Malathion: 28-day oral toxicity study in beagle dogs (MRID 45077703).

9/12/2000

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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

September 12, 2000

SUBJECT: Review of 28-Day Oral Toxicity Study of Malathion in Beagle Dogs (MRID 45077703); Recommendations for Further Testing in Dogs

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Registrant: Cheminova Agro A/S
Chemical: Malathion
Case No.: 818961
DP Barcode: D265565
MRID No.: 45077703

Submission No.: S579174
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ACTION:

In responding to the February 10, 2000 preliminary risk assessment for malathion, which includes a requirement for an additional subchronic study in the dog to address deficiencies in the existing Guideline 1-year chronic toxicity study in dogs (MRID 40188501), most notably the absence of a NOAEL for cholinesterase inhibition, the malathion registrant, Cheminova Ago

A/S, submitted a 28-day study in dogs toward satisfying the requirement in question.

This document constitutes a review of the 28-day study in Beagle dogs, and the response to the registrant's request that the requirement for a 90-day study in the dog be lifted.

Presented below are the Citations and Executive Summary of the reviewed study, the Assessment follows.

CITATIONS:

Tegeris Laboratories, Inc., 1987. One-Year Oral Toxicity Study in Purebred Beagle Dogs with AC 6,601. Tegeris Laboratories, Inc., Laurel, MD. Study No. 85010. April 30, 1987. Unpublished. MRID 40188501

American Cyanamid Company, 1988. AC 6,601: A 28-Day Oral Toxicity Study in Beagle Dogs. American Cyanamid Company, Agricultural Research Division, Princeton, NJ. Project No. 0852, Report No. AX88-3. Study Author: J.E. Fischer. Report Issued April 8, 1988. MRID 45077703.

EXECUTIVE SUMMARY:

At its December 22, 1998 meeting, Health Effects Division's HIARC established a requirement for a 90-day (subchronic) study in the dog. HIARC's rationale is cited in this review. The requirement for a subchronic study was driven by the core supplementary status of the 1-year chronic study in the dog based principally on the absence of NOELs for erythrocyte and plasma cholinesterase inhibition in dogs of either sex. The registrant subsequently submitted a 28-day study in the dog, in an effort to satisfy the requirement for a subchronic study. It is uncertain but this 28-day study may have served initially as a range-finding study for the chronic study. It also did not identify NOELs for the blood borne cholinesterases, since in fact the dose range for the 28-day study was higher and involved fewer animals per dose group. After reviewing the 28-day study, it is concluded that this study standing alone, or considered in conjunction with the chronic study, does not address the question of the absence of NOELs for erythrocyte or plasma cholinesterase inhibition in the dog. The two studies affirm a poor or weak dose response, but leave unanswered the question of NOELs for cholinesterase inhibition and whether the manner of dosing, daily capsule administration, may be less efficacious than dietary administration in eliciting a response, which was one of the concerns expressed by HIARC. It is therefore concluded additional subchronic testing (feeding) in the dog will be necessary in an effort to identify NOELs for plasma and erythrocyte cholinesterase inhibition. Since the identification of NOAELs for cholinesterase inhibition constitutes the focus of concern, the subchronic study could be limited to an investigation of plasma and erythrocyte cholinesterase inhibition.

The registrant should consult with HED the manner of conduct of this study.

DETAILED CONSIDERATIONS:

I - Hazard Identification Assessment Review Committee (HIARC) Requirement for Additional Testing in Dogs

The rationale for requiring additional testing of malathion in the dog was set forth in the December 22, 1998 HIARC report: "The HIARC concluded that a 90-day study in dogs is required based on the rationale provided below: (I) in 1988, the requirement for a subchronic feeding study in dog (82-1b) was waived contingent upon performance of a chronic toxicity study in dogs; (ii) in waiving this study, there is enhanced burden for the Registrant to provide an acceptable chronic study which was not achieved by the present study (MRID No. 40188501); (iii) there are species-related biochemical similarities (absence of plasma carboxylesterase) to anticipate that the dog would respond similarly to man; (iv) since the chronic study was conducted in 1987, cholinesterase methodology may be problematic and should be examined for conformity with the most current Agency standards; (v) the contrast between doses inhibiting cholinesterase in man and in rat serves to indicate more definitive testing is required in a third species; and (vi) a subchronic study could possibly address the question of whether the type of dosing (capsule vs. dietary) is critical in the dog." (pp. 15-16) It should be noted that the 1988 Malathion Registration Standard required a new chronic *feeding* (emphasis added) study in the dog. (p. 121) Additionally, the HIARC recommended that the registrant consult the Agency for study design and protocol prior to initiation of the study.

II - Statement of Registrant's Concern

In responding to the Preliminary Risk Assessment for malathion as it pertains to the issue of testing requirements in the dog, the registrant says the following:

"In its Hazard Profile, Section 3.1, EPA is requiring a 90-day feeding study in dogs because the available 1-year study is unacceptable. EPA classified the 1-year study as core-supplemental mainly because a NOEL for cholinesterase inhibition was not identified.

"Cheminova believes that a 90-day feeding study in dogs is not needed because available data from a 1-year dog toxicity study (using 6 animals/sex/group) and a 28-day dog toxicity study (using 3 animals/sex/group) provide adequate information on the toxicity of malathion in non-rodent species. Each of these studies is discussed below.

"In the 1-year dog study, Cheminova believes that the NOEL is 62.5 mg/kg bw/day based on statistically significant inhibition of plasma and RBC cholinesterase activity at the next higher dose level.

"With these comments, Cheminova is submitting a final report for a 28-day oral toxicity study of malathion (92.4%) in Beagle dogs (Fischer, 1988). In this study, 3 dogs/sex/group were given 0, 125, 250, or 500 mg malathion/kg bw/day by capsule daily for 28 days. One dog died at 500

mg/kg bw/day (became listless and anorexic). Clinical signs (diarrhea and loose and mucoid stool), decreased food consumption, and statistically significant body weight gain were seen at 500 mg/kg bw/day. At 250 and 125 mg/kg bw/day, no significant effects were noted on food intake, body weight gain, organ weights, clinical chemistry and hematological parameters. No microscopic changes were seen in any of the tissues examined at any dose levels tested. Cholinesterase data indicate statistically significant inhibition of plasma and RBC cholinesterase activities at 250 and 500 mg/kg bw/day. Based on statistically significant inhibition of plasma and RBC cholinesterase activity, the NOEL is 125 mg/kg bw/day.

"Cheminova believes that the data provided in these two studies should be sufficient for characterizing the toxicity of malathion in non-rodent species. Conducting an additional 90-day feeding study in dogs will provide no data that would alter the present dietary and non-dietary risk assessments." (p. 27)

III - Review of the 28-Day Dog Study (MRID 45077703)

This study was not submitted to satisfy a Guideline testing requirement, but rather as providing information intended to address a deficiency in the Guideline 1-year Chronic Toxicity Study in Dogs, initially reviewed October 5, 1987 (HED Doc. No. 006349) and subsequently revised for presentation to HIARC (DER # 4 in HIARC package). Since the study was not submitted to satisfy a Guideline testing requirement, it will receive here only a partial review, focused mainly upon the data on cholinesterase inhibition.

According to the study report, the testing protocol is summarized as follows: "Beagle dogs, approximately 7 months of age, received AC 6,601 by gelatine capsule at doses of 0, 125, 250 and 500 mg/kg/day for 28 consecutive days. There were 3 dogs/sex/dose level. The dogs were observed twice daily for signs of overt toxicity, morbidity, and mortality. Detailed observations and individual body weights were recorded weekly. Individual food intake was recorded daily. Hematologic and biochemical tests were performed on all animals at pretreatment (-1 day) and at 15 days and 29 days posttreatment. The weights of the liver, kidney, heart, spleen, brain, thyroid and adrenal glands, testes, and ovaries were recorded. Samples of liver, kidney, heart, spleen, brain, skeletal muscle, sciatic nerve, adrenal glands, thyroid glands, gonads, spinal cord and all abnormal tissues were taken from each dog and submitted for microscopic evaluation." (p. 4 of study report)

General findings are summarized as follows as taken from page 5 of the study report: Overt symptoms of toxicity indicative of treatment (including diarrhea, loose stools, and mucus in the stool) were observed at *all* doses. Anorexia was observed at 500 mg/kg/day, and one male dog in this dose group died on day 24. Weight gains were significantly decreased at the 500 mg/kg/day dose level, with some lesser degree of decrease at 250 mg/kg/day. Weight gain among dogs of receiving 125 mg/kg/day was comparable to control animals. Food consumption was decreased at 500 mg/kg/day, while food consumption in the lower two dose groups and the control group were comparable.

"At the 15-day bleeding statistically significant decreases in hemoglobin, hematocrit, and RBC counts were observed at the 125 mg/kg/day level. A decrease in band cells was observed at termination in the dogs that received the 500 mg/kg/day dose. These changes while statistically significant were still within normal ranges for this age of dog. No other significant changes were observed in any of the hematological parameters measured during the study.

"At the 15-day bleeding, values for albumin and sodium were decreased at the 500 mg/kg/day level. At the terminal bleeding values for BUN (500 mg/kg/day), SGOT (500 and 125 mg/kg/day), albumin (500 and 250 mg/kg/day), and creatinine (500 and 125 mg/kg/day) were significantly decreased but were still within normal limits for this age of dog. No other statistically significant changes were observed in any of the clinical chemistry parameters measured during the study." (p. 5 of study report)

A cursory review of data presented in the study report supports the above assessment of the study author of the parameters in question, but again, the focus of this review is upon the cholinesterase data, a more detailed presentation of which data follows at this point.

Mean cholinesterase activity values as independently calculated for male and female dogs at the pretreatment, 15-day and terminal (28-day) time points are tabulated below:

	Pretreatment		*15-day*		*Termination*	
	RBC (IU/ML)	Plasma (U/L)	RBC	Plasma	RBC	Plasma
Control						
males	2.42	2.9	1.97	2.1	2.38	2.0
females	2.34	3.2	2.05	2.2	2.46	2.3
125 mg/kg/day						
males	2.42	3.4	1.76	1.6	2.01	1.5
females	2.30	4.0	1.84	1.9	1.97	1.9
250 mg/kg/day						
males	2.25	3.1	1.84	1.4	1.97	1.4
females	2.54	3.7	1.84	1.7	1.97	1.8
500 mg/kg/day						
males	2.05	3.3	1.72	1.1	1.91	1.1
females	2.33	4.5	1.56	1.8	2.01	1.8

The above mean values were computed from individual data in Appendix Tables 2.7 - 2.9 (pp. 145-152 of the study report). The means are for three data points each excepting those for males of the 500 mg/kg/day group at term, which are for two animals.

In the opinion of this reviewer, the study incorporates too few animals per dose group and the data are too variable to reach any firm conclusions concerning a NOAEL or NOEL for this study based upon cholinesterase inhibition. In males, the data reveal a tendency for plasma cholinesterase to be inhibited in a dosing related manner (starting at 24-25% at the low dose) both at the 15-day and terminal time points, but the data do not permit a conclusion as to whether there is a NOAEL for this end point, or where within the dosing range a NOAEL/LOAEL resides. It is questionable as to whether there is any effect on plasma cholinesterase among females at any dose, again because the data are too weak and variable to reach a conclusion. Where erythrocyte cholinesterase is concerned, there appears to be a slight tendency for inhibition in dosed groups, but little can be affirmed in terms of a dose response, again for the same reasons of too few animals and variability in the data. Among females, inhibition of erythrocyte cholinesterase at the high dose level of about 24% at the 15-day time point and 18% at term might be meaningful findings, but for the reasons previously stated, this cannot be viewed as anything more than an indication of an effect at the highest dose level. The problem with this data is that it is not suitable to establish with any degree of certainty a NOAEL/LOAEL, nor it is adequate to rule out a meaningful effect in inhibiting cholinesterase *even at the lowest dose level*, as both enzymes were numerically reduced in activity at the lowest dose level in *both sexes* to an overall extent ranging 10-25%.

Discussion

Again, the focus of this review is on the cholinesterase data and whether this 28-day study viewed in concert with the previously submitted and reviewed 1-year chronic toxicity study in the dog would satisfy the 1988 Registration Standard requirement for a chronic feeding study in dogs, i.e. would this 28-day study serve to upgrade the core supplementary status of the Guideline study to core acceptable. It appears as though this 28-day study served as a dose range-finding study for the selection of dose levels employed in the chronic study. Accordingly, the dose levels in the 28-day study were 0, 125, 250, and 500 mg/kg/day, whilst those doses employed in the chronic study were 0, 62.5, 125, and 250 mg/kg/day, suggesting a decision was made for a frame shift of dosing to lower dosages, perhaps in search of a NOEL for cholinesterase inhibition, and perhaps certain other end points.

Where cholinesterase inhibition is concerned, the chronic study did not identify NOELs for either erythrocyte or plasma cholinesterase inhibition in either sex. As indicated in the DER, it is apparent that mean values for erythrocyte and plasma cholinesterases were inhibited in both sexes at all dose and all time points. At the low dose of 62.5 mg/kg/day, both plasma and erythrocyte cholinesterase inhibitions were about 25% compared to control levels. Yet, ".....mean plasma cholinesterase values appeared to reach a steady state reduction in male dogs at the lowest dose level and earliest time point, i.e. there appeared to be no further decrease in activity with either increasing dose or time. This steady state level of activity occurs at about 70-75% of control values. In females there is more evidence of a dose-related (not time-related) trend, where levels of activity decline with increasing dose from about 78% to about 63% of base line value at 1-year." (pp. 5-6) The finding of cholinesterase inhibition in the chronic study was

somewhat more firm, as more animals were evaluated. Like unto the 28-day study, the dose response was weak across doses.

In other words, both of these studies where malathion was administered via capsule similarly reveal a poor dose response, i.e. only gradual increases in inhibition with increasing dose, yet while not identifying a NOEL for cholinesterase inhibition. In the 28-day study, there was numerical inhibition at the lowest dose of 125 mg/kg/day, a finding which was not definitive for either enzyme in either males or females. Yet, when the lowest dose was reduced to 62.5 mg/kg/day in the chronic study, inhibition of both enzymes continued to be obse. ed, perhaps because the power of resolution was increased by the use of a larger number of animals/dose level. So the two studies are not inconsistent, however the 28-day study does not help to address the underlying concerns over: a) the absence of NOAELs for plasma and erythrocyte cholinesterase inhibition, b) uncertainty as to just how low dosing must be in order to preclude cholinesterase inhibition, and c) what might be the reason for such poor dose responses in the dog, i.e is the dog inherently a weakly responding species, or might the problem be an artifact of the manner of dosing.

Conclusions

1) The 28-day study of malathion in Beagle dogs appears to have served as a dose range-finding study for dose selection in the Guideline 1-year chronic study. The 28-day study was conducted at higher doses than those selected for the chronic dog study, in which a NOAEL for cholinesterase inhibition was not identified. Thus, it is not surprising that the 28-day study failed to show a clear NOAEL for cholinesterase inhibition, making it even less definitive than the original chronic dog study it was recently submitted with the intent to satisfy.

2) Both the 28-day and chronic studies yielded similar results where cholinesterase inhibition is concerned, both in not identifying a NOAEL and in yielding evidence of a poor dosing-related response in the dog. So in this sense, the 28-day study not heretofore seen in the Health Effects Division, supports the poor dose response observed in the chronic study. However, this is not particularly surprising given the similarities of dosing procedure and doses employed, and offers little reason to revise the chronic study to a core acceptable status. In other words, it is difficult to embrace just how this less definitive 28-day study could serve to satisfy concerns expressed in regard to the more definitive Guideline study.

3) In yielding poor dosing-related inhibitions of the blood cholinesterases, both studies may serve to indicate that malathion is poorly absorbed from the G.I tract when administered once per day as a bolus dose via capsule. Since the 1988 Registration Standard called for another chronic study, specifically a *feeding* study, and the HIARC report noted that another study, subchronic in this case, "....could possibly address the question of whether the type of dosing (capsule vs. dietary) is critical in the dog", malathion should be tested via the diet. Furthermore, the dietary route would more closely emulate another human exposure route.

Another study of subchronic duration should be performed to evaluate cholinesterase inhibition in the dog during dietary administration of malathion. The registrant should consult with HED the manner of conduct of this study. The purpose will be to identify the NOAEL/LOAEL for plasma and erythrocyte cholinesterase inhibition. Doses should be selected appropriately for this purpose. Periodically during testing, blood samples should be taken for assay of erythrocyte and plasma cholinesterase activities. Should such testing confirm the level of response observed in the 28-day and 1-year studies, and identifies the NOAELs for these end points, no further work would be indicated. On the other hand, if based on the cholinesterase data, the dog appears considerably more responsive, this may raise concerns about the overall adequacy of the previous 1-year study, in which case the registrant and HED should reconsider what course to pursue, particularly if this and perhaps any other available data suggest more remarkable absorption of the test material as administered via the diet.