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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.: 428515-06

TEST MATERIAL: L-660,599 (an MK-0244 plant metabolite)

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

STUDY NUMBER: TT #91-114-0; Lab Project ID: 618-244-TOX43

SPONSOR: Merck & Co.

TESTING FACILITY: Merck Research Laboratories

TITLE OF REPORT: L-660,599: Fifteen Day Dietary Neurotoxicity Study in CF-1 Mice. TT #91-114-0

AUTHOR(S): Ronald J. Gerson

REPORT ISSUED: May 15, 1993

CONCLUSION: Randomized groups of 10/sex/dose CF-1 mice were fed continuously via the diet 4"-epi-(N-formyl-N-methyl)-amino-4"-deoxy-avermectin B1 (L-660,599, 98.9% purity) for 14 days at targeted dietary levels of 0 (untreated feed), 0.10, 0.30, and 0.90 mg/kg/day. All mice were observed daily for clinical signs and mortality. Mice were weighed pretest and once per week thereafter. Food consumption was measured pretest, and once weekly. All mice underwent a limited necropsy. Brain weights were recorded. Samples of brain, spinal cord, and sciatic nerve were fixed in 10% neutral buffered formalin. Sections of the brain, 3 levels of the spinal cord, and sciatic nerve were processed by routine methods from all animals and stained with hematoxylin and eosin. All gross lesions were also examined histologically in all animals.

The NOEL is less than 0.1 mg/kg/day (LDT). One

low-dose male had tremors, hunched posture and piloerection on day 14.

At 0.30 mg/kg/day, beginning at day 3, seven mice (4 males and 3 females) had tremors, lethargy, splayed limbs, hunched posture, piloerection, and ptosis. These signs generally increased in severity with time. Four of these mid-dose mice were sacrificed due to the severity of their clinical signs. One mid-dose female was found dead on day 12. The cause of death was not identified at necropsy.

At the high-dose (0.90 mg/kg/day), beginning at day 2, six mice had clinical signs with tremors appearing first, followed by lethargy, splayed limbs, shallow breathing, and urine staining. Four of these high-dose mice (1 male and 3 female) which had clinical signs were sacrificed from days 3 through 9 due to the severity of their clinical signs.

Mice in the mid- and high-dose groups which had severe clinical signs, especially tremors, lost body weight ranging from 4-8 grams within the first three days of exposure. Most mice with body weight loss were sacrificed in a moribund condition. There were no treatment-related effects in body weight in low-dose mice.

There were no treatment-related effects in gross necropsy findings, brain weights or histopathology in male or female mice from treated groups in comparison to controls.

Core Classification:

SUPPLEMENTARY

This is not a Guideline requirement study.

1. Quality Assurance: A Certification of Good Laboratory Practice was signed by Dr. Ronald J. Gerson, Study Director, and dated April 15, 1993. A Quality Assurance Statement was dated April 6, 1993 and signed by Oksana C. Powzaniuk and Carolyn V. Barthel, Quality Assurance Auditors, and Warren D. Ditzler, Associate Director, Nonclinical Quality Assurance.
2. Test Material: L-660,599-000N004 (the Formyl Methylamino Plant Metabolite of MK-0244); 98.9% purity by HPLC analysis;
3. Animals: Male and female Crl:Cf-1[™]BR mice, approximately 39 days old, weighing 20.4-27.9 g (males) and 17.1-24.6 g (females), purchased from Charles River Laboratories, Inc., Portage, MI, were fed Purina Certified Rodent Chow (meal) and tap water ad libitum and housed individually in polycarbonate boxes. Food was withdrawn overnight prior to scheduled necropsy. Test diet was prepared once immediately prior to initiation of the study and was used for the entire study.
4. Methods: Randomized groups of 10/sex/dose CF-1 mice were fed continuously via the diet for 14 days at dietary levels of 0 (untreated feed), 0.10, 0.30, and 0.90 mg/kg/day. All mice were observed daily for clinical signs and mortality. Mice were weighed pretest and once per week thereafter. Food consumption was measured pretest, and once weekly. All mice underwent a limited necropsy. Brain weights were recorded. Samples of brain, spinal cord, and sciatic nerve were fixed in 10% neutral buffered formalin. Sections of the brain, 3 levels of the spinal cord, and sciatic nerve were processed by routine methods from all animals and stained with hematoxylin and eosin. All gross lesions were also examined histologically in all animals.

RESULTS

Dietary Analyses and Stability: The test material was stable in the rodent diet for 21 days at fortification levels of 0.11 and 10.6 ug/g (recoveries averaged 91.5% and

90.2%, respectively over the course of the study). Homogeneity and concentration analyses showed that at 0.5 ug/g that the average results of two analyses was 89.7% with a C.V. of 1.9% for top, middle, and bottom level for males and 88.7% with a C.V. of 3.2% for top, middle, and bottom levels for females. For 1.4 ug/g concentration, the average results of two assays were 84.3% with a C.V. of 3.0% for bottom, middle, and high levels for males and 88.0% with a C.V. of 0.9% for bottom, middle, and high levels for females. For the 4.4 ug/g concentration, the average results were 85.3% with a C.V. of 2.4% for bottom, middle, and high levels in males and, in females, 86.3% with a C.V. of 1.5% for bottom, middle, and high dose levels.

Clinical Signs and Mortality: One low-dose male (#91-5788) had tremors, hunched posture and piloerection on day 14.

At the mid-dose (0.30 mg/kg/day), beginning at day 3, seven mice (4 males and 3 females) had tremors, lethargy, splayed limbs, hunched posture, piloerection, and ptosis. These signs generally increased in severity with time. Four of these mid-dose mice which had clinical signs were sacrificed from day 3 through day 9 due to the severity of their clinical signs. One mid-dose female (#91-5817) was found dead on day 12. The cause of death was not identified at necropsy.

At the high-dose (0.90 mg/kg/day), beginning at day 2, six mice had clinical signs with tremors appearing first, followed by lethargy, splayed limbs, shallow breathing, and urine staining. Four of these high-dose mice (1 male and 3 female) which had clinical signs were sacrificed from days 3 through 9 due to the severity of their clinical signs.

Body Weight and Food Consumption:

Mice in the mid- and high-dose groups which had severe clinical signs, especially tremors, lost body weight ranging from 4-8 grams within the first three days of exposure. Most mice with body weight loss were sacrificed in a moribund condition. There were no treatment-related effects in body weight in low-dose mice.

Necropsy, Brain Weights and Histopathology

There were no treatment-related effects in gross necropsy findings, brain weights or histopathology in male or female mice from treated groups in comparison to controls. The incidence of focal degeneration of the sciatic nerve was 0/10, 0/10, 1/10, and 0/10 in males of the control, low,

mid, and high-dose groups, respectively. In females, the incidence was 0/10 for control and each treated group. The single mid-dose occurrence of sciatic nerve degeneration in males was not considered treatment-related.

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. A NOEL was not established in this study. The LEL is 0.10 mg/kg/day (LDT) based on tremors, hunched posture and piloerection.