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OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

May 27, 1999

MEMORANDUM

SUBJECT: Review of the Histopathology Re-Assessment of Nasal Tissues for the Malathion 24-Month Oral (Dietary) Combined Toxicity/Carcinogenicity Study in the F344 Rat (MRID 44782301)

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Registrant: Cheminova Agro A/S Submission No.: S560089

Chemical: Malathion P.C. Code: 057701

Case No. 818961

DP Barcode: D255027

MRID No.: 44782301

ACTION:

Expedited Review, 6(a)(2) data. Review the Peer Reviewed histopathology re-assessment of nasal tissues for the malathion 24-month combined chronic toxicity/carcinogenicity study in the rat. This assessment of nasal tissues of the previously submitted and reviewed study was requested by the September/October 1997 meeting of the Cancer Assessment Review Committee (CARC) to consider the malathion data base. Nasal tissues were not completely examined histopathologically in the original study submissin (MRID 43942901).

CONCLUSION:

Presented below are the Citation and Executive summary of the reviewed study, the Review follows.

CITATIONS:

A 24-month Oral Toxicity/Oncogenicity Study of Malathion in the Rat via Dietary Administration. Author: Ira W. Daly, Ph.D., D.A.B.T., February 17, 1996. Sponsor: Cheminova Agro A/S, Lemvig, Denmark (MRID 43942901).

A 24-month Oral Toxicity/Oncogenicity Study of Malathion in the Rat via Dietary Administration: Nasal Tissue Evaluation and Peer Review. Author: James A. Swenberg, D.V.M., Ph.D., March 12, 1999. Sponsor: Cheminova Agro A/S, Lemvig, Denmark (MRID 44782301).

EXECUTIVE SUMMARY:

Toward fulfilling a requirement of HED'S CARC for the histopathology evaluation and peer review of microscopic slides of nasal tissues among rats of both sexes in the combined chronic toxicity/carcinogenicity study (MRID 43942901), the sponsor has submitted the results of this peer review (MRID 44782301). Nasal tissues had not been fully evaluated in the original submission. Accordingly, all animals in the low dose groups had not been examined histopathologically. Furthermore, only two sections of nasal tissues were examined in all animals that were so examined. Thus, the registrant was requested by the CARC to increase to five the number of nasal tissue sections to be examined from all animals in all study groups.

In the Guideline study, F344 rats of both sexes were administered malathion via the diet for a period of 24 months at dietary concentrations of 0, 100/50, 500, 6000 and 12000 ppm. The low dose group was initiated at 100 ppm malathion, whereupon it was discovered at the three months time point that erythrocyte cholinesterase was inhibited across all doses in females. Consequently, the low dose level was reduced at the three months time point to 50 ppm in both sexes in search of a NOEL for cholinesterase inhibition.

The nasal tissue *non-neoplastic* histopathology reported in the original submission of the combined chronic toxicity/carcinogenicity study, based upon examinations of nasal/turbinate

sections 2 and 4, has been confirmed in this more extensive re-evaluation of nasal tissue histopathology, now involving five nasal cavity sections in each rat. Many non-neoplastic histopathologic findings, e.g. hyperplasia of olfactory and respiratory epithelia, olfactory epithelial degeneration/atrophy, cyst formation, edema, congestion, inflammation, etc. attest to the serious compromising effects of malathion on nasal tissues in both sexes, particularly at the 6000 and 12000 ppm doses. Certain of these effects are considered in this review to extend to the 500 ppm in both sexes, but are particularly evident in females. Furthermore, in this review, females are considered to be responding at the lowest dose, 100/50 ppm, as evidenced, for example, by increased incidences of nasal mucosa (respiratory) edema, squamous metaplasia and hyperplasia, nasal mucosa (olfactory) epithelium degeneration/atrophy, nasal mucosa inflammation. Among males, an effect at 100/50 ppm is less certain, though there were increased incidences of nasal mucosal inflammation. This review concludes that for non-neoplastic nasal tissue histopathology, the study LOAEL = 100/50 ppm, NOAEL < 100/50 ppm (females); LOAEL = 500 ppm, NOAEL = 100/50 ppm (males).

In the case of *neoplastic* findings, the original study submission identified single neoplasms of the nasal cavity in two males [6000 ppm dose group (adenoma, olfactory epithelium) and 12000 ppm dose group (carcinoma, olfactory epithelium)] and single neoplasms of the oral cavity in two females [100/50 ppm and 12000 ppm dose groups (squamous cell carcinoma arising from the squamous epithelium lining the alveolus of a tooth)]. In the re-evaluation, additional single neoplasms of the nasal cavity were identified in two females [6000 and 12000 ppm dose groups (adenoma, respiratory epithelium)] in addition to the two previously identified in males. On re-examination, the neoplasm in the 12000 ppm male was revised from the diagnosis of carcinoma of the olfactory epithelium to that of adenoma of the respiratory epithelium, and its site of origin shifted from tissue section 2 to tissue section 1, the latter section not having been available in the original reading. Furthermore, in the re-evaluation two additional single neoplasms of the oral cavity were identified, one in a male rat [100/50 ppm (squamous cell papilloma of the palate)] and one in a female [6000 ppm (squamous cell papilloma of the palate)]. Also the diagnosis of the oral cavity neoplasm of the 12000 ppm group female rat was changed from that of squamous cell carcinoma arising from the squamous epithelium lining the alveolus of a tooth to that of squamous cell carcinoma of the palate. As discussed in this review, all of the neoplasms of both nasal and oral cavities are extremely rare, and in consideration of their rarity, their number in this study and the evidence of extensive nasal tissue non-neoplastic histopathology, *the study is considered positive for neoplastic findings at all doses in both sexes.*

Toward a more adequate assessment, it is to be questioned whether oral cavity tissues received adequate histopathologic assessment for non-neoplastic or neoplastic findings in what essentially amounted to a re-assessment nasal tissues, wherein rare oral tissue neoplasms were identified. FIFRA Guidelines do not require an assessment of the oral cavity, nor was it pursued in this two-year Guideline study. Therefore, a histopathologic examination of the oral cavity is recommended, the results of which may prove useful in the adequate characterization of the oral cavity neoplastic response thus far observed. Furthermore, in the malathion bioassays conducted by the National Cancer Institute in the late 1970s, nasal tissues evidently were not routinely

examined histopathologically. It is thus recommended that, given availability of relevant slides, these be examined for what they may disclose as to histopathology of oral and nasal tissues. This review also notes the high incidences of squamous cell hyperplasia and hyperkeratosis of the forestomach in males at the top two doses and in females at the top dose as identified in the original study submission. As explained in this review, experts in pathology claim that squamous cell histopathology of the oral cavity, esophagus and forestomach are combinable in evaluating squamous cell tumorigenic responses, and it is thus recommended that forestomach histopathology be considered in evaluating the squamous cell tumorigenic response in the oral cavity.

REVIEW OF PATHOLOGY PEER REVIEW REPORT

I. Background Information

The HED Carcinogen Assessment Review Committee (CARC) convened during September and October 1997 to consider the malathion cancer assessment data base, elected to require the histopathologic examination and peer review of microscopic slides of nasal tissues among rats of both sexes in the combined chronic toxicity/carcinogenicity study in the F344 rat (MRID 43942901). The CARC concluded nasal tissues had not been fully evaluated histopathologically in the original submission. This requirement, along with others from the CARC, was recorded in a November 3, 1997 report by Jess Rowland, Executive Secretary, CARC "Malathion: request for reevaluation of tissues/slides by the Cancer Assessment Review Committee (HED Report No. 012374)." These requirements were in turn forwarded to the registrant's sponsor via a January 7, 1998 letter of Walter Waldrop, Chief, Registration Branch III, SRRD. The results of the histopathology examination and peer review of the rat nasal tissue component of the data requirements have now been submitted to the Agency (MRID 44782301), and constitute the subject of this review.

According to this submission, the report contains the results of the evaluation and peer review of the rat nasal tissues that were conducted according to PR Notice 94-5 in response to the January 7, 1998 letter from Walter Waldrop, as mentioned above. Furthermore, the report claims the data are being submitted "....under Section 6(a)(2) because it contains the results of pathology evaluations of tissues not previously evaluated in the original study that was conducted at Huntingdon Life Sciences (HLS)." (From the March 18, 1999 letter of Blane Dahl, Jellinek, Schwartz and Connolly, Inc. to Mr. Phil Poli, Office of Pesticide Programs, USEPA).

Further, according to the sponsor's March 18 letter, Dr. Henry Bolte (the Study Pathologist) of HLS evaluated the nasal tissues from all animals from the original study, and these were peer reviewed by James Swenberg, D.V.M., Ph.D. While there was good agreement between the two pathologists, differences of opinion between them were resolved with agreement on final diagnoses, according to the March letter of Mr. Dahl.

II. The Study Peer Review Report

A. Review Procedure:

As set forth in the January 12, 1998 letter of Walter Waldrop, the five nasal tissue sections required by the Agency to be examined microscopically were to be obtained as decribed in Eldridge, S.R., et al (1995), Fund. Appl. Toxicol. 27, 25-32. Furthermore, the Agency specified the evaluations to be done in compliance with the August 24, 1994 PR Notice 94-5, although the PWG component of the PR Notice was not applicable to this particular data requirement, i.e., in the case of this nasal tissue histopathology requirement, the Agency was not seeking a Pathology Working Group (PWG) assessment. Rather, the Agency was seeking a Peer Review consisting of an initial assessment by the designated Study Pathologist followed by an assessment by a Reviewing Pathologist, and their concurrence or consensus.

Specifically, the following was done: 1) five sections of nasal turbinates were examined for each rat in the control and all dose groups, an increase from but two sections (designated nasal turbinate sections 2 and 4) in the original study; 2) for each animal previously examined, this involved making three additional sections (designated nasal turbinate sections 1, 3 and 5); 3) for animals not previously examined (lower dose groups), a full five sections were prepared and examined histopathologically.

As pursued in this peer review process, the Study Pathologist, Dr. Bolte, initially read each slide histopathologically, and prepared a report which, along with the slides, was provided to the Reviewing Pathologist, Dr. Swenberg, who in turn, interpreted each slide. The Reviewing Pathologist either agreed or rendered an alternative interpretation, following which a meeting of the two pathologists was held for the purpose of reaching consensus, which was also recorded.

B. HED's Review of Results

1. *Non-neoplastic Findings*

An inspection of the Summary of Peer Review Findings (Appendix A, copy appended to this review) as presented in Dr. Swenberg's March 12, 1999 report (MRID 44782301) of the Nasal Tissue Evaluation (p. 12) discloses numerous end points that were identified microscopically from examination of five nasal tissue sections from each rat. The total number of slides examined were said to be 4,580. The following represents the results of HED's review of this data to characterize the nasal toxicologic effects, to identify and substantiate the NOAEL/LOAEL for non-neoplastic findings and to determine what end points, if any, appear to correlate with or may be precursors of any neoplastic findings. In this review, an attempt was not made to compare in detail the non-neoplastic findings for tissue sections 2 and 4 as they appear in the original study submission with the readings for the same sections as conveyed by

the Study Pathologist to the Reviewing Pathologist for purposes of the nasal tissue re-examination. However, it is apparent that certain differences do exist, for which no explanation was offered in the report of the re-evaluation. Just how important these differences of diagnosis are to the conclusions is uncertain. Simply stated, based upon a cursory comparative examinations from the two reports, it appears the histopathology for sections 2 and 4 was not merely conveyed unedited from the original study report to the Reviewing Pathologist for purposes of the re-evaluation when the new slides for sections 1,3 and 5 were obtained and read by the Study Pathologist. Again, for purposes of this review of the re-evaluation report no detailed effort was made to compare the two sets of readings for sections 2 and 4 for non-neoplastic findings beyond noting that certain differences do exist.

In evaluating the effects, the reader must keep in mind that mortality was excessive in males at 6000 and 12000 ppm and in females at 12000 ppm. Hence, in these high dose groups, certain nasal effects may be less numerous because fewer animals were at risk for the duration of the two-year study period.

Among all histopathologic findings of increased incidence, the following are those which appear to be confined to the 6000 and/or 12000 ppm dose levels, often occurring at high incidences for one or more of the five nasal tissue sections.

Females: nasal lumen: "inflammatory cells/cell debris/ metachromatic amorphous material" (section 1); nasal lumen: "inflammatory cells/cell debris/metachromatic-basophilic amorphous material" (sections 2 and 4); nasal lumen: "inflammatory cells/cell debris" (sections 3 and 5); nasal mucosa (olfactory): "epithelium-cysts"; nasal mucosa (olfactory): "olfactory epithelium replaced by ciliated and nonciliated columnar epithelial cells"; nasal mucosa (olfactory) and/or (respiratory): "glands-dilated"; nasal mucosa (olfactory): "glandular epithelium-hyperplasia"; nasal mucosa (respiratory): "epithelium hyperplasia" (section 2); nasal mucosa (olfactory): "epithelium-hyperplasia, multi-focal"; and nasal mucosa (respiratory) and/or (olfactory): "subacute (chronic active)/chronic inflammation".

Males: nasal lumen: "inflammatory cells/cell debris/metachromatic amorphous material" (section 1); nasal lumen: "inflammatory cells/cell debris/metachromatic-basophilic amorphous material" (sections 2 and 4); nasal lumen: "inflammatory cells/cell debris" (section 3); nasal mucosa (respiratory): "epithelium hyperplasia" (section 2); nasal mucosa (respiratory) and/or (olfactory): "congestion"; nasal mucosa (olfactory): "edema"; nasal mucosa (olfactory): "epithelium-cysts"; nasal mucosa (olfactory): "epithelium-hyperplasia, multi-focal"; nasal mucosa (olfactory): "glands dilated"; nasal mucosa (olfactory): "olfactory epithelium replaced by ciliated and nonciliated columnar epithelial cells"; nasal mucosa (olfactory): "epithelium-degeneration/atrophy, multi-focal"; nasoturbinates: "fusion (fusion of nasoturbinates to each)"; and nasal mucosa (olfactory): "erosion".

Collectively, these effects attest to a remarkable nasal tissue toxicity of the test material at the top two doses, in both sexes.

In addition to the above cited effects illustrating the character of nasal toxicity that was essentially confined to the 6000 and 12000 ppm dose levels, the following tabulation (Table 1) of end points were selected from Appendix A as those which *possibly illustrate effects extending below those seen at the top two dose levels.*

TABLE 1: SELECTED FINDINGS CONSOLIDATED FROM THE STUDY REPORT APPENDIX A (MRID 44782301) (TERMINAL SACRIFICE, TISSUE SECTIONS 1 THRU 5)

FEMALES **Dietary Conc. (ppm)** **No. Animals Examined***	**0**	**100/50**	**500**	**6000**	**12000**
Nasal Mucosa (Respiratory) (R) or (Olfactory) (O): Congestion					
1 R	15	17	26	19	33
2 O(R)	3(4)	3(5)	10(11)	15(13)	22(19)
3 O(R)	4(3)	5(5)	5(5)	8(8)	16(16)
4 O	3	3	1	2	12
5 R	3	0	4	0	2
Nasal Mucosa (Respiratory) (R) or (Olfactory) (O): Edema					
1 R	0	4	9	28	38
2 O	0	0	2	48	23
3 O	0	1	1	13	3
4 O	0	0	0	6	19
5 N/A					
Nasal Mucosa (Respiratory): Squamous/Squamoid Metaplasia, Focal					
1	0	2	0	1	0
2	0	2	0	1	0

3 N/A					
4 N/A					
5	0	0	0	1	0
Nasal Mucosa (Respiratory): Squamous/Squamoid Metaplasia, Multi-Focal					
1	1	4	5	7	0
2	0	0	2	1	0
3 N/A					
4 N/A					
5 N/A					
Nasal Mucosa (Respiratory) (R) or (Olfactory) (O): Subacute (Chronic Active/Chronic Inflammation), Multi-Focal					
1 R	4	27	5	7	5
2 O(R)	0(0)	0(1)	1(2)	1(4)	0(1)
3 O(R)	0(1)	0(0)	1(0)	0(0)	3(0)
4 O	0	0	0	1	1
5 N/A					
Nasal Mucosa (Olfactory): Epithelium-Degeneration/ Atrophy					
1 N/A					
2	0	3	3	89	82
3	0	2	1	88	63
4	0	1	1	62	5
5 N/A					

Nasal Mucosa (Olfactory): Epithelium-Degeneration/ Atrophy, Multi-Focal					
1 N/A					
2	0	0	0	0	5
3	0	0	1	0	24
4	2	1	0	26	74
5 N/A					
Paranasal Sinus(es): Maxillary Gland-Atrophy					
1 N/A					
2 N/A					
3	1	0	19	45	12
4	0	0	0	0	1
5 N/A					
Nasal Mucosa (Vestibular): Congestion					
5	8	12	18	16	33
Nasal Mucosa (Vestibular) Squamous Cell Hyperplasia					
5	1	0	1	7	2
Nasal Mucosa (Vestibular) Squamous Cell Hyperplasia, Focal					
5	1	2	1	1	1
Nasal Mucosa (Vestibular) Squamous Cell Hyperplasia, Multi-Focal					
5	1	8	5	10	0

MALES					
Nasal Mucosa (Respiratory)(R) or Olfactory (O): Subacute (Chronic Active)/Chronic Inflammation					
1 N/A					
2 O(R)	1(5)	1(8)	4(18)	33(50)	19(22)
3 O(R)	1(3)	1(6)	23(3)	27(13)	6(3)
4 O(R)	1(0)	0(0)	5(2)	53(0)	30(2)
5 N/A					
Nasal Mucosa (Olfactory): Epithelium-Degeneration/Atrophy					
1 N/A					
2	2	2	7	83	84
3	2	1	3	82	61
4	0	0	4	32	5
5 N/A					

* Essentially 90 animals per Group were examined for all tissue sections except for section 5, where 78-81 per Group for males and 78-85 per Group for females were examined. However, in all five study groups, slides for all five tissue sections were available for most of the 55 rats/group that were on test for the full 2-year study period.

Arguably, effects extending to the 500 ppm dose level include the following, which are supported not only by increased incidences at 500 ppm, but by the same effects occurring in a dose-related manner at the 6000 and 12000 ppm dose levels.

Females

1) nasal mucosa (respiratory): "congestion"

2) nasal mucosa (respiratory): "edema"
3) nasal mucosa (olfactory): "epithelium-degeneration/atrophy"
4) paranasal sinuses: "maxillary gland-atrophy"
5) nasal mucosa (vestibular): "congestion"
6) nasal mucosa (vestibular): "squamous cell hyperplasia"

Males

1) nasal mucosa (olfactory) and/or (respiratory): "subacute (chronic active)/chronic inflammation"
2) nasal mucosa (olfactory): "epithelium-degeneration/atrophy"

Of the above, we inerpret the following to include the lowest dose group, 100/50 ppm, as well. **Females:** nasal mucosa (respiratory): "edema", based on findings in tissue section 1; nasal mucosa (respiratory): "squamous/squamoid metaplasia, multi-focal", again based on tissue section 1; nasal mucosa (olfactory): "epithelium-degeneration/atrophy"; nasal mucosa (vestibular): "congestion", based on tissue section 5; and nasal mucosa (vestibular): "squamous cell hyperplasia, multi-focal", based on section 5. A peculiarity among females is that of a high incidence in the low dose group *only* for nasal mucosa (respiratory): "subacute (chronic active/chronic inflammation), multi-focal", for which there is no explanation. **Males:** nasal mucosa (respiratory): "subacute (chronic active)/chronic inflammation", considered equivocal at 100/50 ppm.

2. *Neoplastic Findings*

In the original study report (MRID 43942901), two neoplastic lesions of the nasal cavity were reported, one in each of two male rats. One such lesion was described as "nasal mucosa (olfactory): carcinoma" in a Group 5 male (# 5040), as identified in nasal tissue section 2 (p. 4100 of the study report). The other finding was described as "nasal mucosa (olfactory): adenoma" in a Group 4 male (# 4033), as identified in nasal tissue section 4 (p. 3805).

Neoplastic findings for nasal and oral tissues identified in the study report are summarized in Table 2 and discussed below for the re-evaluation.

In the nasal tissue re-evaluation (MRID 44782301), the characterization of the lesion in rat # 5040 is not the same. In his submission of pathology findings to the Reviewing Pathologist, the Study Pathologist, reported only "hyperplasia, multi-focal, mild" for section 2, but identified in section 1 "nasal mucosa (respiratory): adenoma". The audience is to be reminded that findings only in nasal tissue sections 2 and 4 were reported in the original study report, so the reading in section 1 is entirely new. When Dr. Swenberg examined the slides, he concurred with Dr. Bolte's assessment of an adenoma in section 1, but discovered an adenoma in sections 2 and 3 as well. By consultation, the two pathologists share the view that the lesion is an adenoma, apparently arising in the respiratory epithelium of section 1, but extending into the lumen of

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sections 2 and 3. *While uncertain, this is interpreted to mean in this HED review* the lesion has no origin or point of growth in sections 2 or 3, but simply intrudes into those spaces. As a matter of interest, according to Boorman et al (1990) such lesions of the respiratory epithelium may be attached by a stalk at one locus, or have a broad base of attachment. (p. 332) One would think the nature of the attachment would be instructive as to where in the nasal cavity it arose when the tumor spans as many as three tissue sections. There must be a certain degree of uncertainty over just what appears in section 2, given that the Study Pathologist first saw a carcinoma, later saw multi-focal hyperplasia described as "mild", only to have the Reviewing Pathologist diagnose an adenoma. Nonetheless, this remains as a neoplasm of the nasal tissues of this rat, though now an adenoma (respiratory) as contrasted with a carcinoma (olfactory), formerly. It would be important to have an unambiguous statement as to just where in the nasal cavity the tumor arose, olfactory or respiratory epithelium. **(Note: a request has been made through the registrant's representative for clarification of the Study Pathologist's diagnosis of tissue section 2 for male rat # 5040)**

No other neoplastic findings were reported among **male** rats in the original study report. However, in the re-evaluation, a squamous cell papilloma of the palate (oral cavity tissue) was identified in section 3 of a Group 2 male (# 2029) (p. B-343 of the study report). This tumor initially was not identified by the Study Pathologist, and when first seen by the Reviewing Pathologist was diagnosed as hyperkeratosis. However, during the two pathologists' consensus review, they agreed the lesion was in fact a squamous cell papilloma.

Among **female** rats, there were no *nasal tissue* neoplastic lesions identified in the original study report. However, in the re-evaluation, both pathologists identified an adenoma of the respiratory epithelium in section 5 of a Group 4 female (# 4539) (p. B-2073), and another adenoma of the respiratory epithelium, similarly in section 5 of a Group 5 female (# 5525) (p. B-2321). Both of these findings were in tissues (section 5) not examined in the original study submission, and were identified by both pathologists.

In the original study report, diagnosed in the nasal tissue sections taken were two female rats with "m-squamous cell carcinoma arising from the squamous epithelium lining the alveolus of a tooth". One of these was identified in section 4 of a Group 5 female (# 5503) (p. 5062), and the other similarly identified in section 4 of a Group 2 female (# 2546) (p. 4574). In the re-evaluation, the Study Pathologist's characterization of the carcinoma in the Group 5 female as submitted to the Reviewing Pathologist was different than in the original study report. In the more recent communication it was described as "m-palate: squamous cell carcinoma" (p. B-2237) in section 4 of female # 5503. The Reviewing Pathologist confirmed this latter diagnosis. However, unexplained is the change of diagnosis of the Study Pathologist from that of a squamous cell carcinoma associated with the squamous epithelium lining the alveolus of a tooth to that of a squamous cell carcinoma of the palate. **[Note: a request has been made through the registrant's representative for clarification of the Study Pathologist's diagnosis of tissue section 4 of female rat # 5503]** The Reviewing Pathologist confirmed the presence of a squamous cell carcinoma arising from the squamous epithelium lining the alveolus of a tooth, as

identified in section 4 of the Group 2 female rat # 2546. In addition to these tumors identified in sections 4 of the original study, both pathologists identified in section 3 (another new section) a squamous cell papilloma of the palate of a Group 4 female rat (# 4518) (p. B-1982).

In summary (Table 2), the total number of neoplastic lesions identified in the nose/turb sections of this study as a whole was eight, three among males and five among females, as tabulated below. In **males** these consisted of one adenoma in sections 1 (respiratory epithelium), 2 and 3 among Group 5; one adenoma in section 4 (olfactory epithelium) among Group 4; and one squamous cell papilloma (palate) among Group 2. In **females** these consisted of one adenoma each in section 5 (respiratory epithelium) of both Groups 4 and 5; one squamous cell carcinoma (palate) among Group 5; one squamous cell papilloma (palate) among Group 4; and one squamous cell carcinoma (squamous epithelium lining the alveolus of a tooth) in Group 2. All adenomas mentioned are considered nasal tumors, while all squamous cell tumors mentioned are considered oral cavity tumors.

TABLE 2 NEOPLASTIC FINDINGS (NASAL AND ORAL TISSUES)

DIAGNOSIS/SEX	Group 1	Group 2	Group 3	Group 4	Group 5
FEMALES					
Nasal Respiratory Epithelium, Adenoma	0	0	0	1	1
Tooth Alveolus, Squamous Cell Carcinoma	0	1	0	0	0
Palate, Squamous Cell Carcinoma	0	0	0	0	1
Palate, Squamous Cell Papilloma	0	0	0	1	0
MALES					
Nasal Olfactory Epithelium, Adenoma	0	0	0	1	0
Nasal Respiratory Epithelium, Adenoma	0	0	0	0	1
Palate, Squamous Cell Papilloma	0	1	0	0	0

3. *Discussion*

In general, females appear to be more sensitive in terms of the variety of nasal tissue responses, particularly as these effects extend to the intermediate and lower dose levels. One peculiarity among females is the high incidence of "subacute (chronic active/chronic inflammation), multi-focal" of the nasal mucosa respiratory epithelium, tissue section 1, where the incidence for the 100/50 ppm Group was 27, versus 4, 5, 7 and 5 in the control, 500, 6000 and 12000 ppm dose groups, respectively. For this particular parameter, in males the effect appears to extend to the lowest dose, although is clearly evident at the higher doses as well. Since this is evidently a finding at the lowest dose level in both sexes, there is no NOAEL, but must be considered with other end points in identifying the study NOAEL for non-neoplastic findings. Another somewhat peculiar data set among females (not tabulated in this review) wherein the 100/50 ppm group differs markedly was that of vomeronasal organ: "eosinophilic material", where the respective incidences were: 31, 7, 30, 32 and 29.

In both sexes, particularly in females, effects appear to extend to all dose levels, or stated differently, the study affords inadequate evidence or assurance that effects do not extend to the lowest dose level, 100/50 ppm, though the evidence of an effect at this level is less substantial than at the other doses. For non-neoplastic findings, it is concluded the LOAEL $\leq$ 100/50 ppm for females, while the NOAEL = 100/50 ppm for males.

In the submission of the full report of this two year study (MRID 43942901), the *study author* acknowledged the two nasal tumors among male rats, one carcinoma of the olfactory epithelium of a Group 5 male, and one adenoma of the olfactory epithelium of a Group 4 male, as constituting positive evidence of carcinogenicity of the test material, predicated upon the rarity of such spontaneous lesions in the F344 rat. Specifically, the study report itself claimed: "Neoplasms which were considered to be related to treatment with malathion were seen in the nasoturbinal tissues and liver. In the nasoturbinal tissues, an adenoma was observed in one male (animal number 4033) from the 6000 ppm dose level and a carcinoma was observed in one male (animal number 5040) from the 12000 ppm dose level. 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* (emphasis added) were identified from nearly 4000 control males and *none* (emphasis added) 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 the original submission) These claims in the original study report were followed-up in HED's review of that study. Accordingly, it was affirmed that Boorman et al (1990) did make the claim as cited, but this published work went on to say that the six neoplasms among historical control male rats were all of the nasal *respiratory* epithelium, while claiming the incidence was *zero* (emphasis added) for neoplasms of the *olfactory* epithelium among some 4000 control males as previously stated. There is a little uncertainty in reading Boorman et al (1990) as to whether the zero incidence refers generally to the olfactory region of the epithelium, or to the neural component of the olfactory epithelium. In either case the incidence is extremely rare. Of course, both nasal tissue neoplasms in males in

the original malathion study were identified as being in the olfactory epithelium, and thus even more rare than referenced in the study report.

As a result of the re-evaluation and peer review of nasal tissues, which is now before us, the character of nasal tissue neoplasms among males has changed somewhat in that one of the two nasal neoplasms is now said to be of the respiratory epithelium (Group 5), while the other remains as of the olfactory epithelium (Group 4). However, further inspection of Boorman et al (1990) discloses that of the six nasal tissue neoplasms identified among male F344 rats, two were of the respiratory epithelium, while the other four were squamous cell tumors. So in essence the relevant historical incidence of neoplasms of the respiratory epithelium is two and of the olfactory epithelium, none, among nearly 4000 control males. It must be acknowledged that nasal tissues in the control animals in this historical data base likely did not receive the level of scrutiny such tissues have now received in the study before us, but by virtue of its largeness it remains perhaps the most relevant historical data base, where rare tumors are concerned, for interpreting this 1996 malathion study. Also noteworthy is that the contemporaneous control group in the present study did receive the same scrutiny as the dose groups, and we understand that of all controls, the contemporaneous control is most important. So the two nasal tissue adenomas among males remain exceedingly rare, whether located in the respiratory or olfactory epithelium. Add to that the two female rats with adenoma of the respiratory epithelium (for which the historical incidence is cited above as zero), one each in Groups 4 and 5, adds further weight to the conclusion of the author of the original study report that nasal tissue neoplasms in this study are related to treatment with malathion. It is now evident in <u>both</u> sexes.

The question of <u>oral</u> tissue squamous cell tumors was not discussed in the original study report, and accordingly there was no presentation or discussion of the historical incidences. Of course, on re-evaluation, the incidence of this tumor type has risen from two to four among treated animals. It should be noted that squamous cell papillomas and carcinomas of oral tissues are nearly as rare as the nasal tumors discussed above. Haseman et al (1990), which served as the source of the nasal tissue historical data as cited in Boorman et al (1990), reported one squamous cell carcinoma and 7 squamous cell papillomas among the nearly 4000 males in untreated dietary and corn oil gavage (gavage) dosed control studies, where six of the seven squamous cell papillomas were seen among the nearly 2000 corn oil gavage controls. Similarly, among females there was one squamous cell carcinoma and seven squamous cell papillomas among nearly 4000 control females in untreated dietary and corn oil (gavage) dosed control studies, and as in the case of males, six of the seven squamous cell papillomas were identified among the nearly 2000 control corn oil gavage dosed females. (p. 557, table 1, Haseman et al, 1990) Since the squamous cell tumors in question are of the oral cavity, the untreated *dietary* historical controls as opposed to the *gavage* dosed group may be expected be the more relevant.

In the nasal tissue re-evaluation report (MRID 44782301), the report's author, Dr. James Swenberg discussed the results. <u>We will quote here in bold print from that report, while inserting in italics qualifying information</u>. The text reads as follows: **"Malathion exposure was associated with clear evidence of nasal toxicity in the two highest exposure groups."** (p. 7)

Further along: **"The nasal toxicity induced by chronic exposure to 6000 or 12000 ppm malathion was characterized by olfactory epithelial degeneration, hyperplasia and cyst formation, goblet cell hyperplasia, congestion, edema and inflammation. The pattern of distribution was somewhat unusual for a dietary study. The lesions were most severe in the dorsal meatus of sections 2 and 3, and were least severe to non-toxic in the lateral scrolls of section 4. Such a pattern is much more reminiscent of inhalation studies. It is possible that the animals received part of their exposure via inhalation at the feeders. There are no known enzyme distribution patterns in the olfactory epithelium of rats that could account for this distribution of lesions."** (pp. 7-8) *We would comment at this point that the uncertainties regarding the possibility of an inhalational component to the nasal exposure in addition to a dietary systemic component is acknowledged, and is mentioned in HED's review of the original study report. Also, we would note that metabolic capabilities of the olfactory epithelium (as contrasted with the other nasal tissues) are remarkable, similar in many ways to those of the liver. It has been speculated this particular capability of the olfactory epithelium serves in the clearing of odorants in maintaining acuteness of olfaction. Interestingly, Boorman et al (1990) say: "Metabolic rate is much higher in the nasal mucosa than in the liver." (p. 336) This background information is also documented in HED's review of the original study report and will not be reiterated at this point. It would have been helpful if Dr. Swenberg had affirmed the metabolic capabilities of the olfactory epithelium per se, and then explained in greater detail what he means by enzyme distributions in the olfactory epithelium in explaining effects seen. We are not quite certain what is being claimed.*

"Based on a review of the histopathology data from the nasal passages, the NOEL for toxicity in the rat nose appears to be 500 ppm malathion in the diet." (p. 8)

"The Study Pathologist and the Reviewing Pathologist had no differences on the diagnosis of animals with neoplasia (table 2)." (p. 8) *Dr. Swenberg goes on to acknowledge the one rat that the Study Pathologist diagnosed as a nasal adenoma in section one, while he, the Reviewing Pathologist, also noted its presence in the lumen of sections 2 and 3. This was the rat (# 5040) first diagnosed by the Study Pathologistas in the original study submission as carcinoma (olfactory) in section 2.* **"There were a total of four primary nasal tumors. All of these were well differentiated nasal adenomas, but they occurred in several locations. There was one in section 5, the most anterior section, in a Group 4 female** *(# 4539, section 5 "respiratory")*, **one in section 1, the second most anterior section in a Group 5 female** *(# 5525, we find this reported also in section 5 "respiratory", rather than in section 1, p. B-2321)*, **one in a lateral scroll of section 4, the most posterior section, in a Group 4 male** *(# 4033, adenoma "olfactory")*, **and one in section 1 in a Group 5 male** *(# 5040)*. **Thus there was no common site for the nasal adenomas.** *This is incorrect in the case of females, where both adenomas were in section 5 "respiratory".* **The Reviewing Pathologist confirmed the presence of a large squamous cell carcinoma arising from the** *(squamous epithelium lining the)* **alveolus of a tooth in a Group 2 female** *(# 2542, section 4)* **and a small squamous cell carcinoma in the palate of the mouth of a Group 5 female** *(# 5503, section 4; this carcinoma was first diagnosed by the Study Pathologist in the original study report as "m-squamous cell carcinoma*

arising from the squamous epithelium lining the alveolus of a tooth." p. 5062 and evidently was changed by the Study Pathologist to that of a squamous cell carcinoma of the palate before submitting the same to the Reviewing Pathologist). **A small squamous cell papilloma in the palate of the mouth in one Group 5 female** *(unable to locate such a tumor in Group 5, but found one in a Group 4 female. #4518, section 3, p. B-1982)* **was diagnosed in the original study** *(presumably meaning as first diagnosed by the Study Pathologist in the new nasal tissue section 3)* **and confirmed by the Reviewing Pathologist. An additional Group 2 male had a similar lesion that was not diagnosed originally by the Study Pathologist, was first called hyperkeratosis by the Reviewing Pathologist, and during the consensus review was diagnosed by both the Study and Reviewing Pathologists as a squamous cell papilloma of the palate** *(# 2029, section 3, p. B-343).*" (pp. 8-9)

"**There is no relationship between the tumor of the tooth** *(we view the language as too loose here, the tumor in question was not of a tooth proper, but of the squamous epithelium of the lining of the alveolus, the socket, if you will, in which a tooth resides, and thus should more properly be considered as an oral cavity squamous cell carcinoma, an assessment concurred in by Dr. Gary Boorman, NTP, in a personal telephone conversation with Dr. Dementi at the time of review of the original study submission)* **and any other neoplasms** *(we disagree and pose that all four squamous cell tumors are tumors of the oral cavity),* **nor is there a relationship between the squamous cell carcinoma and papillomas of the palate with the nasal or tooth** *(again, characterization of this latter tumor as that of a tooth is inappropriate, in our view)* **tumors. These neoplasms arise from different tissues** *(again, we pose all four squamous cell tumors arise in common from the oral squamous epithelium)* **and would not be combined** *(we further pose all eight of these tumors appearing in nasal/turbinate slides are <u>rare</u> in the nasal and oral cavities, a matter of concern within itself which they share in common)* **(McConnell, et al., Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. J. Natl. Cancer Inst. 76: 283-289, 1986).**" (p. 10) *We obtained this reference, and find that with respect to oral cavity tumors, the publication recommends combining squamous cell carcinomas and papillomas of the following tissues: oral cavity, esophagus and forestomach, but is mute with respect to combining tumors of nasal and oral tissues, which we presume should be interpreted to mean the latter would not be combinable. As we understand, according to Boorman et al (1990), the nasal cavity contains respiratory, olfactory and squamous cell epithelia, and had the nasal tumors been identified in the squamous cell epithelium, as opposed to the respiratory and olfactory epithelia, perhaps they then would have been combinable with the oral cavity squamous cell tumors, as we read McConnell et al (1986).*

"**It is difficult to say whether or not the nasal tumors are associated with exposure to Malathion. There was only one benign nasal tumor in any group, so they will clearly not be statistically significant. Likewise, there is no evidence of progression from benign tumor to a malignant neoplasm** *(true insofar as the original carcinoma diagnosis in a Group 5 male is incorrect and remains an adenoma by more recent diagnosis)* **and no common site of occurrence.** *Both nasal tissue adenomas in females were "respiratory", and diagnosed in section 5.* **On the other hand, nasal adenomas are rare neoplasms** *(we have indicated above just how rare)* **and all**

four occurred in noses of the two exposure groups showing considerable nasal toxicity. In my opinion, this would not be a lesion that could be extrapolated to a low dose risk of cancer due to the clear relationship to toxicity that is not present at lower exposures." (p. 10) *We do not concur that nasal toxicity did not extend below the 6000 ppm level, unless he speaks of a specific type of toxicity, and we should note this comment does not address the question of possible risks associated with exposures via the inhalational route, which of course is another subject.*

This last paragraph was devoted to the interpretation of nasal tissue findings. There is no similar discussion by Dr. Swenberg on the rarity, commonality of location, evidence of progression, etc. of the oral squamous cell tumors. Three were of the palate and all four were squamous sell tumors of the oral cavity. Among females, a squamous cell papilloma of the palate in Group 4 and a squamous cell carcinoma of the palate in Group 5 constitutes some evidence of progression. These also, as explained previously, are rare tumors. Two such rare tumors in the low dose group, one in each sex, is of considerable concern, given their rarity, the much less evidence of nasal toxicity at the lowest dose level and the lowness of the dose.

"Dietary exposure of rats to 6,000 and 12,000 ppm Malathion caused significant nasal toxicity characterized by olfactory epithelial degeneration, hyperplasia and cyst formation, goblet cell hyperplasia, congestion, edema and inflammation." (p. 10) *As explained in this review, we conclude evidence of nasal toxicity includes the 500 ppm group, and likely the 100/50 ppm dose group as well.* **"No treatment-related increases in neoplasia were apparent in nasoturbinal and nasopharyngeal tissues."** (pp.10-11) *The phrase "no treatment-related increases in neoplasia" is not to be confused with "no increases in neoplasia related to treatment" and neglects to note that in cases of rare tumors while dose-relatedness strenghtens the conclusion, it is not obligatory before concluding the findings positive. In addition, this statement avoids confronting the evidence of an oral tissue neoplastic response which is as compelling as that of the nasal tissue response, and perhaps of greater concern in that the low dose group is involved, both sexes.*

Additional comments: a) Nasal tumors: given the utter rareness of nasal tumors in historical controls, the four rats with such tumors, two of each sex, one at each of the top two dose levels is considered adequate evidence of carcinogenicity as discussed. In the case of **males**, the tumors were located in tissue sections 1 (Group 5) and 4 (Group 4), both of which tissue sections exhibited other evidence of compound-related toxicity, particularly section 4. In tissue section 1 of Group 5, rats exhibited high incidences of nasal mucosal respiratory epithelial congestion, edema and dilated glands, but no particularly noteworthy increase of incidences of hyperplasia. By contrast, in tissue section 4, more numerous histopathologic findings were increased in Group 4 (and Group 5), e.g. nasal mucosal olfactory epithelial degeneration/atrophy, congestion, edema, hyperplasia, glands-dilated, replacement of the olfactory epithelium by other tissue, etc., such that a more persuasive case for a pre-neoplastic condition existed in tissues represented by section 4. In other words, tissue section 4 was evidently more adversely affected than section 1, and perhaps a more expected site for tumor formation. Concerning nasal cavity tumorigenesis, it is noteworthy that McConnell et al (1986) say "A direct transition from the hyperplastic or dysplastic to the malignant stage has been suggested for *nasal cavity* (emphasis added), glandular stomach, and thyroid C-cell lesions in rats...."

(p. 284) The finding of a neoplasm in section 1 may be of greater concern even though it was seen in the highest dose group. In **females** nasal tumors appearing in both animals, one in Group 4 and the other in Group 5, were identified in sections 5. Among females (and males for that matter), section 5 yielded relatively little to no evidence of toxicity, particularly of the respiratory epithelium (where the tumors appeared), above that of the control and lower dose group. In fact, were section 5 alone examined, apart from the finding of rare tumors, there is virtually no other evidence of a dosing-related toxic response of the respiratory epithelium to the test material. Thus, the argument these tumors are secondary to other toxicologic effects is weak in this case, and with it the argument the tumorigenic effects would not be extapolatable to a low dose risk, except insofar as the low dose in this study is quite a bit lower. In other words, based on evidence of nasal toxicity in section 5, there would be little reason to anticipate the finding of rare nasal tumors. Hence, it is inappropriate to say at what dose level a nasal tumorigenic effect would not be seen. It could be argued that elevated incidences in dose groups of "nasal mucosa (vestibular) squamous cell hyperplasia" seen in section 5 females is possibly another correlate of the incidences of squamous cell neoplasms of the oral tissues identified in females.

b) Oral tumors: Of even greater concern with respect to the possibility of a low dose tumorigenic response rests with the rare oral cavity squamous cell tumors, one of which appeared in each of three dose groups, namely, 100/50, 6000 and 12000 ppm in females, and one at 100/50 ppm in males. Again, these are very rare tumors, and occurred in the absence of any other *claimed* histopathology in the oral cavity. We are not certain oral cavity tissues receive proper evaluation for non-neoplastic or neoplastic effects through examination of nasal/turbinate slides when nasal tissue evaluation is the primary objective. At the very least these rare oral tissue neoplastic findings that were identified buttress a conclusion the agent is tumorigenic based on the nasal tumor incidences.

On examining McConnell et al (1986) as cited in the Reviewing Pathologist's report, and as noted previously, we find the publication advocates combining squamous cell tumors of the *oral cavity, esophagus and forestomach.* The publication also says: "For some neoplasms there is substantial evidence for the sequential progression from the hyperplastic to the benign stage and from the benign to the malignant stage. Progression has been suggested for the following lesions: epidermal skin lesions in mice, alveologenic lesions in mice, *esophageal and forestomach lesions* (emphasis added) in rats, bladder urothelial lesions in rats, testicular interstitial cell lesions in rats, and prostatic lesions in rats." (p. 284). Given this information, we re-examined the summary tables of histopathology findings of the esophagus and forestomach in the 1996 malathion study. In the case of the esophagus, one slide per animal was examined and no squamous cell histopathology was noted. Concerning the forestomach, high incidences of squamous cell hyperplasia and hyperkeratosis at the top two dose levels, *though no tumors, were identified.* [Squamous cell hyperplasia, males: 1, 4, 4, 24 and 47; females: 5, 3, 4, 11 and 36 and hyperkeratosis, males: 1, 4, 4, 23 and 47; females: 4, 3, 4, 11, 36 for the respective dose groups in both cases of 0, 100/50, 500, 6000 and 12000 ppm. The study report notes most of these were in early decedents and the histopathology was attributed in the study report to animals not eating. In considering this data it is noteworthy that squamous cell hyperplasia of the esophagus and forestomach is usually accompanied by hyperkeratosis as noted in Napalkov and Pozharisski (1969), a reference cited in McConnell et al (1986). Also, Brown and

Hardisty(1990) say, in reference to the forestomach: "Focal hyperplasia is characterized by thickening of the epithelial layer that often forms a broad-based lesion with marked hyperkeratosis and infiltration of inflammatory cells in the lumina propria." and "When hyperplasia consists of a diffuse thickening of the squamous epithelium, hyperkeratosis is often a component of the lesion." (p. 21)] While no tumors were found in the forestomach, it is noteworthy that McConnell et al (1986) claim not only that oral, esophageal and forestomach squamous cell tumors are combinable, but that "....the incidence of hyperplasia is taken into consideration in the evaluation of a carcinogenic response..." (p. 286) at these sites. Elsewhere, this publication says: "NTP policy is that certain neoplasms may be combined for statistical assessment of tumor data and that hyperplastic responses may be used as supportive evidence." (p. 283) Hence, squamous cell hyperplasia in one of these organs, in this case the forestomach, might be interpreted as supportive of squamous cell tumor findings in another site, in this case the oral cavity. It is noteworthy that information on oral cavity histopathology in the 1996 Guideline malathion study in the F344 rat is limited to that noted in the nasal tissue slides. FIFRA Guideline testing requirements do not incorporate oral cavity tissues among those required for histopathologic examination. Unfortunately, McConnell et al (1986) do not say how much weight should be given squamous cell hyperplasia, or exactly how to include it in the interpretive process. Also, the finding of squamous cell neoplasms in the oral cavity indicates a need for a closer examination of the squamous cell hyperplasia and hyperkeratosis of the forestomach. Findings of a similar character were evident in the 1979 NCI study of malathion in the F344 rat, as discussed in Huff et al (1985), an NTP Pathology Working Group (PWG) assessment of the study, wherein considerable attention was given to histopathology findings of the forestomach. As rendered in Table 6 (p. 169) of that publication, there were, particularly among male rats at both dose levels, 2000 and 4000 ppm, dosing-related increased incidences of chronic inflammaation, ulcer, acanthosis (diffuse hyperplasia) and hyperkeratosis in the forestomach. Among females the same findings were observed, though were of lower incidence than in males at both dose levels. Among males there was one incident of squamous cell papilloma of the forestomach in the low dose group. The PWG concluded the study was negative for carcinogenicity. In a publication cited by McConnell et al (1986), namely that of Mulay and Firminger (1952), it was demonstrated that squamous cell hyperplasia and hyperkeratosis are precursors of chemical induced squamous cell papillomas and carcinomas of the forestomach. Given the uncertainty that existed initially over the diagnosis of squamous cell papilloma versus hyperkeratosis of the palate in male rat # 2029 according to Dr. Swenberg and considering the fact that Rueber (1985), a pathologist who also examined the 1979 NCI bioassay, interpreted the forestomach histopathology to include dose-related increased incidences of squamous cell papilloma and advised that organs with squamous cells were target sites in the F344 rat, we advise re-examining a number, if not all, of the forestomach lesions diagnosed as hyperkeratosis to be certain they have not been mis-diagnosed in the 1996 study.

Collectively, all of the histopathologic effects tend to support or undergird the findings of four rare neoplasms of the nasal turbinates seen in both sexes at top two dose levels. However, in terms of adverse non-neoplastic effects seen in the respiratory epithelium of tissue section 5 at the top two dose levels in females, there is little that would anticipate the finding of rare neoplasms.

The finding of squamous cell neoplasms of the oral cavity is of particular concern, as these too are very rare, and are distributed at three dose levels among females, the gender exhibiting the greater sensitivity to the test material. One such tumor in the low dose group of both males and females is of particular concern in consideration of rarity, both sexes being involved and the lowness of the dose. The finding is arguably supported among females by evidence of squamous cell hyperplasia even at the lowest dose in nasal tissue section 5, and by extensive squamous cell hyperplasia of the forestomach at the higher dose levels.

In the bioassays on malathion conducted by the National Cancer Institute in the late 1970s, nasal tissues evidently were not routinely examined histopathologically. We are attempting to obtain a definitive answer to this question. However, in the 1978 malathion study in the Osborne-Mendel rat, carcinoma of the nasal sinus was identified in a high dose female rat. It is unclear whether nasal tissues were examined in but this rat alone. Given the current findings in the F344 rat and B6C3F1 mouse, it would be important to have the benefit of nasal tissue histopathology for the NCI studies were the necessary slides available.

In *conclusion*, in this study there is the finding of dosing-related nasal tissue histopathology in the F344 rat study embracing the 500, 6000 and 12000 ppm dose groups, and extending, at least equivocally so, to the lowest dose of 100/50 ppm. Nasal tissue compromise is extensive at the two highest dose levels, and most evident in tissue sections 2,3 and 4. This histopathology, including hyperplasia, would be considered pre-neoplastic and supportive of a positive neoplastic response in terms of the rare nasal tissue neoplastic findings observed, where one neoplasm was identified in each of the two high dose groups of both males and females. The location of the adenomas in females, being of the respiratory epithelium of tissue section 5, places them somewhat out of the principle areas of the nasal tissues in which the most extensive histopathology was seen, namely sections 2,3 and 4, and thus compromises somewhat the argument that these lesions were secondary to nasal pre-neoplastic histopathology. This concern also resides somewhat with the neoplasm in tissue section 1 in the high dose male group where pre-neoplastic histopathology was less evident than in sections 2,3 and 4. So one cannot get around the concern that tumorigenic findings in the nasal cavity are not necessarily captive to pre-neoplastic conditions evident in this study.

Of equal or greater concern is the finding of four rare oral cavity neoplasms. These may be of greater concern because two of the lesions were identified in the lowest dose group, 100/50 ppm, one in males and one in females. Unfortunately, there is no full histopathology assessment of oral cavity tissues for non-neoplastic histopathology, nor for that matter a full assessment of neoplastic findings. The oral cavity tumors that were identified appeared in the nasal turbinate slides. It must be questioned as to whether oral tissues have been adequately examined histopathologically, especially given the rare neoplasms that have been so identified from the formal examination of another tissue, that of the nasal cavity. Publications have been cited in this review indicating that in carcinogenicity assessment, tissues that should be combined for the assessment of squamous cell tumorigenic responses, including hyperplasia, include the oral cavity, esophagus and forestomach. Accordingly, a reinspection of histopathology of the forestomach disclosed increased incidences squamous cell hyperplasia and hyperkeratosis of the two high dose groups, particularly in males. These findings,

to the extent they are combinable with oral tissue findings, tend to support the findings of rare neoplasms of the oral cavity. It is suggested the forestomach hyperplasia and hyperkeratosis be re-examined histopathologically for any evidence of progression to squamous cell papilloma.

As it currently stands, for non-neoplastic nasal tissue histopathology, the study LOAEL = 100/50 ppm (females) and 500 ppm (males); the NOAEL < 100/50 ppm (females) and 100/50 ppm (males).

For neoplastic findings, the study is considered positive at 6000 and 12000 ppm for rats of both sexes based upon the finding of rare nasal tissue neoplasms, as generally supported by extensive nasal histopathology at both of the high doses. The study is considered positive at all doses attributable to rare neoplastic findings, two of which occurred at the 100/50 ppm dose level, one each in rats of each sex, supported by evidence of a dose response for the same rare findings at 6000 and 12000 ppm in females. Additional assessments of oral cavity histopathology may prove useful in characterizing this response more fully, both in terms of neoplastic and non-neoplastic histopathology. The obtaining of nasal and oral tissue histopathology from the NCI studies performed in the late 1970s could also be very helpful to the interpretation.

Additional Comment

This two-year feeding study yielded extensive non-neoplastic nasal tissue histopathology, accompanied by findings of rare neoplastic responses of the nasal cavity. It is uncertain what, if any, direct exposure of nasal tissues may have occurred via inspiration of the test material at the feeders. A matter of concern is whether nasal tissues would be more vulnerable to these effects when exposure at a given dosage level occurs, in fact, by the inhalational route. This remains to be addressed. It also remains to be determined, definitively, the time of onset and progress of nasal tissue responses following inhalational exposure. As matters of interest, the malathion Guideline 13-week subchronic inhalation study in the rat (MRID 43266601) yielded evidence of nasal non-neoplastic histopathology similar to that of this two-year feeding study, namely, degeneration and/or hyperplasia of the olfactory epithelium. These effects were seen after only 13-weeks and occurred at all test concentrations. In fact, another study is required in order to determine the NOAELs for histopathologic effects of the nasal cavity and larynx, as well as for plasma and erythrocyte cholinesterase inhibition, which were also not identified in the current inhalation study. Furthermore, it is noteworthy that essentially the same nasal cavity histopathology was seen in the 2-week range finding inhalation study (MRID 44554301) conducted in preparation for the 13-week inhalation study referenced above. Many questions remain outstanding regarding assessment of nasal tissues responses to malathion via inhalation exposure, and how these may compare to oral feeding studies in terms of dose delivered, time of onset and time course of nasal cavity histopathologic responses. Another question remaining outstanding is to what extent inhalation exposure results from exposure to malathion in the feed. Inhalation exposure might explain or contribute to the presence of rare tumors in the nasal and oral cavities.

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