Date of Approval: July 11, 2018

FREEDOM OF INFORMATION SUMMARY

SUPPLEMENTAL NEW ANIMAL DRUG APPLICATION

NADA 138-952

Maxiban™ 72

narasin and nicarbazin Type A medicated article

Broiler chickens

This supplement revises the tissue residue tolerance for nicarbazin from 4 ppm to 52 ppm, and reduces the withdrawal period for Maxiban™ 72 from five days to zero days.

Sponsored by:

Elanco US Inc.

Table of Contents

I. GENERAL INFORMATION

A. File Number

NADA 138-952

B. Sponsor

Elanco US Inc.
2500 Innovation Way
Greenfield, IN 46140

Drug Labeler Code: 058198

C. Proprietary Name

Maxiban™ 72

D. Product Established Name

narasin and nicarbazin Type A medicated article

E. Pharmacological Category

Anticoccidial

F. Dosage Form

Type A medicated article

G. Amount of Active Ingredient

36 g narasin/lb and 36 g nicarbazin/lb

H. How Supplied

25 kg (55.12 lb) bags

I. Dispensing Status

OTC

J. Dosage Regimen

27 to 45 grams per ton each of narasin and nicarbazin to be fed continuously as the sole ration

K. Route of Administration

Oral

L. Species/Class

Broiler chickens

M. Indication

For the prevention of coccidiosis in broiler chickens caused by *Eimeria necatrix*, *E. tenella*, *E. acervulina*, *E. brunetti*, *E. mivati*, and *E. maxima*.

N. Effect of Supplement

This supplement revises the tissue residue tolerance for nicarbazin from 4 ppm to 52 ppm, and reduces the withdrawal period for Maxiban™ 72 from five days to zero days.

II. EFFECTIVENESS

A. Dosage Characterization

This supplemental approval does not change the previously approved dosage range. The Freedom of Information (FOI) Summary for the original approval of NADA 138-952 dated January 18, 1989, contains dosage characterization information for the use of 27 g/ton narasin and 27 g/ton nicarbazin to 45 g/ton narasin and 45 g/ton nicarbazin in broiler chickens.

B. Substantial Evidence

CVM did not require effectiveness studies for this supplemental approval. The FOI Summary for the original approval of NADA 138-952 dated January 18, 1989, contains a summary of studies that demonstrate effectiveness of the drug for the use of 27 g/ton narasin and 27 g/ton nicarbazin to 45 g/ton narasin and 45 g/ton nicarbazin in broiler chickens.

III. TARGET ANIMAL SAFETY

CVM did not require target animal safety studies for this supplemental approval. The FOI Summary for the original approval of NADA 138-952 dated January 18, 1989, contains a summary of target animal safety studies for use of 27 g/ton narasin and 27 g/ton nicarbazin to 45 g/ton narasin and 45 g/ton nicarbazin in broiler chickens.

IV. HUMAN FOOD SAFETY

This supplemental approval to NADA 138-952 for Maxiban™ 72 (narasin and nicarbazin Type A medicated article) revises the tissue residue tolerance for nicarbazin from 4 ppm to 52 ppm, and reduces the withdrawal period for Maxiban™ 72 from five days to zero days. Maxiban™ 72 is an anticoccidial that consists of narasin and nicarbazin in a fixed 1:1 ratio. Nicarbazin has two components [4,4'-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethylpyrimidine (HDP)]. The human food safety of Maxiban™ 72 was assessed from the perspectives of antimicrobial resistance (Section A), effects of residues on human intestinal flora (Section B), toxicology (Sections C, D, and E) and residue chemistry (Section F and Section G).

Based on general knowledge about the pharmacologic activity and target organisms of these two drugs, there was no need to address antimicrobial resistance risks associated with either narasin or nicarbazin. Further, an acceptable daily intake (ADI) was previously established for both narasin and nicarbazin based on traditional toxicological endpoints rather than a microbiological endpoint.

The toxicology assessment determined ADIs for narasin and nicarbazin (DNC and HDP), and safe concentrations for narasin and nicarbazin (DNC and HDP) residues in foods. The ADI (5 µg/kg body weight *per* day) and safe concentrations for total residues of narasin in edible tissues of broiler chickens (1 ppm for muscle, 3 ppm for liver, and 6 ppm for skin with fat in natural proportions) have been established in the original approval (Sections D and E). To determine the ADI and safe concentrations for nicarbazin (DNC and HDP), a series of studies were conducted to assess subchronic and chronic toxicity, reproductive and developmental toxicity, genotoxicity, carcinogenicity (Table IV.C.3), and the pharmacokinetics/bioavailability of nicarbazin and its components (DNC and HDP).

The toxicity data showed that nicarbazin caused developmental effects in the rabbit developmental toxicity study. The lowest-observed-effect-level (LOEL)/lowest-observed-adverse-effect level (LOAEL) of 30 mg/kg body weight *per* day from this rabbit study was used in the calculation of the ADI. Applying an overall safety factor of 200 to the LOEL/LOAEL, the ADI for nicarbazin (DNC and HDP) is established at 200 µg/kg body weight *per* day (see Section C, 4-5 and Section D). Based on the ADI, the safe concentrations for total residues of nicarbazin (DNC and HDP) in the edible tissues of broiler chickens are 40 ppm for muscle, 120 ppm for liver, and 240 ppm for skin with fat in natural proportions (see Section E).

For residue chemistry, CVM did not require additional studies for narasin. The FOI Summary for the original approval of NADA 118-980 dated August 14, 1986, contains a summary of studies for narasin. The sponsor conducted total radiolabeled residue studies and a comparative metabolism study for nicarbazin and a marker residue depletion study for narasin and nicarbazin. The total radiolabeled residue studies demonstrated that DNC is the marker residue, liver is the target tissue, and that a 52 ppm tolerance for DNC in chicken liver is appropriate to protect the public health. The comparative metabolism study demonstrated that nicarbazin is metabolized similarly in chickens and rats. Although the rabbit was the species used for the toxicology assessment because it is a more sensitive species than the rat, and provided a lower, more conservative ADI, the rat is considered the next most sensitive species. Therefore, the comparative metabolism study conducted in rats showed that rats were exposed to the metabolites that humans can be exposed to as residues in edible tissues from treated chickens. Data from the marker residue depletion study supported a 0-day withdrawal period for Maxiban™ 72. An analytical method is available for monitoring residues of nicarbazin in chicken liver.

A. Antimicrobial Resistance

There are no microbial food safety (antimicrobial resistance) requirements for the use of 27 g/ton narasin and 27 g/ton nicarbazin to 45 g/ton narasin and 45 g/ton nicarbazin in a 1:1 fixed ratio (Maxiban™ 72) in Type C medicated feeds for broiler chickens.

B. Effects of Residues on Human Intestinal Flora

Effects of narasin residues on human intestinal flora

CVM did not require additional information for the effects of narasin residues on human intestinal flora for this supplemental approval. The FOI Summary for a supplemental approval under NADA 118-980, containing narasin alone, dated March 2, 2012, provides a summary of all information used to assess the effects of narasin residues on human intestinal flora. The final ADI for narasin is based on toxicological endpoints.

Effects of nicarbazin residues on human intestinal flora

Determination of the need to establish a microbiological acceptable daily intake (mADI)

Considering the question: Are residues of nicarbazin, and (or) its metabolites, microbiologically active against representative human intestinal flora?

The answer is "no". Literature reports of three studies performed for nicarbazin, and its moieties 4,4'-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethylpyrimidine (HDP), demonstrated no notable antibacterial activity against >100 isolates of various bacterial groups, and the lowest MIC was >128 µg/mL.

Therefore, because nicarbazin has no noticeable antibacterial activity, there is no concern for the effects of nicarbazin residues on human intestinal flora, and subsequently no need to determine a mADI. The final ADI is based on toxicological endpoints.

Effects of both narasin and nicarbazin residues on human intestinal flora

Because nicarbazin has no noticeable activity against bacteria, CVM did not require additional information or further assessment for this supplemental use of Maxiban™ 72 (*i.e.,* a 1:1 combination of narasin and nicarbazin).

C. Toxicology

Maxiban™ 72 consists of narasin and nicarbazin in a fixed 1:1 ratio. The following sections summarize the toxicology information for narasin, nicarbazin and its components [4,4'-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethylpyrimidine (HDP)], and for studies conducted with the combination of narasin and nicarbazin.

1. Summary of Toxicology Studies (**Narasin**)

   Reassessment of the toxicological acceptable daily intake (ADI) for narasin was not needed for this supplemental approval. The FOI Summary for the original approval of NADA 118-980, approval date August 14, 1986, contains a summary of all toxicology studies.

2. Summary of Toxicology Studies (**Nicarbazin and DNC/HDP components**)

Nicarbazin is an equimolar complex of DNC and HDP. Residue chemistry studies submitted to NADA 009-476 for Nicarb® 25% (nicarbazin) demonstrated that nicarbazin dissociates in the intestine of chickens and is present in their edible tissues as DNC and HDP in a ≥ 3:1 (w/w) ratio. Therefore, the following toxicity studies on nicarbazin, DNC and HDP in a 3:1 (w/w) ratio, DNC alone, and Maxiban™ 72 (narasin and nicarbazin), together with the submitted toxicity studies in NADA 009-476 for nicarbazin and NADA 138-952 for Maxiban™ 72 were considered to assess the toxicity of residues in the edible tissues of broiler chickens treated with Maxiban™ 72.

a. Subchronic Oral Toxicity Study in Rodents (DNC)

Title: 4,4'-Dinitrocarbanilide (DNC): A 13-week Oral Gavage Toxicity Study in Rats

Study Number: 130-167

Report Date: October 29, 2009

Study Location (in-life): Mattawan, Michigan

Study Design: In this Organization for Economic Co-operation and Development (OECD) Good Laboratory Practices (GLP) study conducted according to OECD Test Guideline No. 408, DNC (purity 97.9%) was administered *via* oral gavage to CD® [Crl:CD®(SD)] rats (15/sex/dose) for 91 days at dose levels of 106, 284, and 709 mg/kg bw/day. The control animals were administered the vehicle of 0.5% methylcellulose. Clinical signs, body weights, food consumption, water consumption, ophthalmoscope examinations, hematology, functional observational battery, clinical biochemistry, urine analysis, organ weights, and histopathology were evaluated.

Results and Conclusion: No compound-related effects on mortality, clinical signs, food and water consumption, body weight, functional observations, and eye toxicity were observed. There were no clinical manifestations of toxicity or pathological changes that were attributable to the administration of DNC.

The No-Observed-Effect Level/No-Observed-Adverse-Effect Level (NOEL/NOAEL) for DNC in this study was 709 mg/kg bw/day, corresponding to the highest dose tested.

b. Subchronic Oral Toxicity Study in Rodents (Nicarbazin)

Title: Nicarbazin: A 13-Week Dietary Toxicity Study in Rats

Study Number: 130-104

Report Date: September 3, 2009

Study Location (in-life): Mattawan, Michigan

Study Design: This OECD GLP study based on the OECD Test Guideline No. 408 was to evaluate the subchronic toxicity of the test article, nicarbazin, administered to CD rats [Crl:CD®(SD)] *via* dietary admixture for 13 consecutive weeks. Three treatment groups of 15 animals *per* sex were administered nicarbazin (purity 97.9%-100.6%) at initial diet concentrations of 2.20, 6.59, and 10.99 g/kg (equal to target dose levels of 200, 600, and 1000 mg/kg bw/day, respectively). Due to decreased food consumption and body weight after the first week of test article administration, the mid- and high-dose levels were adjusted to the target dose levels of 400 mg/kg bw/day and 600 mg/kg bw/day, respectively. The calculated mean test article intake over the 13-week treatment period was 181.1, 383.7, and 598.6 mg/kg bw/day for males and 189.0, 400.3, and 618.9 mg/kg bw/day for females at 200, 600/400, and 1000/600 mg/kg bw/day, respectively. One additional group of 15 animals *per* sex served as the control and received basal diet. Clinical signs, body weights, food consumption, ophthalmoscope examinations, hematology, functional observational battery, clinical biochemistry, hematology, organ weights, and histopathology were evaluated. Kidneys from all animals and selected tissues from control and high-dose males were microscopically examined.

Results and Conclusions: There was no test article-related effect on mortality. No clear test article-related effects on clinical signs or ophthalmology were observed during the study. Both body weight gain and food consumption were decreased in a dose-proportional manner in all treated groups. The decrease in body weight was considered to be directly related to the decrease in food consumption. The maximum tolerated dose (MTD) was exceeded based on 12.2-25.1% (females) and 24.2-51.6% (males) decreases in body weights across all treatment groups.

There were dose-dependent decreases in erythrocyte mass (erythrocytes, hemoglobin, and hematocrit) in both sexes of all treated groups, and were considered biologically and toxicologically significant at the 600/400 and 1000/600 mg/kg bw/day dose levels. Reticulocyte responses were inconsistent, particularly at the 600/400 and 1000/600 mg/kg bw/day dose levels, indicating erythroid suppression that was either directly or indirectly (decreased food consumption) test article-related. The mild increases in platelets in both sexes were considered test article-related. The sporadic increases in neutrophils in both sexes were likely to be test article-related due to all treated groups showing some degree of change. There were decreases in APTT (activated partial thromboplastin time) in both sexes at the 600/400 and 1000/600 mg/kg bw/day dose levels that were test article-related. No biologically or toxicologically meaningful changes among prothrombin times were observed in either sex.

Both males and females of all treated groups showed dose-dependent increases in urea nitrogen, creatinine and phosphorus. All protein parameters (total protein, albumin, and globulin) were generally decreased in a dose-dependent manner in both sexes of all treated groups. There were increases in triglycerides in both sexes at the 600/400 and 1000/600 mg/kg bw/day dose levels and in cholesterol in both sexes at all dose levels, generally in dose-dependent patterns. In addition, in both sexes at the 600/400 and 1000/600 mg/kg bw/day dose levels, chloride was dose-dependently decreased.

Weight changes in multiple organs including kidneys, spleen, brain, adrenal glands, epididymides, heart, liver, ovaries, pituitary gland, testes, thymus, thyroid/parathyroid glands, and uterus with cervix were associated with low body weights, decreased food consumption, and/or overall debilitation as the MTD was exceeded in this study. However, direct test article effects on these organs could not be excluded. Test article-related macroscopic and microscopic findings were present in the kidneys, testes, and epididymides. Potential test article-related macroscopic findings also were present in the renal lymph node, urinary bladder, prostate gland, seminal vesicles, body as a whole, abdominal cavity, thoracic cavity, spleen, stomach (glandular and non-glandular), thymus, skin, and uterus with cervix; however, these tissues were not examined microscopically and the toxicological significance of these findings was not clear. The kidneys in males and females at all doses were predominantly characterized by the presence of numerous crystals associated with mild to severe tubular degeneration/regeneration, minimal to severe fibrosis, and mild to severe chronic inflammation. Greenish-brown crystals were arranged in sheaves and rosettes in the renal tubules, occasional glomeruli, renal pelvis, and the interstitium of the kidney. Acute inflammation was present in the kidneys of one male at 1000/600 mg/kg bw/day that died on Day 11. The slight increases in incidence and severity of renal tubular mineralization in males and females in all treated groups were considered a potential (direct or indirect) test article effect. The following additional microscopic changes in the kidneys present at a low overall incidence were considered to be potentially test article-related: minimal to moderate hemorrhage in males at 600/400 and 1000/600 mg/kg bw/day, hydronephrosis in males and females at 600/400 and males at 1000/600 mg/kg bw/day, and thrombosis in males and females at 1000/600 mg/kg bw/day. Males at 1000/600 mg/kg bw/day had minimal to severe degeneration/atrophy of the seminiferous tubules in the testes, and mild to severe spermatic granulomas and minimal to severe oligospermia and germ cell debris in the epididymides.

A NOEL/NOAEL could not be established in this study based on multiple effects described above at all dose levels and that the MTD was clearly exceeded. The lowest-observed-effect level (LOEL)/lowest-observed-adverse-effect level (LOAEL) for this study was 200 mg/kg bw/day of nicarbazin, the lowest dose tested.

c. Chronic Oral Toxicity Study in Non-Rodents (DNC and HDP)

Title: Two-Year Chronic Toxicity Studies with Components of Nicarbazin in Dogs

Study Number: 87892

Report Date: June 15, 1969

Study Location: Maspeth, NY

Study Design: Nicarbazin components, DNC and HDP (purity not provided), were administered at a ratio of 3:1 by diet for six days *per* week for 104 weeks to groups of 5 male and 5 female beagle dogs at 0 mg/kg bw/day (control), 60 mg DNC/kg bw/day and 20 mg HDP/kg bw/day (low), 180 mg DNC/kg bw/day and 60 mg HDP/kg bw/day (mid), and 600 mg DNC/kg bw/day and 200 mg HDP/kg bw/day (high). This study was conducted prior to GLP regulations and OECD test guidelines. Daily observations for behavior, physical appearance and survival were recorded. Body weight, food consumption, and reflexes were evaluated weekly. Water intake and urinary output were measured monthly. Clinical determinations including hematology, clinical chemistry, urinalysis, and functional tests were performed pretest and every six months and on four dogs/sex/group at three months. Organ weights and histopathology were performed at necropsy. Two animals were sacrificed at 56 weeks.

Results and Conclusions: A green to yellow hue was found in the urine and feces from all treated dogs. One male dog in the mid-dose group died during week 44 of the study. Elevated serum glutamic pyruvic transaminase (SGPT) (6/10) was observed in the high-dose group during the 12th month of the study. Collection of additional samples during the second year of the study for SGPT determined the elevation to be transitory in females, but still higher in males at 23 months (41 ±27 units compared to control values of 28 ± 6 units). Liver function tests (serum alkaline phosphatase, bromsulfalein retention time, and total protein) showed no abnormal results. Histopathology revealed that one male dog had slight bile duct proliferation at 56 weeks. Several animals including the controls showed cysts of the pituitary. No other treatment-related changes were observed.

The NOEL/NOAEL for this study was the mid-dose level (180 mg DNC/kg bw/day and 60 mg HDP/kg bw/day) due to the change in SGPT and bile duct proliferation noted in the high-dose group (600 mg DNC/kg bw/day and 200 mg HDP/kg bw/day). Because the animals were treated for 6 days *per* week, the NOEL/NOAELs were adjusted based on 7 days *per* week to 154 mg DNC/kg bw/day and 51 mg HDP/kg bw/day or 205 mg nicarbazin/kg bw/day.

d. Oral Developmental Toxicity Study in Rodents (Nicarbazin)

Title: Teratogenicity Test of Nicarbazin with Rats by Oral Administration

Report Number: Not available

Report Date: 1977

Study Location: not specified

Study Design: This oral (gavage) prenatal developmental toxicity study was designed to determine the effects of nicarbazin on pregnant CD/CRJ rats and on the developing conceptuses. This study was conducted prior to GLP regulations and OECD test guidelines. Only a summary report was available. Four groups of pregnant rats (24-25 animals/group) were administered orally by gavage nicarbazin (purity not provided) at 70, 200 and 600 mg/kg bw/day from gestation days (GDs) 7-17. Control animals were administered an unidentified vehicle.

Results and Conclusions: Seven dams in the 600 mg/kg bw/day dose group died during the study. Mean food consumption was reduced and water consumption was increased in the 600 mg/kg bw/day dose group throughout the entire dosing period. Some increases in water consumption occurred in the 200 mg/kg bw/day dose group from the early to mid-gestation period but returned to normal levels towards the end of the gestation period. The mean body weight was drastically reduced at 600 mg/kg bw/day from GDs 8-14 followed by a recovery, but it was still significantly lower than that of the control group.

The rate of implantation in the 600 mg/kg bw/day dose group (94.2%) was greater than the control group (83.3%). However, the mean number of dead fetuses (early, intermediate and late) was increased by 78%, 33%, and 11% in the 600, 200 and 70 mg/kg bw/day dose group, respectively, when compared to the control group. The rate of embryo-fetal mortality at birth was very high at the high-dose level, and the mean number of live fetuses *per* litter was reduced in a dose-dependent manner (14.5, 14.3, 14.2 and 8.5 for the control, low-, mid- and high-dose groups, respectively).

The mean body weight of live fetuses was significantly decreased at 200 mg/kg bw/day and 600 mg/kg bw/day. The major fetal abnormalities that were test article related included: (1) at 600 mg/kg bw/day, decreased ossifications of corpus vertebrae, delayed ossification (cervical vertebrae, caudales, sternum, pharynx prima, os metatarsal), sacralization of the 6th or 7th lumbar vertebrae, cleft palate, hydronephrosis, subcutaneous edema, remained Meckel's diverticulum, and cryptorchismus; and (2) at 200 mg/kg bw/day, decreased ossifications of corpus vertebrae and corpus costae (left).

Based on the effects reported at 600 and 200 mg/kg bw/day in both dams and fetuses, 70 mg/kg bw/day was established as the maternal and embryo-fetal developmental toxicity NOEL/NOAEL for nicarbazin.

e. Oral Developmental Toxicity Study in Non-Rodents (Nicarbazin)

Title: Nicarbazin Developmental Toxicity Study in Rabbits

Study Number: 493768

Report Number: 24696

Report Date: April 17, 2006

Study Location: Edinburgh, United Kingdom

Study Design: This OECD GLP oral prenatal developmental toxicity study was conducted according to the OECD Test Guideline No. 414. It was designed to: (1) evaluate the potential of nicarbazin to cause embryo-fetal developmental toxicity following maternal exposure from GDs 6-28, (2) characterize maternal toxicity at the dose levels tested, and (3) determine a NOEL/NOAEL for maternal toxicity and embryo-fetal developmental toxicity. The test article, nicarbazin (purity 96.6%), was prepared in a vehicle suspension [0.5% (w/v) carboxymethyl cellulose] and administered orally by daily gavage to mated New Zealand White rabbits (24/treatment group) at 30, 60 and 120 mg/kg bw/day from GDs 6-28. The control group of 25-mated female rabbits received the vehicle.

Clinical observations and viability checks were carried out twice daily while excreta trays were checked at the start and end of each day. Food consumption and body weights were recorded daily throughout GDs 6-29. On GD 29, all dams were subjected to gross necropsy, and representative samples of abdominal tissues were excised and fixed. The reproductive tract was dissected out and weighed, uterus was opened and its content was examined. The gravid uterine weights were recorded, and uteri and ovaries were excised and examined; the numbers of fetuses, early and late resorptions, total implantations sites, and the number of corpora lutea were recorded. The fetuses were removed, weighed, sexed, chemically fixed and later examined for internal and external abnormalities, skeletal malformations and extent of ossification.

Results and Conclusions: No treatment-related clinical observations were reported. The test article did not significantly affect body weight, food consumption, and pregnancy performance parameters. Oral prenatal administration of nicarbazin at 120 mg/kg bw/day caused prominent lobulation of the liver in 8% (2/24) of the animals. One of these animals had pale liver lobes and dark foci of the lungs.

The major biologically and toxicologically significant fetal effects observed in all treated groups included increased incidences of incomplete ossification of the skeletal system, mainly for bones originated from the neural crest (odontoid and jugal connected/fused to zygomatic process of maxilla) and lateral plates of the mesoderm (epiphyses of fore and hind limbs, and metacarpal and/or phalanx on pollex), and visceral/soft tissue abnormalities, such as those observed on eyes central corneal opacity, common carotid arteries and right subclavian artery retro-esophageal.

Based on the increased incidence of prominent lobulation of the liver at 120 mg/kg bw/day, the maternal NOEL/NOAEL was established at 60 mg/kg bw/day. A NOEL/NOAEL for embryo-fetal developmental toxicity could not be established for nicarbazin because of increased incidences of incomplete ossification of the skeletal system and alterations of visceral/soft tissue at all doses tested. The lowest-observed-effect level (LOEL)/lowest-observed-adverse-effect level (LOAEL) for embryo-fetal developmental toxicity was established at 30 mg/kg bw/day (the lowest dose tested) based on multiple fetal effects.

f. Multi-Generation Oral Reproductive Toxicity and Lactation Study in Rats (DNC and HDP)

Title: Multigeneration Study and Lactation Studies with 4,4'-Dinitrocarbanilide (DNC) and 2-Hydroxy-4,6-Dimethylpyrimidine Dihydrate (HDP)

Study Number: 88943

Report Date: February 27, 1970

Study Location: Maspeth, NY

Study Design: This study was conducted prior to GLP regulations and OECD test guidelines. Three generations of rats, each allowed for two breeding cycles, were included in this multi-generation reproductive toxicology study. Albino rats (48/sex) of the first generation (F_0), 28 days of age, were assigned to the control and three treatment groups (12/sex/dose). The treatment consisted of dietary admixtures of DNC:HDP at 3:1 ratio (on a weight basis) purity not provided, at the nominal doses of 50 and 17 mg/kg bw (low-dose), 150 and 50 mg/kg bw (mid-dose), and 300 and 100 mg/kg bw (high-dose) of DNC and HDP, respectively. The control group received the basal diet alone. The F_0 rats received their respective treatment diet for 10 weeks before allowed for individually paired male and female cohabitation for 14 days, and the females were allowed to carry the pregnancy to term. The offspring litters were designated as F_0L_1. Each F_0L_1 litters were culled to eight pups and nursed to weaning on postnatal day (PND) 21 before being sacrificed for gross examination. Seven days after the weaning of F_0F_1 litters, the F_0 females were re-mated individually with F_0 males in cohabitation for 14 days, and pregnant females were allowed to carry the pregnancy to term and give birth to the second litters of the F_0 rats (F_0L_2).

Forty-eight (48) males and 48 females of the F_0L_2 rats were designated as the F_1 generation. At weaning on PND 21, the rats were placed on the control diet for seven days before assignment to the four groups (control, low-, mid-, and high-dose groups), 12/sex/dose. The F_0 animals were then sacrificed for gross examination.

Starting on PND 28, the four groups of F_0L_2 rats were placed on their respective diets for 10 weeks before the female and male rats within each group were individually mated in cohabitation for 14 days. The males were then removed from the cage, and the females were allowed to carry out the pregnancy and to give birth to the litters of F_1L_1.

Seven days after weaning of F_1L_1 litters, the F_1 females were mated again with the F_1 males in the same manner as in the first mating, and the F_1 females were allowed to give birth to the litters of F_1L_2.

On PND 21, 48 male and 48 female pups from F_1L_2 were designated as the F_2 generation. The F_2 males and females were subjected to the same experimental procedures as the F_1 males and females were, and produced the F_2L_1 and F_2L_2 litters.

At weaning of F_2L_2 pups, the F_2 parental rats and the F_2L_2 pups were sacrificed and gross examination performed. The testes from F_2 parental males of the control and high-dose groups were collected, processed, and examined microscopically. Tissues from the liver, kidneys, heart, bladder, and gonads from five males and five females of the F_2L_2 animals also were examined microscopically.

Cage-side observations were carried out daily for all animals during the study. All the parental rats in each generation were weighed during premating treatment at weekly intervals and at mating, separation, parturition, and weaning. Food consumption data were collected on a weekly basis. The offspring rats were weighed at birth and on PNDs 4, 12, and 21. For each breeding, the parameters of reproductive performance, including measures of fertility, gestation, pup viability, and lactation, were recorded.

Results and Conclusions: For the F_0 generation, during the 10-week pre-mating treatment period, weekly-measurement of body weight of individual rats indicated that there were no changes in the average body weight of any of the three treatment groups comparing to the control. The 10-week accumulative food intakes of the treatment groups were not different from those of the control group. For reproductive performance, there were no consistent differences among the three treatment groups and the control group in the measurements of fertility (percent of pairings resulting in pregnancy), length of gestation, number of live litters, litter size, live pup survival to 4 days, live pup survival to weaning (PND 21), and pup growth during lactation. The litter means of pup weights were not different among the four groups from birth to weaning. These observations were similar between the two litters of the F_0 rats. The actual DNC intakes, estimated based on the target DNC concentration in the diets and the weekly-measured food intake, were 0, 47, 140, and 284 mg/kg bw/day for the males in the control and low-, mid-, and high-dose groups, respectively, during the 10-week pre-mating treatment; the corresponding doses for the females were 0, 51, 159, 325 mg/kg bw/day.

For the F_1 generation, no differences were found in body weight increases and food consumptions between the four groups during the 10-week pre-mating dosing period. For the offspring of the F_1 rats, there were no differences among the groups in the measurement of fertility, the length of gestation, pup survival and growth during lactation. It was noted; however, higher rates of stillbirth were associated with the treatment in the high-dose group of F_1L_1 litters and in the low dose group of the F_1L_2 litters. The actual DNC intakes for the control and low-, mid-, and high-dose groups were estimated to be 0, 49, 152, and 302 mg/kg bw/day, respectively for males, and 0, 55, 157, 327 mg/kg bw/day, respectively for females, during the 10-week pre-mating treatment.

For the F_2 generation, no treatment-dependent differences were found in body weight and food consumption among the four groups during the 10-week pre-mating dosing period. There were no differences among the groups in length of gestation, birth weight of pups, or pup growth during lactation. However, compared to the control group, the numbers of total live births per group were markedly lower for F_2L_1 at the high-dose, and the numbers of dead births per group were markedly higher for both F_2L_1 and F_2L_2 at the high-dose. Furthermore, the mating efficiency for the treated groups was significantly lower when compared to the control group: the numbers of mating needed to yield 10-12 pregnancies (expressed as number of pregnancies/number of mating) were 12/12 (100%), 11/14 (79%), 12/15 (80%), 10/14 (71%) for the control and low, mid, and high dose groups, respectively, of the first breeding (F_2L_1), and 12/12 (100%), 10/15 (67%), 12/15 (80%), and 10/13 (77%) for the control and low-, mid-, and high-dose groups, respectively, of the second breeding (F_2L_2).

The gross examinations at necropsy for the parental and offspring rats in each of the three generations and the microscopic evaluations of the F_2 parental male testes and selected organ tissues from the F_2L_2 offspring resulted in no consistent test-article related findings.

A NOEL/NOAEL could not be established for this study due to the effects observed at the lowest dose level tested in this study (15 mg/kg bw/day of DNC and 50 mg/kg bw/day of HDP). The 150:50 mg/kg bw/day DNC:HDP dose was considered the LOEL/LOAEL.

g. Genetic Toxicity studies (Nicarbazin)

The findings from the genotoxicity testing for nicarbazin are presented in Table IV.C.1 and described in detail below. Results from the genotoxicity tests indicated that nicarbazin is not a genotoxic compound.

Table IV.C.1. Summary of Nicarbazin Genotoxicity Studies.

Study Type	Report Number	Result
Bacterial Reverse Mutation Assay (Ames Test)	23922	Equivocal (a slight increase in mutation rate in *S.* Typhimurium TA98 strain; negative in all other strains)
In Vitro Mammalian Cell Gene Mutation Test	24032	Negative
Mammalian Erythrocyte Micronucleus Test	20677	Negative
Unscheduled DNA Synthesis Assay in Rat Liver	060902	Negative

(1) Bacterial Reverse Mutation Assay (Ames Test)

Title: Nicarbazin: Testing for Mutagenic Activity with *Salmonella* Typhimurium TA1535, TA1537, TA98 and TA100 and *Escherichia coli* WP2*uvr*A

Study Number: 23922

Report Date: July 2, 2004

Study Location: Tranent, United Kingdom

Study Design: The study was OECD GLP-compliant and conducted according to the OECD Test Guideline No. 471. Nicarbazin (purity 98.0%) was tested for mutagenic activity in *Salmonella* Typhimurium strains (TA1535, TA1537, TA98 and TA100) and *E. coli* WP2*uvr*A at concentrations ranging from 3 to 1000 µg *per* plate. Concurrent negative and positive controls were included in the experiments. The solvent (DMSO) was used as the negative control, and the positive controls were 2-aminoanthracene (2AAN), sodium azide (NaN_3), 2-nitrofluorene (2-NF), and N-ethyl-N-nitro-N-nitrosoguanidine (ENNG). The S9 fraction of the liver tissue homogenate from Aroclor 1254-treated male rats was included in the metabolic activation system. Two independent tests, using the direct plate method and pre-incubation method, were conducted on agar plates with and without metabolic activation. The mutation test was conducted at the nicarbazin doses of 3, 10, 33, 100, 333, and 1000 µg/plate; the toxicity test was conducted in the TA100 strain at the same dose range of nicarbazin.

Results and Conclusions: No overt toxicity to the bacteria cells was observed within the tested dose range, in either the presence or absence of metabolic activation. In the first mutation test, *S.* Typhimurium strain TA98 showed increases of 2.4 and 1.9-fold in revertant colony count over the vehicle control following exposure to nicarbazin at 1000 and 333 µg/plate, respectively, with metabolic activation, and 2 and 1.6-fold increases at the same concentrations without metabolic activation. The corresponding positive controls resulted in a 24-fold increase in revertant count in the assay with metabolic activation, and a 40-fold increase without metabolic activation. There was no increased mutation rate in TA98 at lower nicarbazin concentrations, and none of the other test strains were affected by nicarbazin exposure at any of the concentrations. Similar results were seen in the second mutation test. With metabolic activation, there was a 2.8-fold increase in the number of revertant colonies of the TA98 strain over the vehicle control at 1000 µg/plate. Without metabolic activation, the increases of TA98 revertant colonies over the vehicle were 2.5-fold increase at 100 µg/plate, 2.6-fold at 333 µg/plate, and 5-fold at 1000 µg/plate. No other strains were affected by the treatment. The result of the nicarbazin bacterial reverse mutation assay was considered equivocal due to the weak mutagenic activity at exposure to high concentrations in *S.* Typhimurium strain TA98.

(2) *In Vitro* Mammalian Cell Gene Mutation Test

Title: Nicarbazin: Mouse Lymphoma Cell Mutation Assay

Study Number: 24032

Report Date: June 15, 2004

Study Location: Tranent, United Kingdom

Study Design: The study was OECD GLP-compliant and conducted according to the OECD Test Guideline No. 476. Nicarbazin (purity 98.0%) was tested to determine its potential to induce mutations at the $TK^{+/-}$ locus of mouse lymphoma L5178Y cells. Cytotoxicity test was conducted in the absence and presence of the S9 mix at the nicarbazin treatment concentrations ranging from 0.4 to 100 µg/mL. Two independent mutation tests with the L5178Y cells were performed; each was conducted with and without metabolic activations. In the first test and in the absence of S9 mix, and in both tests in the presence of S9 mix, the cells were exposed for 4 hours (h); in the second test in the absence of S9 mix, the cells were exposed for 24 h. The first test was conducted at the nicarbazin concentrations of 12.5, 25, 50 and 100 µg/mL, with and without S9 mix. The second test was conducted at the nicarbazin concentrations ranging from 0.0625 to 100 µg/mL without the S9 mix, and ranging from 1 to 100 µg/plate with the S9 mix. The vehicle DMSO was used as negative control; ethyl methanesulphonate (EMS) and methyl methanesulphonate (MMS) were used as positive controls in the absence of S9, and 3-methylcholanthrene (3-MC) was used as the positive control in the presence of S9. Relative suspension

growth (RSG), cloning efficiency, mutant selection, and plate readings were determined.

Results and Conclusions: The cytotoxicity test showed that nicarbazin was moderately toxic to the mouse lymphoma cells. In the absence of the S9 mix with a 4-h exposure, RSG was reduced to 63.3% at 50 µg/mL and to 68.5% at 100 µg/mL. In the presence of S9 with a 4 h exposure, the relative RSG values were 37.9% at 50 µg/mL and 32.6% at 100 µg/mL. Nicarbazin was highly toxic to the cells with a 24-h exposure, causing the reduction of RSG to 33.9% at the concentration of 0.4 µg/mL. For the mutagenic tests, RSG values indicated a dose-dependent toxicity associated with nicarbazin treatment. In the absence or presence of the S9 mix with a 4-h exposure, or in the absence of the S9 mix with a 24-h exposure, mutant fraction (MF) values for all the nicarbazin treatment concentrations were similar to those of the vehicle control. In contrast, MF values for 3-MC, EMS and MMS positive controls were significantly higher than those of the vehicle control. These results indicated that mouse lymphoma cells exposed to nicarbazin exhibited mutation rates similar to those of the vehicle control, and nicarbazin was therefore not mutagenic in mouse lymphoma L5178Y cells under the test conditions of this study.

(3) Mammalian Erythrocyte Micronucleus Test

Title: Nicarbazin: Micronucleus Test in Bone Marrow of CD-1 Mice

Study Number: 20677

Report Date: November 23, 2001

Study Location: Tranent, United Kingdom

Study Design: This study was OECD GLP-compliant and conducted according to the OECD Test Guideline No. 474. Nicarbazin (purity 98.5%) was dissolved in the vehicle of 0.5% carboxymethyl cellulose at appropriate concentrations to yield a dosing volume of 10 mL/kg bw, given through gavage to young adult CD-1 mice. In a preliminary toxicity test, mice (3/sex) were given nicarbazin *via* two gavage administrations, at a 24-h interval, at a limit dose of 2000 mg/kg bw. No clinical signs of toxicity were observed post-dosing. In the micronucleus assay, the nicarbazin treatment group (5/sex) received two administrations of 2000 mg/kg bw at a 24-h interval. Animals in the vehicle control group (5/sex) and positive control group (5 males) were dosed at the same time interval. Cyclophosphamide (CPH) at 50 mg/kg bw was used as the positive control. At 24 h following the second dosing, the mice were sacrificed and bone marrow samples were collected from the femurs. Harvested bone marrow cells were processed and smeared on glass slides for staining and scoring. Two slides from each animal were evaluated. Polychromatic erythrocytes (PCE) were scored for micronuclei (2000 *per* animal).

Results and Conclusions: Nicarbazin exposure at 2000 mg/kg bw resulted in micronucleus frequencies in the erythrocytes samples from bone marrow of treated mice similar to those of the vehicle control group. The number of micronucleated PCE in bone marrow erythrocyte was 0.08% and 0.06% in the vehicle control and the nicarbazin treatment groups, respectively; the corresponding value for the positive control was 1.25%. The mean PCE/NCE ratios for the negative control, positive control, and nicarbazin treated samples were 0.98, 0.74, and 0.88, respectively, indicating slight systemic toxicity associated with nicarbazin treatment. Under the study conditions, nicarbazin treatment did not increase micronuclei in bone marrow erythrocytes at the dose of 2000 mg/kg bw in male and female mice.

(4) Other genotoxicity studies

Title: *In vivo/in vitro* Unscheduled DNA Synthesis (UDS) Assay in Rat Hepatocytes with Nicarbazin

Study Number: FSR-IPL 060902

Report Date: April 24, 2007

Study Location: Lille, France

Study Design: This study was OECD GLP-compliant and conducted in accordance with the OECD Test Guideline No. 486. A preliminary toxicity test conducted with Fischer male rats (4 animals/dose) at nicarbazin doses of 800 and 2000 mg/kg bw (purity 98.1%) showed no mortality or adverse clinical signs within the 48-h period of post-dosing observation. For the UDS assay, a single nicarbazin gavage administration (10 mL/kg bw) was given to male rats in 0.5% carboxymethyl cellulose vehicle. The nicarbazin treatment included two dose levels: 1000 and 2000 mg/kg bw, with three male rats in each dose group and for each expression time point. The treated animals were allowed for two expression times: 2 to 4 h and 12 to 16 h after dosing. The positive controls were 10 mg/kg bw dimethylhydrazine (for 2 to 4 h expression time) and 25 mg/kg bw 2-acetamidofluorene (for 12 to 16 h expression time).

Animals were sacrificed 2 to 4 h and 12 to 16 h post-dosing. To determine the incorporation of [^{3}H]-thymidine in the DNA of hepatocytes that were not in the S-phase of DNA synthesis, primary hepatocytes were isolated from the liver tissue using the two-step collagenase perfusion method, and the cultured cells were exposed to [^{3}H]-thymidine, which were processed to produce autoradiography for grain counting. S-phase cells were identified microscopically through stained slides.

Results and Conclusions: For both expression times, there was no difference between nicarbazin-treated groups and the vehicle control group in net nuclear grain count (NNG). In addition, measurements for NNG of cells in repair, percent cells in repair, and percent cells in S-phase were similar among the control and two nicarbazin treatment groups, whereas positive controls groups for both expression times showed significantly elevated values for those parameters. Nicarbazin treatment in rats did not cause increases in unscheduled DNA synthesis in rat liver cells under the experimental conditions of this study.

h. Oral Carcinogenicity Study in Rats (DNC and HDP)

Title: Chronic Toxicity Studies with Nicarbazin Formulation in Rats

Study Number: 87891

Report Date: August 22, 1969

Study Location: Maspeth, New York

Study Design: This study was conducted prior to GLP regulations and OECD test guidelines. FDRL rats (50 animals/sex for the control and high-dose groups, 40 animals/sex for the low and mid-dose groups) received the test item, DNC and HDP at 3:1 (w:w) ratio (purity not provided), at dietary concentrations to provide approximately 0, 50, 150, or 300 mg/kg bw/day of DNC and 0, 17, 50, or 100 mg/kg bw/day of HDP for two years. The dose preparation, homogeneity, stability or concentration verification were not provided. The rats were observed daily for behavior, physical appearance, and survival. Clinical signs, food consumption, water consumption, urinary output, and body weights were recorded periodically. At 3, 6, 9, 12, 18 weeks and 24 months, blood samples were withdrawn from 10 rats/sex/group and limited clinical chemistry and hematology parameters were analyzed. At 6 and 18 months, 5 rats/sex from the control and high-dose group were sacrificed and necropsied. Histopathology was performed only on kidneys. At 56 weeks, 10 rats/sex/group were sacrificed, necropsied, and examined for histopathology. All surviving animals were sacrificed and necropsied after 24 months of treatment. Histopathological examination was performed on selected organs and tissues.

Results and Conclusions: No significant differences were noted in behavior, survival rate, body weight, food consumption, water consumption, urinary output, clinical chemistry, hematology, and histopathology examination. Under the conditions of the study, no toxicological or carcinogenic effects were observed. The NOEL/NOAEL of this study was 300 mg/kg bw/day of DNC and 100 mg/kg bw/day of HDP, the highest dose tested. Because the minimal toxic effect was not demonstrated at any of the dose levels tested in the study, the carcinogenic potential of nicarbazin in rats could not be adequately assessed.

i. Other pivotal studies

Title: Relative Bioavailability of DNC in Rats Administered Alone, Mixed with HDP and as Nicarbazin

Study Number: 130-136

Report Date: August 19, 2009

Study Location (in-life): Mattawan, Michigan

Study Design: This study was to estimate the relative bioavailability of DNC when given either in the form of nicarbazin, a mixture with HDP, or alone to male CD® rats [Crl:CD®(SD)] *via* oral gavage. Three groups of animals (5 animals *per* group) were given a single dose of the each of the three test articles in 0.5% methylcellulose. DNC was administered at dose levels of 150, 450, and 900 mg/kg bw, nicarbazin was given at 50, 150, and 450 mg/kg bw, and the DNC with HDP was given at 50, 150, and 450 mg/kg bw. The purity of the test articles was not provided. Animals were fasted overnight and food was withheld for the first 4 h of blood sample collection. Blood was collected at 0.5, 1, 2, 4, 8, 12, 24, 36, 48, and 72 h post-dose, and at each time point 0.3 mL of 0.9% sodium chloride for injection, USP, was administered to replace the volume of blood collected. Samples were centrifuged and plasma was analyzed for DNC concentration. The lower limit of quantification (LLOQ) was 100 ng/mL. Non-compartmental analysis was used to estimate pharmacokinetic parameter values using WinNonlin Enterprise (Version 5.0.1) software. Relative bioavailability (%F) was calculated relative to the nicarbazin.

Results and Conclusions: No treatment related clinical observations were noted. Following the administration of nicarbazin, DNC was detected in plasma in all animals at 0.5 h post-dose. The median T_{max} was achieved within 2 to 4 h followed by a decrease in DNC concentration over time; however, DNC remained above the LLOQ at 72 h post-dose in most animals. Following the administration of DNC, DNC was detected in plasma in all animals at 0.5 h post-dose. Median T_{max} was achieved within 2 h and then a decrease in DNC plasma concentration; however, DNC remained above the LLOQ at 72 h post-dose in most animals. Following the administration of DNC and HDP mixture, DNC was detected in plasma in all animals at 0.5 h post-dose. Median T_{max} was achieved at 24, 2, and 8 h post-dose for 50, 150, and 450 mg/kg, respectively. The plasma concentration then decreased; however, DNC remained above the LLOQ at 72 h post-dose in most animals. The mean terminal $T_{½}$ was 5.98 to 13.8 h for nicarbazin, 8.71 to 26.4 h for DNC, and 6.49 to 17.3 h for DNC and HDP. Nicarbazin formulation resulted in much higher systemic exposure to DNC compared to DNC alone or DNC mixed with HDP. Systemic exposure to DNC was lower than dose proportional in all three formulations. The bioavailability was lower than 5% when dosed as DNC alone or mixed with HDP, relative to nicarbazin.

3. Summary of Toxicology Studies (Maxiban™ 72)

   a. Subchronic Oral Toxicity Study in Rodents

   Title: A Three-Month Subchronic Toxicity Evaluation of EL-9193 (Narasin + Nicarbazin) Administered Orally to Fischer 344 Rats

   Study Number: R13183

   Report Date: October 1984

   Study Location: Greenfield, Indiana

   Study Design: In this GLP study, Fischer 344 rats (20 animals/sex/group) were administered narasin and nicarbazin orally at equal ratios, at 0, 0.00075, 0.002, and 0.006% of each component in the diet for 90 to 93 days (narasin purity 93%, activity 10.6%; nicarbazin purity 98.8%, activity 98.8%). The animals were examined daily for general observations. Weekly detailed observations, body weight measurements and food consumption determinations were performed. Ophthalmic examinations were conducted at the beginning and end of the study treatment period. Hematology, clinical chemistry, urinalysis, necropsy, organ weights, pathology, and histology were evaluated on all animals at the end of the study.

   Results and Conclusions: Mean body weight and body weight gain were decreased in males for the 0.006% dose group compared to the control during the first 21 days of the study then returned to normal. Mean body weight and body weight gain were decreased in females for the high-dose group (0.006%) compared to the control during the first 42 days of the study but then returned to normal. Decreased average daily food consumption was noted in high-dose males for the first 35 days of treatment but then returned to normal. Average daily food consumption for high-dose females was reduced compared to controls throughout the study. No other compound related effects were observed.

   A NOEL/NOAEL of 0.002% narasin and 0.002% nicarbazin (equivalent to a time-weighted average daily dose for each component of 1.36 mg/kg bw/day for males and 1.57 mg/kg bw/day for females) was established for this study based on the changes in body weight and body weight gain during the beginning of the study.

   b. Oral Developmental Toxicity Study in Rodents

   Title: Teratology study on a combination on narasin and nicarbazin in Wistar rats

   Study Number: R12383

   Report Date: March 30, 1984

   Study Location: Greenfield, Indiana

Study Design: This GLP teratogenic rodent toxicity study was designed to evaluate the potential of narasin/nicarbazin in a 1:1 (based on activity) combination to produce maternal and fetal developmental toxicity. The test article was a combination of narasin dried fermentation product and nicarbazin administered in equal proportion, based on activity of narasin/nicarbazin [narasin: 10.6% activity, and nicarbazin: 98.8% activity]. The test article was prepared in a vehicle suspension [10% aqueous acacia solution] and was administered by daily oral gavage to 25 mated female Wistar rats *per* treatment group at 0.25/0.25, 0.75/0.75 and 2.25/2.25 mg/kg bw/day of narasin/nicarbazin activity from GDs 6 to 15. Animals were sacrificed on GD 20. A control group of 25 mated female rats received vehicle only.

Animals were observed once daily for overt signs of toxicity and survival. Body weights and food consumption were recorded on GDs 0, 6, 11, 16, and 20. Total weight gain (GD 20 body weight minus GD 0 body weight) and net weight gain (total weight gain minus uterine weight) were calculated. At necropsy, laparohystero-oophorectomy was performed on each female rat. The weight of the uterus and contents, the number of corpora lutea in each ovary, the number and distribution of implantations, live and dead fetuses and resorptions were recorded. Live fetuses were examined for gross malformations, sexed and weighed individually. Half of the fetuses were processed for skeletal necropsy and the remaining fetuses were processed for visceral examination. No detailed examinations for soft tissue alterations were conducted.

Results and Conclusions: Food consumption of dams from the high-dose group was reduced throughout the entire treatment period. Mean body weight, total weight gain, and net weight gains during gestation were reduced in the high-dose group. At necropsy, three of the females from the high-dose group were confirmed to have completely resorbed their embryos. The total number of fetuses at birth was significantly decreased in the high-dose group and the following endpoints were significantly affected: mean percentage of live fetuses decreased, the number of implantation sites decreased and the resorption rate was increased.

The body weight of male and female fetuses was reduced in all treatment groups with a statistically significant reduction in the high-dose level group. The percent of normal, variant and abnormal fetuses *per* litter in each of the treated groups were comparable to the control group. No significant gross structural malformations were observed on any of the endpoints examined in any of the treatment groups. Skeletal examination revealed the incidence of incomplete development of dorsal cranial bones increased in the mid- and high-dose groups.

We established the maternal NOEL/NOAEL of narasin/nicarbazin at 0.75/0.75 mg/kg bw/day based on reduced food consumption, reduced mean and net body weight gains in the high-dose group. An embryo-fetal developmental toxicity NOEL/NOAEL was not established because soft tissue alterations were not examined.

c. Genetic Toxicity studies

The findings from the genotoxicity testing for Maxiban™ 72 are presented in Table IV.C.2 and described in more detail below. Results from the genotoxicity tests indicated that Maxiban™ 72 is not a genotoxic compound.

Table IV.C.2. Summary of Maxiban™ 72 Genotoxicity Studies.

Study Type	Study Number	Results
Bacterial Reverse Mutation Assay (Modified Ames Test)	830808GPA2264 830808GPA816 830808GPA1027	Negative
Bacterial Reverse Mutation Assay (Ames Test)	831128AMS816 831205AMS1027 831212AMS2264 840305AMS2264	Negative
In Vitro Mammalian Cell Gene Mutation Test	830927MLA816 831025MLA1027 831026MLA2264	Negative
Mammalian Erythrocyte Micronucleus Test	880405MNT2264	Negative
In Vitro Mammalian Chromosome Aberrations Test	910206CAB2264	Negative
In Vivo Sister Chromatid Exchange Assay in Mammalian Cells	830913SCE816 830922SCE816 831011SCE816 840109SCE816 830922SCE1027 840118SEC2264	Negative
Unscheduled DNA Synthesis Assay in Rat Liver	830927UDS2264 831005UDS2264	Negative

Narasin is a dried fermentation product. Due to the insolubility of mycelial narasin, crystalline narasin (purity 92%) was used in these studies below, in equal parts-by-weight combination with crystalline nicarbazin (purity 98.80%) to represent Maxiban™ 72 (coded as EL-9193 in the genotoxicity studies).

(1) Bacterial Reverse Mutation Assay (Modified Ames Test)

Title: The Effect of EL-9193 on the Induction of Bacteria Mutation Using a Modification of the Ames Test

Study Numbers: 9830808GPA2264, 830808GPA816, 830808GPA1027

Report Date: March 8, 1984

Study Location: Greenfield, Indiana

Study Design: EL-9193, crystalline narasin and crystalline nicarbazin were each evaluated in a GLP bacterial mutation assay using eight strains of *Salmonella* Typhimurium (G46, TA1535, TA100, C3076, TA1537, D3052, TA1538, and TA98) and two strains of *Escherichia coli* (WP2 and WP2*uvr*A) with and without metabolic activation. The assays employed a gradient plate method in which a gradient of test article concentrations was achieved in a test dish. Four concentrations for each test articles were applied in the assay, and their estimated gradient ranges were 0.1-1.0 µg/mL, 1.0-10 µg/mL, 10-100 µg/mL, and 100-1000 µg/mL. DMSO, the solvent for the test articles, was used as the negative control, and the positive controls included N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, for non-activated assays) and 2-aminoanthracene (2AA, for activated assays). The S9 fraction of the liver tissue homogenate from Aroclor 1254-treated male rats was used in the metabolic activation system.

Results and Conclusions: Without metabolic activation, none of the three test articles was associated with increased cytotoxicity at any exposure concentrations. With metabolic activation, all three test articles demonstrated some cytotoxicity, manifested as inhibition of background bacteria growth, in some but not all bacteria strains; the most cytotoxicity was seen in the TA1538 and TA98 strains exposed to nicarbazin, with cytotoxicity observed at the estimated exposure concentration of 500 µg/mL.

In the non-activated assays, exposures to narasin, nicarbazin, or EL-9193 did not slow the growth of the bacterial colonies nor increased the mutation frequency in any of the tester strains. The positive control, MNNG, in the estimated concentration range of 0.05-5.0 µg/mL, produced a dose-related increase in mutation frequency in *S.* Typhimurium strains of G46, TA1535, TA100, C3076, TA1537 and *E. coli* strains of WP2 and WP2*uvr*A. In the activated assays, no increase in mutation frequency was seen in any of the tester strains with any of the three test articles, whereas exposure to 2AA resulted in dose-related mutations in strains TA1535, TA100, D3052, TA1538, TA98, and WP2*uvr*A, at various estimated exposure concentration ranges.

Based on the results, EL-9193, crystalline narasin, and crystalline nicarbazin did not cause increases in the mutation frequency in the test bacterial strains.

(2) Bacterial Reverse Mutation Assay (Ames Test)

Title: The Effect of EL-9193 on the Induction of Reverse Mutations in *Salmonella* Typhimurium using the Ames Test

Study Numbers: 831128AMS816, 831205AMS1027, 831212AMS2264, 840305AMS2264

Report Date: April 18, 1984

Study Location: Greenfield, Indiana

Study Design: This GLP bacterial reverse mutation assay was conducted to test the mutagenic potential of EL-9193, as well as crystalline narasin and nicarbazin individually. The three test articles were tested for mutagenic potential in *S.* Typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100. Each tester strain was tested at five concentrations of the three test articles as well as at two concentrations of each of the positive controls; each assay was done in triplicate plates. Revertant colonies were counted using an automated counter. Metabolic activation was provided by inclusion of the S9 fraction of liver homogenate from Aroclor 1254-treated male Fischer rats. MNNG (for TA1535 and TA100), 2-nitrofluorene (2NF, for TA1538 and TA98) and 9-aminoacridine (9AmAc, for TA1537) served as the positive controls for the non-activated test, while 2AA served as the positive control for the activated assays of all tester strains. The solvent, DMSO, was used as the vehicle control.

Results and Conclusions: Treatment with MNNG, 2NF, 9AmAc and 2AA resulted in a dose-dependent induction of reverse mutations in the corresponding bacteria tester strains in the non-activated and activated assays. Treatment with EL-9193 (50 to 250 µg/plate, and repeated at five concentrations ranging from 125 to 1000 µg/plate), narasin (125 to 1000 µg/plate) or nicarbazin (25 to 500 µg/plate) did not result in the increase of *Salmonella* revertants compared to the vehicle control, with or without metabolic activation.

The results indicated that the test articles were not mutagenic under the test conditions.

(3) *In Vitro* Mammalian Cell Gene Mutation Test

Title: The Effect of EL-9193 on the Induction of Forward Mutation at the Thymidine Kinase Locus of L5178Y Mouse Lymphoma Cells

Study Numbers: 830927MLA816, 831025MLA1027, 831026MLA2264

Report Date: April 11, 1984

Study Location: Greenfield, Indiana

Study Design: EL-9193, narasin and nicarbazin were tested *in vitro* for the induction of mammalian cell mutation in the L5178Y $TK^{+/-}$ mouse lymphoma cell assay with and without liver microsomal mix according to GLP. Eight concentrations each were used for EL-9193 (0.1 to 20 µg/mL with and without metabolic activation), narasin (0.1 to 10 µg/mL), and nicarbazin (1.0 to 1000 µg/mL without activation and from 0.05 to 100 µg/mL with activation). The solvent, DMSO, was used as negative control; ethyl methanesulfonate (EMS) was used as a positive control in the non-activated assay and 3-methylcholanthrene (3-MC) in the activated assay. The S9 fraction of the liver tissue homogenate from Aroclor 1254-treated male rats was used for metabolic activation of the test articles.

Prepared stock L5178Y $TK^{+/-}$ cells were cultured in media (10×10^6 cells/mL) and mixed with either a test article, a positive control compound, or the solvent alone (with or without the S9 activation mix), and incubated for 4 h at 37 °C. Cells then were washed, re-suspended, and cultured for additional 48 h (with or without the TFT-selection medium) to allow mutant expression. Following the expression period, final suspension was adjusted to 100 cells/mL, and approximately 100 cells were plated and incubated for 12±2 days. The number of viable colonies (growth in non-selective cloning media) and the number of $TK^{-/-}$ mutants (growth in TFT-selection media) were counted from triplicate samples, and both small and large colonies were included in the total count.

Results and Conclusions: Treatment with EMS (620 µg/mL) in non-activated assay and 3-MC (2 µg/mL) in activated assay caused increases in cytotoxicity and mutational frequency.

Narasin treatment with and without metabolic activation showed a dose-dependent cytotoxic response, with a suspension growth range from 9% to 87% for non-activated assays and 14% to 92% for the activated assays as compared to their respective solvent controls. There was no significant difference in the mutation frequencies between the treated cultures at any narasin concentration and their respective solvent controls under either non-activated or activated conditions. For nicarbazin, dose-dependent inhibition of suspension growth was observed under both conditions, with the growth values ranging from 2% to 112% in non-activated assays and 17% to 132% in activated assays, as compared to their respective solvent controls. No significant changes in mutation frequency were observed in the nicarbazin-treated cultures under the non-activated conditions. In the activated assay, greater than 2-fold increases in mutational frequency were seen at the nicarbazin concentrations of 1.0, 5.0 and 100 µg/mL, with precipitation observed at the concentration of 100 µg/mL.

Two subsequent replicate assays of nicarbazin under activated conditions at the same doses as the initial assay were conducted, and the resultant suspension growth ranged from 17% to 99% and 12% to 90% of their respective controls. The first replicate did not result in a significant increase in mutation frequency at any dose, whereas the second replicate resulted in a three-fold increase in mutation frequency at 100 µg/mL. Analysis of the combined data from the three assays indicate that only 100 µg/mL was associated with a 2.5-fold increase of mutation frequency, not meeting the criteria of a positive result, which requires at least two treatment concentrations causing greater than a 2-fold increase in mutation frequency.

With EL-9193, eight concentrations were tested at ranges from 0.1 to 20 µg/mL for both the non-activated and activated assays, and suspension growths ranged from 12% to 91% and 10% to 98%, respectively. The treatment at 20 µg/mL caused 2-fold and 3-fold increases in mutation frequency for the non-activated and activated assays, respectively, but the corresponding cell survivals were about 10% in both cases. In two replicates of the assay under the activated condition, treatment with EL-9193 at the same concentrations did not produce the change in mutation frequency as seen in the initial assay.

While there were instances of positive results of increased mutation frequency with treatment of nicarbazin and EL-9193, those findings were isolated and occurred at high concentrations that were associated with very high cytotoxicity and precipitation. Overall, the results indicated that EL-9193, as well as narasin and nicarbazin individually, were not mutagenic under either the non-activated or the activated condition, in the mouse lymphoma cell mutational assay.

(4) Mammalian Erythrocyte Micronucleus Test

Title: The Effect of EL-9193 on the *In Vivo* Induction of Micronuclei in Bone Marrow of CD-1 Mice

Study Number: 880405MNT2264

Report Date: June 17, 1988

Study Location: Greenfield, Indiana

Study Design: This GLP *in vivo* micronucleus test in mice was conducted to evaluate the mutagenic potential of EL-9193, an equal parts combination of narasin and nicarbazin. Male and female CD-1 mice (seven weeks old, 15 mice/sex) were administered a single dose of EL-9193 by oral gavage at 4, 8, and 16 mg/kg bw, cyclophosphamide at 100 mg/kg bw (the positive control), or the dosing vehicle at 0.02 mL/g bw (the negative control). All the animals were sacrificed by cervical dislocation at 24, 48, and 72 h post-dose administration, and bone marrow samples from the femurs were collected from 5 mice *per* sex at each of those time points. Glass slides with bone marrow smears were processed and stained with Wright's stain followed by Weight's-Giemsa stain. One slide from each animal

was scored microscopically for a combined total of 1000 polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE). Additionally, a total of 1000 PCEs from each animal were evaluated for the presence of micronucleated PCEs (MPCE).

Results and Conclusions: The group means of MPCEs in each EL-9193 dose group at each harvest time were not significantly different from their corresponding vehicle control. EL-9193 treatment at any dose did not affect the PCE/NCE ratio (a measure of toxicity) of either sex at any of the harvest times examined. In contrast, treatment with cyclophosphamide resulted in significant increases in MPCE in both males and females at 24 h post-dosing, accompanied by severe toxicity as indicated by depressed PCE/NCE ratio in both males and females at the 48 and 72 h time points; this toxicity also resulted in too few PCEs at the latter time points to enumerate the MPCEs at those times.

The study results indicated that treatment with EL-9193 in mice did not cause increase in micronuclei in the bone marrow erythrocytes, and the test article is not mutagenic under the test condition.

(5) *In Vitro* Mammalian Chromosome Aberrations Test

Title: The Effect of Maxiban®[1] on the *In Vitro* Induction of Chromosome Aberrations in Chinese Hamster Ovary Cells

Study Number: 910206CAB2264

Report Date: April 22, 1991

Study Location: Greenfield, Indiana

Study Design: In this GLP complaint study, Maxiban® was tested for mutagenic activity in cultured Chinese hamster ovary (CHO) cells at the exposure concentrations of 10, 25, 50, 75, and 100 µg/mL, with and without metabolic activation. The S9 fraction of the liver tissue homogenate from Aroclor 1254-treated male rats was used in the metabolic activation system. The solvent (DMSO) was used as the negative control, and the positive controls were cyclophosphamide (with metabolic activation) and mitomycin C (without metabolic activation). Following an initial culture incubation for 24 h, CHO cells (1×10^6 in 10 mL media) were exposed to the prescribed concentrations of Maxiban® for 4 h, after which time the cells were maintained in culture without Maxiban® for additional 19 h. Triplicate cultures were included for each test article concentration: two for chromosome aberration evaluation and one for toxicity evaluation. Following colcemid-induced metaphase arrest, the cells were processed for slide preparation. Collected metaphase figures from each culture were smeared on two slides, and all slides were scored in a blind fashion for the presence of chromosome aberrations. Fifty metaphase figures from each Maxiban® or solvent-treated culture, and 25 metaphase figures from each positive control culture were scored for

[1] Maxiban® is the proprietary name of the drug product administered in this study.

chromosome aberrations, and 100 cells *per* slide were examined for ploidy increases.

Results and Conclusions: Precipitation was observed in cultures at the test article concentrations ≥50 µg/mL without activation and ≥10 µg/mL with activation. Therefore, the highest concentration evaluated for chromosome aberrations was 50 µg/mL. No toxicity to the CHO cells was observed within the tested dose range, either with or without metabolic activation.

Under the test condition of this assay, exposure to Maxiban®, with or without metabolic activation, did not cause an increase in the mutational frequency among the bacteria strains tested.

(6) *In Vivo* Sister Chromatid Exchange Assay in Mammalian Cells

Title: The Effect of EL-9193 on the *In Vivo* Induction of Sister Chromatid Exchange in Bone Marrow of Chinese Hamsters

Study Numbers: 830913SCE816, 830922SCE816, 831011SCE816, 840109SCE816, 830922SCE1027, 840118SEC2264

Report Date: April 23, 1984

Study Location: Greenfield, Indiana

Study Design: In this GLP study, EL-9193, crystalline narasin, and crystalline nicarbazin were each tested for their mutagenic potential in *in vivo* induction of sister chromatid exchange (SCE) in bone marrow of adult female Chinese hamsters. Three animals were included for each test article at each dose; two animals were used in the vehicle control group and one animal was used as the positive control. The oral doses given to the animals were 12.5, 25, 50, and 100 mg/kg bw for EL-9193; 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 mg/kg bw for narasin; and 200, 300, 400, and 500 mg/kg bw for nicarbazin. Cyclophosphamide at 50 mg/kg bw was used as the positive control. An agar-coated BrdUrd tablet (20-30 mg) was implanted into each animal subcutaneously to mark sister chromatid differentiation. Five hours after the implantation of the BrdUrd tablet, the test articles were administered in a single dose *via* gavage in 10% aqueous acacia, followed by an intraperitoneal injection of spindle inhibitor Velban® (1 mg/kg bw) 19 h after the oral administration. The animals were euthanized 2 h after the injection. Bone marrow samples were collected from femurs, and slides with bone marrow cell smears were processed and stained with the fluorescence plus Giemsa method. For each animal, 25 representative metaphases were scored for SCEs. For cytotoxicity evaluations, a total of 100 metaphase figures were scored for characteristic staining representing the first, second, and third division.

Results and Conclusions: For the animals treated with narasin, cytotoxicity, characterized by an increase in the number of first division metaphase figures, was evident at the doses of 40 mg/kg bw and above. Marked cytotoxicity also was seen in the positive control animal. No SCE induction by narasin was evident at any doses. Nicarbazin treatment was not associated with cytotoxicity at any of the doses tested, whereas the positive control animal showed a marked increase in the first division metaphase figures. There were no indications of SCE induction in the bone marrow samples from animals treated with nicarbazin at any of the doses. For the animals treated with EL-9193, no signs of cytotoxicity were detected, whereas the positive control animals showed increased first division metaphase figures. No induction of SCEs by EL-9193 was seen at any of the doses.

While the number of animals *per* test group was small and only one sex was used in the study, the results were consistent with other tests suggesting that the test articles were not genotoxic under the test conditions.

(7) Other genotoxicity studies

Title: The Effect of EL-9193 on the Induction of DNA Repair Synthesis in Primary Cultures of Adult Rat Hepatocytes

Study Numbers: 830927UDS2264, 831005UDS2264

Report Date: February 13, 1984

Study Location: Greenfield, Indiana

Study Design: In this GLP study, crystalline narasin, crystalline nicarbazin, and their equal parts-by-weight combination, EL-9193, were each tested for their potential to cause DNA damage, as measured by unscheduled DNA synthesis (UDS) in cultured rat primary hepatocytes. Two male Fischer rats were used to produce replicate sets of primary hepatocytes. The cells were obtained through the conventional two-step collagenase liver perfusion method, and the yields were 1.7×10^8 and 2.8×10^8 hepatocytes, with viabilities of 88.7% and 81.2%, respectively. The cells were cultured for 2.5 h in a standard medium before being exposed to 10 µCi/mL [^{3}H]-TdR and the appropriate dilutions of the test articles. After a 20-h of incubation, the cells were processed and stained with 1% aceto-orcein. The number of silver grains over the cell nucleus were counted for each sample and the net nuclear grain counts that represent UDS activities were derived. A total of 20 morphologically intact cells with at least 4 nuclear grains were counted for each treatment culture. For each of the test articles, eight concentrations were included in the assays; the ranges were 0.005 to 10 µg/mL for EL-9193, 0.0005 to 1 µg/mL for narasin, and 0.025 to 50 µg/mL for nicarbazin. N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and 2-acetylaminofluorene (2AAF) were used as the positive controls, also at eight concentrations each (MNNG from 0.075 to 150 µg/mL and 2AAF from 0.0125 to 25 µg/mL). Negative control cultures were treated with the solvent DMSO.

Results and Conclusions: Control cultures, treated with DMSO, showed neither cytotoxicity nor induction of UDS. Cytotoxicity was seen in EL-9193-treated cells at doses of 0.5 µg/mL and above in both replicate studies; narasin cytotoxicity was seen at doses of 0.1 µg/mL and above; no cytotoxicity was shown in the nicarbazin-treated cultures at the tested concentrations, including the highest concentration of 50 µg/mL, which is the limit of solubility. UDS induction was detected in the samples treated with either MNNG or 2AAF compared to the vehicle controls, with the highest induction detected at the highest non-cytotoxic concentrations. There was no UDS induction in the samples treated with EL-9193, narasin, or nicarbazin, comparing to the vehicle control.

Overall, the results indicated that treatment with the three test articles did not increase UDS in the test system.

4. Determination of Toxicological NOEL/NOAEL for Chronic Exposure

   a. Narasin

      The NOEL/NOAEL for chronic exposure of narasin is established from a 1-year oral toxicity study in beagle dogs at 0.5 mg/kg bw/day, as published in the original FOI Summary for NADA 118-980, approval date August 14, 1986.

   b. Nicarbazin

      Studies considered for determination of the toxicological NOEL/NOAEL or LOEL/LOAEL for chronic exposure are summarized in Table IV.C.3. Considering the types and quality of the available toxicology studies for Maxiban™ 72, nicarbazin, DNC, or DNC and HDP in combination, the LOEL/LOAEL of 30 mg/kg bw/day for nicarbazin from the oral developmental toxicity study in rabbits (Study Number 493768) is selected to be the most appropriate for the determination of the toxicological acceptable daily intake (ADI) for chronic exposure of total residues of nicarbazin (DNC and HDP) found in chickens to human consumers.

Table IV.C.3. Summary Table of NOELs/NOAELs or LOELs/LOAELs from Toxicology Studies.

Study Type and Year Performed	Study Number	NOEL/NOAEL or LOEL/LOAEL (mg/kg bw/day)
90-day Subchronic Oral Toxicity Study in Rats (2009)	130-104	NOEL/NOAEL cannot be established for nicarbazin LOEL/LOAEL 200 mg/kg bw/day for nicarbazin (exceeded MTD)
90-day Subchronic Oral Toxicity Study in Rats (2009)	130-167	NOEL/NOAEL 709 mg/kg bw/day for DNC
2-Year Chronic Oral Toxicity Study in Dogs (1969)	87892	NOEL/NOAEL 154:51 mg/kg bw/day for the 3:1 mixture of DNC:HDP
Oral Developmental Toxicity Study in Rats (1977)	Not provided*	Maternal and embryo-fetal NOEL/NOAEL 70 mg/kg bw/day for nicarbazin
Oral Developmental Toxicity Study in Rabbits (2006)	493768	Maternal NOEL/NOAEL 60 mg/kg bw/day for nicarbazin Embryo-Fetal LOEL/LOAEL 30 mg/kg bw/day for nicarbazin
Multi-Generation Reproductive Toxicity & Lactation Study in Rats (1970)	88943	NOEL/NOAEL cannot be established LOEL/LOAEL 150:50 mg/kg bw/day for the 3:1 mixture of DNC:HDP
Oral Carcinogenicity Study in Rats (1969)	87891	NOEL/NOAEL 300:100 mg/kg bw/day for the 3:1 mixture of DNC:HDP
Oral Development Toxicity Study in Rats with Maxiban™ 72 (1984)	R12383	Maternal NOEL/NOAEL 0.75/0.75 mg/kg bw/day for narasin/nicarbazin[#] Embryo-fetal NOEL/NOAEL was not established due to study limitations

*only summary report provided
#consistent with the toxicity reported from narasin only treatment

5. Toxicological Acceptable Daily Intake (ADI)

   a. Narasin

      Reassessment of the toxicological ADI for narasin was not needed for this supplemental approval. The FOI Summary for the original approval of NADA 118-980, approval date August 14, 1986, contains a summary of all toxicology studies and information for narasin. The ADI for narasin of 5 µg/kg body weight *per* day, which is based on the toxicological endpoint, is codified in 21 CFR 556.428.

   b. Nicarbazin

      The toxicological ADI for total residues of nicarbazin (DNC and HDP) is calculated using the following formula based on the LOEL/LOAEL of 30 mg/kg bw/day for nicarbazin from the developmental toxicity study in rabbits and a safety factor of 200.

$$\text{Toxicological Acceptable Daily Intake} = \frac{\text{LOEL/LOAEL}}{\text{Safety Factor}}$$

$$= \frac{30\,\text{mg/kg bw/day}}{200}$$

$$= 0.15\ \text{mg/kg bw/day} = 150\ \mu\text{g/kg bw/day}$$

      The overall safety factor of 200 includes a factor of 10 for animal-to-human extrapolation, a factor of 10 for differences in human variation, a factor of 20 for the irreversible developmental effect (common carotid arteries arising separately from the aortic arch) and for the LOEL/LOAEL to NOEL/NOAEL extrapolation, and a safety factor of 0.1 to account for the lower toxicity of either DNC or DNC and HDP in combination than nicarbazin administration to test animals. The use of the reduced safety factor of 0.1 is based on consideration that, although the available toxicology information reveals that nicarbazin is much more toxic than DNC or DNC and HDP in combination, residue data have demonstrated that only DNC and HDP residues are found in the edible tissues of chickens treated with nicarbazin; therefore, humans would be mainly exposed to DNC and HDP residues when consuming edible tissues of treated chickens.

      The toxicological ADI for total residues of nicarbazin (DNC and HDP) is 150 µg/kg bw/day.

D. Establishment of the Final ADI

The toxicology information discussed above suggest that the human food safety for the combination of narasin and nicarbazin can be determined by the toxicological ADIs for the individual new animal drugs in this combination product listed below.

1. Narasin

   The final ADI is the toxicological ADI of 5 µg/kg/ bw/day for total residues of narasin derived from a 1-year oral toxicity study in beagle dogs. The codified ADI for narasin is listed under 21 CFR 556.428.

2. Nicarbazin

   Because nicarbazin (DNC and HDP) is not an antibacterial, the toxicological ADI (150 µg/kg bw/day) is established as the final ADI for total residues of nicarbazin (DNC and HDP). Rounding to one significant figure, the final ADI for total residues of nicarbazin (DNC and HDP) is 200 µg/kg bw/day.

E. Safe Concentrations for Total Residues in Edible Tissues

The calculation of the tissue safe concentrations is based on the General Principles for Evaluating the Safety of New Animal Drugs used in Food-Producing Animals (FDA/CVM, Guidance for Industry #3, revised July 2016). The safe concentration of total residues in each edible tissue of broiler chickens is calculated using the following formula:

$$\text{Safe Concentration} = \frac{\text{ADI} \times \text{Human Body Weight}}{\text{Food Consumption Value}}$$

The average human body weight is approximated at 60 kg. The daily food consumption values for each edible tissue of broiler chickens are 300 g for muscle, 100 g for liver, and 50 g for skin with fat in natural proportions.

1. Safe Concentrations for Total Residues of Narasin in Edible Tissues of Broiler Chickens Using the Food Consumption Values.

   The safe concentrations for total residues of narasin in the individual edible tissues of broiler chickens are summarized in Table IV.E.1.

   Table IV.E.1. Summary Table of Safe Concentrations for Total Residues of Narasin.

Edible Tissue	Amount Consumed Per Day	Safe Concentration
Muscle	300 g	1 ppm
Liver	100 g	3 ppm
Skin with fat in natural proportions	50 g	6 ppm

2. Safe Concentrations for Total Residues of Nicarbazin (DNC and HDP) in Edible Tissues of Broiler Chickens using the Food Consumption Values.

   The safe concentrations for total residues of nicarbazin (DNC and HDP) in the individual edible tissues of broiler chickens are summarized in Table IV.E.2).

   Table IV.E.2. Summary Table of Safe Concentrations for Total Residues of Nicarbazin (DNC and HDP).

Edible Tissue	Amount Consumed Per Day	Safe Concentration
Muscle	300 g	40 ppm
Liver	100 g	120 ppm
Skin with fat in natural proportions	50 g	240 ppm

F. Residue Chemistry

1. Summary of Residue Chemistry Studies

   a. Total Residue and Metabolism Studies

      (1) Narasin

      CVM did not require additional total residue and metabolism studies for this supplemental approval. The FOI Summary for the original approval of NADA 118-980 dated August 14, 1986, contains a summary of total residue and metabolism studies for narasin.

      (2) Nicarbazin

      (a) **Title:** The Absorption, Distribution, Metabolism and Excretion of [^{14}C]-HDP Following Multiple Administrations of Nicarbazin Containing [^{14}C]-HDP to Broiler Chickens (Study No. 805286)

      Study Dates: July 15, 2004, to February 15, 2007

      Study Location: Edinburgh, United Kingdom

      Study Design: In this OECD study, twenty-four 21-day old broiler chickens (12 male and 12 female) were treated with 125 mg nicarbazin/kg of food consumed (125 ppm or 0.0125% nicarbazin, equivalent to 113.5 g/ton of feed) for 7 consecutive days *via* two daily oral capsule doses 12 hours apart. Excreta were collected from six birds from Day -1 until slaughter at 228 hours post-last dose. Plasma samples were collected from six birds before each morning dose and again 24 hours post-last morning dose. Four groups of six birds (3 male and 3 female) were slaughtered at 12, 60, 108 and 228 hours post-last dose. Muscle, liver, kidney and skin/fat were collected. Total radioactivity was measured by combustion followed by liquid scintillation counting. For metabolite profiling, tissues were analyzed by HPLC with on-line radio-detection (excreta) or HPLC with fraction collection and liquid scintillation counting (tissue samples).

Results: The highest concentration of total radiolabeled residues (TRR) for each tissue was observed in tissues collected at 12 hours withdrawal. The highest mean concentration was quantitated in kidneys, followed by skin/fat, liver and muscle. Mean TRR concentrations decreased for all tissues collected at each subsequent withdrawal time.

Table IV.F.1. Mean Total Radiolabeled Residues in Chicken Tissues as ng equivalents/g (ppb).

Withdrawal Time (hours)	Mean Total Residues in muscle (ppb) ± S.D.	Mean Total Residues in Liver (ppb) ± S.D.	Mean Total Residue in Kidney (ppb) ± S.D.	Mean Total Residue in Skin/Fat (ppb) ± S.D.
12	84 ± 37	95 ± 41	134 ± 61	106 ± 36
60	3 ± 1*	8 ± 3	5 ± 3*	27 ± 10
108	2 ± 1*	6 ± 2	2 ± 1*	17 ± 10*
228	0 ± 0*	2 ± 1*	2 ± 2*	6 ± 2*
LOQ	3.5	3.5	3.5	11

*Indicates mean calculated from data less than 30 dpm above background

HPLC established parent HDP to be the principal component of TRR in excreta and chicken tissues. The highest concentration of HDP residues for each tissue was observed in tissues collected at 12 hours withdrawal. The highest mean concentration was quantitated in skin/fat, followed by liver, muscle and kidney.

Table IV.F.2. Mean Total [^{14}C]-HDP Residues in Male and Female Chicken Tissues as ng equivalents/g (ppb).

With-drawal Time (hours)	Mean Total HDP Residues in muscle in male/female chickens (ppb)	Mean Total HDP Residues in Liver in male/female chickens (ppb)	Mean Total HDP Residues in Kidney in male/female chickens (ppb)	Mean Total HDP Residues in Skin/Fat in male/female chickens (ppb)
12	49/53	66/39	38/32	67/51
60	Not analyzed*	Not analyzed*	Not analyzed*	Not analyzed*
108	Not analyzed*	Not analyzed*	Not analyzed*	22/9
228	Not analyzed*	Not analyzed*	Not analyzed*	Not analyzed*
LOQ	3.5	3.5	3.5	11

*HPLC analysis was not performed because of low levels of radio-labeled compound

(b) **Title:** The Residue Depletion and Metabolic Identification of [^{14}C]-DNC in Chickens Following Repeated Administrations of Nicarbazin-Containing [^{14}C]-DNC (Study No. 805129)

Study Dates: July 15, 2004, to March 16, 2007

Study Location: Edinburgh, United Kingdom

Study Design: In this OECD study, eighteen 21-day old broiler chickens (9 male and 9 female) were treated with 125 mg nicarbazin/kg of food consumed (125 ppm or 0.0125% nicarbazin, equivalent to 113.5 g/ton of feed) for 7 consecutive days *via* two daily oral capsule doses 12 hours apart. Excreta were collected from six birds from Day -1 until slaughter at 228 hours post-last dose. Plasma samples were collected from six birds before each morning dose and again 24 hours post-last morning dose. Three groups of six birds (3 male and 3 female) were slaughtered at 12, 108 and 228 hours post-last dose. Muscle, liver, kidney and skin/fat were collected. Radioactivity was measured by combustion followed by liquid scintillation counting. For metabolite profiling, tissues were analyzed by HPLC with on-line radio-detection (excreta) or HPLC with fraction collection and liquid scintillation counting (tissue samples).

Results: The highest concentration of TRR for each tissue was observed in tissues collected at 12 hours withdrawal. The highest mean concentration was quantitated in liver, followed by kidneys, skin/fat and muscle. Mean TRR concentrations decreased for all tissues collected at each subsequent withdrawal time.

Table IV.F.3. Mean Total Radioactive Residues in Chicken Tissues as µg equivalents/g (ppm).

Withdrawal Time (hours)	Mean Total Residues in muscle (ppm) ± S.D.	Mean Total Residues in Liver (ppm) ± S.D.	Mean Total Residue in Kidney (ppm) ± S.D.	Mean Total Residue in Skin/Fat (ppm) ± S.D.
12	4.43 ± 0.57	27.8 ± 1.45	16.8 ± 1.25	5.12 ± 0.32
108	0.07 ± 0.02	0.61 ± 0.2	0.37 ± 0.12	0.15 ± 0.04
228	0.002 ± 0.001*	0.05 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
LOQ	0.0035	0.0035	0.0035	0.011

*Indicates mean calculated from data less than 30 dpm above background

Radio-HPLC and LC-MS/MS analysis confirmed that the major component in all tissue samples was DNC. The highest concentration of DNC residues for each tissue was observed in tissues collected at 12 hours withdrawal. The highest mean concentration was quantitated in liver, followed by kidney, skin/fat and muscle.

Table IV.F.4. Mean Total [^{14}C]-DNC Residues in Male and Female Chicken Tissues as µg equivalents/g (ppm).

Withdrawal Time (hours)	Mean Total DNC Residues in muscle in male/female chickens (ppm)	Mean Total DNC Residues in Liver in male/female chickens (ppm)	Mean Total DNC Residues in Kidney in male/female chickens (ppm)	Mean Total DNC Residues in Skin/Fat in male/female chickens (ppm)
12	0.16/0.89	13.5/10.5	7.2/5.1	2.0/2.8
108	<LOD/0.01	0.17/0.24	0.21/<LOD	Not analyzed*
228	Not analyzed*	Not analyzed*	Not analyzed*	Not analyzed*
LOQ	0.0035	0.0035	0.0035	0.011

*HPLC analysis was not performed because of low levels of radio-labeled compound

b. Comparative Metabolism Study

(1) Narasin

CVM did not require additional comparative metabolism studies for this supplemental approval. The FOI Summary for the original approval of NADA 118-980 dated August 14, 1986, contains a summary of comparative metabolism studies for narasin.

(2) Nicarbazin

Title: [^{14}C]-Nicarbazin Metabolism in Orally Dosed Rats (Study No. ABC-0313)

Study Dates: March 28, 1985, to July 11, 1985

Study Location: Greenfield, Indiana

Study Design: In this GLP study, six Fisher strain rats (3 male and 3 female) were dosed by oral gavage with an aqueous acacia suspension containing 1 mg of [^{14}C]-nicarbazin *per* mL of solution once daily for five consecutive days. Urine and excreta were collected every 24 hours immediately following dosage. Radioactivity was measured by combustion followed by liquid scintillation counting. For metabolite profiling, urine and excreta were analyzed by thin-layer chromatography (TLC) and HPLC.

Results: The fecal extracts contained 77% of the total radioactivity. Urine contained 60% of the total radioactivity. TLC analysis showed that the radioactivity from the feces comprised labeled DNC and metabolite M1. Urine was comprised primarily of both M1 and M3 metabolites. HPLC analysis showed that metabolite M1 was DNC with both nitro groups reduced and acetylated, and metabolite M3 was DNC with one nitro group reduced and acetylated. This study provided data to qualitatively compare the residue profile in the rat with the residue profile in the chicken (Studies 805286 and 805129). Results from Studies 805286, 805129 and ABC-0313 showed that both the major metabolite (DNC) and minor metabolites (M1 and M3) were present in both the chicken and rat.

c. Study to Establish Withdrawal Period

Tissue Residue Depletion Study

Title: Residue Depletion of Nicarbazin and Narasin in Edible Tissues from Chickens Following Administration of Maxiban®[2] G160 *via* Feed (Study No. T4HAUK0703)

Study Dates: October 2007 to May 2008

Study Location: Cumbria, United Kingdom and Edinburgh, Scotland

Study Design: In this GLP study, twenty-four one-day old broiler chickens (12 male and 12 female) received Maxiban® G160 (50 mg nicarbazin + 50 mg narasin *per* kg feed) for 35 days *via* medicated feed. Four groups of six birds (3 male and 3 female) were slaughtered at 0, 3, 5 and 7 days after treatment. Muscle, liver, kidney, skin/fat and fat were collected for analysis of DNC and narasin. Fat samples were not analyzed.

Results: The mean narasin residue concentrations in liver, kidney, and muscle were <LOQ at 0 days withdrawal and 27.2 ppb in skin with fat at 0 days withdrawal. Mean narasin residue concentrations were not detected (ND) in any tissues at 3, 5, and 7 days withdrawal.

[2] Maxiban® G160 is the proprietary name of the drug product administered in this study.

Table IV.F.5. Mean Narasin Residue Concentrations (ppb) in Chicken Tissues.

Group	Withdrawal Period (Days)	Liver	Kidney	Muscle	Skin/Fat
1	0	<LOQ	<LOQ	<LOQ	27.2
2	3	ND	ND	ND	ND
3	5	ND	ND	ND	ND
4	7	ND	ND	ND	ND

LOQ = 25 ppb

The mean DNC residue concentrations were highest in the liver, followed by kidney, skin/fat and muscle at 0-day withdrawal. Mean DNC residue concentrations decreased at each subsequent time point.

Table IV.F.6. Mean DNC Residue Concentrations (ppb) in Chicken Tissues.

Group	Withdrawal Period (Days)	Liver	Kidney	Muscle	Skin/Fat
1	0	9190	4290	1610	2040
2	3	2450	399.5	230*	313
3	5	355	<LOQ	<LOQ	<LOQ
4	7	212*	<LOQ	<LOQ	<LOQ

*Only 1 animal above LOQ
LOQ (liver) = 118 ppb, LOQ (kidney) = 362 ppb, LOQ (muscle) = 195 ppb, LOQ (skin/fat) = 184 ppb

2. Target Tissue and Marker Residue

   a. Narasin

      The marker residue is parent narasin (21 CFR 556.428). The target tissue for narasin is abdominal fat.

   b. Nicarbazin

      Because total residues of DNC are higher than HDP, the marker residue for nicarbazin is DNC (Study Nos. 805286 and 805129). Because DNC residues are highest in chicken liver, the target tissue for nicarbazin is liver.

3. Tolerances

   a. Narasin

      A tolerance of 480 ppb for narasin in chicken abdominal fat was established previously (21 CFR 556.428).

   b. Nicarbazin

      Based on the results from the total residue and metabolism study (Study No. 805129), a tolerance of 52 ppm for DNC in chicken liver was calculated.

4. Withdrawal Period

   Tissue residue data from Study No. T4HAUK0703 were analyzed using a statistical tolerance limit algorithm that determines the upper tolerance limit for the 99th percentile of the population with 95% confidence. The data support assignment of a 0-day withdrawal period for Maxiban™ 72 when used according to label directions in broiler chickens. Parent narasin and DNC residues fall below their respective tolerances at 0-day withdrawal.

G. Analytical Method for Residues

1. Narasin

   The requirement for an analytical method to monitor residues of narasin in chickens was waived because total residues were well below the safe concentrations in the edible tissues of chickens dosed with [^{14}C]-narasin (FOI Summary for NADA 118-980 dated August 14, 1986).

2. Nicarbazin

   Chicken liver tissue residues of DNC were measured using a validated LC-MS/MS method. The method is on file at the Center for Veterinary Medicine, 7500 Standish Place, Rockville, MD 20855. To obtain a copy of the analytical method, please submit a Freedom of Information Summary request to: https://www.accessdata.fda.gov/scripts/foi/FOIRequest/requestinfo.cfm.

V. USER SAFETY

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to Maxiban™ 72:

"When mixing and handling Maxiban™ 72, use protective clothing, impervious gloves, and a dust mask. Operators should wash thoroughly with soap and water after handling. If accidental eye contact occurs, immediately rinse thoroughly with water. Not for human use."

VI. AGENCY CONCLUSIONS

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act (FD&C Act) and 21 CFR part 514. The data demonstrate that Maxiban™ 72, when used according to the label, is safe and effective for the prevention of coccidiosis in broiler chickens caused by *Eimeria necatrix*, *E. tenella*, *E. acervulina*, *E. brunetti*, *E. mivati*, and *E. maxima*. Additionally, data demonstrate that residues in food products derived from species treated with Maxiban™ 72 will not represent a public health concern when the product is used according to the label.

A. Marketing Status

This product can be marketed over-the-counter (OTC) because the approved labeling contains adequate directions for use by laypersons and the conditions of use prescribed on the label are reasonably certain to be followed in practice.

B. Exclusivity

Maxiban™ 72, as approved in our approval letter, does not qualify for a marketing exclusivity under section 512(c)(2)(F) of the FD&C Act.

C. Supplemental Applications

This supplemental NADA required a reevaluation of the safety or effectiveness data in the original NADA (21 CFR 514.106(b)(2)).

D. Patent Information

For current information on patents, see the Animal Drugs @ FDA database or the Green Book on the FDA CVM internet website.