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MEMORANDUM

SUBJECT: **Carbaryl**: Additional Morphometric Measurements, Analyses of Data, and an Age Related Sensitivity Study as supplements to the Developmental Neurotoxicity Study

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Carbaryl
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This memo takes the place of a supplemental DER. The original DER is in TXR#0012858, and the first supplementary DER is in TXR#0050307.

I. Conclusion

This memo is an amendment to memo TXR # 0050307 which evaluates morphometry data submitted regarding study MRID 44393701. The executive summary has been updated (see below). The previous review (TXR# 0012858) delineates 4 deficiencies (see below). Only 1 of these has been addressed in the submission (TXR# 0050307), in relation to the morphometric measurements. The remaining 3 are still to be addressed.

II. Executive Summary

In a developmental neurotoxicity study (MRID # 44393701, 45456701, 45456702, 45456703), 26 pregnant female Sprague-Dawley rats/group were administered carbaryl (99.1% a.i.) by gavage from Gestation Day (GD) 6 through Lactation Day (LD) 10 at doses of either 0, 0.1, 1.0 or 10 mg/kg/day. An additional 6 pregnant females/group were dosed at the same levels for the cholinesterase (ChE) phase of the study. ChE measurements were done pre-dosing (GD 6) and post-dosing at time of peak effect (1 hour post-dosing) on GD 6, 15 and 20 and LD 4 and 10. Functional Observational Battery (FOB) measurements were performed at approximately 0.5 and 2 hours post-dosing on the same days as body weight measurements during the dosing period (GD 0, 6, 9, 12, 15, 18 and 20 and LD 4, 7, 11, 13 and 21). Measures of reproductive performance were evaluated. Offspring were examined for body weight, physical development landmarks (tooth eruption and eye opening), FOB assessments (days 4, 7, 11, 13, 17 and 21) and motor activity (days 13, 17 and 21). On LD 11, 1 animal/sex/litter was sacrificed for brain weights; of these, six/sex were randomly selected for neuropathological evaluation. The eyes from all dose groups were examined. After LD 21, 3 animals/sex/litter were separated from the dams and constituted the F1 adult generation. These animals were evaluated for body weight, physical development (vaginal opening and preputial separation), motor activity (day 60), startle habituation response (days 22 and 60), passive avoidance (day 23) and water maze behavior (day 60). After completion of the behavior test period (at approximately 10 weeks of age), 12 animals/sex/group were anesthetized and perfused for post-mortem examination. Tissues from 6 animals/sex of the control and high dose group were processed for neuropathological evaluation and morphometric measurements; the eyes from the low and mid-dose group of all perfused animals were examined.

For the F0 generation animals, there were no carbaryl-associated deaths. No treatment-related clinical signs of toxicity were observed. There was a statistically significant decrease (92%) in body weight gain for females in the 10 mg/kg/day group for the period GD 6-9. Unfortunately, food consumption was not measured during the study. During the FOB measurements, the incidence of females in the 10 mg/kg/day group with decreased pupil size (pinpoint pupils) was increased on all occasions during the dosing period. An increased incidence of dams with slight tremors affecting the head, body and/or limbs was noted on the majority of assessment occasions in the dosing period. There were also occasional occurrences of ataxic gait/overall gait incapacity which was considered to be of toxicological significance due to other effects upon gait.

For the 10 mg/kg/day group, RBC and whole blood ChE levels were statistically significantly decreased (28% and 32-34%, respectively) on GD 20 and LD 10. Although the plasma ChE levels were not statistically significantly altered, the percentage decreases on GD 20, LD 4 and LD 10 were 32-39%. Brain ChE levels were statistically significantly decreased (42%). There were no treatment-related effects on gross necropsy findings for the F0 generation animals. There were no effects observed on maternal performance parameters of pregnancy rate, gestation index, length of gestation, numbers of live pups, dead or malformed pups, implantation scars, sex ratio or post-implantation loss. There was a slight ($P>0.05$) increase in the number of dead pups

in the 10 mg/kg/day group, however the value was within the historical control range for this strain.

For the F1 generation pups, there were no treatment-related effects on pup weight, pup survival indices, developmental landmarks (tooth eruption and eye opening), FOB measurements or motor activity assessments. At sacrifice on LD 11, there were no treatment-related effects on brain weight and gross or microscopic pathology. Significant differences noted in the morphometric measurements included an increase in Line B of the right forebrain and Line F of the left cerebellum in the 10 mg/kg/day males. In the 10 mg/kg/day females, Line F through both the right and left cerebellum were significantly decreased (15% and 22%, respectively).

For the F1 generation adults, there were no treatment-related effects on clinical condition, body weight, physical development (vaginal opening and preputial separation), motor activity, auditory startle response, passive avoidance and water maze measurements. At sacrifice, there were no gross or microscopic neuropathological lesions observed for animals examined in this study that were attributable to treatment with the test article. There was an increased incidence of retinal fold/rosette in the 10 mg/kg/day group (1/12 for control vs. 4/12 for males; 0/12 for control vs. 2/12 for females). The finding was not considered of toxicological significance since the incidence was within the historical control range for males, occurred at a low rate and was not dose-dependent. For the morphometric measurements, there was a significant bilateral decrease in Line A through the forebrain (7.7-9.8%) and a significant increase in Line F through the right cerebellum of the 10 mg/kg/day males. Increases originally noted in 10 mg/kg adult females in Line G, width of the cerebellum, were found to be based on erroneous measurements, and additional measures were submitted. Now, for the 10 mg/kg/day females, there were significant bilateral increases in Line F through the cerebellum (7.4-15%). Measurements of the size of the thickness of lobes and of the granule cell layers of the cerebellum in high dose pups and adults did not differ from those of controls. While additional statistical analyses by the registrant indicated no treatment related effects, HED's additional statisical analyses did indicate treatment related effects.

The maternal toxicity LOAEL was 10 mg/kg/day based on decreased body weight gain, alterations in Functional Observational Battery measurements and RBC, plasma, whole blood and brain cholinesterase inhibition.

The maternal NOAEL was 1.0 mg/kg/day.

The developmental neurotoxicity LOAEL was 10 mg/kg/day based on a bilateral decrease in the size of the forebrain (Line A) in adult males (7.7-9.8%); a bilateral decrease in the length of the cerebella (Line F) in female pups (15-22%); and a bilateral increase in the length of the cerebella (Line F) in female adults (7.4-15%).

The developmental NOAEL was 1 mg/kg/day. Morphometric assessment at the mid and low doses could not be conducted due to inadequate tissue storage; however, based on the minimal findings at the LOAEL, it is HED's judgment that effects would be unlikely to occur at 1 mg/kg/day, which is 10% of the LOAEL.

This developmental neurotoxicity study is classified **acceptable/guideline** and **does satisfy** the guideline requirement for a developmental neurotoxicity study (OPPTS 870.6300) in rats. The study deficiency related to morphometric measurements (number 1 of 4) has been addressed. Deficiencies 2-4 still remain to be addressed.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Confidentiality Statements were provided.

III. Study Deficiencies (number 1. has already been addressed)

1. The findings in the morphometric measurements are judged to be possibly treatment related. At Day 11, the 10 mg/kg/day females had a significant bilateral decrease in the length of the cerebellum accompanied by a slight decrease ($P>0.05$) in the weight of the cerebellum. At Day 60, these animals had a significant bilateral increase in the width of the cerebellum. Forebrain measurements were also affected. **Therefore, the registrant/study author should conduct additional morphometric measurements on the mid- and low-dose groups to define a NOEL and to describe more fully the cell layers in these areas as well as the thickness of cell layers, as described in the OPPTS.6300 guidelines.**

2. The level of carbaryl administered to the 0.1 mg/kg/day group was significantly less than the nominal amount. At Week 1, the range of the percentage of nominal value for three preparations was 55% - 79%. The acceptance criteria was then changed from ± 15% to ± 25%. At Week 2, the average recovery was 80% of nominal and all other samples were within the revised specification. This reduced dose to the low-dose group could affect the results of the study depending on the morphometric evaluations of the low- and mid-dose groups, as requested in item 1 above. For the 1.0 mg/kg/day group, the average Week 1 recoveries were 84.6 and 74.1%; the remaining weeks were within the original ±15% acceptance criteria. For the 10 mg/kg/day group, the average Week 1 recoveries were 82.3 and 91.3%; the remaining weeks were within the original ±15% acceptance criteria.

3. Positive control data should be submitted for motor activity in young pups to validate the data in this study, which were extremely variable.

4. The registrant/study author should explain the variability in the cholinesterase data.