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

014413

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM December 14, 2000

SUBJECT: Review of FIFRA 6(a)(2) Data Submission for a Dose Range-Finding Study in Sprague-Dawley Rats Intended to Provide Guidance for Dose Selection for the Definitive Developmental Neurotoxicity and Acetylcholinesterase Studies as Required by SRRD for Malathion.

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Registrant: Cheminova Agro A/S
Chemical: Malathion
Case No.: 818961
DP Barcode: D268862; D269706
MRID Nos.: L0000581; L0000598

Submission No.: S585067; S586744
P.C. Code: 057701

ACTION:

Review 6(a)(2) and follow-up data submitted for dose-range finding studies designed for dose

selection for definitive Developmental Neurotoxicity and Acetylcholinesterase studies of malathion in SD rats.

EXECUTIVE SUMMARY:

The registrant, Cheminova A/S, has submitted a FIFRA 6(a)(2) letter presenting preliminary data on a dose range-finding study on malathion designed to provide data for dose selection in definitive Developmental Neurotoxicity and Acetylcholinesterase studies in CD rats. The study was conducted at doses of 0, 7.5, 750 and 1250/1000 mg/kg/day. The study revealed excessive toxicity among dams at the top two dose levels, resulting in the study's premature termination. In order to derive additional information from the study, pups born of dams in the control and low dose groups were re-allocated, and tested at 200 and 450 mg/kg/day. These dose levels also proved to be excessively toxic to pups, and it was necessary to terminate prematurely this portion of the study as well. Among *dams*, plasma, erythrocyte and brain cholinesterases were all inhibited at the top two dose levels. In terms of per cent inhibition, the erythrocyte enzyme was the more remarkably inhibited, for which there may have been a threshold 10% inhibition, not statistically significant, at the low dose level. Among *litters*, plasma cholinesterase was not inhibited at any dose level; brain cholinesterase was inhibited only at the high dose level, and that among females only; erythrocyte cholinesterase was inhibited at the top two dose levels, accompanied by possibly a threshold 16% inhibition, not statistically significant, at the low dose level. The registrant attempted to determine what might have caused the unexpected excess deaths among both adult and juvenile rats, but has been unable to explain the findings as due to anything other than the toxicity of the test material.

In consideration of these findings, a second range-finding study was conducted at the dosage levels of 0, 7.5, 35, 75 and 150 mg/kg/day. Among *dams*, there were no effects on bodyweight or bodyweight change at any dose during gestation. Clinical chemistry parameters assayed were unaltered. Based upon GD 20 data, plasma cholinesterase was inhibited by about 18% at the 150 mg/kg/day dose level. Erythrocyte cholinesterase was inhibited by approximately 10%, 33% and 60% at the 35, 75 and 150 mg/kg/day dose levels, respectively. It is uncertain as to the statistical significance of these findings. Brain cholinesterase was not inhibited at any dose level. Among *litters*, brain cholinesterase was not inhibited in either sex. As derived from pooled blood samples, plasma and erythrocyte cholinesterases were evidently not inhibited, though high variability may have masked a marginal effect on erythrocyte cholinesterase at the higher dose levels.

In the case of pups on PND 21, plasma and brain cholinesterases were reportedly significantly inhibited in both sexes at 150 mg/kg/day. Marginal inhibitions of plasma cholinesterase (males, 11%; females, 16%) were observed at 75 mg/kg/day. Erythrocyte cholinesterase was significantly inhibited in both sexes at 35, 75 and 150 mg/kg/day, while in pups treated at 7.5 mg/kg/day, this enzyme was marginally lower (males, 19%; females, 18%) than control.

No rationale was provided for the selection of dose levels for the second dose range-finding

study. It is unlikely that the highest dose level chosen for the repeat range-finding study, namely 150 mg/kg/day, would be an MTD for dams based upon effects observed in the first range-finding study. Rather, it is estimated that a high dose level more on the order of 650-700 mg/kg/day and appropriately spaced lower dose levels should have been employed in the second range-finding study in search of an MTD for the definitive Developmental Neurotoxicity study, as the first range-finding study does not support a conclusion the MTD for dams would be much below 750 mg/kg/day; nor is there evidence in the second range-finding study to support a conclusion the MTD would not be considerably above 150 mg/kg/day.

The registrant is advised that an acceptable Developmental Neurotoxicity study must incorporate an MTD for dams. Furthermore, the definitive studies should be designed to yield clear NOAELs for cholinesterase inhibition.

DOCUMENTS UNDER CONSIDERATION

1) The 6(a)(2) letter dated July 10, 2000 from Cheminova's Dr. D. O'Shaughnessy addressed to EPA's Docket Processing Desk, reporting "Preliminary data on malathion developmental neurotoxicity study (HLS study CHV062)" This was a dose range-finding study of cholinesterase inhibition intended to provide guidance for dose selection in definitive Developmental Neurotoxicity and Acetylcholinesterase studies required by SRRD as set forth in a September, 1999 DCI.

Please note Toxicology Branch I did not receive this submission until September 11, presumably attributable to formatting issues.

2) A Huntingdon Life Sciences June 29, 2000 protocol entitled: "Malathion : Effects on Cholinesterase in the CD Rat (Adult and Juvenile) by Oral Gavage Administration". The stated objectives of this protocol are two-fold, namely, to assess the effects of acute or prolonged dosing of adult or young pre-weanling rats with malathion on erythrocyte, plasma and brain cholinesterase activity; and secondly, to evaluate recovery of cholinesterase activity 39 days after the end of dosing young rats. (p. 1) While not indicated in the protocol, it appears as though this work is also preliminary or range-finding for the required Developmental Neurotoxicity study on malathion.

3) Additional information provided by SRRD, consisting of a September 20 cover letter of Cheminova's Don O'Shaughnessy containing follow-up information pursuant to our September 19 conference call on the above 6(a)(2) submission, plus information on a second, on-going, range-finding study.

4) An October 13, 2000 letter of Cheminova's Dr. Don O'Shaughnessy to Ms Patricia Moe, SRRD, containing additional follow-up data to the second malathion cholinesterase range-finding study as presented in a September 29 letter of S.M. Fulcher, Huntingdon Life Sciences, to Mette Jensen/Dorrit Sandergaard, Cheminova.

In support of our understanding of the objectives of the studies so far conducted, we obtained in-house a copy of a March 21, 2000 letter of Dr. O'Shaughnessy to Ms. Karen Angulo, SRRD, containing additional information on the conduct of the Developmental Neurotoxicity study and the Cholinesterase study.

HED's interpretation and assessment of these materials is summarized as follows.

In terms of overview, the July 10 letter of Dr. O'Shaughnessy can be understood to convey that the study which was the subject of the 6(a)(2) letter was designed to have served as a dose range-finding study for dose selection for *both* of the subsequent definitive studies, namely, the Developmental Neurotoxicity study and the Acetylcholinesterase study, as required in the SRRD DCI of September 1999. The information submitted does not provide a clear statement of protocol for this range-finding study. Dosage levels employed were 0, 7.5, 750 and 1250/1000 mg/kg/day. Inasmuch as the study was terminated due to unexpected toxicity as the higher dose levels, another dose-range finding study was initiated at lower dose levels, as explained in the September 20 letter of Dr. O'Shaughnessy. Dr. O'Shaughnessy advises in the same more recent letter that the first data from the repeat range-finding study are ".....only now becoming available." He also indicated in the more recent letter that Cheminova has been unable to determine what might have caused the anomalous results in the original range-finding study. Doses chosen for the repeat range-finding study were: 0, 7.5, 35, 75 and 150 mg/kg/day. No rationale was provided for the dose levels chosen, nor any protocol.

It is noteworthy that the substantial lowering of the dose range for the repeat range-finding study would suggest that the inherent toxicity of the test material was concluded by the registrant to be the most likely explanation for those anomalous results.

Again, as evident from the information and data currently available, the more recent study is a repeat dose range-finding study that is designed to serve in dose selection for the definitive Developmental Neurotoxicity and Acetylcholinesterase studies required by SRRD, and that the same dosage levels would be employed in both studies. Although, along these lines, there is a statement in the March 21, 2000 letter of Dr. O'Shaughnessy to Ms. Karen Angulo, SRRD, to the effect that the same doses may not be employed in both studies: ".....it may be useful to have a similar range but not equal doses in the two studies in order to better assess both cholinesterase NOELs and other, potentially persistent, developmental effects." (p. 2) Be it advised at this point that dosage selection for the definitive Developmental Neurotoxicity study must incorporate an MTD for dams.

a) Comments on the initial range-finding study

As recorded in the 6(a)(2) submission, dose levels employed in the original range-finding study were 0, 7.5, 750 and 1250/1000 mg/kg/day. Apparently there were 15 dams each in the control and three dose groups. The high dose level was reduced from 1250 mg/kg/day to 1000 mg/kg/day due to clinical signs and early mortalities. The report does not say how soon, after

commencement of dosing on day 6 post-mating, the dosage level was reduced to 1000 mg/kg/day.

Further, due to excessive toxicity and mortality among dams at the top two dose levels (there were 6 premature deaths in the 1250/1000 mg/kg/day and 2 premature deaths in the 750 mg/kg/day group), it was necessary to terminate the study, evidently on day 20. Dr. O'Shaughnessy says in his July 10, 2000 letter "Due to excessive mortality, the high and mid-dose groups were sacrificed early for humane reasons." This is somewhat confusing in that the July 6, 2000 letter of Huntingdon's Steve Fulcher included in the same 6(a)(2) package says: "All animals were killed on Day 20 of gestation, however for Groups 3 and 4 dosing stopped prior to Day 20 and some animals had a longer period for recovery prior to necropsy than others." This apparent confusion may be reconciled by the fact that as set forth in the protocol for the definitive cholinesterase study, one-half of the dams are sacrificed by GD 20 to obtain dam and fetal cholinesterase data and the remaining one-half of the dams are continued until PND 4. Pups derived from that delivery are then dosed on PND days 11-21. If this was the approach to be taken in the range-finding study, then indeed to sacrifice all dams on GD 20 in the top two dose groups would be premature for at least half of the dams.

In order to reap additional information from this range-finding study, offspring from the control and low dose groups were re-allocated for further evaluation of effects of direct dosing (PND 11-21) and according to the July 10 [6(a)(2)] letter "Doses selected for this evaluation were 450 mg/kg, 200 mg/kg, and 7.5 mg/kg." (rather than only at 0 and 7.5 mg/kg/day for these two groups as originally planned). This aspect of the study was also terminated prematurely due to excessive toxicity and mortality among pups at the top two dose levels. There are no comments regarding the response of the low dose group pups. It is unclear in the documents submitted just how many times pups were actually treated, before dosing was terminated. According to the July 4, 2000 letter of Dr. Fulcher, in reference to pups receiving the 200 mg/kg/day dose: " A number of pups at this dosage showed adverse clinical signs, consistent with that seen previously at 450 mg/kg/day including body tremors, approximately 1-2 hours following dosing and one pup was killed for animal welfare reasons."

Necropsy indicated the finding of "prominent, reddened pancreas" among decedent dams, and that: "Preliminary cholinesterase data in dams sacrificed early from 1250/1000 and 750 mg/kg groups, and from pups sacrificed early from 200 and 450 mg/kg groups appear to be within a range that might be expected from the dose levels of malathion in the study, but would not be expected to cause death." We should note that since dosing was halted for the top two groups earlier in the study, cholinesterase inhibition may have been more remarkable at that time than at term. More specifically, as tabulated below, data provided in the 6(a)(2) submission indicate that among *dams,* plasma, erythrocyte and brain cholinesterases were all clearly inhibited at the top two dose levels, though with not much apparent difference between the top two doses. Erythrocyte cholinesterase was inhibited by about 10% in the low dose group, though not statistically significantly so. It is difficult to say whether this represents a threshold for erythrocyte cholinesterase inhibition. As assayed among *litters* at day 20, plasma cholinesterase

was not inhibited at any dose level. Brain cholinesterase among males was not inhibited at any dose level, while among females brain cholinesterase was inhibited in the high dose group. Erythrocyte cholinesterase was significantly inhibited at the top two dose levels, and by 16%, though not statistically significant, at the low dose. Again, it is difficult to determine whether this finding at the low dose level constitutes a threshold effect, but it may well be. Erythrocyte cholinesterase was the more remarkable responder of the cholinesterases as assayed in both dams and litters.

The cholinesterase data do not suggest an MTD for dams was exceeded.

	Cholinesterase Inhibition (%)		
Dose level (mg/kg/day)	7.5	750	1250/1000
Dams			
Plasma	None	28b	39b
Erythrocyte	10	78b	80b
Brain	None	49b	35b
$b \leq 0.01$			
Litters			
Plasma	None	None	None
Erythrocyte	16	27a	27a
Brain			
Males	None	None	None
Females	None	None	30a
$a \leq 0.05$			

In summary, it appears the 6(a)(2) letter is communicating the facts that clinical signs of excessive toxicity and mortality observed among dams in this range-finding study were unexpected at the dosage levels of 1250/1000 mg/kg/day and 750 mg/kg/day, and as a result the study had to be discontinued. Furthermore, testing of pups at doses of 450 and 200 mg/kg/day was similarly terminated prematurely for the same reason of excessive toxicity. In his September 20 letter, Dr. O'Shaughnessy says: "Due to excess mortality, it was not possible to determine cholinesterase activity in rat pups at 200 and 450 mg/kg/d." This latter quotation is somewhat at variance with the above quotation from Dr. Fulcher's letter concerning the obtaining of cholinesterase data on the re-allocated pups tested at 200 and 450 mg/kg/day.

The registrant has no explanation for these unexpected findings. The 6(a)(2) letter indicates that cholinesterase inhibition obtained for the studies in both dams and pups was not of sufficient magnitude to explain the early mortalities. However, we should note that since dosing was terminated early due to the evidence of excessive toxicity, cholinesterase activity may have recovered somewhat by the time of sacrifice. In terms of cholinesterase inhibition among litters, it appears that plasma and brain cholinesterases were not as remarkably affected as among the

parent dams. However, erythrocyte cholinesterase was inhibited in both dams and litters at the top two doses, and questionably so among litters (16% inhibition) versus dams (10% inhibition) at the low dose level. It is very important that the definitive studies be designed to identify clear NOAELs for cholinesterase inhibition.

Following the September 19 joint SRRD/HED conference call with Cheminova, additional information on this initial range-finding study was provided by Cheminova. Accordingly, as further explained, pregnant dams were administered malathion by oral gavage, at the doses indicated, beginning on day 6 of gestation. By the next day, i.e. day 7 of gestation, salivation was a common clinical sign among all dams at the top two dose levels, a symptom which persisted for the duration of testing, except at the very end of the study period. No clinical observational data were provided for the control and low dose groups, which are assumed to be negative. On day 20, one dam in the 750 mg/kg/day group was "killed *in extremis*". This rat was exhibiting additional adverse clinical signs. Among the 1250/1000 mg/kg/day group, tremors and other clinical signs, in addition to salivation, were beginning to be seen around day 14, becoming more evident with time. By day 19, several rats evidently were either sacrificed *in extremis*, or found dead.

In additional data submitted, during gestation food consumption was reduced in dams of the high dose group. Bodyweight was decreased at the high dose level and bodyweight change evidently so at the top two doses. There was evidently no effect on bodyweight in the low dose group. In terms of bodyweight gain, as recorded in the table entitled "Bodyweight change - group mean values (g) for females during gestation" (page 10, though the pages are not completely numbered in the submission), bodyweight decreases are evident at the 1200/1000 and 750 mg/kg/day. However, the effect at 750 mg/kg/day may not be real given the variability, particularly evident in the control and low dose groups. *Based upon the bodyweight gain data, it appears as though an MTD for dams was not exceeded at the 750 mg/kg/day, as according to the Agency's testing Guidelines for Developmental Neurotoxicity (OPPTS 870.6300; August 1998), the highest dose level ".....should not result in a reduction in weight gain exceeding 20 percent during gestation and lactation." (p. 2)*

There were no evident adverse effects noted for dams in the 7.5 mg/kg/day group, based upon inspection of food consumption, bodyweight and reproduction parameter data provided in the submitted package of materials.

As indicated, pups from the control and 7.5 mg/kg/day groups were re-allocated and tested at the 200 and 450 mg/kg/day dosage levels. According to the September 20 letter of Dr. O'Shaughnessy, those tested at 450 mg/kg/day are designated as group 5 and those tested at 200 mg/kg/day as group 6. This ordering of dosing does not appear to be consistent with data provided on the two groups. For example, individual bodyweight data presented for Groups 5 and 6, would suggest survival was better for Group 5 than for Group 6. Also, post-dose clinical signs and increased mortality would suggest Group 6 was the more severely affected, though the data sheet for most of the Group 5 offspring seems to be missing from the package. It is

assumed the September 20 letter has mistakenly reversed the ordering of the two offspring dose groups. It is apparent the data submitted are preliminary and were assembled in an expedited manner. It is most obvious that the young rats treated at 200 and 450mg/kg/day were severely affected, resulting in pre-mature termination of both groups (see table of "Post-dose signs for offspring")

b) Comments on the second range-finding study

According to the September 8, 2000 letter of Steve Fulcher, Huntingdon Life Sciences to Dr. Mette Jensen, Cheminova A/S, the more recent range-finding study, conducted at dosages of 0, 7.5, 35, 75 and 150 mg/kg/day, yielded findings as presented in the letter, which are briefly summarized as follows.

There were no effects on bodyweight or bodyweight change at any dose during gestation. Other than cholinesterase, clinical chemistry parameters assayed were unaltered. Among *dams* on GD 20, plasma cholinesterase was inhibited by about 18% at the 150 mg/kg/day dose level, but not evidently so at the lower doses. Brain cholinesterase was not inhibited at any dose level. Erythrocyte cholinesterase, on the other hand, was inhibited by approximately 10%, 33% and 60% at the 35, 75 and 150 mg/kg/day dose levels, respectively. Among *litters*, brain cholinesterase was not inhibited in either sex. Based upon assays of pooled blood samples, plasma and erythrocyte cholinesterases were evidently not inhibited, though high variability may have masked a marginal effect on erythrocyte cholinesterase at the higher two dose levels. Results of any assessments of statistical significance for these various findings were not in evidence.

In the October 13, 2000 letter of Dr. O'Shaughnessy, pertaining to the second dose range-finding study, cholinesterase data for PND 21 rat pups are provided. The findings might be summarized as follows.

In the case of pups on PND 21, plasma and brain cholinesterases were reportedly significantly inhibited in both sexes at 150 mg/kg/day. Marginal inhibitions of plasma cholinesterase (males, 11%; females, 16%) were observed at 75 mg/kg/day. Erythrocyte cholinesterase was significantly inhibited in both sexes at 35, 75 and 150 mg/kg/day, while in pups treated at 7.5 mg/kg/day, this enzyme was marginally lower (males, 19%; females, 18%) than control. In consideration of the dose response for erythrocyte cholinesterase inhibition, namely, males 58%, females 67% @ 150 mg/kg/day; males 42%, females 46% @ 75 mg/kg/day; males 30%, females 31% @ 35 mg/kg/day; and males 19%, females 18% @ 7.5 mg/kg/day, it cannot be accepted that the study identifies a NOAEL even though the inhibitions are reported to be statistically significant in all but the 7.5 mg/kg/day dose groups.

In his October 13, 2000 letter, Dr. O'Shaughnessy says: "Please note that these preliminary data have been compiled for EPA in an expeditious manner, and are not formatted per PRN 86-5. While the studies have been conducted according to GLPS, the data presented here have not been

subject to QA. It is anticipated that the range-finding studies will be formally reported in the same time-frame as the principal studies." We therefore are more or less compelled to accept the data submitted at face value, and render our best interpretation of the same.

Again, the rationale for the dose range selection for the repeat range-finding study has not been presented. The highest dose level, 150 mg/kg/day, is insufficient, since it would not be anticipated to be an MTD for pregnant dams, as would be required for the conduct of an acceptable Developmental Neurotoxicity study. The top dose level in a range-finding study would be expected to slightly exceed an MTD, such that among the dosages employed the investigator would have sufficient information to permit selection of a dose that would be reasonably expected to be an MTD. It is suggested that the top dose for the new range-finding study should have been around 650-700 mg/kg/day, with other doses reasonably spaced below this level in the search for an MTD. There is concern that a dose level of 150 mg/kg/day is too low for ferreting out an MTD.

c) Concluding observations

Be it advised that based upon the initial range-finding study, there is evidence of enhanced sensitivity of pups versus dams based in turn upon serious toxicologic effects observed in pups at 200-450 mg/kg/day versus that in dams at 750-1250/1000 mg/kg/day, though dams were not tested at doses between 7.5 and 750 mg/kg/day.

It is also advised that based upon the comparative GD 20 dam and PND 21 pup cholinesterase data in the second range-finding study, it appears pups are more sensitive, and there is no clear NOAEL for erythrocyte cholinesterase inhibition in pups of either sex.

It is essential that dose selection for the definitive studies be designed to 1) secure an MTD, 2) address the question of relative susceptibility, and 3) anticipate the identification of clear NOAELs for cholinesterase inhibition.