fact, Boorman et al (1990) says 'Neoplasms of the olfactory epithelium have occurred in F344 rats exposed to certain carcinogens, but have not been observed in controls.' (P. 332) So the finding in this study of two such tumors of the olfactory epithelium is exceedingly rare indeed, and heretofore unique to carcinogens." (p. 62 of DER)

Given the above, it is important to recognize that in addition to the rare tumors, hyperplasia and other non-neoplastic lesions of the olfactory epithelium were observed with high incidence in rats of both sexes at both 6000 and 12000 ppm. However, these lesions were not increased at or below the 500 ppm dosing level in the chronic toxicity/carcinogenicity study after a full two years. To the extent that hyperplasia was a precursor event to tumorgenic findings of the olfactory epithelium in this study (which we cannot actually say in this case), the fact that hyperplasia of the olfactory epithelium was not observed at or below 500 ppm is of some encouragement that such tumors may not be expected at those doses.

The added concern I have and wish to make note of is that the subchronic inhalation study on malathion (MRID 43266601) revealed hyperplasia of the olfactory epithelium in nearly all animals, both sexes and all dose levels. There was no NOEL, where concentrations employed were 0.1, 0.45 and 2.01 mg/liter. These concentrations when expressed in dosages delivered to the animal as calculated from inhalation concentrations using the best in-house procedure (mathematical formula) available for obtaining

clearly marginal at 8000 ppm and not sufficiently exceeded at 16000 ppm to merit discounting the findings at these doses. I say this in light of the fact that under the FIFRA Subdivision F Guidlines for carcinogenicity testing the limit dose is 5% of the diet, or 50,000 ppm. Only in more recent times has it been revised by internal memorandum to 7000 ppm for mice. If one were designing a study today perhaps the highest concentration of malathion in the food to be tested would be 7000 ppm, but now that the study has been conducted at 8000 and 16000 ppm as required of the registrant specifically to address so called questionable findings in the earlier National Cancer Institute study, I find it incredible that people would elect to discount positive findings at these doses in the current study if they were seriously interested in providing public assurance of the minimal risk. Furthermore, in my judgement, cholinesterase inhibition alone does not satisfy as sufficient reason to discount these findings. From the perspective of public health considerations, a much more compelling argument must be presented before the liver tumor findings are to be discounted. But if the Committee insists upon discounting findings at these two dose levels, there is an encumbency to test at 7000 ppm, viewed as the limit dose for mice. To permit the next lower dose below 8000 ppm, namely 800 ppm, to serve as an adequate high dose for the current study is to deny proper testing in the mouse for carcinogenicity of malathion.

I find it unfortunate to have to express these views after the CARC meetings, but quite frankly I was surprised at the invoking of cholinesterase inhibition as a way of discounting tumorigenic findings and needed additional time to reflect on the issue. At such time as this matter is revisited after the Pathology Working Group has rendered an opinion on the mouse liver tumors, I must put the challenge to CARC members to produce reasonable evidence to substantiate that cholinesterase inhibition, in the absence of other evidence of excessive toxicity, was somehow responsible solely, or even primarily (walking that extra mile), for the tumorigenic findings in the malathion mouse study. **I recommend this question to the Science Advisory Panel, if not previously addressed by that body, the question being the appropriateness of using cholinesterase inhibition in the absence of any other evidence an MTD was exceeded, to discount tumor findings.**

Another matter of considerable importance is that of nasal tissue lesions identified in the new malathion chronic toxicity/carcinogenicity study in the F344 rat (MRID 43942901). As I recall, when the Committee engaged this topic, there was little, or inadequate, discussion of the tumorigenic findings. Rather, the Committee quickly acknowledged that all nasal tissues had either not been examined or not fully examined, and elected to call in the additional histopathology assessments, deferring until such data is received a decision on this tumorigenic endpoint. To the extent that malathion may continue to be used during this interim period, I consider it my responsibility to

such estimates, were 4.7, 21.2 and 94.5 mg/kg/day, or in terms of ppm in the diet, 75, 340 and 1508 ppm, respectively. These are very gross estimates with several qualifiers, as related to me by one of HED's inhalation experts who provided the formula. However, in terms of comparative inhibitions of erythrocyte cholinesterase, inhibitions among female rats in the chronic toxicity/carcinogenicity study after three months were 24% and 30% at 100 and 500 ppm, as compared to 11% and 27%, respectively, at the estimated doses of 75 and 340 ppm in the inhalation study at the same time point, three months. There is fair agreement in these findings in the two studies, suggesting that the estimated dosages delivered in the inhalation study were not far removed from those by the oral route. Thus after only 90 days of treatment, hyperplasia in the inhalation study extended to a much lower dose, estimated to be equivalent to that of 75 ppm in the diet (without a NOEL), than in the oral feeding study where the NOEL/LOEL was 500/6000 ppm. This may not be surprising given the direct application of the agent to vulnerable nasal tissues via the inhalational route. We have no idea how much earlier than 90 days of treatment hyperplasia might have occurred.

Since there was no NOEL in the inhalation study, exposures to low and possibly unknown concentrations by this route are problematic in terms of affirming public safety via the inhalational route of exposure. In my opinion, there is the need for additional review of this topic during the interim period that malathion continues to be used while the nasal tissue effects are being evaluated.

Also, it is my recommendation that when the CARC convenes again to consider the carcinogenicity of malathion, that you re-visit the mononuclear cell leukemia and interstitial cell testicular tumor data. I am concerned that these findings were dismissed too quickly by the Committee at the September/October meetings. In my judgement, in both cases competing toxicity, excessive early mortality and causes of death as matters pertaining to the proper selection of statistical methods of analysis and interpretation were not given adequate attention.

In closing, I would again compliment you and the Cancer Assessment Review Committee for your fine work in evaluating the malathion carcinogenicity data base.

Brian Dementi

Brian Dementi, Ph.D.
Toxicologist

cc Jess Rowland
Richard Brown

ATTACHMENT 9

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

012623

MAY 21 1998

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Supplemental Information - Statistical Analysis of Survivorship and Tumor Incidence Data for Rats from the 24-Month Oral Toxicity and Carcinogenicity Study with Malaoxon (MRID No. 43975201).

DP Barcode: 242506
Submission No.: S536382
Pesticide Chemical No.: 057701

Case No.: 818961
Tox. Chem. No.: 535

FROM: Brian Dementi, Ph.D., DABT
Toxicologist
Toxicologist Branch I
Health Effects Division (7509C)

Brian Dementi 5/19/98

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

Alberto Protzel 5/20/98

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

and

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

A combined chronic toxicity/carcinogenicity study for malaoxon (MRID 43975201) was reviewed by the Health Effects Division. The date of that final review was 7/2/97. In consideration of the overall carcinogenicity assessment for malathion, involving an examination of all studies in the data base, HED decided to request that the registrant provide additional

statistical analyses for two tumorigenic end points in the malaoxon study, namely, testicular interstitial cell tumors (males) and mononuclear cell leukemia (males and females), using the Peto test and incorporating only those animals designated for the full two year dosing period. Animals sacrificed after 12 months in the control and high dose groups were not included in the analysis. Basically, this was intended to be a statistical evaluation of the indicated tumorigenic responses for the 55 rats/sex/group assigned to the full 2-year study protocol. HED was particularly interested in obtaining actual p values for trend and pairwise comparisons between dose groups and control.

An initial response to the request was received under a cover letter of Carol Auletta, Science Director, Toxicology, Huntingdon Life Sciences to Judy Hauswirth of Jellinek, Schwartz and Connolly dated September 22, 1997 (MRID 44479301). In this response, survivorship and tumorigenic analyses were provided. As to survivorship, the following was reported:

> "For the male rats, Cox's Test and the Gehan-Breslow/Kruskal-Wallis Analysis indicated significantly decreasing trend in survivorship with increasing dose at less than the 0.01 level. Pairwise comparisons indicated significantly shorter survivorship in the highest dose group relative to the control group at the 0.01 level by Cox's Test and the Gehan-Breslow/Kruskal-Wallis test. The incidence of early deaths was greater in the highest dose group relative to control by the Fisher Exact test at the 0.01 level and the chi-square test at the 0.05 level."
>
> "For the female rats, Cox's Test and the Gehan-Breslow/Kruskal-Wallis Analysis indicated significantly decreasing trend in survivorship with increasing dose at less than the 0.01 level. Pairwise comparisons indicated significantly shorter survivorship in the two highest dose groups relative to the control group beyond the 0.01 level by cox's Test and the Gehan-Breslow/Kruskal-Wallis test. The incidence of early deaths was greater in the two highest dose groups relative to control by the Fisher Exact test and chi-square test beyond the 0.01 level."

Concerning the tumorigenic end points, the submission claimed "there were no statistically significant increases in incidences of testicular tumors in males or leukemia in females." For mononuclear cell leukemia in males there was a statistically significant difference among the groups and a statistically significant trend ($p \leq 0.05$). When the highest dose group was removed from the analysis the difference among groups "was not statistically significant, the test for trend was marginally significant at $p = 0.054$." The report concluded therefore that malaoxon administration is associated with an increase in mononuclear cell leukemia in males at the 2000 ppm dose level in the diet. A copy of this submission (MRID 44479301) is appended.

This response did not fully address HED's request which was to provide actual p values for trend and pairwise comparisons. A follow-up phone call to Jellinek, et al, requesting this information, resulted in the submission of another report dated January 9, 1998 which incorporated the actual survivorship and tumorigenic data (MRID 44457201). Unfortunately, HED was still unable to

excerpt the p values of interest from this report, which resulted in another call by HED requesting the p values. The request was followed with a February 17, 1998 response from Judy Hauswirth of Jellinek, et al. to B. Dementi in which the requested p values were provided (MRID 44502401), copy appended.

It can now be reaffirmed that based on the methods of Peto, et al, there was no statistically significant increase in interstitial cell testicular tumors or mononuclear cell leukemia in females at any dose level, nor was there a positive dose trend. For mononuclear cell leukemia in males there was a positive trend ($p = 0.03$) and a positive pairwise comparison ($p = 0.05$) for the high dose group (2000 ppm) versus the control group. According to the September 22 statistician's report, "When the highest dose group was removed from the analysis the difference among groups was not statistically significant, (note: $p = 0.07$, per his February 17 correspondance) the test for trend was marginally significant at $p = 0.054$." These statistical findings considered in light of the positive findings at 2000 ppm lead this toxicologist to conclude, conservatively, that increased numerical incidences of leukemia at 1000 ppm cannot be dismissed as a real, biologically relevant, finding at that dose level.

One would have to conclude from this that leukemia was a statistically significant positive response among males at 2000 ppm, and marginally significant among males at 1000 ppm. The method of statistical analysis employed in this case adjusts for increased mortality among dose groups.

Historical control data cited for this end point in the DER are not truly relevant to findings in the mid and high dose male groups, where mortality was increased relative to the control, i.e., historical data are for F344 rats of normal survival. This might be explained as follows: the incidence of leukemia (35%) in this study at 1000 ppm is at the upper end of the historical range of 15-36% for male F344 rats as provided for the performing laboratory. However, fewer animals were actually at risk for their full lifetime in the 1000 ppm group (58% survival) than in the control group (71% survival), even though this difference in survival was not found to be statistically significant. The point being, had survival in the 1000 pm group been somewhat high, the incidence of leukemia likely would have exceeded the 36% upper end of the historical range. Similarly, in the high dose group (2000 ppm) where survival was more severely affected (48% survival) and was statistically significant, and where leukemia incidence was significantly increased, the actual incidence of leukemia (29%) likely would have exceeded the upper end of the historical range had the animals survived normally and all were as at risk, timewise, as the control group to developing leukemia. The historical range for the NTP data base as cited in the DER is much wider (10-72%), but probably is an older and less relevant data base. Of course, the contemporaneous control in any study is generally recognized as the most important control. Among female rats, increased mortality was even more problematical. As shown in the DER (Table 2, p. 10), mortality was excessive at 1000 ppm and 2000 ppm, not only at term, but after 18 months as well. All of these increases in mortality were reportedly statistically significant at $p \leq 0.01$. Also, though not statistically significant, mortality among females was numerically increased at the low dose (20 ppm), where incidences were 24% and 7% at 24 and 18 months,

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respectively, versus 13% and 0% in the controls at the same respective time points. Now although the Peto Test adjusts for decreased survival and can show that a tumorigenic response actually recorded may be greater than the expected in a group because the group survival was low, the test cannot be used as a substitute to predict tumorigenic responses to a test material in animals that were not at-risk because of compromised survival, particularly if the tumorigenic response were late occurring. So decreased survival among females may have markedly precluded full expression of leukemia, and, hence, the study may not be adequate to address the potential for malaoxon to elicit leukemia in females.

The results of this reanalysis should be reflected in the form of an addendum to the DER for the malaoxon combined chronic toxicity/carcinogenicity study in the F344 rat.

Attachments (2)

HED Info - Cancer Assessment Review Committee file - McCatherine

Page _____ is not included in this copy.

Pages 143 through 149 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

__✓__ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

ATTACHMENT 10
(SELECTED PAGES: 1, 2, 10, 11)

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

December 22, 1998

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: ***MALATHION*** **- RE-EVALUATION** Report of the Hazard Identification Assessment Review Committee.

FROM: Jess Rowland, Executive Secretary 12/22/98
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

THROUGH: K. Clark Swentzel, Chairman, 12/22/98
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Diana Locke, Risk Assessor
Reregistration Branch II
Health Effects Division (7509C)

PC Code: 057701

On November 6, 1997, the Health Effects Division's Hazard Identification Review Committee evaluated the toxicology data base, selected doses and endpoints for acute dietary, chronic dietary (RfD) as well as occupational and residential exposure risk assessments, and addressed the sensitivity of infants and children from exposure to malathion as required by the Food Quality Protection Act (FQPA) of 1996. The HIARC's conclusions were presented in the committee report issued on December 17, 1997 (*Memorandum:* J. Rowland to A. Nielsen, HED Document No. 012440)

Following that meeting, the Agency pursued the external peer review mechanism to address a number of issues raised by Dr. Brian Dementi, the malathion, toxicologist following the November 6, 1997 HIARC meeting. This peer review was conducted by soliciting comments from three experts in toxicology chosen by the Agency. The external peer review panel submitted their responses to the Agency in May, 1998. On August 18, 20 and 27, 1998, the HIARC evaluated the comments and responses provided by the external peer review panel.

These responses, the HIARC's evaluation of the panel's responses and the HIARC's conclusions are presented in this report.

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Committee Members in Attendance

Members in attendance were:

William Burnam
Robert Fricke
Karen Hamernik
Susan Makris
Melba Morrow
Kathleen Raffaele
John Redden
Jess Rowland (Executive Secretary)
Clark Swentzel (Chairman)

Data was presented by Brian Dementi of Toxicology Branch 1.

HED staff also in attendance at this meeting were:
E. Budd
S. Dapson
C. Jarvis
M. Lamont
A. Protzel
B. Tarplee
P. Wagner.

Report Preparation: _____
Jess Rowland
Executive Secretary

IV. Subchronic Inhalation Study

Question 1): *Is the use of a UF (uncertainty factor) of 3 to compensate for the absence of a NOEL for cholinesterase inhibition and nasal and laryngeal degeneration/hyperplasia supportable?*

Panel's Response: One member recommended against the use of additional UF, another, recommended a UF of 10, while the third member did not feel qualified to answer this question.

HIARC's Conclusion: The HIARC concluded that a Margin of Exposure of 1000 is required for Short-, Intermediate- and Long-Term inhalation exposures. The MOE of 1000 includes the conventional 100 and an additional 10 for the use of a LOEL and the severity of the nasal lesions.

This decision was based on the results of a two-week range finding study (MRID No. 44554301) which was not available to the Committee at the November 6, 1997 meeting. In that study, there was a dose-related increase in the lesions of the nasal cavity (hyperplasia and respiratory epithelium) which was similar to the laryngeal and nasal cavity lesions seen in the subchronic study.

Question 2): *A two-week range-finding inhalation study, evidently not available to the Hazard ID Committee, did not establish NOELs for cholinesterase inhibition or histopathology findings of nasal and laryngeal tissues at doses as low as 0.54 mg/L. Should this study influence the Hazard ID Committee decision not to evoke an uncertainty factor for acute risk assessment (i.e., 1-7 days) on the basis of cumulative effects?*

Panel's Response: Conclusions from two members suggests that the cholinesterase inhibition is well characterized and that an extra UF is not warranted. The third member recommended against using this study since such studies (range finding) do not provide reliable information.

HIARC's Conclusion: The HIARC concluded that based on the availability of the new data (the range finding study), a MOE of 1000 is required also for Short-term inhalation risk assessment (previously it was determined that a MOE of 100 is adequate for this exposure period).

Question 3): *Should another study be required to identify the NOEL for the end points in question ?*

Panel's Response: One member would like to identify a NOEL, while the other suggests first using bench mark approach. The third does not want an inhalation study with rats.

HIARC's Conclusion: The HIARC determined that a new inhalation study is required based on the results of the two-week range-finding study (MRID No. 44554301) and the lack of a NOAEL for cholinesterase inhibition in the 90-day study (MRID No. 43266601).

Question 4). *Given the findings of nasal and laryngeal degeneration/hyperplasia in both of the recently submitted malathion and malaoxon combined chronic toxicity/carcinogenicity studies and the finding of rare nasal tumors in the malathion study, should the Agency require a carcinogenicity study by the inhalation route (e.g., inhalation exposure for first 90 days of a two year study)?*

Panel's Response: One member said yes to requiring this study, another member does not want this study and the third member would like to see mode of action studies to understand nasal injury and questions the utility of the inhalation study.

HIARC's Conclusion: At its meetings held on September 24, October 8 and October 15, 1997, HED's Cancer Assessment Committee (CARC) determined that in order to conduct an accurate assessment on the relevancy of nasal tumors to malathion exposure, the nasal tissues from all animals from all dose groups in the 2-year carcinogenicity study (MRID No. 43942901) should be evaluated/re-evaluated (*Memorandum:* J. Rowland, to M. Ioannou, dated 11/3/97; HED Document No. 012374). **Therefore, the HIARC concluded that the need for a study will be determined after CARC's review and evaluation of the requested histopathological examinations.**

Question 5): *Other than contributing to the completeness of the malathion data base, does this study provide any support for discounting a 10x safety factor imposed under FQPA for the protection of infants and children?*

Panel's Response: The panel agreed that the study does not provide any support for discounting use of the 10x safety factor imposed by FQPA. One member acknowledged that the study does not evaluate young individuals and asserted that the FQPA 10x factor is a risk management tool and including it in the scientific discussion of database sufficiency is not appropriate.

HIARC's Conclusion: This study is not appropriate for FQPA assessment because: (i) the study was conducted in adult animals; (ii) there was no exposure to pregnant animals nor was there pre/post natal exposure; (iii) this study did not evaluate parameters in fetuses or pups; and (iv) is not appropriate for assessment of increased susceptibility under FQPA provisions.. **Therefore, HIARC concluded that discussion about the FQPA Safety Factor is neither applicable nor appropriate for this study. In addition, the FQPA Safety Factor, when required, is not applied to any single toxicity study but rather for dietary and residential exposure risk assessments.**

ATTACHMENT 11

Jess Rowland, Secretary
Hazard ID Committee

March 10, 1998

This is an addendum to my December 17, 1997 comments to you on the Hazard ID Committee report for the November 6, 1997 meeting on malathion. My comments here pertain to the subchronic inhalation study. I recently requested from the registrant's representative a copy of the range-finding inhalation study. The study is entitled "A 2-Week Toxicity Study of Aerosolized Malathion Administered by Whole-body Inhalation Exposure to the Albino Rat" completed on July 20, 1993. Concentrations evaluated in this study were 0, 0.56, 1.58 and 4.23 mg/L, as contrasted with those employed in the full subchronic study of 0, 0.1, 0.45 and 2.01mg/L. After two weeks of treatment, with respect to upper respiratory findings, the Summary of the study claims that histological findings on the nasal and laryngeal mucosa were observed in most low dose animals and in the majority of the mid and high dose animals. "These findings included a slight to mild loss of goblet cells and similar hyperplasia in the nasal respiratory epithelium, slight leucocyte exocytosis in the nasal squamous and respiratory epithelium and slight to mild epithelial hyperplasia of the laryngeal mucosa." (p. 10) The fact that there was no NOEL for nasal and laryngeal effects after only two weeks of exposure demonstrates a much earlier onset of the nasal effects than could be determined from the subchronic inhalation study with malathion or the chronic feeding studies with malathion and malaoxon, where similar nasal and laryngeal effects were observed.

These histopathologic findings, without a NOEL, in this range-finding study after only two weeks of exposure, taken together with similar findings in the other longer term studies, serve to reinforce my opinion that another inhalation study is needed to identify a NOEL, and to determine the time of onset and ultimate course for nasal and laryngeal effects. Again, I consider inadequate the Hazard ID Committee's decision to employ a UF of 3 to compensate for the absent NOEL for this effect in the subchronic inhalation study. Your February 1997 Guidance Document for the Toxicology Endpoint Selection Process claims that "However, a LOEL may be used if a NOEL is not established in the critical study, when severity of the effects observed at this dose is of negligible concern for human risk, or when there is a data gap. Therefore, when a LOEL is identified for risk assessment, additional modifying factors (range of 3 to 10) may be used in addition to the total Uncertainty Factorof 100 (i.e., 10 for intra- and 10 for inter-species variation)." (p. 12) In response to this, I cannot accept the premise that the severity of the nasal and laryngeal tissue effects are to be viewed as of such "negligible" concern for human risk as to justify use of a modifying factor as explained in your paper. Furthermore, if the committee were inclined on employing a modifying factor of between 3 and 10, what reasoning was invoked to support choosing the low factor? Please be reminded that at the Cancer Assessment Review Committee meeting of last September-October these nasal tissue findings in the chronic feeding studies were considered of sufficient concern as to require additional nasal histopathology in the malathion rat and mouse studies.

Brian Dementi

Brian Dementi, Ph.D.
Toxicologist/HED

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