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DATA EVALUATION REPORT

STUDY TYPE: 82-7; Fifteen Day Dietary Neurotoxicity Study in CF-1 Mice

TOX. CHEM NO: New Chemical; P.C. Code: 122806

MRID NO.: 428515-03

TEST MATERIAL: L-660,599; 4"-epi-(N-formyl-N-methyl)-amino-4"-deoxy-avermectin B1

SYNONYMS: Formyl methylamino derivative of MK-0244

STUDY NUMBER: TT #92-049-0; Lab Project ID: 618-244-TOX39

SPONSOR: Merck & Co.

TESTING FACILITY: Merck Research Laboratories

TITLE OF REPORT: L-660,599: Fifteen Day Dietary Neurotoxicity Study in CF-1 Mice. TT #92-049-0

AUTHOR(S): Ronald J. Gerson

REPORT ISSUED: April 2, 1993

CONCLUSION: Randomized groups of 10/sex/dose CF-1 mice were fed 4"-epi-(N-formyl-N-methyl)-amino-4"-deoxyavermectin B1 (L-660,599, 98.1% purity) continuously in the diet at targeted doses of 0, 0.05, 0.075, 0.10, or 0.30 mg/kg/day for 14 days. All mice were observed daily for clinical signs and mortality. Mice were weighed pretest and once a week during the study. Food consumption was measured prior to study and weekly (food measured over 6 days per week) during the study. All mice underwent necropsies at death or scheduled termination. Weights of brains were recorded. Samples of brain, spinal cord, and sciatic nerve were fixed in neutral buffered 10% formalin, prepared by routine methods and stained with hematoxylin and eosin. Sections of the brain (to include cerebral cortex and subcortical white matter, cerebellum and pons) and spinal cord (cervical, thoracic and lumbar) from all mice in the control, 0.10 mg/kg/day and 0.30 mg/kg/day groups were examined. In addition, sciatic nerve was examined from all animals on study.

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The NOEL is 0.075 mg/kg/day. At the LEL of 0.10 mg/kg/day, there were clinical signs, mortality, decreases in food consumption and body weight in those mice with clinical signs, and degeneration of the sciatic nerve. The degeneration in the sciatic nerve in mice at 0.10 and 0.30 mg/kg/day observed with L-660,599 has not been previously observed in CF-1 mice with MK-0244 or any of its other photoproducts. More specifically, in the 0.10 mg/kg/day group, 3 of 10 mice had tremors beginning on days 3 and 5. In 2 of these mice, tremors were accompanied by ptosis, decreased activity, and hunched posture. One of these mice was sacrificed in a moribund condition due to the severity of the signs on day 8. The remaining 2 mice continued to have tremors until the end of the study.

In the 0.30 mg/kg/day group, 3 of 10 males and 5 of 10 females had tremors on days 2-4. Following the onset of tremors, during days 4-8, additional signs consisting of ptosis, decreased activity, hunched posture, splayed limbs, ataxia, labored breathing, urine staining and lateral recumbency were recorded. Due to the severity of these clinical signs, all 8 of the affected mice were sacrificed on days 4-8. Because of the presence of overt toxicity in this dosage group, the remaining 12 mice were sacrificed on day 8. In those mice which had clinical signs, decreases in food consumption and body weight were observed at 0.10 and 0.30 mg/kg/day. Body weight and food consumption values for the 0.075 and 0.050 groups were comparable to controls.

There were no treatment-related findings in necropsy results or absolute and relative brain weights in treated mice in comparison to controls. Degeneration of the sciatic nerve was present in 1 control female, 4 males of the 0.10 mg/kg/day group, and 1 male in the 0.30 mg/kg/day group. This treatment-related lesion was described as consisting of single or multiple foci of myelin sheath dilation and breakdown ("myelin bubble" formation). There were no treatment-related histologic findings in the brains or spinal cords of treated mice at any dosage level in comparison to controls.

Core Classification: **SUPPLEMENTARY**

This is not a Guideline requirement study,

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1. Quality Assurance: A Certification of Good Laboratory Practice was signed by the Study Director, Dr. Ronald J. Gerson, and dated April 2, 1993. A Quality Assurance Statement was signed by Michelle M. Mace and Terry Wilson, Quality Assurance Associates, Oksana C. Powzaniuk, Quality Associate Auditor, and Warren D. Ditzler, Associate Director of Nonclinical Quality Assurance and dated April 2, 1993.

2. Test Material: L-660,599, identified as L-660,599-000N (Lot #4), 98.1% purity by HPLC analysis.

3. Animals: 50 male and 50 female CF-1 mice (Crl:CF-1 BR), approximately 39 days, weighing 19.8-28.6 g (males) and 17.3-23.7 g (females), purchased from Charles River Laboratories, Portage, MI, were fed Purina Certified Rodent Chow (meal) and drinking water ad libitum and housed individually in polycarbonate boxes. Food was withdrawn overnight prior to scheduled necropsy.

4. Methods: The test material was given continuously in the diet for 14 days (7 days for the 0.30 mg/kg/day group). The treatment groups were arranged as shown below:

	Males	Females
Control (diet without test material)	10	10
L-660,599:		
0.05 mg/kg/day	10	10
0.075 mg/kg/day	10	10
0.10 mg/kg/day	10	10
0.30 mg/kg/day	10	10

All mice were observed daily for clinical signs and mortality. Mice were weighed pretest and once a week during the study. Food consumption was measured prior to study and weekly (food measured over 6 days per week) during the study. All mice underwent necropsies at death or scheduled termination. Weights of brains were recorded. Samples of brain, spinal cord, and sciatic nerve were fixed in neutral buffered 10% formalin, prepared by routine methods and stained with hematoxylin and eosin. Sections of the brain (to include cerebral cortex and subcortical white matter, cerebellum and pons) and spinal cord (cervical, thoracic and lumbar) from all mice in the control, 0.10

mg/kg/day and 0.30 mg/kg/day groups were examined. In addition, sciatic nerve was examined from all animals on study.

RESULTS

Diet Analyses and Compound Intake: Stability analyses showed an average 97.4% recovery at a fortification level of 0.11 ug/kg diet over 21 days at room temperature. For the homogeneity samples in week 1 for males, the average analysis was 89.3% (RSD = 6.7) for all fortification levels. For females in week 1, the average analysis was 89.3% (RSD = 6.7) for all fortification levels. For week 2, the mean recovery for males was 81.7% (RSD = 8.5), while for females, the mean recovery was 80.9% (RSD = 6.4). The mean compound consumption and range of values for day 7 and 14 are presented below.

Average Compound Consumption and Range (mg/kg/day)

L-660,599 (mg/kg/day)	Day 7 Males	Day 7 Females	Day 14 Males	Day 14 Females
0.05	0.06 (.05-.07)	0.07 (.06-.08)	0.05 (.04-.05)	0.05 (.04-.05)
0.075	0.11 (.09-.13)	0.11 (.09-.12)	0.07 (.06-.07)	0.07 (.05-.09)
0.10	0.14 (.12-.18)	0.14 (.12-.18)	0.09 (.08-.10)	0.09 (.08-.11)
0.30	0.42 (.37-.53)	0.37 (.25-.43)	-	-

Because the mice ate more than anticipated for the first week of the study, the average compound intake was 20-47% higher than desired. Adjustments made in compound concentration in the diet gave the desired values in week 2. Treatment-related changes were seen in the 0.10 and 0.30 mg/kg/day groups, but not in the 0.05 and 0.075 mg/kg/day groups.

Clinical Signs and Mortality: In the 0.10 mg/kg/day group, 3 of 10 mice had tremors beginning on days 3 and 5. In 2 of these mice, tremors were accompanied by ptosis, decreased

activity, and hunched posture. One of these mice was sacrificed in a moribund condition due to the severity of the signs on day 8. The remaining 2 mice continued to have tremors until the end of the study.

In the 0.30 mg/kg/day group, 3 of 10 males and 5 of 10 females had tremors on days 2-4. Following the onset of tremors, during days 4-8, additional signs consisting of ptosis, decreased activity, hunched posture, splayed limbs, ataxia, labored breathing, urine staining and lateral recumbency were recorded. Due to the severity of these clinical signs, all 8 of the affected mice were sacrificed on days 4-8. Because of the presence of overt toxicity in this dosage group, the remaining 12 mice were sacrificed on day 8.

There were no reported clinical signs or mortality in the 0.075 and 0.05 mg/kg/day groups.

Body Weight and Food Consumption: In those mice which had clinical signs, decreases in food consumption and body weight were observed at 0.10 and 0.30 mg/kg/day. Body weight and food consumption values for the 0.075 and 0.050 groups were comparable to controls.

Necropsy, Brain Weights, and Histopathology: There were no treatment-related findings in necropsy results or absolute and relative brain weights in treated mice in comparison to controls. Degeneration of the sciatic nerve was present in 1 control female, 4 males of the 0.10 mg/kg/day group, and 1 male in the 0.30 mg/kg/day group. The lesion was described as consisting of single or multiple foci of myelin sheath dilation and breakdown ("myelin bubble" formation). There were no treatment-related histologic findings in the brains or spinal cords of treated mice at any dosage level in comparison to controls.

Discussion: This is a 15-day dietary neurotoxicity study in mice. It is not a Guideline requirement and is therefore classified as a **CORE-SUPPLEMENTARY** study. The NOEL is 0.075 mg/kg/day and the LEL is 0.10 mg/kg/day based on clinical signs, mortality, decreases in food consumption and body weight, and histological degeneration of the sciatic nerve.

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