12

Reviewed by: William Dykstra, PhD, Toxicologist William Dykstra 7/26/94
Review Section I, Tox. Branch I

for Secondary Reviewer: Roger Gardner, Section Head Pamela M. Hurley 7/26/94
Review Section I, Tox Branch I

DATA EVALUATION REPORT

STUDY TYPE: 81-8; Acute Oral Neurotoxicity Study in Rats

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

MRID NO.: 427436-18

TEST MATERIAL: MK-0243 Technical; 96.9% purity

SYNONYMS: Emamectin, Deoxyavermectin

STUDY NUMBER: TT #89-069-0; Lab Project ID: 618-244-TOX14

SPONSOR: Merck & Co.

TESTING FACILITY: Merck Research Laboratories

TITLE OF REPORT: MK-0243. Acute Oral Neurotoxicity Study in Rats. TT #89-069-0

AUTHOR(S): Jeanne M. Manson

REPORT ISSUED: December 21, 1992

CONCLUSION: Emamectin (purity, 96.9%) was tested in an acute oral mammalian neurotoxicity study in Sprague-Dawley rats (10/sex/dose) at the following dose levels: 0, 27.4, 54.8, or 82.2 mg/kg. This study was a range-finding study for the main oral neurotoxicity study. A NOEL was not established in this study, since toxic signs of neurotoxicity as well as histologic lesions in the brain, spinal cord, and sciatic nerve occurred at all doses tested. The LEL is 27.4 mg/kg, for both sexes, as a single oral dose. The LD_{50} values, based on a 21 day mortality response, were:

Sex	LD_{50} (95% Fiducial Limits) mg/kg
Male	67 (54-84)
Female	70 (55-104)

Toxic signs included salivation, tremors, ataxia, bradypnea, loss of righting reflex, and decreased

activity. Neural lesions were seen in the brain, spinal cord, and sciatic nerve of both male and female rats in all three groups treated with MK-0243. Lesions were visible as soon as 2 days after exposure to MK-0243 in early sacrifice animals. Of the 12 rats sacrificed early in a moribund condition (2 and 10 in the 54.8 and 82.2 mg/kg/day groups, respectively), 9 had lesions in neural tissue. The changes in all three tissues were characterized by the presence of focal vacuolation of white matter or nerve fiber (sciatic nerve) with the presence of a few swollen axons or remnants of cell debris.

Core Classification: **SUPPLEMENTARY**

This study was a range-finding study for the main acute study entitled "Acute Oral Neurotoxicity Study in Rats #2" (89-0129-0). The guidelines for the acute mammalian neurotoxicity study were not followed.

1. Quality Assurance Statement: A Certification of Good Laboratory Practice was signed by the Study Director, Dr. Jeanne M. Hanson, and dated December 21, 1992. QUA Inspections and Report Dates were signed by Cindra L. Lohan, Nelly P. Sanjuan, And Michelle M. Nace, Quality Assurance Auditors.

2. Test Material: Mk-0243 Technical; L-656,748-010V003, hydrochloride salt; 96.9% purity by HPLC area.

3. Animals: Male and female Crl:CD™(SD)BR strain (Sprague-Dawley) rats, approximately 6 weeks old, and weighing 112-160 grams, were used in the study. The rats were purchased from Charles River Laboratories, Raleigh, NC, housed individually and fed Certified Purina Rodent Chow and tap water ad libitum. Food was withheld approximately 19 hours before oral administration of the test material.

4. Methods: Randomized groups of 10/sex/dose were orally gavaged by gastric intubation using a metal catheter attached to a syringe with test material in water at the volume of 5 ml/kg. Treatment groups received 0 (vehicle), 27.4, 54.8, or 82.2 mg/kg of test material. Rats were observed at least twice daily (except on weekends) for 21 days. Body weights were taken pretest, and on Days 7, 14, and 21. Calculation of the 21-day LD_{50} values and their 95% fiducial limits was made by Probit Analysis.

   Functional Observational Battery and motor activity were not performed. On day 21, all survivors were sacrificed by exsanguination under ether anesthesia and a gross examination of the brain, spinal cord, optic and sciatic nerves from all animals was performed. A similar limited gross necropsy was performed on all animals that died. Brain weight was taken. The following tissues from all animals were fixed in 10% neutral buffered formalin: brain, spinal cord, optic nerve, and sciatic nerve. Tissues were embedded in paraffin, sectioned and stained with hematoxylin and eosin. The standard procedures recommended in the 81-8 Guidelines were not followed in this study.

RESULTS

Mortality

Sex	Dose	No. Tested	Found Dead	Early Necropsy
M	27.4	10	0	0
F	27.4	10	0	0
M	54.8	10	2	0
F	54.4	10	0	2
M	82.2	10	4	4
F	82.2	10	1	6
M	water	10	0	0
F	water	10	0	0

Deaths occurred from the second to sixth day. Twelve animals were sacrificed moribund from the second to fourth day.

LD_{50} (Males) = 67 (54-84) mg/kg*

LD_{50} (Females) = 70 (55-104) mg/kg*

* Includes rats taken for early necropsy

Number of Animals with Toxic Signs

Dose	Sex	Tremors	Ataxia	Bradypnea	Saliv.	Irrit.	Dec.Act.	Dischar.
0	M	0	0	0	0	0	0	0
0	F	0	0	0	0	0	0	0
27.4	M	8	3	0	0	0	0	1
27.4	F	6	2	0	3	0	0	0
54.8	M	10	9	3	3	10	6	6
54.8	F	9	7	2	6	4	1	2
82.2	M	10	8	8	4	8	6	8
82.2	F	10	10	7	6	6	2	8

Miscellaneous: At 27.4 mg/kg: 1 male was hyperactive for 5 hours
At 54.8 mg/kg: ptosis,loss of righting reflex (3 F & 5M)
At 82.2 mg/kg: ptosis, hypothermia, loss of righting reflex, urine staining (7F & 7M)

At 27.4 mg/kg: Toxic signs in females were seen in 10 minutes and in 5 hours in males.

At 54.8 mg/kg: Toxic signs appeared in females in 5 minutes and in males in 5 hours

At 82.2 mg/kg: Toxic signs appeared in 10 minutes in females and 5 hours in males

Body Weight

In the first week after dosing, in comparison to control rats, body weight gains in treated rats were less. For male rats, the decrease in body weight gain ranged from 25% (82.2 mg/kg) to 17% (27.4 mg/kg) and in female rats, the decrease ranged from 27% (82.2 mg/kg) to 9% (27.4 mg/kg). For the last two weeks of the study, the body weight gains of the treated rats were greater than controls. Based on this inconsistent finding, body weight gain was not substantially affected by the chemical.

Necropsy

At necropsy, examination of rats was limited to the brain, spinal cord, optic nerve, and sciatic nerve. Brain weight was measured on all animals. There were no gross lesions in the animals which died on study with toxic signs, killed moribund, or sacrificed terminally. Additionally, there was no treatment-related effect in absolute or relative brain weight at any dose-level.

Histopathology

Dose (mg/kg/day)	0		27.4		54.8		82.2	
	M	F	M	F	M	F	M	F
No. Examined	10	10	10	10	10	10	10	10
Brain								
No. Autolyzed	0	0	0	0	1	0	4	1
White Matter, Degeneration	0	0	6	5	7	4	5	3
Neuron, Cytoplasmic Vacuolation	0	0	0	0	0	0	0	1
Spinal Cord								
No. Autolyzed	0	0	0	0	1	0	4	1
White Matter, Degeneration	0	0	8	5	9	8	5	6
Nerve								
Sciatic, Degeneration	2	0	8	7	8	8	2	4

Neural lesions were seen in the brain, spinal cord, and sciatic nerve of both male and female rats in all three groups treated with MK-0243. Lesions were visible as soon as 2 days after exposure to MK-0243 in early sacrifice animals. Of the 12 rats sacrificed early in a moribund condition (2 and 10 in the 54.8 and 82.2 mg/kg/day groups, respectively), 9 had lesions in neural tissue. The changes in all three tissues were characterized by the presence of focal vacuolation of white matter or nerve fiber (sciatic nerve) with the presence of a few swollen axons or remnants of cell debris.

CONCLUSIONS

A NOEL was not established in this study, since toxic signs of neurotoxicity as well as histologic lesions in the brain, spinal cord, and sciatic nerve occurred at all doses tested (27.4, 54.8 and 82.2 mg/kg in 10/sex/dose Sprague-Dawley rats). The study is grade

as **CORE-SUPPLEMENTARY** because it does not follow the neurotoxicity testing guidelines for an acute mammalian neurotoxicity study. The functional observational battery and motor activity assessment were not conducted and the tissues were prepared for microscopic examination using traditional methods as opposed to the special methods as suggested in the neurotoxicity testing guidelines.

Reviewed by: William Dykstra, Ph.D. Toxicologist William Dykstra 8/23/93
Review Section I, Tox. Branch I
Secondary Reviewer: Roger Gardner, Section Head Roger Gardner 9/18/94
Review Section I, Tox Branch I

DATA EVALUATION REPORT

STUDY TYPE: 81-8; Acute Oral Neurotoxicity Study in Rats

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

MRID NO.: 427436-18

TEST MATERIAL: MK-0243 Technical; 96.9% purity

SYNONYMS: Emamectin, Deoxyavermectin

STUDY NUMBER: TT #89-069-0; Lab Project ID: 618-244-TOX14

SPONSOR: Merck & Co.

TESTING FACILITY: Merck Research Laboratories

TITLE OF REPORT: MK-0243. Acute Oral Neurotoxicity Study in Rats. TT #89-069-0

AUTHOR(S): Jeanne M. Manson

REPORT ISSUED: December 21, 1992

CONCLUSION: A NOEL was not established in this study, since toxic signs of neurotoxicity as well as histologic lesions in the brain, spinal cord, and sciatic nerve occurred at all doses tested (27.4, 54.8 and 82.2 mg/kg in 10/sex/dose Sprague-Dawley rats). The LEL is 27.4 mg/kg, for both sexes, as a single oral dose. The LD_{50} values, based on a 21 day mortality response, were:

Sex	LD_{50} (95% Fiducial Limits) mg/kg
Male	67 (54-84)
Female	70 (55-104)

Toxic signs included salivation, tremors, ataxia, bradypnea, loss of righting reflex, and decreased activity. In the first week after dosing, control male rats had increased body weight gains of 65% and control female rats had increased body weight gains of 45%. In comparison, body weight gains in treated rats were less than controls. For male rats, the increase in body

weight gain ranged from 40% (82.2 mg/kg) to 47% (27.4 mg/kg) and in female rats, the increase ranged from 18% (82.2 mg/kg) to 36% (27.4 mg/kg). For the last two weeks of the study, the body weight gains of the treated rats were greater than controls. At necropsy, examination of rats was limited to the brain, spinal cord, optic nerve, and sciatic nerve. Brain weight was measured on all animals. There were no gross lesions in the animals which died on study with toxic signs, killed moribund, or sacrificed terminally. Additionally, there was no treatment-related effect in absolute or relative brain weight at any dose-level. Neural lesions were seen in the brain, spinal cord, and sciatic nerve of both male and female rats in all three groups treated with MK-0243. Lesions were visible as soon as 2 days after exposure to MK-0243 in early sacrifice animals. Of the 12 rats sacrificed early in a moribund condition (2 and 10 in the 54.8 and 82.2 mg/kg/day groups, respectively), 9 had lesions in neural tissue. The changes in all three tissues were characterized by the presence of focal vacuolation of white matter or nerve fiber (sciatic nerve) with the presence of a few swollen axons or remnants of cell debris.

Core Classification: **ACCEPTABLE**

This study was a range-finding study for the main acute study entitled "Acute Oral Neurotoxicity Study in Rats #2" (89-0129-0). Although neither of these two studies performed the required functional observational battery of measurements, the 14-week rat subchronic neurotoxicity study demonstrated that clinical signs and measurements in the functional observational battery occurred at the same dose level. Therefore, the requirement for a functional observational battery in the acute oral neurotoxicity studies is not needed.

1. Quality Assurance Statement: A Certification of Good Laboratory Practice was signed by the Study Director, Dr. Jeanne M. Hanson, and dated December 21, 1992. QUA Inspections and Report Dates were signed by Cindra L. Lohan, Nelly P. Sanjuan, And Michelle M. Nace, Quality Assurance Auditors.

2. Test Material: Mk-0243 Technical; L-656,748-010V003, hydrochloride salt; 96.9% purity by HPLC area.

3. Animals: Male and female Crl:CD™(SD)BR strain (Sprague-Dawley) rats, approximately 6 weeks old, and weighing 112-160 grams, were used in the study. The rats were purchased from Charles River Laboratories, Raleigh, NC, housed individually and fed Certified Purina Rodent Chow and tap water ad libitum. Food was withheld approximately 19 hours before oral administration of the test material.

4. Methods: Randomized groups of 10/sex/dose were orally gavaged by gastric intubation using a metal catheter attached to a syringe with test material in water at the volume of 5 ml/kg. Treatment groups received 0 (vehicle), 27.4, 54.8, or 82.2 mg/kg of test material. Rats were observed at least twice daily (except on weekends) for 21 days. Body weights were taken pretest, and on Days 7, 14, and 21. Calculation of the 21-day LD_{50} values and their 95% fiducial limits was made by Probit Analysis.

RESULTS

Mortality

Sex	Dose	No. Tested	Found Dead	Early Necropsy
M	27.4	10	0	0
F	27.4	10	0	0
M	54.8	10	2	0
F	54.4	10	0	2
M	82.2	10	4	4
F	82.2	10	1	6
M	water	10	0	0
F	water	10	0	0

Deaths occurred from the second to sixth day. Twelve animals were sacrificed moribund from the second to fourth day.

LD_{50} (Males) = 67 (54-84) mg/kg*

LD_{50} (Females) = 70 (55-104) mg/kg*

* Includes rats taken for early necropsy

Number of Animals with Toxic Signs

Dose	Sex	Tremors	Ataxia	Bradypnea	Saliv.	Irrit.	Dec.Act.	Dischar.
0	M	0	0	0	0	0	0	0
0	F	0	0	0	0	0	0	0
27.4	M	8	3	0	0	0	0	1
27.4	F	6	2	0	3	0	0	0
54.8	M	10	9	3	3	10	6	6
54.8	F	9	7	2	6	4	1	2
82.2	M	10	8	8	4	8	6	8
82.2	F	10	10	7	6	6	2	8

Miscellaneous: At 27.4 mg/kg: 1 male was hyperactive for 5 hours
At 54.8 mg/kg: ptosis, loss of righting reflex (3 F & 5M)
At 82.2 mg/kg: ptosis, hypothermia, loss of

righting reflex, urine staining (7F & 7M)

At 27.4 mg/kg: Toxic signs in females were seen in 10 minutes and in 5 hours in males.

At 54.8 mg/kg: Toxic signs appeared in females in 5 minutes and in males in 5 hours

At 82.2 mg/kg: Toxic signs appeared in 10 minutes in females and 5 hours in males

Body Weight

In the first week after dosing, control male rats had increased body weight gains of 65% and control female rats had increased body weight gains of 45%. In comparison, body weight gains in treated rats were less than controls. For male rats, the increase in body weight gain ranged from 40% (82.2 mg/kg) to 47% (27.4 mg/kg) and in female rats, the increase ranged from 18% (82.2 mg/kg) to 36% (27.4 mg/kg). For the last two weeks of the study, the body weight gains of the treated rats were greater than controls.

Necropsy

At necropsy, examination of rats was limited to the brain, spinal cord, optic nerve, and sciatic nerve. Brain weight was measured on all animals. There were no gross lesions in the animals which died on study with toxic signs, killed moribund, or sacrificed terminally. Additionally, there was no treatment-related effect in absolute or relative brain weight at any dose-level.

Histopathology

Dose (mg/kg/day)	0		27.4		54.8		82.2	
	M	F	M	F	M	F	M	F
No. Examined	10	10	10	10	10	10	10	10
Brain								
No. Autolyzed	0	0	0	0	1	0	4	1
White Matter, Degeneration	0	0	6	5	7	4	5	3
Neuron, Cytoplasmic Vacuolation	0	0	0	0	0	0	0	1
Spinal Cord								
No. Autolyzed	0	0	0	0	1	0	4	1
White Matter, Degeneration	0	0	8	5	9	8	5	6
Nerve								
Sciatic, Degeneration	2	0	8	7	8	8	2	4

Neural lesions were seen in the brain, spinal cord, and sciatic nerve of both male and female rats in all three groups treated with MK-0243. Lesions were visible as soon as 2 days after exposure to MK-0243 in early sacrifice animals. Of the 12 rats sacrificed early in a moribund condition (2 and 10 in the 54.8 and 82.2 mg/kg/day groups, respectively), 9 had lesions in neural tissue. The changes in all three tissues were characterized by the presence of focal vacuolation of white matter or nerve fiber (sciatic nerve) with the presence of a few swollen axons or remnants of cell debris.

CONCLUSIONS

A NOEL was not established in this study, since toxic signs of neurotoxicity as well as histologic lesions in the brain, spinal cord, and sciatic nerve occurred at all doses tested (27.4, 54.8 and 82.2 mg/kg in 10/sex/dose Sprague-Dawley rats). The LEL is 27.4

mg/kg, for both sexes, as a single oral dose. The LD_{50} values, based on a 21 day mortality response, were:

Sex	LD_{50} (95% Fiducial Limits) mg/kg
Male	67 (54-84)
Female	70 (55-104)

Toxic signs included salivation, tremors, ataxia, bradypnea, loss of righting reflex, and decreased activity. In the first week after dosing, control male rats had increased body weight gains of 65% and control female rats had increased body weight gains of 45%. In comparison, body weight gains in treated rats were less than controls. For male rats, the increase in body weight gain ranged from 40% (82.2 mg/kg) to 47% (27.4 mg/kg) and in female rats, the increase ranged from 18% (82.2 mg/kg) to 36% (27.4 mg/kg). For the last two weeks of the study, the body weight gains of the treated rats were greater than controls. At necropsy, examination of rats was limited to the brain, spinal cord, optic nerve, and sciatic nerve. Brain weight was measured on all animals. There were no gross lesions in the animals which died on study with toxic signs, killed moribund, or sacrificed terminally. Additionally, there was no treatment-related effect in absolute or relative brain weight at any dose-level. Neural lesions were seen in the brain, spinal cord, and sciatic nerve of both male and female rats in all three groups treated with MK-0243. Lesions were visible as soon as 2 days after exposure to MK-0243 in early sacrifice animals. Of the 12 rats sacrificed early in a moribund condition (2 and 10 in the 54.8 and 82.2 mg/kg/day groups, respectively), 9 had lesions in neural tissue. The changes in all three tissues were characterized by the presence of focal vacuolation of white matter or nerve fiber (sciatic nerve) with the presence of a few swollen axons or remnants of cell debris.

13544

R057909

Chemical:	**4"-Epimethylamino-4"-deoxyavermectin B**
PC Code:	**122806**
HED File Code	**13000 Tox Reviews**
Memo Date:	**07/26/94 12:00:00 AM**
File ID:	**00000000**
Accession Number:	**412-04-0140**

HED Records Reference Center
04/01/2004