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Malathion: Registrant Comments on Agency review of prenatal developmental toxicity study in rabbits (MRID 45626801) Supplemental to MRIDs 00152569 and 40812001.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

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MEMORANDUM

SUBJECT: Malathion (PC Code 057701).- Registrant comments on Agency review of prenatal developmental toxicity study in rabbits (MRID 00152569 and 40812001)

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Supplemental to:

Siglin, J. (1985) A teratology study with AC 6,601 in rabbits. Food and Drug Research Laboratories, Waverly, NY. FDRL Study No. 8171, February 28, 1985. MRID 00152569 and 40812001. Unpublished.

Note: MRID 00152569 includes: Siglin, J. (1985) A range-finding teratology study with AC 6,601 in rabbits. Food and Drug Research Laboratories, Waverly, NY. FDRL Study No. 8170, February 28, 1985. Unpublished.

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EXECUTIVE SUMMARY: A previous Agency review of the definitive prenatal developmental toxicity study in rabbits with malathion (MRID 00152569, 40812001; TXR 007376) concluded that there was a biologically significant treatment-related increase in the number and percent of resorptions at the mid- and high-dose levels (50 and 100 mg/kg/day). In response to registrant comments, the cesarean section findings from the range-finding and definitive studies were reexamined. Detailed reevaluation of the study data, and an examination of historical control data reported in the literature, supported the previous Agency conclusions. The developmental NOAEL (25 mg/kg/day) and LOAEL (50 mg/kg/day, based upon increased mean resorptions) that were established in a previous review of this study (TXR 007376) have not been revised.

Introduction: The registrant, Cheminova A/C, submitted an evaluation of the embryo-lethality of malathion in the rabbit, providing tables of individual and summary ovarian and uterine parameters derived from the cesarean section data originally reported in Siglin, 1985. This study was previously reviewed by Dr. Brian Dementi in TXR 007376, dated 7/10/87. The conclusion of this Agency review was that the developmental NOAEL was established at 25 mg/kg/day, based upon an increased incidence of resorptions at 50 and 100 mg/kg/day. In the current submission, the registrant argues that the apparent increase in resorptions at these dose levels is not related to treatment.

Cesarean section data: Table 1 provides a detailed summary of the cesarean section data from the prenatal developmental toxicity study in rabbits with malathion (MRID 00152569, 40812001); this information is supplemental to the previous Data Evaluation Record (DER) (TXR 007376). Individual litter data from the range-finding prenatal developmental toxicity study with malathion (reported in MRID 00152569) are presented in Table 2.

As noted in the previous review of the cesarean section data (TXR 007376), there are no statistically significant differences from control values in any of the parameters that are summarized in Table 1. However, there is an apparent doubling of the number of resorptions reported at 50 and 100 mg/kg/day, as compared to control. This is demonstrated in calculations of total resorptions per group, mean number of resorptions per litter, and the mean percent postimplantation loss (which includes both dead fetuses and resorptions). However, the dose-response curve at 50 and 100 mg/kg/day was relatively flat. Additionally, the mean number of live fetuses per dam is only slightly decreased at the 50 and 100 mg/kg/day dose level as compared to control (i.e., 5.8 and 6.0 live fetuses/litter at 50 and 100 mg/kg/day versus 6.2 live fetuses/litter in control).

Range-finding study data: The study author claimed that there was no evidence of an increase in the number or percent of resorptions or dead fetuses following doses of 200 and 400 mg/kg/day. The individual litter data from the range-finding study (Table 2) demonstrate that there is an apparent increase in the number of resorptions observed in litters with resorptions at 50 mg/kg/day; this trend appears to be continued at 100 mg/kg/day, but the low number of does available for evaluation precludes a definitive conclusion. Likewise, at 200 and 400 mg/kg/day, few litters were available for analysis, due primarily to maternal deaths. Therefore, the data at 200 and 400 mg/kg/day from the range-finding study are not considered sufficiently robust to support the claim that there was no increase in fetal wastage at these doses.

In this new submission (MRID 45626801), the study author presented a table of cesarean section

results in which the data from the range-finding and main developmental toxicity studies in rabbits were combined. Specifically, the data from four litters each were added to the control and 50 mg/kg/day groups, while the data from two litters each were added to the 25 and 100 mg/kg/day groups. While for most parameters, the inclusion of the range-finding data in the mean calculations has little effect, a more striking result is observed in the post-implantation loss data. When the range-finding study litters are not included, the mean post-implantation loss values for the 0, 25, 50, and 100 mg/kg/day dose groups, respectively, are: 18.0%, 14.9%, 29.2%, and 30.7% (Table 1). However, when the range-finding study litters are included in the calculation, the mean post-implantation loss for the same dose groups are: 24.7%, 14.9%, 29.2%, 30.0% (MRID 45626801, not shown). The apparent disparity between the two calculations of mean post-implantation loss for the control group appears to result from the following factors. The four control litters from the range-finding study (Table 2) had individual post-implantation losses of 66.7%, 22.2%, 100%, and 16.7%. In the 16 control litters from the main study, there was one incident of total litter loss (100%), one litter with 50% post-implantation loss, seven litters with from 11.1-28.6% post-implantation loss, and seven litters with 0% loss. In other words, 50% (2 of 4) of the range-finding litters had incidences of postimplantation loss that exceeded 50%, while only 12.5% (2 of 16) of the definitive study litters had postimplantation losses of that magnitude. In the two litters with 100% post-implantation loss, the range-finding study litter had 3 dead fetuses and 3 resorptions, and the main study litter had 4 resorptions. Overall, this comparison strongly suggests that the populations of animals were not essentially comparable, and argues that they should not be combined for data analysis. On that basis, this approach to data manipulation and reanalysis (as presented in MRID 45626801) was rejected by the Agency reviewers; it was concluded that the range-finding litter data should not be included in the statistical analysis of cesarean section data from the main prenatal developmental toxicity study in rabbits. It is noted, however, that the difference between the populations in the two studies is somewhat puzzling, since the animals were of the same strain and purchased from the same supplier, the studies were conducted in close temporal proximity (the range-finding study was conducted from 8/1/84-9/4/84 and the main developmental study was conducted from 9/11/84-10/16/84), they were conducted in the same laboratory facility (presumably with the same standard operating procedures and technical staff), and the protocols specified identical in-life procedures.

Historical control data: Historical control data from the performing laboratory were not submitted by the registrant. Instead, the registrant cites historical control data compiled by the Middle Atlantic Reproduction and Teratology Association (MARTA) and reported in Hood (1997). Examination of the data presented in Appendix B of this reference (page 725) revealed that the source of the summarized historical control data in New Zealand white rabbits was a publication by Lang (1993). Rather than using these extracted data, the Agency reviewers decided to access the original reports of cesarean section data that were compiled by MARTA and the Midwest Teratology Association (MTA) in order to confirm the historical incidence of *in utero* death for New Zealand White rabbits. The relevant historical control data are summarized in Tables 3A and 3B.

It is noted that FDRL Study No. 8171 (MRID 00152569, 40812001) was conducted from 9/11/84-10/16/84. The New Zealand white rabbits used in this study were supplied by New York State Rabbit Development, Hartwick, NY. Natural mating was utilized (versus artificial insemination) and cesarean sections were conducted on gestation day 29. The 1993 MARTA historical control data were collected in 1989-1993, and the 1994 MARTA and MTA data were collected in 1992-1994. Both sets of historical control data utilized New Zealand white rabbits, from Hazleton Research

Products, (HRP), Inc., Denver, PA. The data were organized by the method of insemination (natural breeding versus artificial insemination), and by the day of cesarean section. These data represent a relatively robust historical control data base with which to compare the cesarean section data from the malathion developmental toxicity study in New Zealand white rabbits, although it is recognized that the populations may have innate differences due to temporal and source variations.

In the latest submission (MRID 45626801), the registrant cites the 1997 Hood data as showing "ranges for the % resorptions up to 47.3% and the number of resorptions up to 4," arguing that the resorption incidence data from the malathion study fall within the historical control range and are therefore not likely to be treatment-related. It was noted by the Agency reviewers, however, that these values were inappropriate for use in comparison to the malathion study, since they represented cesarean section results from rabbits that had been sacrificed on gestation day 28, not on gestation day 29 (as in the malathion study). More appropriate historical control data (i.e., from does that were cesarean sectioned on gestation day 29) were utilized by the Agency reviewers in assessing the malathion study data (Tables 3A and 3B). An analysis of these data follows. The mean number and percent of resorptions for the control and low-dose (25 mg/kg/day) groups from the malathion study are similar to the historical control data. However, the mean number and percent of resorptions for the mid- and high-dose group (50 and 100 mg/kg/day) are substantially greater than (i.e., approximately double) the mean historical values, although they are similar to the maximum individual values recorded historically for these parameters. Mean postimplantation loss values for the control and 25 mg/kg/day dose groups are somewhat higher than those reported historically, presumably due to relative absence of dead fetuses in the historical control data and the presence of 3 dead fetuses each in the control and low-dose groups of the malathion study. And again, the mean percent postimplantation loss for the 50 and 100 mg/kg/day dose groups is approximately 3-times that reported historically, although the values are similar to the maximum values reported for the historical database. The mean number of fetuses per litter for the control and 25 mg/kg/day dose groups is similar to that reported historically; however, the mean number of fetuses for the 50 and 100 mg/kg/day dose groups is slightly lower than historical control. In conclusion, a comparison of the fetal data from the prenatal developmental toxicity study in rabbits with malathion and two historical control databases compiled by MARTA and/or MTA (1993, 1996) indicate that the mean number and percent of resorptions (and mean percent postimplantation loss) at 50 and 100 mg/kg/day in the malathion study exceed the reported average historical incidences of these findings and support the conclusion that they are treatment-related.

Conclusion: A previous Agency review of the definitive prenatal developmental toxicity study in rabbits with malathion (MRID 00152569, 40812001; TXR 007376) concluded that there was a biologically significant treatment-related increase in the number and percent of resorptions at the mid- and high-dose levels (50 and 100 mg/kg/day). In response to registrant comments, the cesarean section findings from the range-finding and definitive studies were reexamined. Detailed reevaluation of the study data, and an examination of historical control data reported in the literature, supported the previous Agency conclusions. The range-finding study data were not found to be particularly useful in this analysis due to a) the small number of litters examined at each dose level, b) the lack of comparability between the populations of control animals in the range-finding study and the definitive study, and c) the high maternal mortality at the two highest dose levels (200 and 400 mg/kg/day). The developmental NOAEL (25 mg/kg/day) and LOAEL (50 mg/kg/day, based upon increased mean

resorptions) that were established in a previous review of this study (TXR 007376) have not been revised.

References:

Lang, P.L. (ed.) (1993) Historical control data for development and reproductive toxicity studies using the New Zealand white rabbit. Compiled by MARTA (Middle Atlantic Reproduction and Teratology Association). Published and distributed by Hazleton Research Products (HRP) Inc., September 1993.

MARTA and MTA (1996) Historical control data (1992-1994) for developmental and reproductive toxicity studies using the New Zealand white rabbit. Published and distributed by Hazleton Research Products (HRP), Inc., March, 1996.

Hood, R.D. (1997) Appendices. B - Historical Control Data in: Handbook of Developmental Toxicology, CRC Press, Inc., Boca Raton, pp. 713-733.

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TABLE 1 Cesarean Section Observations a

Observation	Dose (mg/kg bw/day)			
	0	25	50	100
# Animals Assigned (Mated)	20	20	20	20
# Animals Pregnant	17	18	17	18
Pregnancy Rate (%)	85	90	85	90
# Not Pregnant	3	2	3	2
Maternal Wastage				
# Died Pregnant	0	4	3	2 b
# Died Nonpregnant	0	0	0	0
# Aborted (GD 21 to 26)	1	0	1	2
# Premature Delivery	0	0	0	0
Total # Corpora Lutea c	202	183	136	168
Mean Corpora Lutea/Dam c	12.6 ± 3.4	13.1 ± 3.0	10.5 ± 3.8	12.0 ± 4.1
Total # Implantations c	117	116	106	116
Mean Implantations/Dam c	7.3 ± 2.5	8.3 ± 2.1	8.2 ± 2.8	8.3 ± 3.0
Total # Litters	16	14	13	14
Total # Live Fetuses	99	104	76	77
Total # Dead Fetuses	3	3	0	3
Mean Fetuses/Dam	6.4 ± 3.0	7.6 ± 2.8	5.8 ± 3.5	6.2 ± 3.7
Mean Live Fetuses/Dam c	6.2 ± 2.7	7.4 ± 2.9	5.8 ± 3.5	5.9 ± 3.5
Mean Dead Fetuses/Dam	0.2 ± 0.5	0.2 ± 0.8	0.0	0.2 ± 0.6
Total # Resorptions d	14	10	30	28
Mean Resorptions/Dam c	0.9 ± 1.2	0.7 ± 1.0	2.3 ± 2.7	2.0 ± 2.6
Mean Percent Resorptions c	15.6 ± 26.1	12.3 ± 19.9	29.2 ± 32.9	28.4 ± 33.6
Litters with Total Resorptions	1	0	2	2
No. Does with ≥2 Resorptions c	5	4	6	6
Mean Live Fetal Weight (g) [range]	41.3[35.2-50.5]	41.4[27.8-55.4]	37.3[31.3-48.7]	40.4[28.9-54.4]
Mean # Stunted Fetuses e	0.1 ± 0.4	0.2 ± 0.4	0.1 ± 0.3	0.4 ± 0.7
Mean Fetal Length (cm) [range]	7.4 [7.0-8.7]	7.3 [6.4-8.4]	7.3 [6.7-8.5]	7.3 [6.8-8.1]
Sex Ratio (Mean % Males)	46.9 ± 17.3	50.9 ± 15.6	38.5 ± 18.6	51.4 ± 15.1
Preimplantation Loss (%) c	40.4 ± 16.1	34.3 ± 18.7	29.0 ± 16.9	34.7 ± 16.9
Postimplantation Loss (%) c	18.0 ± 25.4	14.9 ± 21.7	29.2 ± 32.9	30.7 ± 34.2

a Data obtained from Table 4 and Appendices IV and V, MRID 00152569 and 40812001.
b Cause of death attributed to intubation error.
c Calculated by reviewers.
d Early and late resorptions were not presented separately in study report.
e Stunted fetus = any live fetus weighing less than 2/3 of the mean weight of its larger litter mates.
f Litters with (implantation sites > corpora lutea) were considered biologically implausible and not included in calculations.

TABLE 2. Individual Cesarean Section Data - Range-finding Study a

Dose (m/k/d)	Animal Number	Observations (total number)				
		Corpora Lutea	Implantations	Live	Dead	Resorptions d
Control	84-2113	10	3	1	0	2 (66.7)
	84-2114	13	9	7	0	2 (22.2)
	84-2115 b	-	-	-	-	-
	84-2116	15	6	3	3	3 (50.0)
	84-2117	10	6	5	0	1 (16.7)
25	84-2118	23	13	13	0	0 (0)
	84-2119	12	10	8	0	2 (20.0)
	84-2120 b	-	-	-	-	-
	84-2121 b	-	-	-	-	-
	84-2122 b	-	-	-	-	-
50	84-2123	9	9	9	0	0 (0)
	84-2124	13	10	3	0	7 (70.0)
	84-2125	16	12	8	0	4 (30.0)
	84-2126 b	-	-	-	-	-
	84-2127	13	9	9	0	0 (0)
100	84-2128 b	-	-	-	-	-
	84-2129 b	-	-	-	-	-
	84-2130 b	-	-	-	-	-
	84-2131	13	7	7	0	0 (0)
	84-2132	11	10	5	0	5 (50)
200	84-2133	17	9	9	0	0 (0)
	84-2134 b	-	-	-	-	-
	84-2135 c	11	10	-	-	-
	84-2136 c	13	10	-	-	-
	84-2137	11	9	8	0	1 (11.1)
400	84-2138 c	6	6	-	-	-
	84-2139 c	8	5	-	-	-
	84-2140	15	7	7	0	0 (0)
	84-2141 c	10	6	-	-	-
	84-2142 c	18	11	-	-	-

a Data obtained from pp. 188-192, MRID 00152569.
b Not pregnant.
c Found dead during dosing.
d Number (percent); early and late resorptions were not presented separately in the study report.

Table 3A. Historical Control Data - Lang, 1993 (MARTA)

Natural Mating, C-Section GD 29 a				
	Avg	SD	Min	Max
No. resorptions	0.6	0.5	0	2.4
% resorptions	9.2	7.4	0	29.2
No. dead	0.02	0.1	0	0.2
% postimplantation loss	9.5	7.5	0	29.2
No. live fetuses	7.3	1.3	4	10
All GD 29 Studies (Includes AI) b				
	Avg	SD	Min	Max
No. resorptions	0.6	0.5	0	3.2
% resorptions	8.2	6.5	0	29.2
No. dead	0.02	0.07	0	0.7
% postimplantation loss	7.8	6.6	0	34.4
No. live fetuses	7.0	1.5	0.3	10.7

a Total no. females = 530; total no. pregnant = 452; total no. studies = 39
b Total no. females = 2113; total no. pregnant = 1818; total no. studies = 143

Table 3B. Historical Control Data - MARTA and MTA (1996)

Natural Mating, C-Section GD 29 a					
	Avg	SD	Min	Max	No. Studies
No. resorptions	0.42	0.27	0	1.1	20
% resorptions	4.88	3.48	0	8.3	5
No. dead	0	0	0	0	20
% postimplantation loss	7.29	2.92	2.80	11.6	6
No. live fetuses	7.9	0.60	6.6	9.1	20
All GD 29 Studies (Includes AI) b					
	Avg	SD	Min	Max	No. Studies
No. resorptions	0.58	0.38	0	2.3	88
% resorptions	6.83	4.63	0	17.0	26
No. dead	0.01	0.07	0	0.6	64
% postimplantation loss	8.11	4.30	0.56	17.0	25
No. live fetuses	7.0	1.28	3.4	9.9	88

a Total no. females = 382; total no. pregnant = 362; total no. studies = 20
b Total no. females = 1711; total no. pregnant = 1570; total no. studies = 88