

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY  
DEPARTMENT OF PESTICIDE REGULATION  
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

Emamectin Benzoate

Chemical Code # 4020, Tolerance # 52082

12/2/99

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap; possible adverse effect
Chronic toxicity, dog:	No data gap; possible adverse effect
Oncogenicity, rat:	No data gap; possible adverse effect
Oncogenicity, mouse:	No data gap; possible adverse effect
Reproduction, rat:	No data gap; possible adverse effect
Teratology, rat:	No data gap; no adverse effect
Teratology, rabbit:	No data gap; possible adverse effect
Gene mutation:	No data gap; no adverse effect
Chromosome effects:	No data gap; no adverse effect
DNA damage:	No data gap; no adverse effect
Neurotoxicity:	No data gap; possible adverse effect

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Toxicology one-liners are attached.

All record numbers through 169725 were examined.

\*\* indicates an acceptable study.

Bold face indicates a possible adverse effect.

## indicates a study on file but not yet reviewed.

File name: T991202

Duncan, 12/2/99

## II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

### **SUBCHRONIC STUDIES, Emamectin Benzoate and Emamectin HCl**

#### **Subchronic oral, rats**

52082-025 142032 821 "L-656,748. Three-Week Dietary Range-Finding Study in Rats" by G.R. Lankas, Merck Research Laboratories, Merck & Co., Inc., West Point, PA and Rahway, NJ (618-244-TOX16; 12/18/92). 10/sex/dose were exposed for 20 days to L-656,748-010V (lot #3; 96.9% emamectin HCl) in the diet at 0, 5, 25, 50 & 100/200 ppm (due to a lack of physical signs during wk 1, the HD was increased to 200 ppm in wk 2). The anticipated doses were 0, .5, 2.5, 5 & 10/20 mg/kg/day. Exams were performed daily, body wts. were determined weekly, food consumption was determined over 5-6 day pds. during wks. 1 & 2, hematology, serum chemistry & urinalysis were performed on day 14 or 15. Blood samples were taken from fasted animals. Necropsies were not performed. A few days after increasing the HD, fine tremors & decreased activity (**possible adverse effects**) were observed in all HD rats, w/salivation on the last day of the study. Urine staining was also noted in 2 females. 1 HD male died during wk 3, possibly due to test article. 2 other animals died of non-test article-related causes. Both sexes gained slightly less weight at the HD than controls during wk 1 and lost weight during wk 2. Food consumption was also markedly less than controls after the HD was raised. Lymphocyte & leukocyte counts were statistically lower at the HD in both sexes and in females at 50 ppm. Female neutrophil counts were also lower at the top 2 doses. These effects at the HD were attributed to stress (MD effects were considered as possibly not treatment-related, though this was not clear). Increased RBC parameters and numerous serum chemical changes were probably secondary to decreased food consumption, weight loss & dehydration. There were no effects on urine parameters. NOAEL/NOEL (M/F) = 50 ppm (~5 mg/kg/day; fine tremors, decreased activity, salivation, weight & food consumption decrements, hematologic effects). **Supplemental.** (Rubin, 5/22/96)

52082-026 142041 821 "L-656,748. Fourteen-Week Dietary Toxicity Study in Rats" by G.R. Lankas, Merck Research Laboratories, Merck & Co., Inc., West Point, PA and Rahway, NJ (project #618-244-TOX17; 12/18/92). 20/sex/dose were fed diets containing 0, .5, 2.5 or 12.5/8/5 mg/kg-bw/day (batch #L-656,748-010V003; 96.9% purity) for 14 wk. The HD (high dose) was lowered in wks 3 & 9 to counter decrements in body wt. & food consumption. Standard parameters were measured. Unless indicated, all effects were seen at the HD. 9/20 HD males were killed in extremis between wks 3-11 due to emaciation and/or poor physical condition evidenced by ↓ body wt. & food consumption. Clinical signs: fine whole-body tremors in most HD animals (commencing wk 1 & continuing throughout in several animals), hind limb splay in several animals (commencing wk 7) & genital urine staining in several animals. 20-30% reductions in mean serum glucose, probably due to ↓ food consumption & tremors, occurred throughout, as did ↓ creatinine concentrations due to muscle atrophy. Several effects were secondary to dehydration: ↑ RBC parameters & mean serum urea nitrogen, ↓ urine volume and ↓ urine specific gravity, protein & urobilinogen concentrations. Necropsies revealed emaciation & ↓ muscle mass (particularly in the hind leg). Histopath. revealed brain & spinal cord lesions consisting of white matter degeneration & neuronal cytoplasmic vacuolation, sciatic & optic nerve lesions characterized by vacuolation & debri,

neurogenic skeletal muscle atrophy and trabecular atrophy (femur). Brain & optic nerve lesions were noted also at the MD. Neurologic lesions and resultant clinical signs are considered **adverse effects**. NOAEL/NOEL (M) = 0.5 mg/kg-bw/day (neural lesions); NOAEL/NOEL (F) = 2.5 mg/kg-bw/day (neural & muscular lesions, clinical signs, weight decrements). **Acceptable**. (Rubin, 5/31/96)

#### **Subchronic oral, mice**

52082-027 142042 821 "MK-0243. Thirteen-Week Dietary Toxicity Study in Mice" by R.J. Gerson, Merck Research Laboratories, Merck & Co., Inc., West Point, PA & Three Bridges, NJ (project #618-244-TOX18; 12/18/92). Test article (batch #L-656,748-038W002; 96.2% purity) was administered in the diet to 15/sex/dose for ~13 wk at 0, 0.5, 1.5, 4.5 & 15 mg/kg/day (~0, 3, 9, 27 & 90 ppm). For weeks 8-13 the dose for the 1.5 mg/kg group was raised to 10 mg/kg (~60 ppm). Standard parameters were measured. 2 HD deaths (1 F, wk 5; 1 M, wk 10) occurred w/o signs. However, treatment relatedness is considered probable in these cases due to their incidence at the HD. HD males & females gained 41% and 21% less weight than controls over the entire period, with final body weights equal to 89.3% & 95.3% of controls, respectively. No other dose groups exhibited weight decrements. Food consumption, ophthalmology, hematology, serum chemistry and organ weights were unaffected by exposure. Gross necropsies and histopathology did not reveal abnormalities. NOAEL (M/F) = 10 mg/kg/day (death). NOEL (M/F) = 10 mg/kg/day (weight decrements). **Acceptable**. (Rubin, 6/3/96)

#### **Subchronic oral, dogs**

52082-028 142043 821 "L-656,748. Three-Week Oral Range-Finding Study in Dogs" by G.R. Lankas, Merck Research Laboratories, Merck & Co., Inc., West Point, PA and Three Bridges, NJ (618-244-TOX19; 12/18/92). 2 beagles/sex/dose were initially exposed to test article (batch #L-656,748-010V003; 96.9% emamectin HCl) via the diet at doses of 0, .5, 2.5, 12.5 & 25 mg/kg/day (~0, 15, 75, 375 & 700 ppm). Dietary exposure was halted on day 4 due to low food intake (attributed to poor palatability) and consequent low test article intake at 12.5 & 25 mg/kg/day. Mydriasis & ataxia were observed in 1 HD male on day 5. Dosing was resumed on day 8 (day 9 for 1 HD male), this time by gavage (2 ml/kg-bw). The MHD (mid-high dose) & HD were reduced to 5 & 7.5 mg/kg/day. Mydriasis, severe ataxia and/or tremors progressing to sedation & lateral recumbency were observed at the top 3 doses following the switch to oral gavage. Dosing was terminated for all HD animals by day 10, for all MHD animals by day 11, for all MLD (mid-low dose) animals by day 16. These animals were laterally recumbent for several days following dose termination, but survived. Sporadic food emesis occurred throughout the study at the top 3 doses. Clear body wt. losses occurred at the 3 top doses. No clinical signs or effects on food consumption or body wt. were noted at the LD. Slight changes in hematology and serum chemistry at the MLD (no data are available on the 2 top doses because the animals were withdrawn from treatment) were probably secondary to stress, anorexia & dehydration resulting from test article exposure. Urinalyses and electrocardiograms appeared normal for all remaining animals. NOAEL/NOEL (M/F) = 0.5 mg/kg/day (mydriasis, ataxia, tremors, sedation). **Supplemental**. (Rubin, 7/1/96)

52082-029 142044 821 "L-656,748: Fourteen-Week Oral Toxicity Study in Dogs" by G.R. Lankas, Merck Research Laboratories, Merck & Co., West Point, PA and Three Bridges, NJ (study TT# 88-060-0; 6/29/94). 4 beagles/sex/dose received daily oral gavage doses of 0 (deionized water control, 2

ml/kg), .5/.25, 1/.5 or 1.5/1 mg/kg/day for 14 wks (MD & HD dose reductions occurred at end of wk 2, LD at start of wk 3, due to HD toxicity). 1M/1F at the HD developed tremors, mydriasis, anorexia, lethargy & were recumbent prior to in extremis sacrifice in wk 2. 1 of these animals also developed ataxia. 1F (HD) was sacrificed w/similar symptoms in wk 6. Fine whole-body tremors occurred in 6/8 HD animals, persisting despite dose reduction. Decreased food consumption & wt. gain also occurred at the HD, w/improvement after the dose reduction (except for the dog sacrificed in wk 6). No effects were seen on hematology, serum chemistry, urinalysis, ophthalmology, electrocardiography, organ weights or gross necropsy. Histopathology revealed multifocal white matter degeneration (6/8 HD & 4/8 MD dogs, more prominent in survivors), neuronal degeneration (6/8 HD dogs, more prominent in early sacrifices), multifocal spinal degeneration (8/8 HD & 1/8 MD dogs), sciatic and/or optic nerve degeneration (8/8 HD dogs) and skeletal muscle atrophy consistent w/neurogenic atrophy (7/8 HD & 2/8 MD dogs). The neural/neurologic effects constitute **adverse effects**. NOAEL/NOEL (M/F) = 0.25 mg/kg/day (neural degeneration). **Acceptable**. (Rubin, 7/5/96)

#### **Repeated dose dermal, rabbits**

\*\*151; 169702; 822; "MK-0244 E.C. (0.16 lbs/gal): Twenty-Two Day Dermal Toxicity Study in Rabbits" (Walter J. Bagdon; Merck Institute for Therapeutic Research, Merck Research Laboratories, Merck & Co., Inc., West Point, PA; Lab Report No. TT #95-2656; 2/12/96); MK-0244 E.C. (0.16 lbs/gal) (L-656,748-049C, Lot #11, 2.5% MK-0244 Technical), was applied undiluted at doses of 0 (formulation inerts), 250, 500, and 1000 mg/kg/day to groups of 5 NZW rabbits/sex/dose level for 21 days; a sham or negative control group was not included; exposure period was 6 h/day, wrap was semi-occlusive; no mortality or signs of toxicity, except dermal irritation; dermal irritation ranged from mild, in the 250 mg/kg/day group, to severe, in the vehicle and high-dose groups; the results indicate that the inert ingredients in the formulation contribute significantly to the irritancy of the product; observations included erythema, dryness, cracking, alopecia, wrinkling, brown and hardened areas, and bleeding; **no adverse effects**; systemic NOEL = 1000 mg/kg/day (no effect at HDT); a NOEL for dermal effects was not established; **Acceptable**. (Duncan, 9/28/99)

52082-030 142045 822 "MK-0244 0.16 EC. 24-Day Dermal Toxicity Study in Rabbits" by J-P. Gillet, Laboratories Merck Sharp & Dohme-Chibret, Centre de Recherche, Riom, France (project #618-244-TOX22; 12/18/92). MK-0244 0.16 EC (lot #L-656,748-049C002; 2.3% emamectin benzoate). 5/sex/dose received non-occluded dorsal dermal doses of 0 (vehicle was L-930,776, a formulation equivalent to the formulation inerts; applied at .277 ml/kg/day, equivalent to HD group), .05, .1 or .25 g formulation/kg/day (6 hr/day) for 21-23 days. Standard parameters were assessed. Neither deaths nor systemic signs were observed. Body wt., food consumption, ophthalmology, hematology, serum chemistry & organ wts. were unaffected by exposure. Erythema (moderate to severe in some animals, peaking in 5-8 days), edema (moderate in 1 animal, peaking in 6-7 days), fissuring, wrinkling, discoloration & greater hair density were local effects attributed to the vehicle. Histopathology revealed very slight or slight sciatic axonal degeneration in 2/5 HD males & 2/5 HD females and very slight axonal degeneration in the spinal white matter of 1/5 HD males (this animal had the most severe peripheral axonal degeneration). These neural effects are considered **adverse effects**. Very slight atrophy of the seminiferous tubules was seen in 2/5 HD males. Dermal effects (hyperkeratosis, acanthosis, mixed cellulitis and increased # of hair follicles

& adnexa) were attributed to the vehicle. NOAEL/NOEL (M/F) = 0.1 g formulation/kg/day (axonal degeneration). **Acceptable.** (Rubin, 7/9/96)

#### **Subchronic oral neurotoxicity, mice**

52082-043 142118 827 "MK-0243. Sixteen-Day Dietary Neurotoxicity Study in the CF-1 Mouse" by R.J. Gerson, Merck Research Laboratories, Merck & Co., Inc., Three Bridges, NJ (study #618-244-TOX27; 12/18/92). MK-0243 (lot #L-656,748-038W002; 96.9% emamectin HCl). 10/sex were exposed via the diet to MK-0243 at nominal doses of 0, .05, .1, .3 & .9 mg/kg/day (mean intake values were 0, .06, .12, .34 & 1.02 mg/kg/day) for 15 days. Physical signs at 0.3 mg/kg/day occurred in 2/10 females & 4/10 males and included tremors, decreased motor activity, slow righting reflex, ptosis, splayed hindlimbs, irregular breathing, urine staining, bradypnea & hunched appearance (**possible adverse effects**). In extremis sacrifice was carried out on 3M/1F at this dose between days 6-15. Physical signs at the HD occurred in 3/10 females & 1/10 males and included tremors, decreased motor activity, slow righting reflex, ptosis, splayed hindlimbs, irregular breathing, bradypnea & coldness to touch. In extremis sacrifice was carried out on 1M/3F at this dose between days 1-4. Weight gain in survivors was unaffected by treatment except for 0.3 mg/kg/day males, which sustained mean gains of 0.6 g vs. 3.4 g in controls. This difference was due largely to substantial weight loss in two mice. Mean food consumption was somewhat reduced in HD females and MHD males, a result that was effected by particularly low intakes in 2 individuals in each of these groups. Test article relatedness is unclear in this case. Gross nervous system necropsies and histopathology did not reveal abnormalities. There was no evidence of reaction to test article in the lower 2 dose groups. NOAEL/NOEL (M/F) = 0.1 mg/kg/day (tremors & other neurologic signs). **Supplemental.** (Rubin, 7/29/96)

#### **Subchronic oral neurotoxicity, rats**

52082-041 142101 827 "Fourteen-Week Dietary Neurotoxicity Study in Rats" by R.J. Gerson, Merck Research Laboratories, Merck & Co., Inc., West Point, PA & Three Bridges, NJ (study #618-244-TOX25; 12/18/92). 10/sex/dose received nominal doses of 0 (untreated feed), 0.25, 1 & 5 mg/kg/day MK-0244 (lot #L-646,748-052-S002; 95.9% pure). All rats survived. Tremors were seen in both sexes at the HD (8/10 males, onset wk 7; 2/10 females, onset wk 11). Decreased body wt gain & wt loss occurred in HD males in conjunction with, and possibly as a result of, tremors. The final mean male HD body wt was 75% of controls (females were unaffected). Food intake depressions were noted in wk 9, 12 & 13 HD males, most notably in those showing physical signs. Functional observational batteries revealed the following: tremors (males, wks 9 & 13/14; females, wk 13/14), convulsions (males, wks 9 & 13/14; females, wk 13/14), rear leg splay (males, wk 13/14), soiled fur (males, wks 9 & 13/14), decreased rearing (males, wk 13/14), excessive salivation (males, wks 9 & 13/14), hindlimb paresis (males, wk 13/14), impaired grip strength (males & females, wks 9 & 13/14), impaired righting reflex (males, wks 9 & 13/14; females, wk 13/14). Motor activity was normal in all animals. Neuronal cytoplasmic vacuolation, primarily in brain stem but also in midlateral to ventral spinal cord (gray & white matter), occurred in all HD animals. Spinal cord white matter degeneration was present in 4/6 HD females & 6/6 HD males. Myelinated fiber degeneration in the sciatic nerve occurred in all HD males. Skeletal muscle atrophy (probably neurogenic) occurred in 3/7 HD males. The neurologic signs & histopath. are considered **possible adverse effects**, w/stronger effects in males. NOAEL/NOEL (M/F) = 1 mg/kg/day (clinical & histological neurotoxicity). **Acceptable.** (Rubin, 7/24/96)

**Subchronic oral neurotoxicity, dogs**

52082-039 142076 827 "MK-0243 (L-656,748 [4"epi-methylamino avermectin]), L-682,901 [4"epi-methylamino ivermectin], L-653,648 [4"epi-acetyl avermectin], L-653,649 [4"epi-amino avermectin], L-655,372 [4"epi-dimethylamino avermectin]. Exploratory Comparative Neurotoxicity Study in Dogs" by G.R. Lankas, Merck Research Laboratories, Merck & Co., Inc., West Point, PA (project #618-244-TOX23; 12/18/92). 2/sex/test article received 13 (males [& females receiving L-682,901]) or 14 (other females & controls) daily gavage doses of 1.5 mg/kg/day (due to a shortage the L-682,901 dose was reduced to 0.5 mg/kg on day 11 and to 0.71 mg/kg on days 12 & 13). 2/sex received vehicle only (propylene glycol:glycerol formal, 60:40 v/v, 1 ml/kg). All animals survived treatment. The following signs were noted: mydriasis (L-653,648 & L-653,649), tremors (MK-0243, L-653,649, L-655,372) and drooling progressing to lateral recumbency (1 dog, L-653,649). Physical signs not observed in dogs receiving L-682,901. Mean body wts declined in all treated dogs. Decreased food consumption resulted from exposure to L-653,648, L-653,649 & L-655,372. Gross necropsies were negative. Neuronal (pontine nuclei, reticular formation & cranial nerve nuclei w/i the medulla), spinal white matter and sciatic nerve/spinal nerve root degeneration were evident in dogs treated w/MK-0243, L-653,649 & L-655,372, the latter 2 groups also showing white matter degeneration in cerebellar peduncles and swollen neurons in midbrain & medulla possibly containing neurofilament aggregates. Optic nerve degeneration was noted in 1 dog receiving L-653,649. These clinical & neuropathologic signs are considered **possible adverse effects**. L-653,649 & L-655,372 produced clinical & histologic signs similar to MK-0243. L-682,901 & L-653,648 did not produce the histologic signs, at least at an equivalent dose. **Supplemental.** (Rubin, 7/25/96)

52082-040 142098 827 "MK-0243 (L-767,748). Exploratory Five-Week Neurotoxicity Study in Dogs" by G.R. Lankas, Merck Research Laboratories, Merck & Co., Inc., West Point, PA (study #618-244-TOX24; 12/18/92). MK-0243 (lot #L-656,748-010V003; 96.9% emamectin HCl). Beagles were dosed daily by gavage at 0 (vehicle control, 1/sex), 0.5 (2/sex) & 1.5 mg/kg/day (3/sex) in a volume of 2 ml water/kg. Sacrifices were planned after 7 (1 control male & 1/sex/dose), 13 (1/sex/dose) or 33 doses (terminal). One HD male exhibited tremors/ataxia in wk 3, progressing to lateral recumbency in wk 4 and in extremis sacrifice on day 23. All other dogs survived to scheduled sacrifice. Except for slight salivation in 1 LD male (of uncertain relation to treatment), clinical signs were noted at the HD only. They included tremors, mydriasis & ptialism beginning in wk 2 and becoming generally continuous by wk 4. Food consumption & body wt were significantly decreased by wk 3 in the remaining HD dogs. Gross necropsies were negative. Histopathology revealed changes only in HD dogs treated for 13 days or more. These consisted of neuronal degeneration in brain (pons & medulla oblongata) & spinal cord (ventral horn to intermediate regions), white matter degeneration (vacuolation) in brain (cerebellar peduncles) & spinal cord (ventromedial, ventral & lateral white substance) and peripheral nerve degeneration. Aggregates of SMI-31 staining material appeared in many affected neuronal cell bodies indicating the presence of phosphorylated epitopes on neurofilaments (neurofilament phosphorylation normally occurs during transport into the axon hillock). Examination with the electron microscope of sections from affected brains did not reveal abnormalities, though this may be due to the focal nature of the damage. The clinical and neuropathologic signs are considered **possible adverse effects**. NOAEL/NOEL (M/F) = 0.5 mg/kg/day (clinical & neuropathologic signs). **Supplemental.** (Rubin, 7/25/96)

**Developmental oral neurotoxicity, rats**

52082-037 142066 836 "MK-0244: Oral Developmental Neurotoxicity Study in Female Rats" by D. Wise, Merck Research Laboratories, Merck & Co., Inc., West Point, PA (study #618-244-TOX45; 4/29/93). MK-0244, lot #L-656,748-052S002, 97% emamectin benzoate. 25 pregnant rats received gavage doses of 0 (d.i. water control, 5 ml/kg), .1, .6 or 3.6/2.5 mg/kg/day (HD reduced between GD17-20 due to pup tremors at 3.6 mg/kg/day in a concurrent expt.) from GD6-LD20. F0 effects: Neither deaths nor abortions occurred, nor were physical signs observed. MD & HD dams gained 11% & 15% more weight ( $p \leq .05$ ) than controls over the GD6-20 period. No effects on reproductive performance. F1 effects: No effects on survival or external morphology. Physical signs at the HD included tremors (onset, post natal day 6), hindlimb extension (PND10) and hindlimb splay (PND15) [**possible adverse effects**]. PND11 HD pup wts. were less than control by 10-14% ( $p \leq .05$ ), expanding to 40-42% by PND21. Postweaning HD wt. gain over a 7-wk period was 17-18% less than control. Vaginal canalization & preputial separation were delayed 3.6-3.7 days [**possible adverse effects**]. Behavioral tests revealed the following significant effects ( $p \leq .05$ ) in HD F1 rats:  $\uparrow$  avg. horizontal activity on PND13 (both sexes),  $\downarrow$  avg. horizontal activity on PND17 (both sexes) & PND59%1 (females) and  $\downarrow$  auditory startle  $V_{max}$  on PND22 & 59%1 (both sexes).  $\downarrow$  horizontal activity ( $p \leq .05$ ) also occurred in 0.6 mg/kg/day PND17 females, though data interpretation is obscured by large individual variation. Brains & PNS structures showed no gross or microscopic effects. Maternal NOAEL > 2.5 mg/kg/day. Developmental NOAEL/NOEL (M/F) = 0.6 mg/kg/day (tremors, behavioral effects & developmental delay in F1 pups). Maternal NOEL = 0.6 mg/kg/day (weight gain). **Acceptable.** (Rubin, 7/18/96)

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**SB 950-MANDATED STUDIES, Emamectin Benzoate and Emamectin HCl**

**Chronic oral, rats**

\*\*52082-046 142121 831 "MK-0244: Fifty-Three-Week Toxicity Study in Rats" by R.J. Gerson, Merck Research Labs., West Point, PA (project #618-244-TOX48; 12/18/92). 20/sex/dose were exposed via the diet to MK-0244 (lot #L0656,748-052S002; 97.2-97.8% emamectin benzoate) at concentrations sufficient to achieve intake levels of ~0, .1, 1 or 2.5 mg/kg/day (HD females received 5 mg/kg/day until wk 18 when the dose was lowered due to toxicity). There were no treatment-related deaths. Tremors (often accompanied by urine staining, unkempt appearance and/or unsteady gait) were observed in 9 HD females between wks 9-21. 8/20 females sustained weight losses between weeks 9-18 compared to none in controls. However, mean food consumption in HD females appeared unaffected by treatment. After the dose was lowered, body wt returned to control levels by week 25. Wt gain in MD & HD females was greater than controls over the entire period by 14% & 12%. Wt gain increases in MD & HD males were less clearly treatment-related. Ophthalmology, hematology, serum chemistry & urinalysis were unaffected by treatment. Functional observational batteries (FOB) revealed 30% & 9% decreases in forelimb grip strength in HD females in wks 14 & 24. One HD female had decreased hindlimb grip strength in wks 24 & 38. Suggestions of other effects in the FOB were not clearly treatment-related. Neuronal degeneration occurred in brain (reticular formation & olivary nucleus) & spinal cord at the HD in both sexes. Behavioral & structural neurotoxicity are **possible adverse effects**. NOAEL (M/F) &

NOEL (M) = 1 mg/kg/day (tremors, neuronal degeneration in brain & spinal cord); NOEL (F) = 0.1 mg/kg/day (weight increase). **Acceptable.** (Rubin, 8/12/96)

### **Chronic oral, dogs**

\*\*52082-044 142119 831 "MK-0244: 53-Week Toxicity Study in Dogs" by J.P. Gillet, Laboratoires Merck Sharp & Dohme-Chibret, Riom, France (project #618-244-TOX247; 12/18/92). 4 beagles/sex/dose received oral gavage doses of MK-0244 (L-656,748 038W batch #002; >97% emamectin benzoate) at 0, .25, .5 & 1 mg/kg/day. Control, .25 & .5 mg/kg animals received 364-366 doses. 1 mg/kg animals received 19 doses before in extremis sacrifice. Because the HD was so severely toxic (signs occurring in virtually all animals included tremors, mydriasis & decreased motor activity), an additional group at .75 mg/kg/day was added. Males at this dose received 49 doses before in extremis sacrifice. Females received 364 doses. Signs at .75 mg/kg included tremors, hindleg stiffness & mydriasis, leading to sacrifice of all males and progressing to more pronounced tremors & difficulty getting up in 1 female and ataxia, hyperreactivity & difficulty getting up in another. There were no deaths or clinical signs in the 0 or .25 mg/kg groups, and no deaths in the .5 mg/kg group though 1 female showed tremors w/transient hindleg stiffness. Marked mean weight losses were detected at 1 & .75 mg/kg (males only at the latter dose). Depressed food consumption was noted at 1 (females only) & .75 (males only) mg/kg. Hematology revealed decreased leukocyte counts (both lymphocytes & neutrophils) with time in 0.75 mg/kg females. Some changes were apparent with serum chemistry and urine evaluations, but were not convincingly treatment-related. Ophthalmology & organ wts were not noticeably affected. Gross necropsies did not reveal obvious changes. The following histopath. changes were detected at .5 mg/kg and above: neuronal (pons) & axonal (pons, medulla, spinal cord and peripheral & optic nerve) degeneration, retinal ganglion cell degeneration and muscle fiber degeneration (probably neurogenic). The clinical and neuropathologic effects are considered **adverse**. NOAEL/NOEL (M/F) = 0.25 mg/kg (clinical & histologic neuropathology). **Acceptable.** (Rubin, 8/1/96)

### **Combined chronic toxicity/oncogenicity, rats**

\*\*52082-047 142122 835 "MK-0244: One-Hundred-Five-Week Dietary Carcinogenicity/Toxicity Study in Rats" by G.R. Lankas, Merck Research Laboratories, West Point, PA (study #91-017-0; 8/2/94). 75/sex/dose (130 in controls) were exposed via the diet to MK-0244 (#L-656,748-O52S, lot #2; 95.9%-98.6% emamectin benzoate) at concentrations sufficient to achieve intake levels of ~0, .25, 1 or 5/2.5 mg/kg/day (HD was lowered for males starting wk 6 and for females starting wk 10 due to toxicity in a parallel study [#91-006-0]). Except for the interim deaths & sacrifices (hematology, serum chemistry & urinalyses were performed on 10/sex/group in wks 13, 26, 52, 79 & 105), terminal sacrifices were performed in wk 105 (days 728-732). Necropsies, organ wts & histopath were performed at that time. There were no treatment-related deaths or clinical signs. Female wt gains were greater than controls at the MD (10-20%) & HD (10-28%), attaining statistical significance for wks 1-52 & 53-85. Males also gained significantly more wt than controls (HD, wks 1-52; MD, wks 52-85). HD males began losing wt after wk 45 and had statistically decreased wt gains for wks 52-85. MD & HD females had 3% & 6% elevated food consumption, MD males had 5% elevations and HD males had a 2% drop. Hematology, urinalysis, necropsies & organ wt determinations did not show treatment-related effects. Mean serum triglyceride and bilirubin values were increased in MD/HD females in wks 26, 52, 79 & 105 (the affected individuals also had generally greater body wts than others in those dose groups). No statistically

significant trend in neoplasms was detected. Bilaterally symmetrical clusters of vacuolated neurons were seen in 83/43% (M/F) of HD brains (pons) and 37/5% of HD spinal cords (motor horn). These are considered **possible adverse effects**. 17% of HD males showed chronic proliferative cystitis of the urinary bladder (7% at each of the other doses). NOAEL (M/F) = 1 mg/kg/day (neurotoxicity). NOEL (M/F) = 0.25 mg/kg/day (weight effects, serum chemistry effects in females). **Acceptable**. (Rubin, 8/28/96)

### **Oncogenicity/carcinogenicity, mice**

\*\*52082-048 142123 832 "MK-0244: 79-Week Dietary Carcinogenicity Study in Mice" by G.R. Lankas, Merck Research Laboratories, West Point, PA (study #91-033-0,-1,-2,-3; 8/2/94). 50/sex/dose were exposed to dietary MK-0244 (#L-656,748-052S, lot #2; 97.8% emamectin benzoate) at 0 (2 control groups), .5, 2.5 & 12.5 mg/kg/day (HD reduced in males to 7.5 mg/kg in wk 9 & to 5 mg/kg in wk 31 and in females to 7.5 mg/kg in wk 48 due to toxicity) for 79 wk (an additional 15/sex in each group were examined only for hematology at 1 yr). Mortality exams were conducted daily, clinical signs weekly and palpations for masses every 4 wks. 6 HD males died between wks 3-11 of treatment-related causes. Total mortality in HD M/F was 68%/60%, compared to 34%/25% in controls. HD mortality in the 2nd year was largely due to systemic infections secondary to skin lesions. 3 HD males exhibited tremors starting in wks 5-8. Chirping was heard from 5 HD males starting in wks 8-9. After the HD dose reduction, no further animals developed these signs, which had ended in the affected animals by wk 16. 5 HD females chirped starting in wk 16 (lasting 1 day to several wks & not heard after wk 34). Fine forequarter tremors w/occasional sharp myoclonic movements were noted in several HD animals when they were suspended by the tail. Incidence increased after wk 18. Skin lesions w/heavy bacterial growth occurred in HD M/F @ >80%/>70% (M/F controls: ≤56%/≤8%). Blepharitis, corneal scarring & neutrophilia/lymphocytosis, all secondary to dermal infections, were elevated at the HD. Decreased wt gains at the HD were manifest by 2 wk, with final wts 75-80% of controls. Mean HD male wts declined noticeably after wk 22. Body wt effects were likely partly due to tremors. 2 HD mice exhibited slight sciatic degeneration. Several histologic lesions indicative of infectious processes were also evident. No treatment effect on tumor incidence was detected. **Possible adverse effects:** tremors, infectious lesions, sciatic nerve degeneration. NOAEL/NOEL (M/F) = 2.5 mg/kg/day (tremors, sciatic degeneration, secondary infections, wt loss, death). **Acceptable**. (Rubin, 9/6/96)

### **Teratology, rats**

52082-045 142120 833 "MD-0243. Oral Range-Finding Study in Pregnant Rats" by J.M. Manson, Merck Research Laboratories, Three Bridges, NJ (project #618-24-TOX28; 12/22/92). 10 pregnant rats/dose were treated w/MK-0243 (lot #L-656,748-038W002; 96.2% emamectin HCl) by gavage 1x/day between gestation days (GD) 6-17 at 0, 1.25, 2.5, 5 & 10 mg/kg body weight. All HD animals were sacrificed on GD12-14 due to adverse clinical signs (tremors in 8/10 on days 10-14, unkempt coat in 5/10 on days 11-14 & nasal discharge in 2/10 on days 13-14) & loss of body weight (≤32 g). Clinical signs were not observed at other doses. The 2.5 & 5 mg/kg groups gained 35% & 23% more weight than controls over the GD6-14 period. Surviving animals were sacrificed on GD20 for analysis of reproductive & fetal status. Hematologic & serum chemical parameters, # of implants, resorptions & live fetuses, live

fetal weight and fetal external exams were unaffected at and below 5 mg/kg. Maternal NOEL = 5 mg/kg/day (clinical signs & weight loss). Fetal NOEL > 5 mg/kg/day. **Supplemental.** (Rubin, 8/2/96)

\*\*52082-050 142124 833 "MK-0243. Oral Developmental Toxicity Study in Rats" by J.M. Manson, Merck Research Laboratories, West Point, PA & Three Bridges, NJ (project #618-244-TOX29; 12/22/92). 25 pregnant rats/dose received daily oral gavage doses of MK-0243 (lot #L-656,748-038W002; 94.2% emamectin HCl) of 0 (deionized water vehicle control, 5 ml/kg), 2, 4 or 8 mg/kg between gestation days (GD) 6-19. Animals were sacrificed on GD20 for maternal & fetal exams. No deaths or abortions occurred during the study. Clinical signs occurred only at the HD and included tremors in 15/25, unkempt coat in 3/25, few or no feces in 2/25 & convulsions in 2/25. Slight statistically significant ( $p \leq .05$ ) increases in food consumption in all treated groups between days 6-11 correlated with slight non-significant increases in mean wt gain in the 2 & 4 mg/kg groups (12-21% above control) between days 6-14. Mean maternal wt gains for GD14-20 were significantly reduced at 4 & 8 mg/kg (13 & 35%, respectively). This correlated with a 13-17% reduction in food consumption for GD15-20 at 8 mg/kg ( $p \leq .05$ ). Preimplantation loss, resorptions + dead fetuses, implants & live fetuses per pregnant animal were unaffected by treatment. A decrease in mean fetal weight of 4%-M/6%-F at 8 mg/kg, while not statistically significant, was considered treatment-related. 8 mg/kg fetuses also showed skeletal variations (wavy rib [litter mean = 1.6% vs. 0% in controls], and supernumerary rib [litter mean = 22% vs. 10% in controls,  $p \leq .05$ ]) and sites of incomplete ossification (areas of cervical vertebra [litter mean = 5% vs. 1% in controls], skull [litter mean = 3% vs. 0% in controls,  $p \leq .05$ ] and pelvic bone [litter mean = 12% vs. 0.6% in controls,  $p \leq .05$ ]). These effects were probably a function of the decreased fetal weight at this dose. Maternal NOAEL = 4 mg/kg/day (tremors & convulsions). Maternal NOEL = 2 mg/kg/day (decreased weight gain). Fetal NOAEL/NOEL = 4 mg/kg/day (fetal weight decrement, skeletal variations & incomplete ossification). **Acceptable.** (Rubin, 8/6/96)

### **Teratology, rabbit**

52082-051 142125 833 "MK-0243. Oral Range-Finding Study in Non-Pregnant Rabbits" by J.M. Manson, Merck Research Laboratories, West Point, PA (project #618-244-TOX31; 12/22/92). This project was intended to identify a dose range for a preliminary teratology study in pregnant rabbits. 6 females/dose received oral gavage doses of MK-0243 (lot #L-656,748-010V003; 92.8% emamectin HCl) at 0 (vehicle control, 4 ml water/kg), 1.37, 2.74, 5.48 or 10.96 mg/kg/day for up to 13 consecutive days (the original intent was to dose animals w/emamectin benzoate at 0, 1.25, 2.5, 5 & 10 mg/kg/day, but emamectin HCl was mistakenly delivered and used; this was the reason for the unorthodox dose levels). Except for the HD animals (which were euthanized on days 6-8 due to **possibly adverse physical signs** including tremors in 6/6 animals, lethargy & decreased activity in 1/6 each, mydriasis in 3/6, recumbency in 2/6, and weight losses of 61-370 g in 6/6; other signs at the HD included decreased food consumption in 6/6, decreased water intake in 3/6, no urine, soft feces & dyspnea in 2/6 each, & lack of motor coordination in 1/6), all animals were bled and euthanized on day 14 following collection of ear arterial blood for hematology & serum chemistry. Body wts. were determined on days 1, 3, 5, 7, 9, 11, 13 & 14. There were no deaths nor were there adverse signs at any dose but the HD. Mean body weight gain and hematologic & serum biochemical parameters appeared unaffected at doses up to 5.48 mg/kg/day (the discarded HD animals were not examined for hematology & serum chemistry). These data

indicate that a high dose between 5 & 10 mg/kg/day would be appropriate for the preliminary study in pregnant rabbits. **Supplemental.** (Rubin, 10/7/96)

52082-052 142126 833 "MK-0243. Oral Range-Finding Study in Pregnant Rabbits" by J.M. Manson, Merck Research Laboratories, Three Bridges, NJ (project #618-244-TOX32; 12/22/92). This project was intended to identify a dose range for a full teratology study in pregnant rabbits. 10 artificially inseminated females/dose received oral gavage doses of MK-0243 (lot #L-656,748-010V002; 96.2% emamectin benzoate) at 0 (vehicle control, 4 ml water/kg), 2, 4, 6 or 8 mg/kg/day from gestation days (GD) 6-18. Body weights were determined on GD 0, 6, 8, 10, 12, 14, 16, 18, 19, 22 & 28 and food consumption every 3rd day over a 24-hr period. Medial auricular blood was drawn on GD 19 for hematology & serum chemistry. Animals were observed daily during the dosing pd for physical signs and were euthanized on GD 28 to determine reproductive status and examine the fetuses. Neither deaths nor abortions were observed. Treatment-related physical signs occurred only at the HD and included tremors (1/10, days 19-28; **possible adverse effect**), soft feces (5/10, GD 0-28), small feces (2/10, GD 20-23), mucoid discharge (1/10, GD 22), and no urine (1/10, GD 20). Maternal weight losses occurred in 5/9 at 6 mg/kg & in 9/10 at 8 mg/kg between GD 14-19 vs. 1/9 in controls (mean values: -55 & -172 g vs. +71 g in controls,  $p \leq .05$ ). These were accompanied by decreases in food consumption at those doses on days 16-19 (17-45% below control,  $p \leq .05$  only at the HD) and at the HD on day 22 (13% below control,  $p \leq .05$ ). There were no clear hematologic or serum chemical effects. One HD fetus had cleft palate & hydrocephaly and another from a different litter had only hydrocephaly. Both fetuses were from dams that lost the most weight during dosing, with one of these dams exhibiting tremors. Because of the occurrence of these fetal signs at the HD in affected dams, they are considered test article-related (**possible adverse effects**). Maternal NOAEL = 6 mg/kg/day (tremors). A HD of 6 mg/kg/day is appropriate for a full teratogenicity study. Maternal NOEL = 4 mg/kg/day (weight loss, decreased food consumption). Developmental NOAEL/NOEL = 6 mg/kg/day (hydrocephaly, cleft palate).

**Supplemental.** (Rubin, 10/8/96)

\*\*52082-053 142127 833 "MK-0243. Oral Developmental Toxicity Study in Rabbits" by J.M. Manson, Merck & Co., Inc., West Point, PA & Three Bridges, NJ (project #618-244-TOX33; 12/22/92). 18 artificially inseminated does received daily gavage doses of 0 (4 ml water/kg), 1.5, 3 or 6 mg/kg/day MK-0243 (lot #L-656,748-038W002; 94.2% emamectin benzoate) between gestation days (GD) 6-18. Body weights were determined on GD 0, 6, 8, 10, 12, 14, 16, 18, 19, 22 & 28. Food consumption was measured every 3rd day over a 24-hr pd. Observations for physical signs were made daily. Animals were euthanized on GD 28. Uteri were examined for reproductive status. Fetuses were weighed & examined for external, visceral & skeletal abnormalities. There were no deaths. One control female aborted on GD 19. Treatment-related physical signs occurred only at the HD and included mydriasis in 9/18 animals (GD 11-17) and decreased pupillary reaction in 16/18 (GD 11-23). Mean weight gain at the HD was significantly less than control by 52% between days 12-19 ( $p \leq .05$ ), after which some recovery was evident. Mean food consumption was slightly suppressed compared to control at the HD on GD 10 & 22 ( $p \leq .05$ ). Necropsy of the does did not reveal abnormalities. No clear reproductive or teratologic effects were detected. However, the number of fetuses with skeletal malformations rises from 2/120 in the controls to 5/143, with litter incidence rates at ascending doses of 2/15, 2/17, 2/15 and 5/17 (**possible adverse effect**). A treatment effect at the HD cannot be ruled out.

Maternal NOAEL > 6 mg/kg/day. Maternal NOEL = 3 mg/kg/day (weight gain/food consumption suppression & physical signs). Developmental NOAEL/NOEL = 3 mg/kg/day (skeletal malformations). **Acceptable.** (Rubin, 10/10/96)

### **Two-generation reproduction, rats**

52082-054 142128 834 "MK-0243. Oral Range-Finding Reproduction Study in Female Rats" by L.D. Weiss (project #618-244-TOX30; 12/22/92). 12 pregnant dams/dose were exposed between gestation day (GD) 0 and lactation day (LD) 21 to 0, .1, .7 or 5 mg/kg/day MK-0243 (lot #L-656,748-038W002; 94.2% emamectin HCl) by gavage in 5 ml H<sub>2</sub>O/kg or to 0, 1, 7 or 50 ppm by diet (target doses: .1, .7 & 5 mg/kg; actual drug consumption ranged from 40% below to 70% above target). Clinical exams, body wts, food consumption, litter data & neurologic necropsies & histology were determined on appropriate numbers of dams & pups. There were no F0 deaths, abortions or treatment-related signs. Between GD0-4 MD & HD gavage-treated (GT) and HD diet-treated (DT) dams gained statistically more wt than controls (by 33%, 42% & 68%) & consumed 9-17% more food. HD dams gained statistically less wt than controls by 19-32% between GD8-16. Significantly lower HD wt gains & food consumption were also noted during lactation. There were no gross or histomorphologic effects on dams. Reproductive performance (gestation length & % dams w/live pups) appeared unaffected. Treatment-related pup deaths occurred among HD GT animals (9%, 21% & 42% between PND 1-4, 5-7 & 8-14) resulting in premature euthanasia at LD8-15. Slightly ↑ pup mortality may also have occurred among MD GT & HD DT animals. Tremors occurred in ~1/3 of HD GT and all of HD DT pups (PND11-termination) w/some pups appearing pale, cold, weak and/or shallow of breath. HD GT pups weighed significantly less (by 5% at birth) than controls, progressing to 62% below control by PND14. HD DT pups did not show a difference until PND 14 & 21 (34% & 52% less than controls). 12/16 HD DT pups showed brain/spinal cord neuronal degeneration, though w/o sciatic degeneration. 5 mg/kg/day (gavage or diet) produces unacceptable toxicity for further testing. **Supplemental.** (Rubin, 9/10/96)

\*\*52082-055 142129 834 "MK-0244: Two-Generation Reproduction Study in Rats" by G.R. Lankas, Merck Research Laboratories, West Point, PA (project #618-244-TOX; 5/12/93). 33/sex received oral doses of 0, .1, .6 or 3.6/1.8 mg/kg/day (HD dropped following the 2nd F0 mating for F0 females and following the only mating for F1a females to examine the reproducibility of a fertility decline at the HD in the 1st mating). F0 dosing commenced at 9 wks of age & continued for 24 (M) or 25-27 (F) wks. Animals were mated after 9 wks of treatment. F1a were treated starting at weaning and continuing until necropsy at 21 (M) or 22-24 (F) wks. F0 animals were re-mated (different mates w/i the group) 3 wks after weaning of F1a to produce F1b, which were euthanized between post natal day (PND) 21-24. F1a non-siblings were mated at PN wk 18 to produce F2 litters, which were culled to 4/sex on PND 4 & euthanized on PND 21. F0: HD effects included slight-to-moderate ↑ & ↓ body wt, ↓ fecundity & fertility indices (**possible adverse effect**), and brain, spinal & sciatic neuronal degeneration (4/33 males) (**possible adverse effect**). F1: HD effects included tremors & hindlimb extension splay persisting into 2nd postweaning wk (more pronounced in F1a than F1b due to ↓ HD), ↓ preweaning F1a pup wt (possibly due to maternal hindlimb immobility), ↓ F1a wt gain/food consumption during postweaning & gestational periods, marked ↓ fecundity/fertility in F1a adults (from 80/80% to 52/48%, p<.05, **possible adverse effect**, possibly progressive from the F0 to the F1 generation), brain/spinal neuronal degradation (**possible adverse effect**). F2: HD effects included 1/11 litters w/tremors & hindlimb extension and ↓

pup wts during lactation. NOAEL/NOEL (maternal & developmental) = 0.6 mg/kg/day (↓ F0-F1-F2 wts, F0-F1-F2 neurologic signs, F0-F1 neurologic pathology, ↓ F0 & F1a fertility/fecundity).

**Acceptable.** (Rubin, 10/3/96)

### **Gene mutation**

\*\*52082-056 142148 842 "L-656,748. Microbial Mutagenesis Assay" by G.R. Lankas, Merck Research Laboratories, West Point, PA (project #618-244-TOX34; 12/22/96). Salmonella typhimurium tester strains T1535, 97a, 98 & 100, and Escherichia coli tester strains WP2, WP2 uvrA & WP2 uvrA pKM101 were exposed in triplicate to L-656,748 (lot #L-656,748-010V003; 96.9% emamectin HCl) at 2, 8, 19, 85, 211 & 953 ug/plate +/- S9 microsomes (the high dose was based on previous observations of bacterial toxicity at that approximate level) for 48 hr. Despite the success of the positive controls, no concentration of L-656,748 induced a reversion to histidine (S. typhimurium) or tryptophan (E. coli) independence regardless of the absence or presence of a microsomal activating system. Salmonella lawn growth inhibition occurred at 953 ug/plate in most strains and revertant growth inhibition was noted in all Salmonella strains (except TA98 +S9) at varying concentrations (possibly as low as 85 mg/plate) and at the HD in E. coli strain WP2 pKM101 -S9. L-656,748 is not considered mutagenic in this system under the conditions tested. **Acceptable.** (Rubin, 10/23/96)

\*\*52082-057 142150 842 "L-656,748. V-79 Mammalian Cell Mutagenesis" by G.R. Lankas, Merck Research Laboratories, West Point, PA (project #618-244-TOX35; 12/22/96). Duplicate flasks of Chinese hamster V-79 lung fibroblasts were exposed in Expt. 1 to (-S9) 0, .005, .01, .02 & .04 mM L-656,748 (lot #L-656,748-010V003; 96.9% emamectin HCl) and (+S9) 0, .005, .01, .04 & .06 mM L-656,748, and in Expt. 2 (-S9 only) to 0, .001, .002, .004, .006, .008 & .01 mM L-656,748. In Expt. 1,  $1.23 \times 10^7$  cells/T150 flask were exposed to L-656,748 followed by an expression period of 7 & 12 days. In Expt. 2,  $1.02 \times 10^7$  cells/T150 were exposed followed by an expression period of 9 days. Exposure was for 3 hr. Resistant mutants were counted 6 days after plating  $3 \times 10^5$  of these cells/100 mm plate in the presence of 11 ug/ml 6-thioguanine (9 replicate dishes) and incubating for 6 days. Initial dosing was based on a preliminary cytotoxicity expt. in which plating efficiency after test article exposure of 300 cells/100 mm dish had clearly dropped +/- S9 at .01 mM (54% & 80% of control) and had massively dropped at .1 mM (0% & 15% of control). Cytotoxicity evaluation done concurrently with the mutagenesis test indicated that relative survival +S9 ranged from 70% to 2% between .005 & .06 mM, sufficient for mutation analysis, but ranged from 18% to .4% -S9, necessitating a repeat test (Expt. 2) in which relative survival between .001 & .01 mM -S9 ranged from 101% to 4%. In Expt. 2 the HD and LD were not carried through for mutation analysis. Despite a very slight apparent rise in mutant fraction +S9 at .06 mM (mean day 7 MFs of  $3.43 \times 10^{-6}$  vs.  $1.46 \times 10^{-6}$  in controls), a highly cytotoxic condition (2% relative survival), statistical analysis did not indicate positivity (defined as a significant positive trend and an induced MF  $> 5 \times 10^{-6}$  [value based 2.21 x the SD of current historical controls]) at this or at any other dose +/- S9. L-656,748 is not considered mutagenic under the conditions tested. **Acceptable.** (Rubin, 10/24/96)

### **Structural chromosome aberration**

\*\*52082-060 142166 843 "MK-0244. Assay for Chromosomal Aberrations in Mouse Bone Marrow" by S. Galloway, Merck Research Laboratories, West Point, PA (project #618-244-TOX51; 4/28/93). MK-0244 (L-656,748-052S002; 95.9% emamectin benzoate) was administered to male mice by oral gavage in 0.5% methylcellulose at 0, 8, 26 or 80 mg/kg (dosing based on a preliminary acute study showing an LD50 of 107 mg/kg). Mice were sacrificed for harvest of bone marrow at 6, 24 & 48 hr post treatment (12 controls, 8 LD & MD, & 10 HD mice/sacrifice time). 8 positive controls given 1 mg/kg & 4 given 3.5 mg/kg mitomycin C (MMC) by intraperitoneal injection were sacrificed @ 24 hr. Clinical signs included tremors and ptosis at 26 mg/kg, and tremors ptosis, decreased activity, erect tails, bradypnea, hypothermia, and moribund appearance at 80 mg/kg with one animal found dead at 24 hr. Slides were prepared from bone marrow cell preparations at each sacrifice time. Despite the success of MMC in producing highly significant increases in chromosome aberrations, no such increases occurred in response to MK-0244 exposure. MK-0244 is not considered to be clastogenic in this assay under the conditions tested. **Acceptable.** (Rubin, 11/1/96)

\*\*52082-059 142159 843 "MK-0244. Assay for Chromosomal Aberrations In Vitro in Chinese Hamster Ovary [CHO] Cells" by S. Galloway, Merck Research Laboratories, West Point, PA (project #618-244-TOX50; 4/27/93). Aberrations were assayed after a 3-hr exposure of CHO cells (subclone WBL;  $1.2 \times 10^6$  cells/75 cm<sup>2</sup> flask seeded on the day before treatment) to MK-0244 (L-656,748-052S002; 97.8% emamectin benzoate) at (-S9) 0, 2, 4 & 6 uM and (+S9) 0, 6, 7, & 8 uM. Top doses were chosen such that a 50% reduction in cell growth was not exceeded (based on a preliminary cytotoxicity assay). Cell growth inhibition relative to controls at the highest dose that aberrations were scored was (-S9) 28% and (+S9) 54%. 200 cells/dose were scored for aberrations. The maximum aberration levels (-S9, 2.0% at 6 uM; +S9, 2.5% at 8 uM) were within the historical control range (0.00-5.50%). Despite the success of the positive controls, there were no statistically significant increases in chromosomal aberration frequencies at any test article concentration +/- S9 microsomes. MK-0244 is thus not considered to be clastogenic in this assay under the conditions tested. **Acceptable.** (Rubin, 10/31/96)

#### **Other genotoxic effects**

\*\*52082-058 142154 844 "L-656,748. In Vitro Alkaline Elution/Rat Hepatocyte Assay" by G.R. Lankas, Merck Research Laboratories, West Point, PA (project #618-244-TOX36; 12/22/96).  $2.2 \times 10^6$  hepatocytes isolated from male rats per duplicate dish were exposed for 3 hr to 0, .003, .006, .01 or .02 mM L-656,748 (lot #L-656,748-010V003; 92.8% emamectin HCl), after which they were tested for cytotoxicity (by trypan blue exclusion) and DNA strand breaks (by alkaline elution). The positive control, 1 uM aflatoxin B1, gave a 10.27x increase in mean elution slope with 99% viability compared to negative controls. Experimentals at ascending doses yielded relative elution slopes/relative viabilities of 1.00/100%, 1.36/100%, 1.32/94%, 2.86/76% & 7.77/4%. Because the value of 2.86 at .01 mM approached the 3x level considered indicative of a positive response (the higher value at the HD is associated with severe toxicity), a repeat assay was run at 0, .006, .008, .010, .012 & .014 mM. 1 uM aflatoxin B1 produced an 18.75x increase in mean elution slope with 99% relative viability. Experimentals at ascending doses yielded relative elution slopes/relative viabilities of 1.00/100%, 1.50/92%, 1.75/92%, 2.25/68%, 4.63/59% and 18.38/59%. The report considers these data to be negative since relative elution slopes >3 occurred only in the presence of relative viabilities <70%. In addition, DNA recoveries at these doses

were considerably reduced compared to controls, indicating the presence of cytotoxicity-associated DNA breakdown. Thus dose responsiveness combined with high slope values associated with relative viabilities of just under 70% at .012 & .014 mM (59% in both cases) in Expt. 2 is at best equivocal evidence for genotoxicity. **Acceptable.** (Rubin, 10/25/96)

## METABOLISM, MAB1a Benzoate

### Metabolism, rats

52082-066 142173 851 "[14C]4"-Deoxymethylamino Avermectin B1a: Determination of [<sup>14</sup>C]CO<sub>2</sub> in Exhaled Air of Male and Female Rats After [<sup>14</sup>C]4"-Deoxy-4"-epimethylamino Avermectin B1a (MAB1a) Benzoate Administration (A Preliminary Report)" by M. Mushtaq, Merck Research Laboratories, Three Bridges, NJ (project #ARM-5; 6/29/93). This study was designed to determine if [<sup>14</sup>C]MAB1a metabolism is of sufficient magnitude that [<sup>14</sup>C]CO<sub>2</sub> can be detected in exhaled air. 2 male and 2 female rats were placed individually into glass metabolism cages designed for the capture of exhaled CO<sub>2</sub> into absorbing towers containing 1N NaOH. [<sup>14</sup>C]MAB1a benzoate in propylene glycol:saline (1:1) was administered by gavage at 0.38 (males) or 0.45 (females) mg/kg (0.32 and 0.20 ml, respectively). The alkaline capture solutions were collected at 24 & 48 hr for radiochemical analysis. Trapped radioactivity after 24 & 48 hr exposure was approximately the same as in samples collected 21 hr before dosing or in blank samples. [<sup>14</sup>C]MAB1a benzoate was not eliminated as [<sup>14</sup>C]CO<sub>2</sub> in exhaled air of rats. Such a measurement is thus not considered necessary in a full metabolism study. **Supplemental.** (Rubin, 10/18/96)

52082-065 142172 851 "The Tissue Distribution, Metabolism, and Excretion of [<sup>14</sup>C]-4"-Deoxy-4"-epimethylamino Avermectin B1a (MAB1a) Benzoate in Rats" by M. Mushtaq, Merck Research Laboratories, Three Bridges, NJ (project #ARM-6; 6/29/93). MAB1a benzoate is the major component (>90%) of MK-0244. The following 4 groups were examined: 1. Single high dose gavage (20 mg [<sup>3</sup>H/<sup>14</sup>C]MAB1a benzoate/kg; 6/sex), 2. Multiple low dose gavage (0.5 mg MK-0244/kg daily for 14 days followed by a single dose of 0.5 mg [<sup>14</sup>C]MAB1a benzoate/kg; 9/sex), 3. Single low dose gavage (0.5 mg [<sup>14</sup>C]MAB1a benzoate/kg; 6/sex), 4. Single low dose intravenous (0.5 mg [<sup>14</sup>C]MAB1a benzoate/kg; 6/sex). MAB1a peaked in the plasma of male rats at 12 hr and in female rats at 4 hr after low dose oral administration, dropping to background levels by around 96 hr. Regression analysis indicated exponential clearing with half-lives (M/F) of 34.4/51.1 hr for oral dosing and 28.6/40.7 hr for iv dosing. Comparison of the oral and iv plasma levels indicates that the bioavailability (area under the curve for the oral group divided by that for the iv group) was 54.6%/74.3% (M/F). More than 90% of the administered dose was eliminated by day 5, with nearly all (94-104%) accounted for in the feces regardless of exposure route, dose, or pretreatment. Total recoveries were in the range of 96-104%, with 0.1-0.3% in the urine and 0.1-1.6% in the tissues and remaining carcass. Fecal & urinary residues peaked on day 1 post dose (though fecal residues were on the order of 1000x higher than urinary) in all phases of the study, dropping quickly after that. Residues had a short tissue half-life as indicated by the drop from 5-800 ppb on day 1 to 0-44 ppb on day 7 following low dose exposure. The major metabolite detected in tissues & feces was AB1a, an N-demethylation product of the parent molecule. **Acceptable.** (Rubin, 10/17/96)

\*\*152; 169704; 851; “(<sup>3</sup>H)-MAB1a: Metabolism, Pharmacokinetic Profile, Excretion, Tissue Distribution and Biliary Elimination in the Rat” (P. Powles; Hazleton UK, Harrogate, North Yorkshire, England; Lab Report No. 453/6-1011; 4/20/95); Groups of 4-12 Crl:CD rats/sex were treated with single doses of 0.5 mg/kg or 20 mg/kg, or 14 daily doses of 0.5 mg/kg/day of (<sup>3</sup>H)-MAB1a (97.63% or 98.24% radiochemical purity) prepared as a mixture in saline and propylene glycol. Plasma pharmacokinetic, biliary excretion, and tissue distribution/excretion studies were performed on separate groups of animals. In the tissue distribution studies, 34 different organs/tissues were analyzed. Results showed that at least 20% of an oral dose was absorbed, maximum plasma concentrations were achieved in 5-15 h depending on dose and sex, and plasma half-life ranged from 19.5-36.3 h. The compound was found in all organs/tissues analyzed, but only very low concentrations were found in brain and spinal cord. Highest concentrations were found in secretory glands such as Harderian, adrenal, pituitary, thyroid + parathyroid, and sublingual. The parent compound was generally eliminated unchanged in the feces (renal elimination accounted for less than 0.3% of the dose). A small amount of metabolite AB1a was found most extracts. Bile cannulation appeared to impair the excretion of the test article in the bile. **Acceptable.** (Duncan, 11/8/99)

### **ADDITIONAL STUDIES, Bioequivalence**

#### **Bioequivalence, benzoate & HCl salts**

52082-068 142186 "MK-0243. Bioequivalence Study of Benzoate and HCl Salts in Dogs" by J.M. Manson, Merck Research Laboratories, West Point, PA & Three Bridges, NJ (project #618-244-TOX37; 12/21/92). This study was designed to determine if the benzoate & hydrochloride salts of MK-00243 are bioequivalent after oral administration. There were 2 dosing groups, each consisting of 2 male beagles. Group 1 received 0.5 mg/kg of [<sup>3</sup>H]MK-0243 benzoate (1 ml/kg in 5% ethanol; L-656,748-051P001; 0.239 mCi/mg; 98.8% radiochemically pure) on day 1 and 0.5 mg/kg of [<sup>3</sup>H]MK-0243 HCl (1 ml/kg in deionized water; L-656,748-050M001; 0.229 mCi/mg; 98.7% radiochemically pure) on day 15. Dosing was reversed for Group 2. Body weights were determined before each dose. 2 ml of blood was withdrawn for drug level determinations following each dose at 0.5, 1, 2, 4, 6, 8, 24, 48, 96 & 168 hr. Urine & feces were collected for drug level analysis at 0-24 & 72-96 hr. There was no evidence of drug effects. The mean plasma half lives for the benzoate and HCl salts were 35.7%3.4 hr and 35.5%4.4 hr, respectively. The mean plasma AUC for the benzoate & HCl salts was 4479%1476 & 4574%1514 ng/g plasma/7days. The mean peak plasma MAB1a (the major component of MK-0243 at 90-95%) levels were ~100 ng equivalents/g plasma, occurring at ~6 hr for either salt. Combined fecal & urine recoveries during the 1st & 4th days were ~40% and 0.01% of the dose, respectively. It is concluded that the 2 salts are bioequivalent in male beagle dogs. **Supplemental.** (Rubin, 10/18/96)

#### **Bioequivalence, benzoate MBTE and benzoate monohydrate solvates**

52082-069 142187 "MK-0243 Benzoate MBTE Solvate/MK-0243 Benzoate Monohydrate Bioequivalence Study in Dogs" by R.J. Gerson, Merck Research Laboratories, West Point, PA & Three Bridges, NJ (project #618-244-TOX38; 12/21/92). This study was designed to determine if the MBTE (benzoate-methyl t-butyletherate) and the benzoate monohydrate solvates of emamectin are bioequivalent after oral administration. There were 2 dosing groups, each consisting of 2 male beagles. Group I received

0.5 mg/kg of MK-0243 benzoate MBTE (5 ml/kg in 0.5% methylcellulose; L-656,748-038W002; 96.9-98.0% pure) on day 1 and 0.5 mg/kg of MK-0243 benzoate monohydrate (5 ml/kg in 0.5% methylcellulose; L-656,748-052S001; 96.9-98.0% pure) on day 15. Dosing was reversed for Group II. Body weights were determined before each dose. ~2 ml of blood was withdrawn for drug level determinations (done by HPLC analysis after fluorescent derivatization) following each dose at 0.5, 1, 2, 4, 6, 8, 24, 48, 96 & 168 hr. There were no deaths and no evidence of drug effects. The approximate area under the curve (AUC) for the benzoate MBTE and benzoate monohydrate salts from 0 to 168 hr averaged 1502.6 & 1546.0 ng-hr/ml, respectively. Mean peak plasma concentrations of 33.0 & 35.2 ng/ml were achieved after 2 & 4 hr. The plasma decay profiles for the 2 compounds were virtually identical. Plasma kinetics are thus very similar for these compounds. The Report concludes that the 2 salts are bioequivalent in male beagle dogs. **Supplemental.** (Rubin, 10/21/96)

52082-070 142189 "MK-0244. Fifteen-Day Acute Oral Bioequivalence Study in Female Mice" by W.J. Bagdon, Merck Research Laboratories, West Point, PA (project #618-244-TOX56; 5/5/93). This study was designed to determine if the MBTE (benzoate-methyl t-butyletherate; L-656,748-038W, lot #2; 96.4% pure) and the benzoate monohydrate (L-656,748-052S, lot #2; 99.1% pure) solvates of emamectin are bioequivalent after oral gavage administration. 5 females in each group received 60, 90, 135 or 202 mg/kg of the MBTE or monohydrate solvates of emamectin as 2% suspensions in 0.5% methylcellulose. There were no negative controls. Daily observations were made for mortality & clinical signs. Body weights were recorded weekly. Mortality for the MBTE solvate was 0/5, 0/5, 0/5 & 5/5 (days 2-12), and for the monohydrate solvate was 0/5, 0/5, 2/5 & 5/5 (~4 hr-day 4). Clinical signs for both compounds included decreased activity (predominantly in wk 1), ataxia (wk 1), bradypnea (predominantly wk 1), loss of righting reflex (both wks for MBTE, wk 1 for monohydrate), ptosis (wk 1), tremors (wk 1), and cyanosis (MBTE only, 1 HD animal in wk 2). No clinical sign was noted below 90 mg/kg. Weight losses were manifested in the survivors at all doses during week 1, though recoveries occurred during wk 2. Gross necropsies did not reveal abnormalities. LD50 (female), MBTE solvate = 165 mg/kg (Toxicity Category II); LD50 (female), monohydrate solvate = 141 (115-172) mg/kg (Toxicity Category II). There was no substantial difference in the response to these 2 solvates in the female mouse. They are considered by the Report to be bioequivalent in the female mouse.

**Supplemental.** (Rubin, 10/21/96)

52082-071 142192 "MK-0244. Fifteen-Day Acute Oral Bioequivalence Study in Female Rats" by W.J. Bagdon, Merck Research Laboratories, West Point, PA (project #618-244-TOX57; 5/5/93). This study was designed to determine if the MBTE (benzoate-methyl t-butyletherate; L-656,748-038W, lot #2; 96.4% pure) and the benzoate monohydrate (L-656,748-052S, lot #2; 99.1% pure) solvates of emamectin are bioequivalent after oral gavage administration. 5 females in each group received 40, 60, 90, or 135 mg/kg of the MBTE or monohydrate solvates of emamectin as 6% suspensions in 0.5% methylcellulose. There were no negative controls. Daily observations were made for mortality & clinical signs. Body weights were recorded weekly. Mortality for the MBTE solvate was 0/5, 4/5, 5/5 & 5/5 (days 2-6), and for the monohydrate solvate was 0/5, 3/5, 5/5 & 5/5 (days 2-6). Clinical signs for both compounds (expressed at as low as 40 mg/kg and all occurring during wk 1) included ataxia, bradypnea, decreased activity, tremors, ptosis, salivation, laying on side, blood-like staining (eyes, nose or mouth), urine staining, loss of righting reflex, and soft stools. All survivors gained weight during both wks post

dose. Gross necropsies did not reveal abnormalities. LD50 (female), MBTE solvate = 53 (45-63) mg/kg (Toxicity Category II); LD50 (female), monohydrate solvate = 58 (47-70) mg/kg (Toxicity Category II). There was no substantial difference in the response to these 2 solvates in the female rat. They are considered by the Report to be bioequivalent in the female rat. **Supplemental.** (Rubin, 10/22/96)

### **ADDITIONAL STUDIES, Metabolites and photodegradates**

#### **15-day dietary neurotoxicity, L-930,905**

52082-036 142052 825 "L-930,905: Fifteen-Day Dietary Neurotoxicity Study in CF-1 Mice" by R.J. Gerson, Merck Research Laboratories, Merck & Co., Inc., West Point, PA (project #618-244-TOX44; 4/13/93). 10/sex/dose were exposed to test article (a mixture of emamectin benzoate photodegradates; L-930,905-000D002; 100% pure) at 0 (0.5% methyl cellulose control; 10 ml/kg), 3, 6, 12 or 18 mg/kg/day by oral gavage for 2 wk. There were no deaths. Food consumption appeared unaffected by exposure. Males receiving 12 mg/kg/day gained 70% more weight than controls. Because neither HD males nor females at any dose exhibited a similar weight increase, this observation was considered not to be toxicologically significant. HD males gained noticeably less weight than controls (0.9 g vs. 4.0 g in controls), but the lack of dose trend for both males and females suggests that this is also of no toxicologic significance. There were no treatment-related clinical signs or changes in brain weight. Gross necropsies and histopathology did not reveal abnormalities. NOEL/NOAEL (M/F) > 18 mg/kg/day; **Unacceptable** (the high dose was not sufficient to elicit a response). (Rubin, 7/16/96)

#### **15-day dietary neurotoxicity, L-695,638**

52082-034 142050 825 "L-695,638: Fifteen-Day Dietary Neurotoxicity Study in CF-1 Mice" by R.J. Gerson, Merck Research Laboratories, Merck & Co., Inc., West Point, PA (project #618-244-TOX41; 3/31/93). 10/sex/dose were exposed to target dietary doses of test article (an emamectin metabolite; L-695,638-001C001; 93.7% pure) at 0, .05, .075, .1 or .3 mg/kg/day (actual consumption was 87-107% of the target for wk 1; wk 2 consumption was unacceptably low, necessitating a future repeat study) for 2 wk. There were no deaths. Weight gains and food consumption appeared unaffected by exposure. There were no treatment-related clinical signs or changes in brain weight. Gross necropsies and histopathology did not reveal abnormalities. NOEL/NOAEL (M) > 0.26 mg/kg/day (avg. actual concentration); NOAEL/NOEL (F) not determined. **Unacceptable** (actual doses failed to approximate target doses for wk 2 females; no effects were noted at the HD). (Rubin, 7/12/96)

52082-035 142051 825 "L-695,638: Fifteen-Day Dietary Neurotoxicity Study in CF-1 Mice" by R.J. Gerson, Merck Research Laboratories, Merck & Co., Inc., West Point, PA (project #618-244-TOX42; 3/31/93). 10 females/dose were exposed to target dietary doses of test article (an emamectin metabolite; L-695,638-001C001; 93.7% pure) at 0, .05, .075, .1 or .3 mg/kg/day (actual consumption was 76-86% of the target) for 2 wk (males were exposed in record #142050). There were no deaths. Weight gains and food consumption appeared unaffected by exposure. There were no treatment-related clinical signs or changes in brain weight. Gross necropsies and histopathology did not reveal abnormalities. NOEL/NOAEL (F) > 0.243 mg/kg/day (avg. actual concentration); **Unacceptable** (the high dose was not sufficient to elicit a response). (Rubin, 7/16/96)

### **15-day dietary neurotoxicity, L-657,831**

52082-033 142049 825 "L-657,831: Fifteen-Day Dietary Neurotoxicity Study in CF-1 Mice" by R.J. Gerson, Merck Research Laboratories, Merck & Co., Inc., West Point, PA (project #618-244-TOX40; 5/7/93). 10/sex/dose were exposed to target dietary doses of test article (the formyl amino photodegradate of emamectin; L-657,831-000S008; 89.4% pure) at 0, .05, .075, .1 or .3 mg/kg/day (actual consumption was 67-80% of the target for the 2-wk period) for 2 wk. There were no deaths. Weight gains were inhibited by 17% and 43% compared to controls in HD males and females, respectively. Food consumption appeared unaffected by exposure. There were no treatment-related clinical signs or changes in brain weight. Gross necropsies and histopathology did not reveal abnormalities. NOAEL (M/F) > 0.23-0.24 mg/kg/day (avg. actual concentration). NOEL (M/F) = 0.07-0.08 mg/kg/day (avg. actual concentration; weight gain decrement). **Acceptable**. (Rubin, 7/12/96)

### **15-day dietary neurotoxicity, L-660,599**

52082-031 142047 825 "L-660,599: Fifteen-Day Dietary Neurotoxicity Study in CF-1 Mice" by R.J. Gerson, Merck Research Laboratories, Merck & Co., Inc., West Point, PA (project #618-244-TOX39; 4/2/93). 10/sex/dose were exposed to target dietary doses of test article (the formyl methylamino photodegradate of emamectin; L-660,599-000N, lot #4; 98.1% pure) at 0, .05, .075, .1 & .3 mg/kg/day for 2 wk. All treatment-related changes occurred at the top 2 doses (HD & MHD). Observations at the HD: 3/10 males & 5/10 females had tremors on days 2-4. 2-5 days following the onset of tremors the following signs were seen: ptosis, decreased activity, hunched posture, splayed limbs, ataxia, labored breathing urine staining and/or lateral recumbency. All 8 affected mice were sacrificed on days 4-8. 1 male from this group showed very slight sciatic nerve degeneration. The remaining mice were sacrificed on day 8, showing no PNS lesions. Decreased food consumption & wt. losses occurred only among the affected animals at the HD and MHD. Observations at the MHD: 3/10 males had tremors beginning on days 3 or 5 accompanied in 2 of these mice by ptosis, decreased activity and/or hunched posture. 1 of the mice that showed severe clinical signs was sacrificed on day 8 also exhibiting sciatic nerve degeneration. Tremors continued in the other 2 mice until termination. 3 other MHD males (as well as 1 control female) exhibited sciatic nerve degeneration as well. The sciatic nerve lesion at both doses consisted of myelin sheath dilation & breakdown. This, combined with the neurologic clinical signs, is considered an **adverse effect**. NOAEL/NOEL, M = 0.075 mg/kg/day, F = 0.1 mg/kg/day (neural effects). **Acceptable**. (Rubin, 7/10/96)

52082-032 142048 825 "L-660,599: Fifteen-Day Dietary Neurotoxicity Study in CF-1 Mice" by R.J. Gerson, Merck Research Laboratories, Merck & Co., Inc., West Point, PA (project #618-244-TOX43; 5/1/93). 10/sex/dose were exposed to target dietary doses of test article (the formyl methylamino photodegradate of emamectin; L-660,599-000N004; 98.9% pure) at 0, .1, .3 or .9 mg/kg/day for 2 wk. Mortalities: 4 MD (1M/3F) & 4 HD (1M/3F) animals were sacrificed between days 3-9 w/severe clinical signs and 1 MD female was found dead on day 12 (cause of death unknown). Clinical signs, LD: 1 male had tremors, hunched posture, piloerection (all appearing on day 14) & wt. gain depression. MD: tremors (onset day 3) were observed in 2M/5F and other signs including hunched posture, decreased activity, piloerection, coldness, recumbency, labored breathing, splayed limbs & prolapsed penis. HD: tremors (onset day 2) in 3M/3F and other signs including decreased activity, labored breathing, splayed limbs and urine staining. While the time of symptom onset was dependent on dose, there was no clear difference in

symptom severity between the MD & HD. Weight loss occurred in MD & HD animals showing severe clinical signs, most of which were ultimately sacrificed in extremis. There were no treatment-related histopathologic findings at any dose. Brain weights were unaffected. The neurologic clinical signs are considered **adverse effects**. NOAEL/NOEL, M < 0.1 mg/kg/day, F = 0.1 mg/kg/day (neurologic effects). **Unacceptable** (low dose was not free of adverse signs). (Rubin, 7/11/96)

#### **Gene mutation, L-660,599**

52082-061 142167 842 "L-660,599: Microbial Mutagenesis Assay" by J.F. Sina, Merck Research Laboratories, West Point, PA (project #618-244-TOX52; 3/5/93). Histidine dependent Salmonella typhimurium tester strains T1535, 97a, 98 & 100, and tryptophan dependent Escherichia coli tester strains WP2, WP2 uvrA & WP2 uvrA pKM101 were exposed in triplicate to L-660,599 (L-660,599-000N004; 98.9% purity) at 0, 100, 300, 1000, 3000 & 10,000 ug/plate +/- S9 microsomes for 48 hr. No increase in revertants was noted despite the success of the positive controls. A precipitate formed at 10,000 ug/plate, precluding plate scoring. No inhibition of bacterial lawn or revertant growth was noted at any concentration. L-660,599 is not considered mutagenic in this system under the conditions tested. **Supplemental.** (Rubin, 11/4/96)

#### **Gene mutation, L-657,831**

52082-062 142168 842 "L-657,831: Microbial Mutagenesis Assay" by J.F. Sina, Merck Research Laboratories, West Point, PA (project #618-244-TOX53; 5/3/93). Histidine dependent Salmonella typhimurium tester strains T1535, 97a, 98 & 100, and tryptophan dependent Escherichia coli tester strains WP2, WP2 uvrA & WP2 uvrA pKM101 were exposed in triplicate to L-657,831 (L-657,831-000S008; 89.4% purity) at 0, 100, 300, 1000, 3000 & 10,000 ug/plate +/- S9 microsomes for 48 hr. No increase in revertants was noted despite the success of the positive controls. A precipitate formed at 3000 ug/plate and above, but did not interfere with plate scoring. At 10,000 ug/plate the precipitate was sufficient to preclude scoring on all plates. There was no inhibition of bacterial lawn growth. L-657,831 is not considered mutagenic in this system under the conditions tested. **Supplemental.** (Rubin, 11/4/96)

#### **Gene mutation, L-695,638**

52082-063 142169 842 "L-695,638: Microbial Mutagenesis Assay" by J.F. Sina, Merck Research Laboratories, West Point, PA (project #618-244-TOX54; 4/13/93). Histidine dependent Salmonella typhimurium tester strains T1535, 97a, 98 & 100, and tryptophan dependent Escherichia coli tester strains WP2, WP2 uvrA & WP2 uvrA pKM101 were exposed in triplicate to L-695,638 (L-695,638-001C001; 93.7% B1a purity) in Expt. 1 at 0, 100, 300, 1000, 3000 & 10,000 ug/plate +/- S9 microsomes for 48 hr. No increase in revertants was noted despite the success of the positive controls. A precipitate formed at 3000 ug/plate and above, but did not interfere with plate scoring. Inhibition of bacterial lawn growth sufficient to interfere with plate scoring occurred in Salmonella strains TA100, 1535 & 97a at as low as 1000 ug/plate (revertant growth was also inhibited in all cases with the possible exception of WP2). This necessitated a repeat test in Salmonella (Expt. 2) at 0, 3, 10, 30, 100 & 300 ug/plate for 48 hr. Again no increase in revertants was noted, though slight inhibition of background lawn growth (TA97 +S9 @ 300 ug/plate) and of revertant growth (TA100 & 97a) were noted. Discrepancies in the positive controls, however (TA97a: lower than acceptable increases in revertants for ICR-191 & methyl methanesulfonate; TA98: higher than acceptable increases for ICR-191 and lower than acceptable

for daunomycin) forced yet a 3rd repeat for these 2 Salmonella strains (Expt. 3). The same concentration series as Expt. 2 was run. No increase in revertants was noted, though there was slight inhibition of background lawn growth in TA97a @ 300 ug/plate along with some inhibition of revertant growth in this strain. Positive controls responded appropriately. L-695,638 is not considered mutagenic in this system under the conditions tested. **Supplemental.** (Rubin, 11/4/96)

### **Gene mutation, L-930,905**

52082-064 142170 842 "L-930,905: Microbial Mutagenesis Assay" by J.F. Sina, Merck Research Laboratories, West Point, PA (project #618-244-TOX55; 4/13/93). Salmonella typhimurium tester strains T1535, 97a, 98 & 100, and Escherichia coli tester strains WP2, WP2 uvrA & WP2 uvrA pKM101 were exposed in triplicate to L-930,905 (L-930,905-000D001; 85.0% purity) at 0, 100, 300, 1000, 3000 & 10,000 ug/plate +/- S9 microsomes for 48 hr. A precipitate formed at the HD in TA98 and WP2 -S9. There was no inhibition of bacterial lawn growth at the HD. There was slight inhibition of revertant growth in TA97a -S9. Despite the success of the positive controls, no concentration of L-930,905 induced a reversion to histidine (S. typhimurium) or tryptophan (E. coli) independence regardless of the absence or presence of a microsomal activating system. L-930,905 is not considered mutagenic in this system under the conditions tested. **Supplemental.** (Rubin, 11/1/96)