Date: January 19, 2016

FREEDOM OF INFORMATION SUMMARY

IMPORT TOLERANCE

I. GENERAL INFORMATION:

A. Established Name: monepantel

B. Food-Animal Species: sheep

C. Tolerances/tissues: 7 parts per million (ppm) monepantel sulfone in fat
5 ppm monepantel sulfone in liver
2 ppm monepantel sulfone in kidney
0.7 ppm monepantel sulfone in muscle

D. File Number: VMF 005-967

E. Petitioner: Elanco Animal Health,
A Division of Eli Lilly & Co.,
Lilly Corporate Center,
Indianapolis, IN 46285

II. HUMAN FOOD SAFETY:

A. Toxicology:

Toxicity tests determining the human food safety of monepantel are summarized in items 1-5 below:

1. Genetic Toxicology Studies:

a. Study Title: Mutagenicity test using *Salmonella typhimurium*

Study No.:	0512002
Study Investigator:	Martus, H.J.
Study Location:	Department of Safety Profiling and Assessment, Novartis, Basel, Switzerland
Study Completion Date:	September 19, 2005

Experimental Design and Conclusion: Two procedures were used for assessing the mutagenicity of the test article, AHC2102225 (monepantel). The first procedure was based on the direct plate method of Ames *et al.* (Mutation Research 31, 347-364. 1975). The second procedure was based on the preincubation modification of the plate incorporation method of Yahagi *et al.*, (Cancer Letters 1:91-96, 1975), where the test article (or vehicle, or positive control compound), bacteria and S9 or phosphate buffer were incubated at 37°C for 20 minutes before plating on minimal agar plates.

Five *S. typhimurium* strains (TA1535, TA97a, TA100, TA98 and TA102) were used in these assays. The metabolic activation system (S9 mix) consisted of liver homogenate (S9) from Aroclor-1254 induced male Sprague-Dawley rat liver and the necessary cofactors. Solvent used for the test article was dimethylsulfoxide (DMSO). Positive control substances used in the assays included sodium azide (-S9; TA1535, TA-100), benzo(a)pyrene (+S9; TA98), 2-aminoanthracene (+S9; for all strains), 9-aminoacridine (-S9; TA97a), 2-nitrofluorene (-S9; TA98), and mitomycin C (-S9; TA102).

Doses for the main assay were derived from a preliminary dose range-finding assay. Five doses ranging from 8 to 5000 µg/plate were used in the plate incorporation method and five doses ranging from 312.5 to 5000 µg/plate were used in the preincubation assay. The test article precipitated at 5000 µg/plate in the plate incorporation assay and at 2500 and 5000 µg/plate in the preincubation assay. Preincubation assay was repeated using 5 doses ranging from 25 to 400 µg/plate. In this repeat assay, the test article precipitated at top two doses of 200 and 400 µg/plate, but the precipitate did not interfere with colony counting.

No positive responses were observed in the plate incorporation assay or in the preincubation assay in the presence and absence of metabolic activation. It was concluded that AHC2102225 did not show evidence of a mutagenic potential in the bacterial mutation assay under the conditions of the test.

b. Study Title: Chromosome Aberration Test with Cultured Human Peripheral Blood Lymphocytes

Study No.: 0512102
Study Investigator: Elhajouji, A.
Study Location: Department of Safety Profiling and Assessment, Novartis, Basel, Switzerland
Study Completion Date: December 23, 2005

Experimental Design and Conclusion: Human peripheral blood lymphocytes were used in the assay. DMSO was used as solvent for the test article, AHC 2102225. Metabolic activation system (S9 mix) consisted of liver homogenate (S9) from Aroclor 1254 induced rat liver and the necessary cofactors.

Duplicate cultures were treated with the vehicle or with the test article (+/- S9). Additional duplicate cultures were treated with positive control chemicals ethylmethane sulfonate (EMS; -S9) and cyclophosphamide (CP; +S9).

Two experiments were conducted with the test article. For both experiments, eleven doses of the test article ranging from 0 to 200 µg/mL (without S9, 20 hour treatment, 0 hour recovery), and eleven doses ranging from 0 to 600 µg/mL (with S9, 3 hour treatment, 17 hour recovery) were used.

In Experiment A, the test article produced a concentration dependent decrease in the mitotic index (MI). The relative MI at 43.1 µg/mL was 26.7 and 90.0 at 25.8 µg/mL (-S9, 20 hour treatment). The relative MI was 59.4 at 129.3 µg/mL; and 121.9 at 77.5 µg/mL (+S9, 3 hour treatment, 17 hours recovery). Based on the MI data, the following concentrations were selected for analysis in the chromosomal aberration assay: 25.8, 33.4, 43.1 µg/mL (-S9; 20 hour continuous treatment); and 77.5, 100.1, 129.3 µg/mL (+S9; 3 hour treatment, 17 hour recovery).

In Experiment B, the test article produced a concentration dependent decrease in the MI. The relative MI was 48.6 at 97.8 µg/mL, and 90.5 at 57.2 µg/mL (-S9; 3 hour treatment, 17 hour recovery). The relative MI was 55.2 at 139.9 µg/mL, and 87.4 at 81.8 µg/mL (+S9, 3 hour treatment, 17 hours recovery). Based on the MI data, the following concentrations were selected for analysis in the chromosomal aberration assay: 57.2, 81.8, 97.8 µg/mL (- S9; 3 hour treatment, 17 hour recovery); and 81.8, 117.0, 139.9 µg/mL (+S9; 3 hour treatment, 17 hour recovery).

In the absence and presence of metabolic activation, and at two different times of exposure times, there were no significant differences on the number of abnormal cells compared to the concurrent controls at any of the concentrations evaluated. No increase in polyploidy cells was observed in the absence as well as in the presence of S9. It was concluded that AHC 2102225 did not show any clastogenic potential under the test conditions used in the chromosomal aberration test with human peripheral blood lymphocytes.

c. Study Title: Bone Marrow Micronucleus Test by Oral Route in Mice

Study No.: 30319 MAS
Study Investigator: Hasnaa Haddouk

Study Location: CIT, Evreux, France
Study Completion Date: February 10, 2006

Experimental Design and Conclusion: A formulated solution of AHC210225 was prepared in 0.5% methylcellulose. In the preliminary toxicity study, 3 male and 3 female Swiss Ico: OF1 (IOPS Caw) mice were dosed twice with 2000 mg/kg bw/dose at an interval of 24 hours. Clinical signs and any mortality were recorded for a period of 48 hours. No clinical signs and no mortality were observed in animals treated at the dose level of 2000 mg/kg bw/dose until the end of the observation period.

Since 2000 mg/kg bw/dose produced no observable toxic effects, for the main test, a limit test was performed at this dose level. One group of five male and five female Swiss Ico: OF1 (IOPS Caw) mice (mean body weight of 32.1 g for males and 25.8 g for females) was given oral administrations of AHC 2102225 at the dose level of 2000 mg/kg bw/dose. The frequency of treatment was two treatments separated by 24 hours. One group of five males and five females received the vehicle (0.5% methylcellulose) under the same experimental conditions as the test article, and served as a control group. One group of five males and five females received the positive control test item (cyclophosphamide) once by oral route at the dose-level of 50 mg/kg bw. The age of the animals were approximately 6 weeks on the day of treatment. The animals of the treated and vehicle control groups were sacrificed 24 hours after the last treatment and the animals of the positive control group were sacrificed 24 hours after the single treatment. Femurs of the animals were removed and the bone marrow was extracted. The bone marrow smears prepared and stained with Geimsa were scored for micronucleated polychromatic erythrocytes (MPCE), polychromatic (PE) and normochromatic erythrocytes (NE). For each animal, the number of MPCE was counted in 2000 PEs. The PE and NE ratio was established by scoring a total of 1000 erythrocytes (PE + NE). Means and standard deviations of the frequency of MPCE/1000 PE and PE/NE ratio were calculated. The Mean value of MPCE obtained for males (1.5 MPCE/1000 PE) was within the vehicle control historical control value (0.1 – 2.8 MPCE/1000 PE). For females, the mean MPCE value (2.2 MPCE/1000 PE) was slightly higher than the historical control range of 0.2 – 1.6 MPCE/1000 PE. This increase was considered biologically not relevant since all individual values in the test article treated group were within the historical control values. There were no statistically significant differences in the MPCE mean values as well as the PE/NE ratio in the group treated with the test article, in comparison to the concurrent control groups. Under the experimental conditions, the test article AHC 2102225 did not induce aberrations in the mouse bone marrow cells after two oral administrations, at a 24-hour interval, at 2000 mg/kg bw/dose.

d. Study Title: In Silico prediction of Potential Toxicological Properties. Computational toxicology letter report

Study No.: 0519336
Study Investigator: Mueller, A.
Study Location: Department of Safety Profiling and Assessment, Novartis, Basel, Switzerland
Study Completion Date: November 30, 2005

Experimental Design and Conclusion: This is a computational toxicology report on the test article AHC 2155367, an impurity found in the parent compound AHC 2102225. Percentage of impurity in the parent compound is less than 3%.

The impurity, AHC 2155367, was evaluated using DEREK for Windows version 8.0.1 (Lhasa Ltd) and MCASE. DEREK predicts the toxicity end points for species that include, among others, humans. The toxicity end points include, among others, carcinogenicity, and genotoxicity that includes gene mutations and clastogenicity.

The analysis by DEREK concluded that the presence of the structural alerts in the molecule indicated that:

- AHC 2155367, as well as AHC 2102225, has a low likelihood to behave as genotoxicants in experimental systems,

- AHC 2155367 and AHC 2102225 have some likelihood to result in a tumorigenic response in rodents following life-long treatment.

- Substitution of –CH3 group in AHC2102225 by Cl atom results only in changes of the predicted hERG channel activity and the skin sensitization potential. However, these results are uncertain due to the presence of three unknown fragments for both AHC 2102225 and AHC 2155367 structures. Both structures exhibit a structural alert for cyanide type effects.

It was concluded that the AHC 2155367 as well as the AHC 2102225 structures do not exhibit any structural alerts for genotoxic and carcinogenic response. Only the aliphatic nitrile group present in both structures is an alert for cyanide type adverse effects.

e. Study Title: Microscreen Ames test. Genetic Tox letter report

Study No.:	0413029
Study Investigator:	Glowienke, S.
Study Location:	Preclinical Safety, Europe. Novartis Pharma AG. Basel, Switzerland
Study Completion Date:	March 10, 2004

Experimental Design and Conclusion: This is a non-GLP pre-screening study to assess the mutagenic potential of the test article using *Salmonella typhimurium in vitro*, with and without the addition of a mammalian metabolizing system. The brief report does not contain detailed materials, methods and data.

The main metabolite observed in tissues, the sulfone AHC2092404 (racemate), was tested in 5 strains of *Salmonella typhimurium* (TA1535, TA97a, TA98, TA100 and TA102). Liver S9 mix from male rats treated with Aroclor 1254 was used as a metabolic activation system. Concentrations tested were 30, 100, 300 and 1000 µg/well. The test article neither precipitated nor showed signs of bacteriotoxicity.

Under the testing conditions and applying standard mutagenicity criteria, AHC2092404 did not show evidence of a mutagenic potential.

f. Study Title: Micronucleus test *in vitro* using TK6 cells. Genetic Tox letter report

Study No.: 0414029
Study Completion Date: March 08, 2004
Study Investigator: Frieauff, W.
Study Location: Preclinical Safety, Europe. Novartis Pharma AG. Basel, Switzerland.

Experimental Design and Conclusion: This is a non-GLP pre-screening study to determine the genotoxicity of the test article. The brief report does not contain detailed materials, methods or data.

The sulfone metabolite, AHC 2092404 (racemate), was tested in the *in vitro* micronucleus test with cultured TK6 cells at concentrations of 64.6, 107.8 and 179.8 µg/mL. At the highest concentration, some precipitation of the compound was observed. AHC 2092404 was soluble in DMSO up to 538 mg/mL.

Based on this very brief report, it was concluded that AHC 2092404 did not induce increased numbers of cells containing micronuclei after 3-hour treatment with S9, nor after 20-hour treatment in the absence of metabolic activation.

2. Sub-Chronic Toxicity Studies:

a. Study Title:13-week toxicity study by oral route (diet admixture) in beagle dogs followed by a 4-week treatment free period

Report Number: 29215 TCC
Report Date: September 22, 2006
Study Director: V. Haag
Study Location: CIT, Evreux, France

Experimental Design and Conclusion: This study was conducted to evaluate the potential systemic toxicity of AHC 2102225 (monepantel) when administered daily in diet to beagle dogs for 90 days at constant concentrations of 0, 300, 3,000, and 30,000 ppm (equivalent to 0, 9.9, 106.8, 963.0 mg/kg bw/day for the males and 0, 10.7, 96.8, and 1176.1 mg/kg bw/day for the females, respectively. Following completion of treatment, a set of animals was subjected to a 4-week wash-out period to see if any of the treatment related effects were reversible. Forty (20 male and 20 female) beagle dogs approximately 6 months of age at the beginning of the study were included. Body weights were measured weekly, food consumption and clinical observations were recorded daily, and blood and urine samples for hematology and biochemical determinations were collected and analyzed at pretest, 7 weeks after initiation and at termination. At the termination of the study (90 days), animals were sacrificed and a full macroscopic *post mortem*

examine was performed. Organs were weighed, preserved and subjected to microscopic evaluation.

No test article-related findings were noted for clinical observations or food consumption. The liver was noted as the target organ based on increased liver weight, hepatocellular hypertrophy, brown pigment in Kupffer cells and hepatocytes, and elevated alkaline phosphatase activity. The no-observed-effect level (NOEL) of this study could not be determined because elevated liver weights, hepatocellular hypertrophy, decreased leukocyte/neutrophil counts, and dilation of glands in the intestine, and increased apoptosis in the pancreas were noted at all dose levels tested at the end of the treatment period.

b. Study Title: 90-day oral toxicity study followed by a 4-week recovery period in the Wistar Rat

Study Number:	RCC 858125
Report Date:	December 14, 2005
Study Initiation Date:	December 21, 2004
Study Director:	Dr.M. Bachmann
Study Location:	RCC Ltd, toxicology, a) CH-4452 Itingen/Switzerland, b) CH -4414 Füllinsdorg/Switzerland

Experimental Design and Conclusion: AHC-2102225 (monepantel) was administered in diet to groups of 10 male and 10 female Wistar rats for 90 days at constant concentrations of 0, 50, 200, 1,000, and 12,000 ppm (equivalent to 0, 4, 15, 74, and 900 mg/kg bw/day for males and 0, 4, 15, 82, and 947 mg/kg bw/day for females) Additional 5 rats per sex in the control and high dose group were followed by a 28-day recovery period. Clinical signs, food consumption, body weights, water consumption, ophthalmoscopic examinations, functional observational battery, hematology, clinical biochemistry, urine analysis, organ weights, histopathological examination were obtained.

No compound related effects on mortality, clinical signs, food and water consumption, body weight, or eye toxicity were reported. Changes were observed in liver and lipid metabolism. Liver, adrenal and ovary weights were elevated at 947 mg/kg bw/day in females. Decreased total bilirubin concentration and centrilobular hepatocellular hypertrophy were noted in females at 82 and 947 mg/kg bw/day. Minimal hypospermatogenesis and moderately increased intratubular cellular debris in epididymides in males was reported at 900 mg/kg bw/day. Minimal sex cord stromal hypertrophy occurred in females at 947 mg/kg bw/day. The NOEL for this study was 15 mg/kg bw/day based on the decreased bilirubin concentration and centrilobular hepatocellular hypertrophy at 82 mg/kg bw/day in females at the end of the treatment period.

c. Study Title: 13-week oral toxicity study in the CD-1 mice

Study Number:	RCC 858126
Report Date:	September 15, 2005

Study Initiation Date: January 15, 2005
Study Director: Dr. M. Bachmann
Study Location: RCC Ltd, toxicology, a) CH-4452 Itingen/Switzerland, b) CH -4414 Füllinsdorg/Switzerland

Experimental Design and Conclusion: AHC-2102225 (monepantel) was administered with diet to groups of 10 male and 10 female CD-1 mice for 90 days at constant concentrations of 0, 30, 120, 600, and 6,000 ppm (equivalent to 0, 5, 18, 98, and 959 mg/kg bw/day for males and 0, 5, 22, 115, and 1213 mg/kg bw/day for females. Clinical signs, food consumption, body weights, water consumption, ophthalmoscopic examinations, functional observational battery, hematology, clinical biochemistry, urine analysis, organ weights, and histopathological examination were obtained.

No compound related effects on mortality, clinical signs, food and water consumption, body weight, or eye toxicity were reported. Changes were observed in liver and lipid metabolism based on clinical chemistry, organ weight and histopathology results. Increased cholesterol was found at 115 and 1213 mg/kg bw/day in females. Increased bilirubin levels and liver weights were reported at 98 and 959 mg/kg bw/day in males. Fatty change, increased focal necrosis and lymphoid cell infiltrates in liver was observed at 115 and 1213 mg/kg bw/day in females. Decreased albumin and increased testes to body weight ratio in males at 22 mg/kg bw/day was considered incidental in the absence of a dose-relationship and corroborating histopathology findings. The NOEL for this study was 18 mg/kg bw/day in males and 22 mg/kg bw in females based on increases in cholesterol and bilirubin concentration, liver weight increases, liver pathological findings (fatty changes, increased incidences of focal necrosis and lymphoid cell infiltrates) at 98 mg/kg bw/day in males or 115 mg/kg bw/day in females.

3. Chronic Toxicity Studies:

a. Study Title: 52-week toxicity study by oral route (dietary admixture) in beagle dogs

Report Number: 30240 TCC
Report Date: December 10, 2007
Study Director: V. Haag
Study Location: CIT, Evreux, France

Experimental Design and Conclusion: This study was conducted to evaluate the effects of AHC 2102225 (monepantel) when administered daily in diet to Beagle dogs for 52 weeks at constant concentrations of 0, 100, 300, and 3,000 ppm (equivalent to 0, 2.96, 8.21, 91.03 mg/kg bw/day for the males and 0, 3.16, 10.18, and 99.39 mg/kg bw/day for the females, respectively). Thirty-two (16 males and 16 females) beagle dogs, approximately 6 months of age at the beginning of the study, were included. Following completion of the study, animals were sacrificed and examinations were performed on body weight, food consumption, hematology, blood chemistry, and urinalysis. Selected Organs were weighed, preserved and subjected to microscopic evaluation.

All clinical observations, food consumption, and urinalysis were similar to the control animals or did not show dose-relationship, and were therefore not considered to be treatment related. Treatment-related effects on body weight were only observed at the high dose groups for both males and females. Hematology comparison of the pre-treatment and control values to the treated animals revealed a decrease in the activated partial thromboplastin time (APTT) in males in the mid- and high dose groups, while only in the high dose group in females. The decrease was statistically significant and ranged from a 19 to 23% decrease. No other treatment-related hematology affects were noted in this study. Clinical chemistry revealed several treatment related effects. Lower protein levels, albumin levels, albumin/globulin ratio, calcium, alkaline phosphatase levels were observed in males throughout the study at 3000 ppm. Lower albumin levels and albumin/globulin ratio were observed in females at 300 and 3000 ppm. Increases in alkaline phosphatase activity were observed at 3000 ppm in males, and at 300 and 3000 ppm in females. Alanine aminotransferase activity was increased at 3000 ppm in males and females. γ-glutamyl transferase activity was increased in males at the high dose. Both liver and thyroid weights were increased in the mid and high dose groups.

The liver was noted as the target organ based on liver weight increases, hepatocellular hypertrophy, brown pigment in Kupffer cells and hepatocytes, bile duct hyperplasia, and increases in liver enzymes. The NOEL of this study was determined to be 3 mg/kg bw/day based on the significant decrease in APTT time, the elevated liver and thyroid weights, increased alkaline phosphatase activity, decreased albumin concentration, decreased albumin/globulin ratio and increased incidence and severity of brown pigmentation in the liver and kidney cells at mid-dose levels.

b. Study Title: 52-week chronic oral toxicity (feeding) study in Wistar Rats

Study Number: AHC-210225
Report Date: July 25, 2007
Study Initiation Date: August 15, 2005
Study Director: Dr. K. Broich
Study Location: RCC Ltd, toxicology, a) CH-4452 Itingen/Switzerland, b) CH -4414 Füllinsdorg/Switzerland

Experimental Design and Conclusion: AHC-2102225 (monepantel) was administered with diet to groups of 20 male and 20 female Wistar rats for one year at constant concentrations of 0, 50, 200, 1,000, and 12,000 ppm (equivalent to 0, 3, 11, 55, and 656 mg/kg bw/day for males and 0, 3, 14, 67, and 778 mg/kg bw/day for females). Clinical signs, food consumption, body weights, water consumption, ophthalmoscopic examinations, functional observational battery, hematology, clinical biochemistry, urine analysis, organ weights, and histopathological examination were obtained. No compound related effects on mortality, clinical signs, food and water consumption, body weight, or eye toxicity were reported. Changes were observed in liver weight and lipid metabolism. Cholesterol, phospholipids and triglycerides concentration were elevated at 778 mg/kg bw/day in females. Liver to body weight ratio increased at 656 mg/kg bw/day in males. Increased serum concentration of total protein and globulin and increased liver weight were

reported at 67 and 778 mg/kg bw/day in females. Increased kidney to body weight ratio was reported at 778 mg/kg bw/day in females. No histopathological changes were found in organs and tissues. The NOEL for this study was 11 mg/kg in males and 14 mg/kg in females based on the increased serum concentration of total protein and globulin and increased liver weight at 67 mg/kg in females.

4. Developmental and Reproductive Studies

a. Study Title: Prenatal Developmental Toxicity Study in the Himalayan Rabbit

Study No.: 858649
Report Date: March 8, 2006
Study Director: Dr. R. Gerspach
Study Location: RCC Ltd Toxicology
CH4414 Follinsdorf / Switzerland

Experiment Design and Conclusion: In order to detect effects on embryonic and fetal development in pregnant rabbits, AHC-2102225 (monepantel) was administered to Himalayan rabbits by oral gavage, once daily, from day 6 through day 27 *post coitum* at dose levels of 0, 100, 300 or 1,000 mg/kg bw/day. All groups received a dose volume of 5 mL/kg bw with a daily adjustment of the individual volume to the actual body weight. Control animals were similarly dosed with the vehicle alone (30% polyethylene glycol in purified water). On day 28 *post coitum*, females were killed by an intravenous injection of sodium pentobarbital and the fetuses were removed by Caesarean section. Clinical signs, food consumption and body weights were recorded daily. Treatment with monepantel was well tolerated and did not cause toxicity, neither on dams nor on fetuses. Under the conditions of the study, monepantel did not reveal a teratogenic potential at the doses tested. A NOEL for maternal and fetal toxicity of 1,000 mg/kg bw/day was established.

b. Study Title: Prenatal Developmental Toxicity Study in the Han Wistar Rat

Study No.: 858651
Report Date: March 7, 2006
Study Director: Dr. R. Gerspach
Study Location: RCC Ltd, Toxicology,
CH-4414 Fullinsdorf / Switzerland

Experiment Design and Conclusion: In order to detect effects on embryonic and fetal development in pregnant rats, AHC-2102225 (monepantel) was administered to Wistar rats by oral gavage, once daily, from day 6 through day 20 *post coitum* at dose levels of 0, 100, 300 or 1,000 mg/kg bw/day. Control animals were similarly dosed with the vehicle alone (30% polyethylene glycol in purified water). All females were sacrificed on day 21 *post coitum* and the fetuses were removed by Caesarean section. Clinical signs, food consumption and body weights were recorded daily. Monepantel was well tolerated by the pregnant rats. There were no treatment-related effects on post-implantation

loss or number of fetuses. External, visceral, and skeletal examinations did not reveal treatment-related effects. Under the conditions of the study, monepantel did not reveal a teratogenic potential at the doses tested. A NOEL for maternal and fetal toxicity of 1,000 mg/kg bw/day was established.

c. Study Title: Two Generation Reproduction Study in the Han Wistar Rat

Study No.: 858651
Report Date: December 22, 2006
Study Director: Dr. R. Gerspach
Study Location: RCC Ltd, Environmental Chemistry & Pharmanalytics, CH-4452 Itingen / Switzerland

Experiment Design and Conclusion: Two generations of rats were exposed in the diet to 0, 200, 1500, and 12,000 ppm monepantel. The mean dose levels achieved are presented in the tables below.

Table 1. Achieved Dose Levels in the P generation

Generation P	Concentration (ppm)	Mean achieved dose level (mg/kg bw/day)
Males prepairing	0	0
	200	13.3
	1,500	99.8
	12,000	798
Males postpairing	0	0
	200	10.5
	1,500	79.3
	12,000	647
Females prepairing	0	0
	200	15.8
	1,500	119
	12,000	950
Females gestation	0	0
	200	13.5
	1,500	103
	12,000	863
Females lactation	0	0
	200	32.3
	1,500	245
	12,000	2055

Table 2. Achieved Dose Levels in the F1 generation

Generation F1	Concentration (ppm)	Mean achieved dose level (mg/kg bw/day)
Males prepairing	0	0
	200	16.8
	1,500	125
	12,000	1014
Males postpairing	0	0
	200	11.2
	1,500	81.7
	12,000	694
Females prepairing	0	0
	200	18.6
	1,500	141
	12,000	1109
Females gestation	0	0
	200	15.1
	1,500	114
	12,000	918
Females lactation	0	0
	200	30.8
	1,500	241
	12,000	2028

Monepantel induced changes in the liver and adrenal glands of P parental females, and in the liver, adrenal glands and ovaries of F1 parental females. In the P generation, centrilobular hepatocellular hypertrophy was observed in females at 1,500 and 12,000 ppm. In the adrenal glands, cortical cell hypertrophy of the zona glomerulosa was observed in higher incidence in females at 1,500 and 12,000 ppm. Sex cord stromal hyperplasia of the ovaries of unknown origin was also seen in high dose females. In the F1 generation, male and female liver weights were increased at 12,000 ppm. Liver weights were also significantly increased at 200 and 1,500 ppm in F1 females. Sperm parameters, estrus cyclicity, and additional reproductive parameters such as insemination, fertility, gestation, and litter numbers were not affected. A lowest-observed-effect level (LOEL) of 200 ppm for maternal and fetal toxicity was established based on increased liver weights in females at all dose levels. Given the lack of a histological correlation for effects in the liver at 200 ppm, a safety factor of 3 was used to establish a NOEL of 66 ppm or 3 mg/kg bw/day (the lowest equivalent dose level of 10.5 mg/kg bw/day divided by 3) for maternal and fetal toxicity. A NOEL for reproductive effects was established at 12,000 ppm.

5. Carcinogenicity Studies

a. Study Title: 78-Week Oncogenicity (Feeding) Study in CD-1 Mice

Study No.: A23207
Report Date: March 28, 2008

Study Director: Dr. M. Bachmann until August 23, 2006; Dr. L. Fischer from August 24, 2006
Study Location: RCC Ltd, Zelgliweg 1, 4052 Itingen, Switzerland

Experiment Design and Conclusion: SPF-bred CD-1 mice (50/sex/group) were administered AHC-2102225 (monepantel) in the diet at constant concentrations of 10, 30, 120, and 500 ppm (equivalent to 1.3, 4.2, 16.2 and 69.1 mg/kg bw/day for males, and 1.8, 5.5, 22.8, and 91.7 mg/kg bw/day for females) for at least 78 consecutive weeks. A control group was fed untreated diet. Clinical signs, food consumption and body weights were recorded periodically. At Weeks 52 and 78, blood samples were taken for hematology analyses. After Week 78, all animals were sacrificed, necropsied, and examined *post mortem*. Histopathological examinations were performed on organs and tissues from all control and high dose animals and on all gross lesions from all animals. The liver was examined in low and mid dose groups due to test item-related findings noted in the high dose group. The nasal cavity was also examined in the intermediate groups.

There were no treatment-related effects on clinical observations, food consumption, body weight, and macroscopic findings. The female group at 500 ppm had higher mortality rate compared to the control. Treatment-related effects on hematology parameters were noted at 500 ppm. The relevant treatment-related changes on pathology were non-neoplastic in nature, consisting of increased liver weights (females at 120 and 500 ppm, and males at 500 ppm), centrilobular hepatocellular hypertrophy (both sexes of all treated groups) and hepatocellular fatty change (both sexes at 120 and 500 ppm). Therefore, it was concluded that monepantel did not demonstrate carcinogenic potential in mice under the conditions of the study. Because the histopathological findings (hepatocellular hypertrophy) were noted in both sexes of the lowest dose tested, a NOEL could not be established from this study.

b. Study Title: 104-Week Oncogenicity (Feeding) Study in Wistar Rats

Study No.: A23218
Report Date: March 5, 2008
Study Director: Dr. K. Broich
Study Location: RCC Ltd, Zelgliweg 1, 4052 Itingen, Switzerland

Experiment Design and Conclusion: SPF-bred Wistar rats (50/sex/group) were administered AHC-2102225 (monepantel) in the diet at concentrations of 100, 1,000 and 12,000 ppm (equivalent to 4.6, 47.4, and 578.2 mg/kg bw/day for males, and 5.6, 56.7, and 706.7 mg/kg bw/day for females) for at least 104 weeks. A control group was fed untreated diet. Clinical signs, outside cage observations, food consumption and body weights were recorded periodically. At Weeks 53, 78 and 104, blood samples were withdrawn for hematology analyses. After Week 104, all animals were sacrificed, necropsied and examined *post mortem*. Histological examination was performed on an extensive set of organs and tissues from all control and high dose animals, all gross lesions and all animals that died spontaneously or were killed *in extremis*.

Most of the non-neoplastic and all the neoplastic findings are considered incidental findings commonly noted in rats of this strain and age. The only non-statistically significant finding noticed was an increase in bile duct hyperplasia in the high dose group compared with the control group. It was concluded that monepantel did not demonstrate carcinogenic potential in rats under the conditions of the study. A NOEL of 100 ppm was determined based on the changes observed in females at the higher doses, which included statistically significant increases in liver, kidney and heart weights in females at 1,000 and 12,000 ppm, and a slight reduction in mean body weight in females at 12,000 ppm. These changes were not associated with non-neoplastic or neoplastic changes, but they are considered to be a possible treatment-related effect.

6. Determination of No Observed Effect Level (NOEL) for chronic exposure.

The lowest NOEL for monepantel in toxicity studies was 3 mg/kg bw/day. This was based on elevated liver and thyroid weights, increased alkaline phosphatase activity, decreased albumin levels, decreased albumin/globulin ratio and increased incidence and severity of brown pigmentation in the liver and kidney cells at higher doses in the one-year study in dogs. In addition, a NOEL of 3 mg/kg bw/day was established in the two-generation reproduction toxicology study in rats.

7. Acceptable Daily Intake (ADI)

With lack of antibacterial activity for monepantel, a microbiological ADI was not needed. Therefore, the toxicological ADI was set as the final ADI. Applying a safety factor of 100 to the NOEL of 3 mg/kg bw/day derived from the one-year study in dogs and two-generation reproduction study in rats, a toxicological ADI is calculated as shown below.

$$ADI = \frac{NOEL}{SafetyFactor} = \frac{3\ mg/kg\ bw/day}{100} = 0.03\ mg/kg\ bw/day = 30\mu g/kg\ bw/day$$

8. Safe Concentrations for Total Residues (edible tissues and injection sites, if applicable)

The safe concentration (SC) for total residues of monepantel in muscle is calculated from the ADI, assuming the average weight of a man to be 60 kg and the daily human intake of muscle to be 300 g, as follows:

$$SC = \frac{ADI \times HumanWeight}{FoodFactor} = \frac{30\ \mu g/kg\ bw/day \times 60\ kg}{300\ g/day} = 6\mu g/g = 6\,ppm$$

The safe concentration for total residues of monepantel in liver, kidney and fat are determined, using food factors of 100 g, 50 g and 50 g for these tissues respectively, as follows:

Liver: 18 ppm

Kidney: 36 ppm

Fat: 36 ppm

B. Residue Chemistry:

1. Total Residue and Metabolism Study

ADME and Residue Depletion Study of [^{14}C]-AHC 2101115 in Sheep (Study CRA 05/51)

This study was conducted with adherence to OECD Good Laboratory Practices (GLPs) and Compliance Monitoring, Swiss Ordinance on GLP, and OECD Principles as set forth by the United Kingdom Department of Health.

Study Director: Martin Jung, PhD, Novartis Centre de Recherche Sante Animale, Switzerland
Study Dates: November 16, 2005 to December 3, 2007
Study Facility: Novartis Centre de Recherche Sante Animale, Switzerland

Thirty-four Suffolk cross breed sheep (17 males, 17 females) were orally dosed with monepantel radiolabeled either on the 2-cyano position, on the 3-amide position, or with a 1:1 mixture of both label positions. Total radioactivity was measured by combustion and liquid scintillation counting. Metabolic profiling was performed by HPLC with UV detection. Metabolite identification was performed by analysis with LC/MS and comparison to standards. NMR was used when necessary. The edible tissue with the highest concentration of radioactivity was fat. The sulfone metabolite was the predominant compound found in tissues. Parent monepantel was a minor component and was found only at early time points in liver, kidney, and muscle. A cyclized metabolite (G32) was found in fat and muscle. Two or three minor metabolites below 10% of total radioactivity were found in liver and kidney. In all matrices except feces and urine, the metabolite profiles were the same irrespective of label position.

Table 1. Study Design for Total Residue and Metabolism Study

Sheep Number	Sacrifice Day	Specific Activity in MBq/mg	Metabolite Profiling
Study Design for sheep administered ^{14}C-AHC 210225 labeled on the 2-cyano position			
1-4	2	0.103	tissues and wool
5-8	7	0.103	fat
9-10	14	1.21	tissues, wool, excreta
19 & 12	14	0.103	fat
13-14	21	1.21	fat
15-16	21	0.103	fat
17-18	28	1.21	tissues, wool, blood
11 & 20	28	0.103	fat
21-24	35	0.103	fat
Study Design for sheep administered ^{14}C-AHC 210225 labeled on position 3			
27-28	14	0.111	none
29-30	14	1.14	tissues, wool, excreta

Sheep Number	Sacrifice Day	Specific Activity in MBq/mg	Metabolite Profiling
Study Design for sheep administered ^{14}C-AHC 210225 labeled on positions 2 and 3			
31-34	21	0.1	excreta
Control Sheep			
25-26	--	--	--

Table 2. Depletion of total radioactive residues from tissues of sheep dosed orally with 5 mg monepantel/kg

Withdrawal Day/Label Position	Residues in mg equivalents/kg (mean ±SD)				
	Fat Tissue	Pure Fat	Liver	Kidneys	Muscle
2/2	15.5±4.0	19.3±5.2	6.7±0.23	2.4±0.15	1.5±0.34
7/2	5.8±2.9	7.3±2.3	2.7±0.75	0.81±0.26	0.45±0.23
14/2	2.2±1.2	2.9±1.4	1.5±0.71	0.38±0.24	0.22±0.15
14/3	1.7±0.83	2.1±0.90	1.1±0.47	0.32±0.16	0.14±0.08
21/2	1.1±0.50	1.3±0.57	0.77±0.37	0.16±0.10	0.11±0.06
21/2+3	0.74±0.52	0.99±0.52	0.50±0.33	0.12±0.08	0.06±0.05
28/2	1.1±0.62	1.3±0.67	0.71±0.55	0.18±0.17	0.09±0.06
35/2	0.46±0.26	0.56±0.28	0.33±0.25	0.06±0.06	0.03±0.03

Table 3. Depletion of monepantel in ppm from sheep tissues

Withdrawal Day/Label	Fat	Pure Fat	Liver	Kidneys	Muscle
2/2	3.5±1.4	5.1±2.0	0.39±0.12	0.14±0.05	0.28±0.08
7/2	0.61±0.41	0.92±0.61	<0.05	<0.05	0.81 plus 3@<0.05
14/2,3	0.09±0.02 plus 3@<0.05	0.13±0.04 plus 3@<0.05	<0.05	<0.05	<0.05
21/2,2+3	<0.05	<0.05	<0.05	<0.05	<0.05

Table 4. Depletion of AHC-2144670 in ppm from sheep tissues

Withdrawal Day/Label	Fat	Pure Fat	Liver	Kidneys	Muscle
2/2	10.2±2.1	13.4±3.1	5.2±0.13	1.5±0.23	1.4±0.32
7/2	4.2±2.0	5.7±2.5	1.9±0.57	0.60±0.26	0.47±0.26
14/2,3	1.6±1.0	2.2±1.2	0.87±0.49	0.30±0.08 plus 2@<0.05	0.22±0.10 plus 2@<0.05
21/2,2+3	0.60±0.56	0.76±0.67	0.34±0.30	0.13±0.07 plus 3@<0.05	0.l5±0.06 plus 5@<0.05
28/2	1.1±0.47 plus 1@<0.05	1.5±0.73 plus 1@<0.05	0.55±0.32 plus 1@<0.05	0.18±0.10 plus 1@<0.05	0.15±0.07 plus 2@<0.05
28/2	0.60±0.02 plus 2@<0.05	0.70±0.09 plus 2@<0.05	0.26±0.04 plus 2@0.05	0.10±0.01 plus 2@<0.05	0.06±0.01 plus 2@<0.05

Table 5. Distribution of parent and sulfone metabolite in sheep tissues (as % of total radioactivity)

Withdrawal Time (days)	Fat		Liver		Kidney		Muscle	
	Parent	Sulfone	Parent	Sulfone	Parent	Sulfone	Parent	Sulfone
2	22	62	6	73	6	57	19	88
7	10	68	nd	68	nd	68	12	94
14	2	67	nd	58	nd	62	2	77
21	nd	50	nd	44	nd	64	nd	90
28	nd	59	nd	56	nd	75	nd	67
35	nd	45	nd	43	nd	76	nd	99

Distribution of G32 metabolite in sheep tissues (as % of total radioactivity)

Withdrawal Time (days)	Fat	Liver	Kidney	Muscle
2	nd	nd	nd	nd
7	6	--	--	--
14	21	nd	nd	14
21	30	--	--	--
28	27	nd	nd	9
35	35	--	--	--

--samples not analyzed

2. Comparative Metabolism Study

Study Title: Metabolism and Kinetic Study with Oral Administration in Rats (Study A89493)

This study was conducted with adherence to OECD Good Laboratory Practices (GLPs).

Study Director: M. Glassen, Ph.D.
Study Dates: February 5, 2007 to December 11, 2007
Study Facility: RCC Ltd, Itingen, Switzerland

Twenty-five rats (17 males and 8 females) were dosed by oral gavage with 10 mg ^{14}C-monepantel/kg body weight. Four male and four female rats were dosed for 7 days with monepantel radiolabeled at the cyano position. Four male and four female rats were dosed for 7 days with monepantel radiolabeled at the amide position. Urine and feces were collected during dosing. Tissues, organs, and bone were collected after dosing. Total radioactivity was measured by liquid scintillation counting. Metabolites were measured by HPLC and were identified by LC-MS/MS. Nine male rats were dosed once with monepantel labeled at the amide position to determine pharmacokinetics in blood. All major metabolites in the edible tissues of sheep were found in the rat tissues.

Comparison of metabolites found in rat and sheep tissue shown by percentages of total residues.

Tissue	Rats	Sheep
Blood	Parent (23%), AHC 2144670 (45%)	AHC 2144670 (100%)
Muscle	Parent (24%), AHC 2144670 (62%)	Parent (5.2%), AHC 2144670 (93%), Mu1 (2%)
Liver	Parent (19%), AHC 2144670 (41%), M3 (14%), M9 (6.8%), L21 (4.2%), M6 (1.1%)	Parent (1.1%), AHC 2144670 (92%), M9 (4.5%), L21 (1.4%)
Fat	Parent (44%), AHC 2144670 (54%)	Parent (14%), AHC 2144670 (77%), Fa1 (7.4%)
Kidney	Parent (20%), AHC 2144670 (48%), K3 (2%)	Parent (5.8%), AHC 2144670 (73%), K3 (22%)

GLP Study No. C02946 was conducted with rat samples from the 104-week oncogenicity study to measure metabolite G32 that was found in sheep tissue.

Concentrations of G32 metabolite (ppm) in rat fat samples.

Feeding level (ppm)	Renal Fat	Peritoneal Fat
0	<100	<100
100	148-424	246-463
1000	1030-1642	1058-1894
12000	1314-2543	1725-2241

3. Target Tissue and Marker Residue Assignment

The target tissue is fat because the highest concentration of residues is found in that tissue. The marker residue is the sulfone metabolite because that is the major compound found in the edible tissues.

4. Tolerance Assignment

Import tolerances of 7 ppm monepantel sulfone in sheep fat, 5 ppm monepantel sulfone in sheep liver, 2 ppm monepantel sulfone in sheep kidney, and 0.7 ppm monepantel sulfone in sheep muscle are assigned.

5. Withdrawal Period

A withdrawal period is not assigned in establishing import tolerances.

6. Microbial Food Safety:

Monepantel is not considered to be an antimicrobial product and therefore a microbiological safety assessment was not needed.

C. Analytical Method for Residues:

A validated HPLC method for measuring the sulfone metabolite in sheep edible tissue was provided. The method is available from CVM, FDA, 7500 Standish Place, Rockville, MD 20855.

D. Conclusions

We conclude that we have the appropriate information for us to assign an import tolerance for monepantel in sheep. We assign import tolerances of 7 ppm monepantel sulfone in sheep fat, 5 ppm monepantel sulfone in sheep liver, 2 ppm monepantel sulfone in sheep kidney, and 0.7 ppm monepantel sulfone in sheep muscle.

III. AGENCY CONCLUSIONS:

These data support the establishment of import tolerances of 7 ppm monepantel sulfone in sheep fat, 5 ppm monepantel sulfone in sheep liver, 2 ppm monepantel sulfone in sheep kidney, and 0.7 ppm monepantel sulfone in sheep muscle as provided under Sec. 512(a)(6) of the Food, Drug, and Cosmetic Act .