014544

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

MEMORANDUM **FILE** April 19, 2001

SUBJECT: Review of a February 13, 2001 FIFRA 6(a)(2) Submission of Preliminary Data from a Developmental Neurotoxicity Study on Malathion Performed in CD Rats.

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THRU: Alberto Protzel, Ph.D. 4/20/01
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PM Team 53
Special Review and Reregistration Division (7508W)

Registrant: Cheminova Agro A/S
Chemical: Malathion
Case No.: 818961
DP Barcode: D273313
MRID No.: None

Submission No.: S593717
P.C. Code: 057701

ACTION:

Review 6(a)(2) preliminary data submitted from a Developmental Neurotoxicity (DNT) Study of malathion to determine if it is appropriate for use in determining impacts on the Malathion Risk Assessment.

EXECUTIVE SUMMARY:

Findings reported in the registrant's February 13 submission of data under 6(a)(2), taken in concert with previously submitted 6(a)(2) data as reviewed in HED on December 14, 2000 do not

support a conclusion that a dose as low as 150 mg/kg/day would satisfy as an MTD for dams in a definitive DNT study. Though direct oral administration of malathion at dosage levels of 200 and 450 mg/kg/day to pups was quite toxic as reported under 6(a)(2) for the initial range-finding study, evidence does not support a conclusion that such dosage levels either achieve or exceed an acceptably high dose level, or maximum tolerated dose (MTD), for dams. Furthermore, since under DNT testing Guidelines *only dams* are administered the test material, specifically during gestation and early lactation, GD 6 through PND 10, and since the most recent 6(a)(2) submission indicates limited or compromised conveyance of malathion to offspring, either placentally or via the milk, there is no reason to conclude the developing individual will experience exposures to malathion at dosages close to those administered to dams. Hence, there is no justification for selecting a dosage range no higher than 150 mg/kg/day for the definitive DNT study.

Evidence presented in both 6(a)(2) submissions clearly reveal enhanced susceptibility of the young versus adults to the effects of orally administered malathion in terms of both general toxicity and cholinesterase inhibition. Though the cholinesterase findings provided in the most recent 6(a)(2) submission are described as preliminary, a March 19, 2001 letter of Ms Diane Allemang, JSC, advises that the cholinesterase component of the combined DNT/Cholinesterase testing has been completed, and no additional new cholinesterase data is to be expected. Hence, the earlier findings and the existing cholinesterase data, which represents all of the testing expected from the cholinesterase aspect of the combined DNT/Cholinesterase study, demonstrate enhanced pup susceptibility, and should be considered at this time, pending the final report, in any deliberations to revise the Risk Assessment's conclusion concerning both endpoints and the susceptibility of the young and developing individual toward imposition of the 10X safety factor as required under FQPA.

Where the DNT study component is concerned, the top dose level evaluated should be an MTD for dams. For the cholinesterase component, which involves direct oral dosing of both young (developing) animals and mature animals, it is essential dosage levels chosen be expected to have the full potential to exploit any differences in susceptibility. Nothing less should be sought to satisfy requirements under FQPA toward the protection of infants and children. Should the oral MTD for dams exceed the oral MTD for offspring as anticipated from the data submitted under 6(a)(2), it may be necessary to conduct the two components (DNT; cholinesterase) at different dose ranges.

Cheminova's September 20, 2000 letter to Patricia Moe, says in reference to the initial range-finding study, the Agency is reminded of the ".....unexpected excess deaths among both adult and juvenile rats in the first such study at doses lower than observed in other malathion toxicity studies." While Cheminova offers assurances every effort is being made to explain these anomalous findings, the absence of an adequate explanation remaining at this time is unsettling. To the extent the surprising initial findings of excessive toxicity to pups and dams do not hold up in subsequent studies, there is to that extent, even less justification to pursue the DNT study at a lower dose range than originally intended.

The lack of evidence of malathion's presence in dam's milk as reported in the most recent 6(a)(2) submission should trigger a revisit to HIARC's decision to discount evidence of enhanced pup susceptibility in the Guideline Reproduction Study based upon a generic assumption of the agent's presence in the milk.

HED's REVIEW and INTERPRETATION

The February 13, 2001 submission of Jellinek, et al, covering preliminary cholinesterase data obtained in conjunction with the DNT study being performed on malathion at Huntingdon Labs, was submitted as required by law under Section 6(a)(2) of FIFRA, presumably because the registrant felt sufficient concern that the data may constitute "..... additional factual information regarding unreasonable adverse effects on the environment of the pesticide....." (FIFRA, March 1997, p. 59) So the question posed by SRRD is whether the findings rise to that level, and whether the findings in question would impact the Malathion Risk Assessment as it stands at this time. In an effort to respond to this question, Toxicology Branch will attempt first to review the data as presented in the February 13 submission, taking into consideration the December 14, 2000 Toxicology Branch/HED review of a previous 6(a)(2) data submitted July 10, 2000, and follow with comment regarding the level of importance to ascribe to the findings as they currently stand.

The following are considered noteworthy observations:

1) **Status of Completion of Cholinesterase Component of Study**
The February 13 submission is described by the registrant's representative as preliminary cholinesterase data for the definitive DNT and cholinesterase studies required by the Agency. However, as explained in the March 19, 2001 letter of Ms Diane Allemang to the OPP Docket Processing Desk-6(a)(2), the cholinesterase component of the combined DNT/Cholinesterase study is complete, i.e. the Agency has received via this February 13, 2001 6(a)(2) submission the complete set of statistically analyzed cholinesterase mean values. No additional animal studies are contemplated. Individual animal data are expected to be included as well as the present data in the formal submission of the study report. The registrant is apparently proceeding with a definitive DNT study where the highest dose employed is 150 mg/kg/day, while as explained in the December 14 HED review of the earlier 6(a)(2) submission on the range-finding studies, 150 mg/kg/day would not be expected to be an MTD in dams.

2) **Lack of MTD for Dams in the Definitive DNT Testing Protocol**
In the current 6(a)(2) submission, comparative cholinesterase data for dams versus fetuses on GD20 after dosing mothers from GD6 to GD20 (see Attachment, first table, p. 1) indicate no effect for fetuses or dams in terms of brain cholinesterase inhibition. Plasma cholinesterase was significantly though marginally inhibited at 50 and 150 mg/kg/day in fetuses, but not so in dams. RBC cholinesterase was significantly inhibited at 50 and 150 mg/kg/day in dams and fetuses. ***For reasons expressed in this review, these data do not support a conclusion that 150***

mg/kg/day would serve as an MTD for dams in the definitive DNT study, nor does it reveal much of an effect on fetuses. The 6(a)(2) *text* argues that this data illustrate fetal dosing via transplacental exposure, but that the effect is less remarkable in fetuses due to "distribution and metabolism in dams". Also, we should note that to the extent fetuses are inherently more susceptible than dams, as the 6(a)(2) suggests is true for pups relative to dams based on findings when pups and adults were both dosed orally, transplacental exposure may be even less than one would conclude if fetuses and dams were equally susceptible. ***This could mean that effective dosing of fetuses in reproduction and developmental toxicity studies is actually less than in the dams as administered, and should be factored into the conclusion as to whether such studies do or do not reveal enhanced susceptibility of the young. The comparative cholinesterase responses in pups versus young adults following oral dosing of both represents a recent effort to assess relative susceptibility of the young. The finding that pups are inherently more sensitive in the case of malathion introduces a new factor in the interpretation of whether developmental toxicity studies truly assess relative susceptibility of the developing individual versus the dam. Also, these findings indicate the developing individual is less exposed, systemically, than dams and hence are afforded a level of protection while in utero.***

3) **Evidence Indicating Absence of Appreciable Amounts of Malathion in Dam's Milk**
In an Agency review of DNT protocols for five organophosphates (including malathion) (Susan Makris, HED to Michael Nieves, SRRD, January 8, 2001) mention is made of the need for assessments of the test chemical in dam's milk during postnatal days 0-10 in order to determine likelihood of transfer of the chemical during lactation. Yet the protocol evidently indicated these measurements would not be performed. So the January 8 letter says in response, "It is, nevertheless, expected that the study report for each chemical will discuss these issues." It appears the registrant considers the data on cholinesterase findings presented in this component of the malathion cholinesterase study as serving to address the Agency's desire for information regarding the presence/absence of malathion in dam's milk.

Absence of cholinesterase inhibition in pups whose mothers were dosed at doses as high as 150 mg/kg (single dose) on PND4, four hours post-dosing is illustrated in the second table on the appended page 1 of the 6(a)(2) letter. It is understood, based upon the 3/29/00 correspondence from Cheminova's Don O'Shaughnessy, that the PND4 pups were culled from dams treated all along from GD6 thru PND4, and thus dam dosing was not restricted to but the one PND4. (p. 8) It is not clear which dams were sacrificed on PND4 in the 6(a)(2) letter. The 6(a)(2) letter concludes the test material does not enter mother's milk at sufficient quantity to inhibit pup cholinesterase in any compartment. One might add here, "after multiple daily dosing of rat dams". It is to be noted that the lack of cholinesterase inhibition in any compartment for dams at 150 mg/kg in this particular data set ***again does not support the proposition that a dose as low as 150 mg/kg could serve as an MTD for dams in the DNT study.***

4) **Acute (One Dose) Toxicity More Remarkable in Pups than Young Adult Rats**
As illustrated in the third table on page 1 (Attachment) and clinical signs data for neonates presented on page 2 (Attachment), at the single dose of 450 mg/kg as administered orally to both

pups and young adults on PND 11, toxicity is clearly more pronounced in pups than in adults. Brain cholinesterase was substantially inhibited in pups at 150 [44% (M); 48% (F)] and 450 [84% (M); 81% (F)] mg/kg, as contrasted with no such inhibition in adults of either sex (pup NOAEL = 50 mg/kg; adult NOAEL $\geq$ 450 mg/kg). Also, rbc cholinesterase was more remarkably inhibited in pups, where for males, inhibition was statistically significant at all doses and among females was inhibited across the 50-450 mg/kg dose range, while inhibition in adults was confined to the 450 mg/kg dose level [pup NOAEL = 5 mg/kg (F), < 5 mg/kg (M); adult NOAEL = 150 mg/kg (M, F)]. Plasma cholinesterase was also more remarkably inhibited in pups [pup NOAEL = 5 mg/kg (M, F); adult NOAEL = 150 (M), $\geq$ 450 (F) mg/kg]. ***These data indicate enhanced pup susceptibility to malathion.*** As before, the marginal rbc cholinesterase inhibition in adult rats of both sexes at 450 mg/kg, taken in concert with a similar marginal inhibition of plasma cholinesterase among males only and the absence of brain cholinesterase inhibition in either sex, ***does not support a conclusion that a dose as low as 450 mg/kg/day, and certainly not as low as 150 mg/kg/day, could serve as an MTD for dams in the DNT study.***

5) **Repeated Dosing Toxicity in Pups (PND 11-21) More Remarkable Than in Young Adult Rats Dosed 10 Consecutive Days**

In the daily dosing study during PNDs 11-21 presented in the first table on page 2 (Attachment), brain cholinesterase was inhibited in pups (both sexes) at 150 mg/kg/day. The magnitude of inhibition was not as great as that following the single 150 mg/kg dose as administered on PND 11 shown in the previous table. A possible reason is that of adaptation taking place during the course of the eleven days of administration in the daily dosing study. On the other hand, one might expect a more remarkable effect following the multiple dosing scenario. In any case, the more remarkable inhibition noted in the previous study adds support to the finding of brain cholinesterase inhibition here as real at 150 mg/kg/day. Furthermore, it would appear even less likely that brain cholinesterase inhibition would have been observed in adults at 450 mg/kg/day in the present case had that dose level been included. Among adult rats, there were no statistically significant inhibitions of any of the cholinesterases at any dose level. The substantial rbc numerical inhibitions in adults [43% (M); 48% (F)] at 150 mg/kg/day certainly suggest a positive response, but the absence of statistical significance in both cases would indicate such high variability as to render the data inconclusive. In other words, there is no clear finding in this preliminary data of a positive effect of the daily dosing study in adult animals. The inclusion of a 450 mg/kg/day dose level in this eleven day study, as was done in the one day study, could have proved very beneficial in assessing the magnitude and character of pup enhanced susceptibility now evident in this study, and particularly so in the preceding one day study. ***So again, the multiple daily dosing study across PNDs 11-21 illustrates both enhanced susceptibility of the young and that a dose as low as 150 mg/kg/day would not be an MTD for dams, and hence could not be expected to serve as an adequate high dose for a definitive DNT study.***

In summary, for the sake of comparison, as discussed in the December 14 HED review, doses employed in the first range-finding study were 0, 7.5, 750 and 1250/1000 mg/kg/day. Excessive toxicity among dams at the top two dose levels forced premature termination of the study. Pups

from the control and 7.5 mg/kg/day dose groups were re-allocated, and dosed directly at 200 and 450 mg/kg/day. Both of these levels proved excessively toxic to pups, resulting in premature termination of this aspect of the study as well. However, there is no reason to expect such toxicity among pups when their mothers are treated at such doses as 200-450 mg/kg/day, especially in view of the evidence in the latest 6(a)(2) submission of limited transfer of the test material placentally, or via the milk.

A second range-finding study was conducted at 0, 7.5, 35, 75 and 150 mg/kg/day. In the December 14 HED review of the July 10, 2000 6(a)(2) submission of this range-finding data, it was concluded, among other matters, that the second range-finding study should have included dose levels in excess of 150 mg/kg/day, as the data in the first range-finding study did not support a finding that a dose as low as 150 mg/kg/day would be an MTD for dams.

6) Additional Evidence that the MTD is Predictably Higher than 150 mg/kg/day

Additional reasons to be concerned that a dose level of 150 mg/kg/day would not be an MTD for dams in the DNT study aspect are summarized as follows:

a) There is only RBC ChEI, but no Overt Maternal Toxicity .

In the second dose range-finding study as reported in the first 6(a)(2) letter, wherein doses were 0, 7.5, 35, 75 and 150 mg/kg/day, there were among dams no clinical signs or effects on food consumption or bodyweight or bodyweight gain. Other than cholinesterase inhibition, clinical chemistry parameters 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. The 60 % inhibition at 150 mg/kg/day compares favorably with the 50% inhibition observed in dams at 150 mg/kg/day in the more recent range-finding study as reported in the last 6(a)(2) letter. Other parameters that might help identify an MTD were not provided for the latter range-finding study. So the question is whether erythrocyte cholinesterase inhibition (50-60%) standing alone in the absence of any other evidence of an MTD, so serves to identify an MTD. In the absence of clear guidelines for the use of cholinesterase data alone in establishing an MTD, this toxicologist cannot accept that erythrocyte cholinesterase inhibition is adequate to that end.

The DNT Guidelines say with respect to dose selection: "**Dose levels and dose selection.**

(i) At least three dose levels of the test substance plus a control group (vehicle control, if a vehicle is used) should be used.

(ii) If the test substance has been shown to be developmentally toxic either in a standard developmental toxicity study or in a pilot study, the highest dose level should be the maximum dose which will not induce in utero or neonatal death or malformations sufficient to preclude a meaningful evaluation of neurotoxicity. (We should note the Guideline being quoted is for a study in which only dams are dosed, specifically GD6 through PND 10, and would not pertain to any possible consequences of direct dosing of pups.)

(iii) If a standard developmental toxicity study has not been conducted, the highest dose level, unless limited by the physicochemical nature or biological properties of the substance, *should induce some overt maternal toxicity* (emphasis added), but should not result in a reduction in weight gain exceeding 20 percent during gestation and lactation.

(iv) The lowest dose should not produce any grossly observable evidence of either maternal or developmental neurotoxicity.

(v) The intermediate doses should be equally spaced between the highest and lowest doses used." (OPP Health Effects Test Guidelines OPPTS 870.6300 Developmental Neurotoxicity Study, August 1998, pp. 2-3)

Again, since there was no evidence in the range-finding study of any effects on dams at the 150 mg/kg/day, including clinical signs, food consumption or bodyweight gain, and in the absence of any guidelines for the use of erythrocyte cholinesterase inhibition in identifying an MTD, this toxicologist does not accept that 50-60% erythrocyte cholinesterase inhibition, standing alone, satisfies as adequate evidence of *overt toxicity*. Granted, such inhibition is to be viewed as an effect, indeed as an adverse effect, but of not sufficient import to conclude dams are adequately challenged to serve effectively in addressing the purpose of the DNT study. In the absence of any other effects in dams, erythrocyte cholinesterase inhibition, if so used, could serve as a roadblock to effective testing of cholinesterase inhibiting compounds versus other classes of agents.

b) <u>The Highest Dose Exceeded 150 mg/kg/day in Previous Developmental and Reproduction Toxicity Studies</u>

The proposed dose range for the DNT study (0-150 mg/kg/day) appears quite low in comparison with the **Guideline Developmental Toxicity** and **Reproduction studies** of malathion that are a matter of the record. Presumably, the high dose levels in those studies were satisfactory in terms of MTD. For **Developmental Toxicity studies**, the Guideline (OPPTS 870.3700) indicates, where dose levels are concerned "....the highest dose should be chosen with the aim to induce some developmental and/or maternal toxicity but not death or severe suffering. In the case of maternal mortality, this should not be more than approximately 10 percent. The intermediate dose levels should produce minimal observable toxic effects. The lowest dose level should not produce any evidence of either maternal or developmental toxicity (i.e., the no-observed-adverse-effect level, NOAEL) or should be at or near the limit of detection for the most sensitive endpoint." (p. 2) In the malathion Developmental Toxicity study in the Sprague Dawley rat (MRID 41160901), doses employed were 0, 200, 400 and 800 mg/kg/day. The review (HED Doc No. 008384) says: "No treatment-related mortalities occurred during the study. Clinical signs of toxicity were observed only at 800 mg/kg/day and consisted of urine stained abdominal fur in 5/25 dams and chromodacryorrhea and chromorhinorrhea in 1 dam. Statistically significant decreases in mean body weight gains were observed only in the 800 mg/kg/day dams during the period of treatment with malathion, particularly during days 6-12 of gestation. During days 6-12, mean bodyweight gains were 33.8, 36.0, 36.7 and 27.3 grams for the control, 200, 400 and 800 mg/kg/day groups, respectively. There was a rebound effect (increased body weight gain) in these dams during the post-dosing period. Statistically significant decreases in mean food consumption were also observed in the 800 mg/kg/day dams during the period of treatment

with malathion. During days 6-12, mean food consumption was 20.4, 20.8, 20.8 and 18.7 grams/day for the control, 200, 400 and 800 mg/kg/day groups respectively." (pp 1-2) Cholinesterase assays are not a requirement and were not performed in the Developmental Toxicity study. It is quite likely that one or more of the cholinesterases would have proven to have been significantly inhibited, in a dose-related manner, in this study had such assays been performed. So a significant question concerns the degree to which cholinesterase inhibition, had it been the most sensitive end point, would have overridden the findings used above to conclude dosing was in fact conducted in dams at proper dosage levels. Dams must be adequately challenged in order to provide a meaningful assessment.

Similarly, for the sake of comparison, in the malathion **Guideline 2-Generation Reproduction Study** (MRID 41583401), maternal doses employed were 0, 51, 153, 451 and 703 mg/kg/day. There were no treatment-related mortalities, clinical signs of toxicity, effects on food consumption, or gross or microscopic pathology, at any dose level. There were statistically significant decreases in body weight at the 703 mg/kg/day dose level, but the effect on body weight was not excessive, though it served as the basis for the designated LOEL/NOEL of 703/451 mg/kg/day. As in the case of the Developmental Toxicity study, cholinesterase assays were not included, which could have posed a similar concern as previously mentioned.

The Developmental Toxicity and Reproduction studies reveal malathion to be well tolerated at 700-800 mg/kg/day on the basis of criteria employed according to the Guidelines for those studies. Likewise, the range-finding study for the DNT study indicates malathion to be well tolerated at 150 mg/kg/day on the basis of the same parameters, absent additional testing between 150 and 750 mg/kg/day, wherein it may also have been well tolerated. Yet, in the initial range-finding study, at 750 mg/kg/day dams did exhibit substantial adverse effects, in a study that gave surprising and, as yet, inexplicably high toxicity.

It is difficult to accept a high dose level of but 150 mg/kg/day for the definitive DNT study as an MTD and sufficiently challenging dose based upon rbc cholinesterase inhibition alone, in the absence of clinical signs such as those observed at substantially higher doses in the Developmental Toxicity and Reproduction studies, without some explanation. Such a dose is below the lowest dose, 200 mg/kg/day, employed in the Developmental Toxicity, and below the NOEL of 451 mg/kg/day in the Reproduction study, based upon observations not including cholinesterase data.

Inherent in the December 14 review was the concern that testing at doses no higher than 150 mg/kg/day might preclude discovery of evidence of enhanced pup susceptibility already evident in the initial range-finding study. Now, in support of the earlier expressed concern, this preliminary cholinesterase data provided under the February 13 6(a)(2) submission indicate that neither 150 nor even 450 mg/kg/day would be an MTD in dams in the conduct of a definitive DNT study.

It is not clear in the 6(a)(2) letters how dosing will be conducted in the definitive DNT versus

cholinesterase studies. According to the 1998 Health Effects Test Guidelines OPPTS 870.6300 for the Developmental Neurotoxicity Study, only dams are administered the test material. The dosing period is from day 6 of gestation through day 10 postnatally. So in the conduct of the DNT study, where offspring receive the test material only via the dam (transplacentally or during nursing), data on the toxicity of malathion to pups obtained via range-finding direct dosing of pups is of little value in identifying dosage levels to be employed in the DNT study. The high dose in such studies should be an MTD for dams. On the other hand, such studies would be of value in selecting dosage levels for a definitive study of the comparative cholinesterase enzyme responses, and toxicity in general, of pups versus older animals from direct oral exposure to given doses of malathion. So there needs to be a clear statement as to how the two studies will be conducted, and it appears dosage levels employed might be expected to differ for the definitive DNT and cholinesterase studies.

In the 6(a)(2) submissions, much of the comparative cholinesterase data in pups versus adults derives from direct oral dosing of both. As to the question of relative susceptibility of pups versus adult animals, it is certainly important to assess the relative toxicity *and* degree of cholinesterase inhibition in offspring versus adult animals following direct dosing of both.

7) <u>Impact of the Data on the Risk Assessment</u>

As to the question of whether this preliminary data is appropriate for use in determining the impacts on the Malathion Risk Assessment, pending submission of the final report, the data certainly appear to be sufficiently rigorous to provide compelling evidence of enhanced susceptibility of the young versus adult animals as the result of direct oral dosing. The more remarkable effect on brain cholinesterase in pups following single doses of malathion at 150 and 450 mg/kg is most compelling evidence of a differential effect. This effect was also seen at 150 mg/kg/day, though less remarkably so, following daily dosing for 11 days. That aspect of the testing should also have included a dose at least approximating 450 mg/kg/day. The findings were statistically significant. In addition, the absence of a NOEL for RBC cholinesterase inhibition in male pups in both the single and multiple dosing scenarios based upon statistically significant findings across all doses, constitutes compelling evidence of enhanced susceptibility. The data in the latter multiple dose comparison is more equivocal though, given the numerical inhibitions noted in adult males at 50 and 150 mg/kg/day. The indication of enhanced toxicity among pups in the earlier 6(a)(2) submission as discussed in the December 14 HED review is supportive of enhanced pup susceptibility. Hence, the earlier findings and the existing cholinesterase data, which represents all of the testing expected from the cholinesterase aspect of the combined DNT/Cholinesterase study, demonstrate enhanced pup susceptibility, and should be considered at this time in any deliberations to revise the Risk Assessment's conclusion concerning both endpoints and the susceptibility of the young and developing individual toward imposition of the 10X safety factor as required under FQPA.

In closing, we should note that in Cheminova's September 20, 2000 letter to Patricia Moe, in reference to the initial range-finding study, the Agency is reminded of the ".....unexpected excess deaths among both adult and juvenile rats in the first such study at doses lower than

observed in other malathion toxicity studies." While Cheminova offers assurances every effort is being made to explain these anomalous findings, the absence of an adequate explanation remaining at this time is unsettling. To the extent the surprising initial findings of excessive toxicity to pups and dams do not hold up in subsequent studies, there is to that extent, even less justification to pursue the DNT study in a lower dose range than originally intended.

THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE
ELECTRONICALLY ------- SEE THE FILE COPY

ENCLOSURES FROM (2 PAGES) CHEMINOVA LETTER
DATED FEBRUARY 13, 2001 (From Jellinek, Schwartz & Connolly, Inc.)

Cholinesterase activity as percent less than control in dams and fetuses on GD20 after dosing of mother with malathion technical from GD6 and onwards

Dose	Fetuses			Dams		
	Plasma	RBC	Brain	Plasma	RBC	Brain
5	7	4	-	12	-	1
50	14**	11*	5	6	19**	1
150	15**	19**	-	13	50**	4

-: *level of activity increased compared with control*
*: *P = 0.005*
**: *P = 0.05*

Cholinesterase activity as percent less than control in neonates on PND 4 four hours after dosing of the mother.

Dose	Fetuses			Dams		
	Plasma	RBC	Brain	Plasma	RBC	Brain
5	3	-	-	4	2	2
50	-	2	3	3	4	1
150	-	8	5	1	4	1

-: *level of activity increased compared with control*
*: *P = 0.005*
**: *P = 0.05*

Cholinesterase activity as percent less than control in young adults and pups two hours after a single oral dose of malathion technical

Dose	Males						Females					
	Pups, PND 11			Adults			Pups, PND 11			Adults		
	Plasma	RBC	Brain	Plasma	RBC	Brain	Plasma	RBC	Brain	Plasma	RBC	Brain
5	7	16*	1	0	-	5	3	7	4	10	-	-
50	19**	25**	6	-	-	5	16**	23**	10	-	-	-
150	36**	55**	44**	1	2	7	35**	48**	48**	8	6	-
450	54**	72**	84**	24**	25**	3	52**	61**	81**	11	17**	4

-: *level of activity increased compared with control*
*: *P = 0.005*
**: *P = 0.05*

12.

Cholinesterase activity as percent less than control in young adults and pups following 11 days of oral treatment with malathion technical

Dose	Males						Females					
	Pups, PND 11			Adults			Pups, PND 11			Adults		
	Plasma	RBC	Brain	Plasma	RBC	Brain	Plasma	RBC	Brain	Plasma	RBC	Brain
5	13	17*	1	3	4	-	10	15	1	5	2	2
50	19*	39**	0	10	20	-	19*	34**	-	15	20	-
150	24**	67**	16**	13	43	1	32**	68**	16**	13	48	5

-: *level of activity increased compared with control*
*: *P = 0.005*
**: *P = 0.05*

Clinical signs observed for neonates following a single dose at 450 mg/kg/day

Dam No.	Pup No.	Clinical signs observed
77	5	Slight tremors approximately 1 hour after dosing
	12	Slight tremors approximately 1 hour after dosing
78	5	No signs observed
	12	No signs observed
79	5	No signs observed
	10	No signs observed
80	5	Moribund, killed early approximately 1 hour after dosing
	11	Spasmodic period of marked head nodding approximately 1¾ hour after dosing, tremors approximately 2 hours after dosing
81	5	No signs observed
	13	No signs observed
1082	5	No signs observed
	10	Slight tremors approximately 1 hour after dosing
83	5	No signs observed
	13	No signs observed
84	5	No signs observed
	11	Tremors approximately 2 hours after dosing

No clinical signs were observed at lower dosages.

Pup No. 5 was killed earlier than planned after being found in a moribund condition approximately one hour after dosing. no evidence of any reaction to treatment (i.e. body tremors) had been observed prior for this animal prior to this. It is not clear whether the condition of this animal was due to an effect of treatment or perhaps a dosing error. The pup was killed by decapitation (for blood sampling), so it was not possible to confirm the aetiology.

DP BARCODE: D273313 REREG CASE # 0248

CASE: 818961 DATA PACKAGE RECORD DATE: 04/20/01
SUBMISSION: S593717 BEAN SHEET Page 1 of 1

* * * CASE/SUBMISSION INFORMATION * * *

CASE TYPE: REREGISTRATION ACTION: 625 6(A)(2)
CHEMICALS: 057701 Malathion (ANSI) 100.00 %

ID#: 057701
COMPANY:
PRODUCT MANAGER: 53 BETTY SHACKLEFORD ROOM: CS1
PM TEAM REVIEWER: CARMELITA WHITE ROOM: CM2
RECEIVED DATE: 02/16/01 DUE OUT DATE: 03/18/01

* * * DATA PACKAGE INFORMATION * * *

DP BARCODE: 273313 EXPEDITE: Y DATE SENT: 03/09/01 DATE RET.: / /
CHEMICAL: 057701 Malathion (ANSI)
DP TYPE: 102 Phase V Review
CSF: N LABEL: N

ASSIGNED TO	DATE IN	DATE OUT	ADMIN DUE DATE: 04/06/01
DIV : HED	03/09/01	/ /	NEGOT DATE: 04/06/01
BRAN: TOX	03/09/01	04/20/01	PROJ DATE: / /
SECT: IO	03/09/01	04/20/01	
REVR : BDEMENTI	03/09/01	04/20/01	
CONTR:	/ /	/ /	

* * * DATA REVIEW INSTRUCTIONS * * *

Please review this preliminary data to determine if it is appropriate for use in determining the impacts on the RA. This is the third dose-range finding study Cheminova has performed. No MRID is given since it is preliminary.308-7038 (White).

* * * DATA PACKAGE EVALUATION * * *

No evaluation is written for this data package

* * * ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION * * *

DP BC	BRANCH/SECTION	DATE OUT	DUE BACK	INS	CSF	LABEL