



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

013720

6/9/97

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Review of the Histopathology Re-assessment of Pituitary and Uterus Tissues for the Malathion 24-Month Oral (Dietary) Combined Toxicity/Carcinogenicity Study in the F344 Rat (MRID 44744201)

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Registrant: Cheminova Agro A/S
Chemical: Malathion
Case No.: 818961
DP Barcode: D255027
MRID No.: 44744201

Submission No.: S560089
P.C. Code: 057701

ACTION: Review the Peer Reviewed histopathology re-assessment of tissues of the pituitary and uterus for the malathion 24-month combined chronic toxicity/carcinogenicity study in the rat. This assessment of these 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. These tissues were not adequately examined histopathologically in the original study submission (MRID 43942901).

CONCLUSION: Presented below are the Citations 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: Pituitary and Uterus Tissue Evaluation and Peer Review. Author: James A. Swenberg, D.V.M., Ph.D. January 15, 1999. Sponsor: Cheminova Agro A/S, Lemvig, Denmark (MRID 44782301).

A 24-Month Oral Toxicity/Oncogenicity Study of Malathion in the Rat via Dietary Administration: Pituitary and Uterus Tissue Evaluation and Peer Review. Author: James A. Swenberg, D.V.M., Ph.D. January 15, 1999. Sponsor: Cheminova Agro A/S, Lemvig, Denmark. PQA Review Summary Table (MRID 44792302).

EXECUTIVE SUMMARY: Toward fulfilling a requirement of HED's CARC for the histopathology evaluation and peer review of microscopic slides of tissues of the pituitary gland and uterus among rats in the combined chronic toxicity/carcinogenicity study (MRID 43942901), the sponsor has submitted the results of this peer review.

In this 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 re-examination and peer review of tissues of the pituitary gland in both sexes and uterus did not reveal any neoplastic responses in either tissue related to treatment with malathion. While not the primary objective of this re-examination, the only non-neoplastic findings were increased incidences of "congestion" of the pituitary gland among male rats in the 6000 and 12000 ppm dose groups and among females in the 12000 ppm dose group.

This histopathology re-examination of tissues of the pituitary gland and uterus is **ACCEPTABLE/NON-GUIDELINE (PENDING SUBMISSION OF AFFIRMATION THAT TISSUE SECTIONS WERE PREPARED ACCORDING TO PROCEDURES PRESCRIBED BY HED)**. This is a special study not designed to satisfy a Guideline testing requirement.

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 pituitary gland (both sexes) and uterus in the combined chronic toxicity/carcinogenicity study in the F344 rat (MRID 43942901). The CARC concluded these tissues had not been adequately 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 pituitary and nasal tissue component of the data requirements have now been submitted to the Agency (MRID 44744201), and is subject of this review.

According to this submission, the report contains the results of the evaluation and peer review of the pituitary and uterus 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 January 27, 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 January 27 letter, Dr. Henry Bolte (the Study Pathologist) of HLS evaluated pituitary glands from all animals and the uterus from females of all dose groups 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 January letter of Mr. Dahl.

II. The Study Report

A. Review Procedure:

The study report claims the peer review consisted of the re-examination of all slides and all diagnoses for the tissues in question. The report claims the criteria used for diagnosing *pituitary* tumors were those described in *Guides for Toxicologic Pathology*:

Proliferative Lesions of the Pituitary Gland (1990) and *Pathology of the Fischer Rat: Pituitary Gland* (1990) (pp. 7-8). Evidently the Study Pathologist, Dr. Henry Bolte, rendered to the Reviewing Pathologist, Dr. James Swenberg, work sheets containing his diagnoses for each tissue section, while Dr. Swenberg noted either "Agree" or an alternative interpretation. The two pathologists subsequently met and reached consensus diagnoses for each slide. There were a total of 1,359 such slides. According to the text, differences of opinion primarily related to the distinction between hyperplasia, adenoma and carcinoma in the pars distalis of the pituitary gland. As to the distinction between adenoma and carcinoma, additional slides of brain and sphenoid bone from animals with original diagnoses of carcinoma were requested and supplied by the performing laboratory. Employing strict criteria for making the calls, i.e. presence or absence of metastasis or aggressive local invasion of adjacent brain or bone, adenoma versus carcinoma diagnoses were made.

Concerning histopathology of *uterine* tissues, no particular criteria for diagnosis was offered or references cited. As in the case of the pituitary gland, the Study Pathologist provided the Reviewing Pathologist his diagnoses on work sheets, while the Reviewing Pathologist either agreed or rendered an alternative interpretation, followed by a meeting of the two for consensus diagnoses.

B. HED's Review of Submission

Appended as attachments 1 and 2, respectively, are summary tables of neoplastic findings (including hyperplasia) for the pituitary gland and neoplastic findings for the uterus as reproduced from the study report. Examination of these tables and the individual animal data do not disclose any treatment-related neoplastic findings for either the pituitary, in either sex, or the uterus. One reason for this re-evaluation in the case of the pituitary gland was to examine all animals in the study, as there was evidence of a possible increase of carcinoma in female groups III and IV, even though all animals were not examined. High mortality in group V females may have explained the lesser incidence in that group attributable possibly to fewer animals at risk for their lifetime and/or competing toxicity. By re-examination, which included all animals, fewer carcinomas were observed in the re-examination than in the original study submission. The incidence of carcinomas in Group III declined from 3 to 2, and in Group IV from 4 to 1, even though more animals were examined in the re-evaluation. So perhaps due to more restrictive diagnostic criteria for carcinoma in the re-evaluation, there were fewer carcinomas than originally of the pituitary gland among female rats. In fact there is no evidence of an effect of dosing on this tumorigenic response in females. Also, there is no evidence of a dosing-related increase in carcinomas among males. Similarly, there is no evidence of an effect on adenoma incidences with dosing in either sex. The study must be viewed as negative for carcinogenesis of the pituitary gland.

In the case of non-neoplastic findings of the pituitary gland apparent in this re-

examination, an inspection of data in appended PQA review Summary (MRID 44792302), attachment 3, from the study report discloses increased incidences of "congestion" in male rats of Groups IV and V, and in females of Group V. Otherwise, there are no particularly noteworthy dosing-related non-neoplastic histopathology findings.

Concerning the re-examination of the uterus, while the tumorigenic findings in the original study report led the CARC to have the uterus re-evaluated, and this report is intended to focus on that issue, an inspection of the same PQA Review Summary, attachment 3, does not disclose any remarkable treatment-related non-neoplastic findings.

C. Discussion

Procedurally, this re-assessment of histopathology of pituitary gland was required primarily to address the incidences of neoplasia (pars distalis adenomas and carcinomas) in all animals. The re-assessment of neoplastic responses of the uterus was driven primarily by a number of differing neoplastic findings, none of which was of high incidence in the original study report. Also, in the case of both tissues, all animals from all dose study groups had not been examined in the original study submission, and in light of the neoplastic findings that were identified originally, it was concluded all animals should be examined. In addition to the conditions prescribed for the re-examination as set forth in the January 7, 1998 letter of Walter Waldrop, the registrant was advised by a March 6, 1998 facsimile of HED's William Burnam to Judy Hauswirth of Jellinek, Schwartz & Connolly, Inc. (attachment 4) that in the case of the pituitary gland, sectioning should be through the widest region of the gland such that both lobes would be represented. In the case of the uterus, it was recommended that three sections be examined, one from each uterine horn plus one from the cervix of each rat. There is no mention in the study report submission affirming that sections of the tissues in question were prepared according to procedures set forth in Mr. Burnam's fax. An examination of the original submission of the combined chronic toxicity/carcinogenicity study (MRID 43942901) shows that in the case of the pituitary gland, one section was taken with no further elaboration. If it is customary among pathologists that the *one* section taken samples both lobes, there should be no question that HED's request has been met. However, it would be helpful if the report of the re-examination made note of HED's recommendation and affirmed it had been met. In the case of the uterus, the original study submission (MRID 43942901) says *two* sections were taken: "uterus (body/horns) with cervix" (p. 46 of the study report), which would be interpreted as likely satisfying HED's recommendation. Still, it would have been better if in the report of the re-examination, HED's recommendations had been acknowledged and affirmed as satisfied.

Assuming sections of pituitary gland and uterus were re-evaluated as requested, there is no evidence of a neoplastic response of the test material. Concerns noted by the CARC in the case of the original study submission have been satisfactorily addressed.

Attachments 1 and 2, reproduced from pages 10 and 11, respectively, of the study report (MRID 44744201), attachment 3 (MRID 44792302) and attachment 4 (letter from William Burnam, EPA, to Judy Hauswirth, JSC) are not available electronically.

See the file copy for hard copy of these three attachments.

SignOff Date:	6/9/97
DP Barcode:	D255027
HED DOC Number:	013720
Toxicology Branch:	TOX1