Date of Approval: April 26, 2024

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-582

MULTIMIN® 90

(zinc, copper, manganese, and selenium injection)

Injectable solution

Cattle. Not for use in pregnant cows and heifers during their first trimester because reproductive safety testing has not been done in these animals. Do not use in beef calves less than 2 months of age, dairy calves, and veal calves because safety has not been established.

To provide a supplemental source of zinc, copper, manganese, and selenium in cattle. Not for use in pregnant cows and heifers during their first trimester because reproductive safety testing has not been done in these animals. Do not use in beef calves less than 2 months of age, dairy calves, and veal calves because safety has not been established.

Sponsored by:

Warburton Technology Ltd.

Executive Summary

MULTIMIN® 90 (zinc, copper, manganese, and selenium injection) is approved as a supplemental source of zinc, copper, manganese, and selenium in cattle. The drug is an injectable solution of the four minerals and administered subcutaneously. MULTIMIN® 90 is not for use in pregnant cows and heifers during their first trimester, beef calves less than 2 months of age, dairy calves, and veal calves.

Safety and Effectiveness

Each of the four minerals in MULTIMIN® 90 contributes to the drug's effectiveness because individual sources of zinc, copper, manganese, and selenium are necessary to increase the blood concentration of each mineral.

The sponsor conducted a clinical field study to demonstrate that a single injection of MULTIMIN® 90 is effective to provide a supplemental source of zinc, copper, manganese, and selenium in cattle. Holstein replacement dairy heifers sourced from commercial dairy herds were enrolled in the study. The heifers were administered either saline or MULTIMIN® 90 by subcutaneous injection at the labeled dose, and blood samples were collected at multiple timepoints post-injection. Compared with the control group, cattle in the treatment group had higher mean plasma concentrations of all four minerals, as calculated by the area under the curve from the time of injection (time zero) to 8 hours post-injection (AUC_{0-8}).

The sponsor conducted a margin of safety study in young, healthy, purebred and crossbred beef cattle. Both non-pregnant females and intact males were enrolled. Each animal was injected at three administration sites with MULTIMIN® 90 subcutaneously at 0x, 1x, 3x, or 5x the labeled dose for 3 consecutive days (three times the labeled duration). The 0x group received saline at the 5x dose volume.

The drug was well-tolerated in the 1x and 3x groups. Cattle in all treatment groups had injection site swelling and inflammation. Cattle in the 3x and 5x groups had decreased feed consumption and decreased serum cholesterol compared with the 0x group. The 5x group also had hepatic centrilobular necrosis and associated serum chemistry changes caused by copper toxicity. Selenium and copper are known to be toxic if administered in excess. Therefore, if MULTIMIN® 90 is overdosed or used with other sources of copper or selenium, cattle may show clinical signs associated with copper or selenium toxicity, including death.

The sponsor conducted an injection site irritation study in young, healthy, purebred and crossbred beef cattle of both sexes. Each animal was administered both MULTIMIN® 90 and saline on different sides of the neck. MULTIMIN® 90 caused injection site reactions including pain during the injection, injection site swelling, and injection site induration (thickening and hardening of the skin). Injection site lesions were seen grossly on necropsy and ranged from mild to severe. Abnormal microscopic findings were consistent with a marked local irritant effect. These reactions may result in trim loss of edible tissue at slaughter.

The sponsor evaluated the reproductive safety of MULTIMIN® 90 in male and female cattle in a weight of evidence approach using scientific literature and pharmacovigilance reports. However, there is a lack of studies that evaluated the administration of

MULTIMIN® 90 during the first trimester. Therefore, the Food and Drug Administration (FDA) concluded that the sponsor demonstrated reproductive safety in both male and female cattle except in pregnant cows and heifers during their first trimester.

Human Food Safety
FDA evaluated the microbial food safety of MULTIMIN® 90 using a qualitative risk assessment. Zinc, copper, manganese, and selenium are metals, and FDA identified the hazard as bacteria of human health concern that become resistant to antimicrobials important in human medicine as a direct result of exposure to the trace metals in MULTIMIN® 90. The assessment described the drug's antimicrobial characteristics with respect to (1) promoting the emergence or selection of antimicrobial-resistant bacteria of public health concern in or on treated cattle; (2) the relative consumption quantities and bacterial contamination rates of food products derived from treated cattle; and (3) the importance of metals in human clinical medicine. Results from these components were integrated into an overall risk estimation of low for the intended use of MULTIMIN® 90 in cattle. The conditions of use of MULTIMIN® 90 in cattle are compatible with the Agency's risk management strategies for a drug with an estimated low risk.

FDA determined the toxicological safety of the intended use of MULTIMIN® 90 in cattle through a margin-of-exposure/margin-of-safety approach. Using this approach for each mineral in MULTIMIN® 90, FDA considered both its reference value and the potential human exposure to residues. The Agency also considered the high background levels of copper and selenium in the liver of untreated beef cattle.

The reference values for zinc, copper, manganese, and selenium are their tolerable upper intake levels (ULs) set by the Institute of Medicine (IOM). Data from the sponsor's tissue residue depletion study (see next paragraph) showed that the potential human exposure to residues of the four minerals through consuming edible tissues derived from cattle treated with MULTIMIN® 90 would generally be well below their respective reference values. Therefore, FDA determined that there are sufficient margins between the reference values and human exposure to the residues to ensure the safety of people who consume edible tissues derived from treated cattle. FDA's assessment also factored in people consuming zinc, copper, manganese, and selenium from other food sources.

The sponsor conducted one tissue residue depletion study to assess the quantity and nature of the residues of each mineral in MULTIMIN® 90 in edible tissues derived from treated cattle. Selenium residues in injection site muscle were above the UL or acceptable daily intake (ADI) at 0 days withdrawal. Because selenium accumulated in injection site muscle, the sponsor conducted a milk residue depletion study to evaluate baseline selenium levels in milk and serum of dairy cows and the effect of a single injection of MULTIMIN® 90 on milk and serum selenium levels. Selenium levels in milk returned to baseline within 24 hours after treatment, and selenium levels in milk from all animals were very low compared to its ADI.

FDA did not assign tolerances for any of the four minerals in MULTIMIN® 90. Tissue residues of zinc, copper, and manganese are evaluated in reference to their respective ULs and tissue residues of selenium are evaluated in reference to its ADI. For monitoring selenium in muscle, including at the injection site, FDA assigned a target

testing level of 1.3 parts per million. FDA also assigned a withdrawal period of 14 days and a milk discard time of 0 days. A validated analytical method is not needed because FDA did not assign a tolerance for selenium.

FDA determined that there is a reasonable certainty of no harm for residues of zinc, copper, manganese, and selenium in the edible tissues of treated cattle following human consumption when MULTIMIN® 90 is used according to the labeling.

User Safety
Due to the high mineral concentrations in MULTIMIN® 90, there is a potential risk of zinc, copper, manganese, and selenium toxicity in people who handle or are exposed to the drug. Symptoms of toxicity in people include aches, chills, nausea, vomiting, diarrhea, tachycardia, epigastric pain, tremors, and irritability.

Conclusions
Based on the data submitted by the sponsor for the approval of MULTIMIN® 90, FDA determined that the drug is safe and effective when used according to the labeling.

Table of Contents

I. GENERAL INFORMATION

A. File Number

NADA 141-582

B. Sponsor

Warburton Technology Ltd.
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Dublin 2, D02 HX82, Ireland

Drug Labeler Code: 066679

U.S. Agent Name and Address:

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C. Proprietary Name

MULTIMIN® 90

D. Drug Product Established Name

zinc, copper, manganese, and selenium injection

E. Pharmacological Category

Minerals

F. Dosage Form

Injectable solution

G. Amount of Active Ingredients

zinc (60 mg/mL) as zinc oxide
copper (15 mg/mL) as copper carbonate
manganese (10 mg/mL) as manganese carbonate
selenium (5 mg/mL) as sodium selenite

H. How Supplied

100 mL and 500 mL vials

I. Dispensing Status

Prescription (Rx)

J. Dosage Regimen

Cattle up to 1 year, 1 mL/100 lb bodyweight
Cattle from 1-2 years, 1 mL/150 lb bodyweight
Cattle over 2 years, 1 mL/200 lb bodyweight

To be administered as a single dose. The maximum volume per injection site is 7 mL. Allow a minimum of 30 days before considering repeat dosing.

K. Route of Administration

Subcutaneous injection

L. Species/Classes

Cattle. Not for use in pregnant cows and heifers during their first trimester because reproductive safety testing has not been done in these animals. Do not use in beef calves less than 2 months of age, dairy calves, and veal calves because safety has not been established.

M. Indication

To provide a supplemental source of zinc, copper, manganese, and selenium in cattle. Not for use in pregnant cows and heifers during their first trimester because reproductive safety testing has not been done in these animals. Do not use in beef calves less than 2 months of age, dairy calves, and veal calves because safety has not been established.

II. EFFECTIVENESS

A. Dosage Characterization

MULTIMIN® 90 is a fixed combination of zinc, copper, manganese, and selenium in an injectable solution. The dosages for MULTIMIN® 90 were chosen based on 1) published levels for mineral supplementation for zinc, copper, manganese, and selenium in cattle and 2) doses below toxic concentrations. Based on higher trace mineral requirements for growing animals, higher doses were chosen for younger animals compared to older animals. The dosages for MULTIMIN® 90 are a single dose (in some cases multiple injections given at a single timepoint are needed to administer large doses >7 mL) in cattle according to the following doses in different ages (Table II.1.):

Table II.1. MULTIMIN® 90 dosages for different ages of cattle

Cattle age	Dose of MULTIMIN® 90* per BW**	Zinc mg/kg dose	Copper mg/kg dose	Manganese mg/kg dose	Selenium mg/kg dose
Up to 1 year of age	1 mL/100 lb. (45 kg)	1.33	0.33	0.22	0.11
From 1-2 years of age	1 mL/150 lb. (68 kg)	0.88	0.22	0.15	0.075
Over 2 years of age	1 mL/200 lb. (91 kg)	0.66	0.165	0.11	0.055

*Each 1 mL of MULTIMIN® 90 contains 60 mg zinc, 15 mg copper, 10 mg manganese, and 5 mg selenium.
**BW = bodyweight

B. Substantial Evidence

1. Contribution of Each Active Ingredient of the Combination

   MULTIMIN® 90 is a combination new animal drug because it contains four active ingredients. Each active ingredient makes a contribution to the indication, "to provide a supplemental source of zinc, copper, manganese, and selenium in cattle", because individual sources of zinc, copper, manganese, and selenium are necessary to increase blood concentrations of zinc, copper, manganese, and selenium. For a supplementary source of zinc, an injection of copper, manganese, or selenium would not be expected to increase zinc blood concentrations, and thus would not provide a supplementary source of zinc. For a supplementary source of copper, an injection of zinc, manganese, or selenium would not be expected to increase copper blood concentrations, and thus would not provide a supplementary source of copper. For a supplementary source of manganese, an injection of zinc, copper, or selenium would not be expected to increase manganese blood concentrations, and thus would not provide a supplementary source of manganese. For a supplementary source of selenium, an injection of zinc, copper, or manganese would not be expected to increase selenium blood concentrations, and thus would not provide a supplementary source of selenium.

2. Type of Study: Multi-site Field Study

   Title: A randomized, masked, negatively controlled multi-site clinical field study to evaluate the effectiveness and safety of a single injection of a trace mineral solution containing selenium, manganese, copper, and zinc in replacement dairy heifers. (Study No. Warb2021-1)

   Study Dates: October 2022 to June 2023

   Study Locations: Parma, ID; Oakland, NE; and Nyssa, OR

Study Design:

Objective: The objective of this pivotal, randomized, masked, negatively controlled, multi-site clinical field study was to demonstrate the effectiveness of MULTIMIN® 90 when administered as a single subcutaneous injection to replacement dairy heifers to provide a supplemental source of zinc, copper, manganese, and selenium in cattle.

Study Animals: 112 Holstein replacement dairy heifers, 499 to 695 lb. bodyweight (BW) and 243 to 296 days of age, were sourced from local commercial dairy herds.

Experimental Design: A total of 90 heifers were randomized to treatment (thirty animals per site, fifteen animals/group). Within site, animals were randomized to two cohorts:16 in cohort 1 and 14 in cohort 2. Within cohort, animals were randomized to treatment groups. This resulted in 8 animals per treatment group in cohort 1, and 7 per treatment in cohort 2. Within treatment group, animals were randomized to pens. Treatment groups evaluated were saline (Group 1) or MULTIMIN® 90 (Group 2) at the dose of 1 mL/100 lb. (45 kg) BW at each study site. Animals were group housed. Only treatment administration personnel had access to treatment records during the study. All other personnel involved in the conduct of the study were masked to treatment assignments until the end of the animal phase of the study. Personnel responsible for the bioanalytical sample analysis were masked to treatment group during the bioanalytical phase of the study. The study was conducted in accordance with Good Clinical Practice guidelines.

Drug Administration: Animals were injected with saline or MULTIMIN® 90 via subcutaneous injection at a dose of 1 mL/100 lb. (45 kg) BW.

Measurements and Observations: General health observations (GHO) were conducted once daily at the time of feeding, from Day -14 to the last day of the in-life phase. The animals were individually weighed on Day -2. A physical examination was conducted on Day -2 and on Day 0 following the last blood sample collection at 8 hours. Injection sites and demeanor were evaluated once prior to treatment and at 0.5-, 1-, 3-, 6-, and 8-hours post-treatment. Blood samples were obtained for mineral analysis within 1 to 2 hours prior to dosing, and 0.5 hours (±10 minutes), 1 hour (±10 minutes), 3 hours (±15 minutes), 6 hours (±15 minutes), and 8 hours (±15 minutes) post-treatment.

The primary effectiveness outcome variable was the partial area under plasma concentration curve from dosing to eight hours (AUC_{0-8}).

Statistical Methods: The experimental unit for statistical analysis was the individual animal. The primary effectiveness outcome variable AUC_{0-8} was calculated using the linear trapezoidal method. The actual sampling time was used in the AUC_{0-8} calculation. All values below the lower limit of quantification (LLOQ) were imputed as LLOQ/2. AUC_{0-8} values were natural log transformed prior to the statistical analysis. The statistical model included treatment group as a fixed effect, pre-treatment concentration value as a covariate, and site, site-by-

treatment interaction as random effects. Cohort was not included as a random effect because two cohorts were treated on the same day (Day 0).

Treatment with MULTIMIN® 90 was considered successful if, for all four minerals, the difference in the mean AUC_{0-8} between the MULTIMIN® 90 group and the saline group was significant at a two-sided alpha of 0.05 and favored the MULTIMIN® 90 group.

Results:
Mineral Concentrations

The mean AUC_{0-8}, as a measure of the plasma concentrations of zinc, copper, manganese, and selenium was significantly different for each mineral and higher in the MULTIMIN® 90 treated group vs. the saline treated group. See Table II.2.

Table II.2. Area under Plasma Mineral Concentration Curves

Mineral*	**Least Square Mean for saline treated group (AUC_{0-8})****	**Least Square Mean for MULTIMIN® 90 treated group (AUC_{0-8})**	**95% Confidence Interval for saline treated group (AUC_{0-8})**	**95% Confidence Interval for MULTIMIN® 90 treated group (AUC_{0-8})**	**P-value**
Zn (hr*mg/L)	7.74	16.36	6.82–8.78	14.43–18.56	0.0031
Cu (hr*mg/L)	7.05	8.34	6.81–7.31	8.05–8.64	0.0041
Mn (hr*µg/L)	10.89	253.79	6.35–18.68	147.83–435.71	0.0023
Se (hr*µg/L)	790.25	2734.03	617.41–1011.47	2136.07–3499.39	0.0042

*Zn = zinc; Cu = copper; Mn = manganese; Se = selenium
** AUC_{0-8} = area under concentration curve from dosing to 8 hours

Injection Site Reactions

Table II.3. shows the results from the injection site evaluations:

Table II.3. Injection Site Observations

Treatment Group	**Number Positive for Swelling/edema Post Treatment for at least 1 timepoint (out of 45)**	**Number Positive for Swelling/edema at 8 hours Post Treatment (out of 45)**	**Number Positive for Erythema Post Treatment for at least 1 timepoint (out of 45)**	**Number Exhibiting Signs of Pain Post Treatment (out of 45)**
Saline treated group	10	0	0	0
MULTIMIN® 90 treated group	42	37	3	20

All swellings resolved within 12 days of onset, with the exception of one animal in the MULTIMIN® 90 treated group, where swelling at the injection site was still present without treatment at 14 days post-treatment. Injection site irritation was further evaluated as part of the Target Animal Safety evaluation (see Section III).

Adverse Reactions: Other than injection site reactions described above (Table II.3), no adverse reactions were reported in this study from the use of MULTIMIN® 90.

Conclusion: This study demonstrates that MULTIMIN® 90 is effective to provide a supplemental source of zinc, copper, manganese, and selenium in cattle when administered as a single injection.

III. TARGET ANIMAL SAFETY

A. Injection Site Irritation Study

Title: Target Animal Local Safety of Multimin Solution for Injection for Cattle (Copper Carbonate/Benzyl Alcohol Formulation), After A Single Subcutaneous Administration in Cattle. (Study No. V8163)

Study Dates: November 2017 to February 2018

Study Location: Fondettes, France

Study Design:

Objective: The objective of this study was to assess the injection site irritation of the test article, MULTIMIN® 90 injectable solution for cattle when administered subcutaneously at the dose rate of 1 mL/50 kg BW. Local reaction at the test article injection site, gross pathology and histopathology findings were compared to those of a saline solution (control article) co-administered to the study animals on the opposite side of the neck.

Study Animals: Sixteen (eight females and eight males) Salers, Charolais, Limousine and crossbred beef cattle, ranging in age from 193 to 342 days and ranging in BW from 255 kg to 364 kg.

Experimental Design: Each animal received both the test and control article. Treatment with the test article was given at random in the left or right aspect of the neck. For randomization, the animals were ranked by sex and increasing ear tag number and were assigned a random number in blocks of four (random numbers 1 to 4). Two random numbers were assigned to treatment with the test article in the right aspect of the neck and the other two random numbers were assigned to treatment with the test article in the left aspect of the neck. Treatments with the control article were consequently given on the other side of the neck. Treatments were performed under masked conditions. This experimental study was carried out in compliance with Good Laboratory Practice regulations [Organization for Economic Cooperation and Development (OECD), Paris 1998, ENV/MC/CHEM(98)17] and the Application

of the OECD Principles of Good Laboratory Practice to the Organization and Management of multi-site studies (ENV/JM/MONO (2002)9).

Drug Administration: The test article was MULTIMIN® 90 and the control article was 0.9% sodium chloride. Eleven animals were administered a dose of 1 mL/50 kg BW and the other 5 animals received the maximum dose per injection site of 7 mL. Eight animals (4 males and 4 females) received the test article on the left aspect of the neck and 8 (4 males and 4 females) on the right aspect of the neck. Each 1 mL of the test article included 60 mg zinc (as zinc oxide), 15 mg copper (as copper carbonate), 10 mg manganese (as manganese carbonate), and 5 mg selenium (as sodium selenite).

Measurements and Observations: Animals were administered test and control article on Day 0. Animals were acclimated for 7 days (starting on Day -7). On Day 14 animals were euthanized and the gross pathology and histopathology of injection sites were evaluated. Injection site observations included evaluation of pain during injection and evaluation of injection site pain, swelling, erythema, and induration on Day 0 at a pre-injection timepoint, a 1 hour post-injection timepoint, and a 4 hour post-injection timepoint and then, daily from Day 1 to Day 14.

Injection Site Evaluation Criteria:

See Table III.1. through Table III.4., below.

Table III.1. Scoring system for pain at the injection site (palpation of the site)

Score	Description
0	Nil, no pain
1	Mild pain (twitching of the skin)
2	Moderate pain (defensive reaction)
3	Severe pain (lameness)

Table III.2. Scoring system for swelling at the injection site (palpation of the site)

Score	Description
0	Nil, no swelling
1	Mild swelling (≤10 cm)
2	Moderate swelling (>10 cm)
3	Severe swelling (all around the neck)

Table III.3. Scoring system for erythema at the injection site (visual inspection)

Score	Description
0	Nil, no erythema
1	Mild erythema (mild redness)
2	Moderate erythema (redness and heat)
3	Severe erythema (non-delimited redness and heat)

Table III.4. Scoring system for induration at the injection site (palpation of the site)

Score	Description
0	Nil, no induration
1	Mild induration (≤1 cm)
2	Moderate induration (between 1 cm and 5 cm wide)
3	Severe induration (>5 cm)

Other parameters evaluated during the study included daily clinical observation during acclimation (Days -7 to -1), physical examinations (Day -2 and Days 0 to 14), and BW measurements (on Days -4 and -2).

Statistical Methods: Pain upon injection, local examination of the injection sites, gross pathology, and histopathology findings were presented as the total number and the percentage of animals presenting the abnormality (detailed per abnormality). Animal details (breed, sex, age, BW), treatment details (article administered, volume administered, treatment side, time of treatment, route), and size of lesion at necropsy (surface and volume) were summarized in tables and figures. Physical examination data were only descriptive.

Results:

Administration of the test article was not attributed to any negative effects on general animal health.

Injection Site Observations:

Pain During Injection

There was no pain observed during injection of the control article. One animal demonstrated pain during injection of the test article with a score of 1 (scale of 0 to 3, 0=no pain and 3=severe pain).

Pain Post Injection

After injection, there was no pain observed upon palpation at any of the control article injection sites. One animal demonstrated pain upon palpation of a test article injection site. This was only observed on Day 0 at the 1 hour post-injection timepoint.

Erythema

No erythema was observed at any injection site during the study.

Swelling

For the control article injection sites, only 1 animal had swelling (on Day 0 at the 1 hour post-injection timepoint with a score of 1).

For the test article injection sites, swelling was only present on Day 0 through Day 1 (see Table III.5. below). From Day 2 on, no animals had any swelling at any injection site.

Table III.5. Swelling at the test article injection sites for observations Day 0 through Day 2

Observation Timepoint	Swelling Score 0	Swelling Score 1	Swelling Score 2	Swelling Score 3
Day 0 (pre-injection)	16	0	0	0
Day 0, 1 hour post-injection timepoint	11	1	4	0
Day 0, 4 hours post-injection timepoint	2	0	7	7
Day 1	4	0	5	7
Day 2	16	0	0	0

Mild to moderate swelling was observed at the test article injection sites on Day 0 at the 1 hour post-injection timepoint in 5 out of 16 animals. At 4 hours post-injection, test article injection sites had moderate to severe swelling in 14 out of 16 animals. Swelling was still detectable for the test article injection sites on Day 1 in 12 out of 16 animals. Swelling was no longer detectable from Day 2 on.

Induration

For the control article injection sites, two animals had mild induration (score 1): one animal on Day 0 at the 1 hour and 4 hour post-injection timepoints, and the other animal on Day 0 only at the 4 hour post-injection timepoint.

For the test article injection sites, induration was observed starting on Day 0 at the 1 hour post-injection timepoint in 10 out of 16 animals. The incidence of induration slightly decreased on Day 1, before reaching its peak on Day 2 and Day 3 (mild to severe induration on all animals). Then, the incidence and severity of induration slowly decreased over time. The test article injection sites still exhibited mild to moderate induration at the end of the observation period (Day 14) in 14 out of 16 animals (see Table III.6. below).

Table III.6. Induration at the test article injection sites for observations Day 0 through Day 14

Observation Timepoint	Induration Score 0	Induration Score 1	Induration Score 2	Induration Score 3
Day 0 (pre-injection)	16	0	0	0
Day 0, 1 hour post-injection timepoint	6	0	10	0
Day 0, 4 hours post-injection timepoint	12	2	1	1
Day 1	12	0	3	1
Day 2	0	0	8	8
Day 3	0	0	8	8
Day 4	0	1	11	4
Day 5	0	3	10	3
Day 6	0	2	9	5
Day 7	0	2	12	2
Day 8	0	2	12	2
Day 9	1	3	8	4
Day 10	1	4	10	1
Day 11	3	1	12	0
Day 12	1	5	10	0
Day 13	1	8	7	0
Day 14	2	5	9	0

Gross Pathology Findings:

All control article injection sites were absent of lesions at necropsy (Day 14).

All test article injection sites had presence of a lesion at necropsy. See Table III.7. below for distribution of lesion scores at the test article injection sites.

Table III.7. Lesion scores of test article injection sites at necropsy

Scores	Description	Number of Animals
0	Absence of lesion	0
1	Hardly Detectable lesion	5
2	Slight Irritation	6
3	Moderate Irritation	3
4	Severe Irritation	2

Lesions at the test article injection sites were hardly detectable grossly (gross pathology score of 1) in 5 out of 16 animals. Test article injection sites in 6 out of 16 animals presented with slight irritation (score of 2) consisting of discrete subcutaneous fibrosis at the injection site without muscle involvement. Test article injection sites in 3 out of 16 animals presented with moderate irritation (score of 3) consisting of extensive subcutaneous fibrosis with mild muscle degeneration (grey discoloration on the surface of the underlying muscle). Test article injection sites in 2 out of 16 animals presented with severe subcutaneous irritation with deep muscle degeneration (grey to green discoloration of the underlying muscle).

The areas of surface subcutaneous lesions from the test article injection sites ranged from 18 to 140 cm^2. The volume of lesions from the test article injection sites ranged from 3.6 to 190.4 cm^3.

Histopathology Findings:

All control article injection sites were absent of histological findings.

Test article injection site histological findings included abnormalities such as necrosis, subacute to chronic inflammation and/or hemorrhage in the subcutis and/or in the adjacent muscle indicative of marked local irritant effect (see Tables III.8. through III.13., below). Subcutaneous necrosis was characterized by loss of adipose tissue and connective tissue that were replaced by an amorphous fibrinoid material; blood vessels were occasionally involved. The necrotic process was associated with subacute to chronic inflammation characterized by infiltration of mixed inflammatory cells including macrophages, lymphocytes, plasma cells, neutrophils, and eosinophils with interstitial edema. Vascular thrombosis and granulomatous foci with giant cell macrophages around crystallized necrotic fat material were occasionally seen. Subacute to chronic inflammation and/or necrosis were sometimes present in the muscle adjacent to subcutaneous changes. Fibrosis/fibroplasia, associated with neovascularization, was mainly seen at the leading edge between necrotic area and surrounding tissue.

Table III.8. Histological observation of subcutaneous necrosis at test article injection sites

Scores	Description	Number of Animals
0	No Finding	0
1	Minimal	0
2	Slight	2
3	Moderate	4
4	Marked	10
5	Severe	0

Table III.9. Histological observation of muscle necrosis at test article injection sites

Scores	Description	Number of Animals
0	No Finding	10
1	Minimal	4
2	Slight	1
3	Moderate	0
4	Marked	1
5	Severe	0

Table III.10. Histological observation of subacute to chronic inflammation in the subcutis at test article injection sites

Scores	Description	Number of Animals
0	No Finding	0
1	Minimal	0
2	Slight	14
3	Moderate	2
4	Marked	0
5	Severe	0

Table III.11. Histological observation of subacute to chronic inflammation in the muscle at test article injection sites

Scores	Description	Number of Animals
0	No Finding	4
1	Minimal	3
2	Slight	8
3	Moderate	1
4	Marked	0
5	Severe	0

Table III.12. Histological observation of fibrosis/fibroplasia at test article injection sites

Scores	Description	Number of Animals
0	No Finding	0
1	Minimal	0
2	Slight	11
3	Moderate	2
4	Marked	3
5	Severe	0

Table III.13. Histological observation of hemorrhage at test article injection sites

Scores	Description	Number of Animals
0	No Finding	6
1	Minimal	4
2	Slight	6
3	Moderate	0
4	Marked	0
5	Severe	0

Conclusion: This study supports the injection site safety of MULTIMIN® 90 in cattle. An injection of MULTIMIN® 90 might result in injection site reactions including pain during the injection; injection site swelling that appears the day of injection and resolves 2 days post-injection; and injection site induration that appears on the day of injection and may be present for at least 14 days post-injection. Injection site lesions after an injection of the test article were evident grossly on necropsy evaluation ranging from hardly detectable to severe irritation with discrete subcutaneous fibrosis to extensive subcutaneous fibrosis with deep muscle

degeneration. Microscopic examination included abnormalities such as necrosis, subacute to chronic inflammation and/or hemorrhage in the subcutis and/or in the adjacent muscle indicative of marked local irritant effect. These reactions may result in trim loss of edible tissue at slaughter. These reactions and the potential for trim loss are described on product labeling.

B. Margin of Safety Study

Title: Evaluation of the Margin of Safety of Multimin® 90 after Repeated Subcutaneous Administration in Cattle. (Study No. N2101BT)

Study Dates: December 2021 to March 2023

Study Location: Parma, ID

Study Design:

Objective: The objective of this study was to evaluate the margin of safety (MOS) of MULTIMIN® 90 when administered subcutaneously to cattle over 3 consecutive days at dose groups of 1x [1 mL/100 lb. (45 kg) BW], 3x [3 mL/100 lb. (45 kg) BW], or 5x [5 mL/100 lb. (45 kg) BW] compared to a 0x control [sterile saline (0.9% NaCl)] at the 5x dose volume.

Study Animals: Thirty-two English and/or Continental pure or mixed beef breed cattle (16 non-pregnant females and 16 intact males, 4 animals per sex per treatment group), ranging in weight from 289-387 kg prior to treatment on Day -1 and less than 10 months of age.

Experimental Design: Within each sex, animals were ranked by animal identification number, then randomly assigned to treatments (4 animals per treatment per sex). Within treatment group and sex, animals were randomized to one of two necropsy cohorts, two animals per sex and treatment group cohort, such that 16 animals (8 from each sex, 2 per treatment group per sex) were assigned to each of the 2 necropsy days. Animals were then randomized to pens without regard to sex or treatment group. Animals were housed individually. Animals were administered MULTIMIN® 90 or saline for 3 consecutive days. The study veterinarian and pathologist who performed necropsies and any other study personnel who made or recorded objective/subjective measurements and observations (*e.g.*, general health observations) were kept masked to treatment allocation. For adverse events or other unscheduled events, masking was maintained for all personnel who collected or recorded subjective or objective data. Individuals involved in animal dosing were unmasked and therefore did not assist in other study activities. This study was conducted in compliance with Good Laboratory Practice (GLP) Regulations (21 CFR Part 58).

Drug Administration: Animals were injected with saline or MULTIMIN® 90 via subcutaneous injection according to Table III.14. below. Day 0 was the first day of administration. Each animal received injections at three different sites. Each 1 mL of the test article included 60 mg zinc (as zinc oxide), 15 mg copper (as copper

carbonate), 10 mg manganese (as manganese carbonate), and 5 mg selenium (as sodium selenite).

Table III.14. Summary of Treatment Groups

Dose Group	Treatment	Number of Animals
0x	Treated with saline at the dose of 5 mL/100 lb. (45 kg) BW** for 3 consecutive days	8 (4M/4F*)
1x	Treated with test article at the dose of 1 mL/100 lb. (45 kg) BW for 3 consecutive days	8 (4M/4F)
3x	Treated with test article at the dose of 3 mL/100 lb. (45 kg) BW for 3 consecutive days	8 (4M/4F)
5x	Treated with test article at the dose of 5 mL/100 lb. (45 kg) BW for 3 consecutive days	8 (4M/4F)

*M = male; F = female
**BW = bodyweight

Measurements and Observations: General health observations (GHO) were conducted twice daily from Day -14 until the last day of the in-life phase, except on Day 5 when only one GHO was required prior to final necropsy. The animals were individually weighed on Day -6, Day -1, and the morning of necropsy day, Day 4 or Day 5. Physical examinations were completed on Day -6, Day 0 (before dosing), Day 1 (before dosing), Day 3, and before necropsy on Day 4 or 5 depending on assigned necropsy day. Injection sites were evaluated at least once on Day -6 (±1 day), and twice on Days 0, 1, 2, and 3. Individual animal feed consumption was recorded daily beginning on Day -14 and continued through necropsy. Blood for clinical pathology (hematology, coagulation, and serum chemistry) was collected on Days -6, -1 (the day prior to the start of dosing), Day 1 (prior to dosing on Day 1), Day 3, and Day 4 or 5 (prior to necropsy). Free catch urine was collected into urine cups or similar holding devices once per animal on Days -6 or -5, 1, and 3. At necropsy (Day 4 or 5), urine samples were collected by cystocentesis using a sterile syringe and needle and transferred to a urine cup. Blood for toxicokinetic samples were collected on Day 0 and Day 2. Samples were collected within 90 min prior to dosing, and post-dosing at 0.5 hours (±10 min), 1 hour (±15 min), 1.5 hours (±15 min), 3 hours (±15 min), 6 hours (±20 min), and 12 hours (±30 min). Samples were analyzed for zinc (Zn), copper (Cu), manganese (Mn), and selenium (Se). On Day 4 or 5 animals were euthanized for gross pathology evaluation and sampling of selected tissues for histopathological evaluation. The selected tissues included kidneys*, jejunum, uterus (if applicable), abomasum, brain*, skeletal muscle and skin (gluteal), injection sites including skin and underlying musculature, heart*, cecum, liver*, ileum, mammary gland (if applicable), duodenum, ovaries* (if applicable), pancreas, prostate, lung, rectum, spleen*, colon, testes* (if applicable), epididymis, omasum, reticulum, prescapular lymph node, and rumen. Organs marked with asterisk (*) in the above list were weighed. Paired organs were weighed together.

Statistical Methods: The experimental unit was the individual animal.

Injection site observations were evaluated using Fisher's exact test by comparing the 0x dose group to the non-zero dose groups in a pair-wise fashion.

Where the observations were categorical, observations in the 0x dose group were compared to the non-zero dose groups in a pair-wise fashion using Wilcoxon's rank sum test.

Temperature, respiratory rate, heart rate, and daily feed consumption were analyzed using a repeated measures analysis of covariance. The pretreatment consumption values were averaged to provide the baseline as the covariate in the model. The fixed factors in the models were treatment group, day, sex, the two-way interactions of treatment group by sex, treatment group by day, and sex by day; and the three-way interaction of treatment group by day by sex. The following procedure were used to assess the terms in the models:

> If treatment group by sex by day interaction was significant at α=0.05, no further hypothesis testing would be conducted. The clinical evaluation was based on the calculated means. If this term was not significant, then treatment group by sex and group by day would be evaluated.
>
> If treatment group by sex interaction was significant at α=0.10, then pair-wise comparisons would be performed for each of the treatment groups against the control group within sex. These contrasts would be obtained from linear contrasts on the treatment group by sex interaction at an unadjusted α=0.10.
>
> If treatment group by day was significant at α=0.10, regardless of the significance of treatment group by sex, then pair-wise comparisons would be performed for each of the treatment groups against the control group within day. These contrasts would be obtained from linear contrasts on the treatment group by day interaction at an unadjusted α=0.10.
>
> If none of these interactions were significant, then the main effect of treatment group at α=0.10 would be evaluated. If this term was significant, then pair-wise comparisons between each of the treatment groups and control group would be performed using linear contrasts at an unadjusted α=0.10.

The appropriate variance-covariance matrix structures were selected from among compound symmetry (CS), heterogeneous compound symmetry (CSH), first order autoregressive (AR(1)), and heterogeneous first order autoregressive (ARH(1)) based on Akaike information criterion (AIC). The structures providing the lowest AIC were used in the analysis.

Clinical pathology (hematology, serum chemistry, and coagulation) parameters were analyzed using the model including the pre-treatment value nearest to the first dose as a covariate and fixed factors: treatment group, day, sex, treatment group by sex, treatment group by day, sex by day, treatment group by day by sex. Given unequal measurement intervals (Days 1, 3, and 4 or 5), the covariate structure in the

repeated measures analysis were investigated using three structural assumptions, namely CS, CSH, and spatial power (SP(POW)). The assumption giving the minimum value of the AIC were selected in the final analysis. Unscheduled diagnosis tests were not included in the statistical analysis. The procedure to assess the terms in the model was the same as daily feed consumption.

BWs were analyzed by analysis of covariance; using this model included the pre-treatment value nearest to the first dose as a covariate and fixed factors: treatment group, sex, treatment by sex. The following procedures were used to assess the terms in the model:

> If treatment by sex was significant at α=0.10, then pair-wise comparisons would be performed for each of the treatment groups against the control group within sex. These contrasts would be obtained from linear contrasts on the treatment by sex interaction at an unadjusted α=0.10.
>
> If the interaction was not significant, then the main effect of group at α=0.10 would be evaluated. If this term was significant, then pair-wise comparisons between each of the treatment groups and control group would be performed using linear contrasts at an unadjusted α=0.10.

Urinalysis numerical parameters (specific gravity and pH) were analyzed in the same way as the clinical pathology parameters. Categorical parameters were summarized using descriptive statistics.

Organ weights were analyzed using the model including treatment group and sex and treatment by sex (where applicable). The following procedures would be used to assess the terms in the models when sex was included as a factor:

> If treatment by sex was significant at α=0.10, then pair-wise comparisons of each of the treatment groups and control group within sex would be performed. These contrasts would be obtained from linear contrasts on the treatment by sex interaction at an unadjusted α=0.10.
>
> If this interaction was not significant, then the main effect of treatment group at α=0.10 would be evaluated. If this term was significant, then pair-wise comparisons would be performed between each of the treatment groups and control group using linear contrasts at an unadjusted α=0.10.

The following procedure would be used to assess the terms in the models when sex was not included as a factor:

> The main effect of treatment group at α=0.10 would be evaluated. If this term was significant, then pair-wise comparisons would be performed between each of the treatment groups and control group using linear contrasts at an unadjusted α=0.10.

For toxicokinetic analysis for each animal, the area under the curve from time zero to the last quantifiable concentration ($AUC_{0\text{-}tlast}$) was calculated using the linear trapezoidal method. The maximum plasma concentration (C_{max}) and time to C_{max}

(T_{max}) were determined directly from the data. $AUC_{0\text{-}tlast}$ and C_{max} values were ln-transformed prior to statistical analysis. Differences in plasma concentrations for Zn, Cu, Mn, and Se following the first and last dose were assessed by Analysis of Variance (ANOVA) on $AUC_{0\text{-}tlast}$, C_{max}, and T_{max}.

Results:

General Health Observations and Physical Examinations

Two animals, both in the 5x dose group, experienced clinical signs that were considered treatment related. Starting on Day 1 at the scheduled physical examination, a male in the 5x dose group presented as depressed and reluctant to rise. Approximately 8 hours later at an unscheduled physical examination, this animal's clinical signs progressed to excessive salivation and drooling and abnormal expiratory effort, in addition to presenting as depressed and reluctant to rise. The next day, Day 2, clinical signs progressed further to include head shaking, circling to the left, and slight placement deficit on the front foot. The animal died on Day 2 shortly after receiving the third 5x dose of test article. Clinical signs at the time of death included severe depression, weakness, ataxia, salivation, drooling, and tachypnea.

On Day 3, a female in the 5x dose group presented with clinical signs of depression and dark colored urine. On Day 4, at necropsy, clinical signs were still noted as severely depressed.

Prior to the second treatment on Day 1, both animals had clinically important increases in liver associated chemistry analytes including alanine aminotransferase (ALT), alkaline phosphatase (AP), aspartate aminotransferase (AST), bilirubin (total, direct, and indirect), gamma-glutamyl-transferase (GGT), and lactate dehydrogenase (LDH). Based on gross necropsy findings and histopathology, the early death of the male in the 5x dose group on Day 2 was attributed to marked liver centrilobular necrosis that appeared associated with test article administration. The clinical adverse events for both animals are consistent with elevated copper blood levels. For the male in the 5x dose group, on Day 0, the 0 hours pre-treatment plasma Cu value (916 ng/mL) was similar to the other 5x males (mean: 953.5 ng/mL, range of individual pre-treatment Day 0 Cu plasma concentrations: 835-1, 140 ng/mL). For the six post-treatment time points on Day 0 (0.5, 1, 1.5, 3, 6, and 12 hours), the individual Cu value for this animal was only the maximum in the group at 0.5 hours (1,490 ng/mL) and 6 hours post-treatment (1,900 ng/mL). In contrast, on Day 2, from 0 hours until the last available sample at 1.5 hours (immediately prior to death), this animal consistently had the highest individual Cu plasma value of the four 5x males. At 1 hour post-treatment on Day 2 the individual value for this animal (11,800 ng/mL) was more than 2 times the group mean (mean: 4,830 ng/mL, range of individual Cu plasma concentrations at this timepoint: 2, 180-11,800 ng/mL). This is consistent with the observed abnormal clinical signs on Day 2 for this animal.

For the female in the 5x dose group with clinical signs, the pre-treatment Day 0 individual plasma Cu value (905 ng/mL) was the lowest in the four females in the 5x group (mean: 1,116.25 ng/mL, range of individual pre-treatment Day 0 Cu plasma

concentrations: 905-1,370 ng/mL). On Day 0 all six post-treatment Cu values were similar to the other three 5x females and at no point did it have the highest individual value. In contrast, on Day 2, this animal had the highest individual Cu plasma value of the 5x females at every time point except 0.5 hours post-treatment. The highest plasma Cu value for this was at 3 hours post-treatment on Day 2 (8,290 ng/mL) which was approximately 1.5 times the group mean (mean: 5,230 ng/mL, range of individual Cu plasma concentrations at this timepoint: 2,460-8,290 ng/mL). Comparing the individual Cu results for both of these animals to the 5x treatment group gender means does indicate that both animals had plasma Cu levels on Day 2 (including pre-injection level which revealed high residual plasma levels after the second injection) that were higher than other animals in the treatment group and are consistent with the abnormal clinical signs observed in these animals.

Other than the physical examination findings described above, there were no other abnormal physical examination or general health observation findings that were considered treatment related.

Feed Consumption and BWs

There was a significant treatment effect for dry matter intake (DMI). The 3x (11.35 lb/day) and 5x (7.47 lb/day) treatment groups had significantly different and lower DMI compared to the 0x group (16.21 lb/day).

For both genders in the 5x treatment group, daily mean DMI consistently decreased from the start of treatment on Day 0 to Day 2. On Day 3, for both genders in the 5x treatment group, daily mean DMI then slightly increased. On Day 4, data was only available for half the 5x animals due to Day 4 necropsies. In the 5x male treatment group, pre-treatment mean DMI on Day -1 was 16.65 lb. (range 15.31-19.63 lb.) compared with Day 0 at 7.42 lb. (5.49-10.30 lb.) and Day 2 at 5.70 lb. (3.99-8.31 lb.). On Day 3 the mean DMI then increased to 6.93 lb. (3.74-9.57 lb.) for the male 5x treatment group. A similar but less prominent decrease in daily DMI was also evident in the 3x treatment group for both genders.

Despite differences in DMI, the statistical analysis of BWs found no statistically significant difference between treatment groups.

Injection Site Evaluations

No heat, erythema, or pain was observed in any of the injection sites for any animals in any treatment group.

Test article administration, regardless of dose, was associated with a rapid increase in injection site swelling. All animals in the 3x and 5x dose groups and 7 out of 8 animals in the 1x dose group experienced injection site swelling in at least 1 injection site. Only 1 animal in the control group experienced injection site swelling. That injection site swelling resolved within 24 hours of appearing.

Hematology, Coagulation, and Clinical Chemistry

Clinically relevant treatment related abnormalities were found for the following parameters. Day 4/5 indicates samples taken on the assigned necropsy day (Day 4 or 5 depending on assigned necropsy day).

Serum Chemistry Parameters Indicative of Liver Damage

Alanine aminotransferase (ALT)

Alanine aminotransferase values in the 5x dose group (47.10 U/L) were significantly different (P=0.0058) and higher than values in the 0x dose group on Day 1 (24.76 U/L). No other within day differences between the non-zero dose groups and the 0x dose group were detected.

Aspartate aminotransferase (AST)

Aspartate aminotransferase values were significantly different in the 5x dose group (1057.45 U/L, P=0.0348) and higher as compared to the 0x dose group (82.47 U/L). There were no differences between the other non-zero dose groups and the 0x dose group.

Alkaline phosphatase (AP)

Alkaline phosphatase values in the 5x group (Day 1: 313.39 U/L and Day 3: 295.03 U/L) were significantly different (Day 1: P=0.0017 and Day 3: P=0.0220) and higher than values in the 0x dose group on Day 1 and 3 (Day 1: 149.31 U/L and Day 3: 177.69 U/L). No other within day differences between the non-zero dose groups and the 0x dose group were detected.

Sorbitol dehydrogenase (SDH)

Sorbitol dehydrogenase values in the 5x dose group (294.46 U/L) were significantly different (P<0.0001) and higher than values in the 0x dose group on Day 1 (33.10 U/L). No other within day differences between the non-zero dose groups and the 0x dose group were detected.

Bilirubin (direct, indirect, and total)

Direct bilirubin values were significantly different (P=0.0003) in the 5x dose group (0.33 mg/dL) and higher as compared to the 0x dose group (0.01 mg/dL). There were no differences between the other non-zero dose groups and the 0x dose group for direct bilirubin. For indirect bilirubin, values in the 5x dose group (Day 3: 1.58 mg/dL and Day 4/5: 1.89 mg/dL) were significantly different (Day 3: P=0.0113 and Day 4/5: P=0.0024) and higher than values in the 0x dose group (Day 3: 0.16 mg/dL and Day 4/5: 0.16 mg/dL) on Days 3 and 4/5. No other within day differences between the non-zero dose groups and the 0x dose group were detected for indirect bilirubin.

For total bilirubin, values in the 5x dose group (Day 3: 1.59 mg/dL and Day 4/5: 1.86 mg/dL) were significantly different (Day 3: P=0.0516 and Day 4/5: P=0.0190) and higher than values in the 0x dose group (Day 3: 0.31 mg/dL and Day 4/5: 0.29 mg/dL) on Days 3 and 4/5. No other within day differences between the non-zero dose groups and the 0x dose group were detected.

Lactate Dehydrogenase (LDH)

Lactate dehydrogenase values were significantly different (P=0.0089) in the 5x dose group (4970.34 U/L) and higher as compared to the 0x dose group (1072.90 U/L). There were no differences between the other non-zero dose groups and the 0x dose group.

Gamma-glutamyl-transferase (GGT)

Gamma-glutamyl-transferase values were significantly different (P=0.0037) in the 5x dose group (61.12 U/L) and higher as compared to the 0x dose group. There were no differences between the other non-zero dose groups and the 0x dose group (20.36 U/L).

Other Serum Chemistry Treatment Related Findings

Cholesterol

Compared to controls, during the treatment period cholesterol was significantly different and lower in males in all three treatment groups, 1x, 3x, and 5x. Likewise, cholesterol was also significantly different and lower for females in the two higher dose groups, 3x and 5x (see Table III.15 below).

Table III.15. Summary of the treatment-by-gender interactions for cholesterol

Dose Group	Sex	Cholesterol Value (mg/dL)	P-value
0x	Male	103.65	
1x	Male	85.95	0.0004
3x	Male	75.46	<0.0001
5x	Male	70.29	<0.0001
0x	Female	99.63	
3x	Female	82.79	0.0006
5x	Female	66.11	<0.0001

In addition to a statistically significant treatment-by-sex dose dependent difference in cholesterol, with higher doses associated with decreased cholesterol, a similar dose dependent difference in cholesterol was observed on study Days 1, 3, and 4/5. This dose dependent decrease was consistent with increasing dose from 1x to 5x and with time from Day 1 to Day 4/5 prior to necropsy (see Table III.16 below).

Table III.16. Summary of the treatment-by-day interactions for cholesterol

Dose Group	Day	Cholesterol Value (mg/dL)	P-value
0x	1	98.97	
3x	1	90.21	0.0490
5x	1	87.50	0.0110
0x	3	101.97	
1x	3	90.62	0.0128
3x	3	73.83	<0.0001
5x	3	57.00	<0.0001
0x	4/5	103.97	
1x	4/5	88.12	0.0007
3x	4/5	73.33	<0.0001
5x	4/5	60.10	<0.0001

On Day 4/5 the mean for the 5x dose group had decreased by more than 40% compared to controls (5x: 60.10 mg/dL, 0x: 103.97 mg/dL), compared to a mean value of 88.12 mg/dL in the 1x group and 73.33 mg/dL in the 3x group. The 1x dose was associated with a minor, clinically insignificant, dose and time dependent decrease in cholesterol.

Calcium

Calcium values in the 5x group (Day 1: 9.36 mg/dL, P=0.0282; Day 3: 8.60 mg/dL, P<0.0001; and Day 4/5: 9.31 mg/dL, P<0.0001), 3x group (Day 1: 9.23 mg/dL, P=0.0082; Day 3: 8.95 mg/dL, P<0.0001; and Day 4/5: 9.76 mg/dL, P=0.0011), and 1x group (Day 3: 9.54 mg/dL, P=0.0190; and Day 4/5: 10.16 mg/dL, P=0.0672) were significantly different and lower than values in the 0x dose group on multiple days (Day 1: 9.92 mg/dL, Day 3: 10.14 mg/dL, and Day 4/5: 10.62 mg/dL). However, the lowest reported mean value for calcium (8.60 mg/dL, 5x, Day 3) was only slightly below the reference range of 9.2 to 11.8 mg/dL. The differences between 0x control group and the 1x dose group and 0x control group and the 3x dose group were considered non-treatment related. While the differences between the 0x control group and the 5x dose group were most likely treatment related due to the number of animals below the normal reference range, the change appears to have minimal clinical significance.

Creatine kinase (CK)

Creatine kinase values were significantly different (P=0.0012) in the 5x dose group (1330.13 U/L) and higher as compared to the 0x dose group (294.87 U/L). The individual animal profile plots for CK displayed that increases in this analyte were only observed in the 5x dose group beginning with Day 1 post-treatment and were often above the reference range.

Urinalysis

Other than the discoloration of the sample from the female in the 5x dose group on Day 4, there were no clinically important dose-dependent findings identified.

Gross Pathology and Histopathology Examination

Liver

There were 9 animals with gross liver lesions. Only two of those animals (both in the 5x dose group) had gross liver lesions that were attributed to test article toxicity.

There were test article related microscopic liver findings in 6 out of 8 animals in the 5x group (2 animals in the 5x group did not have microscopic liver findings that were considered test article related), which consisted of minimal to marked centrilobular necrosis and mild hemorrhage. Centrilobular necrosis was characterized by coagulative to lytic necrosis of centrilobular hepatocytes or loss of centrilobular hepatocytes with collapse or sinusoids and infiltration of mononuclear inflammatory cells. Hemorrhage occurred in more severely affected regions of necrosis. Test article related liver findings are summarized in Table III.17. below. There were no microscopic liver findings that were considered test article related in the 0x, 1x, and 3x groups. Other microscopic liver findings were considered not test article related. These findings included chronic inflammation, mononuclear cell infiltrate, fibrosis, and abscesses.

Table III.17. Number of test article related hepatic lesions

Dose Group	**0x**	**1x**	**3x**	**5x**
Number Examined	8	8	8	8
Necrosis, centrilobular, number affected	0	0	0	6
Minimal	0	0	0	1
Mild	0	0	0	3
Marked	0	0	0	2

The increases in liver enzyme values and bilirubin values in 5x dose group were consistent with observed liver necrosis.

Injection Site Findings

In the 0x dose group, 3 animals did not have any injection sites with a gross lesion, 3 animals had a gross lesion at 1 injection site, and 2 animals had a gross lesion at 2 injections sites. For the test article treatment groups, other than 1 animal in the 1x group with a gross lesion at only 2 injections sites, every animal had a gross lesion at every injection site. Test article-related macroscopic observations at the injection sites included subcutaneous edema (correlating microscopically to edema, subcutis), red/brown discoloration (correlating microscopically to hemorrhage), tan discoloration (correlating microscopically to skeletal muscle necrosis) and firm skeletal muscle (correlating microscopically to skeletal muscle necrosis). Microscopically, in the 0x group 3 of the 8 animals had minimal injection site subcutis edema with 1 of the 8 animals positive for minimal injection site hemorrhage and no cases of skeletal

muscle necrosis. In contrast, all 8 animals in each test article treatment group were positive for injection subcutis edema with severity scores ranging from minimal to severe. Similar incidence was observed for injection site hemorrhage and skeletal muscle necrosis. Test article injection site findings were mild to severe subcutaneous edema, mild subcutaneous neutrophilic inflammation, minimal to moderate subcutaneous vessel necrosis, mild to moderate hemorrhage, minimal to marked skeletal muscle necrosis, minimal to mild skeletal muscle degeneration/regeneration, minimal to mild mixed cell inflammation of skeletal muscle, and presence of thrombi.

Pharmacokinetics

Overall, $AUC_{0\text{-tlast}}$ of Zn, Cu, Mn, and Se was significantly increased in all treated groups on Day 0 in comparison with the control group. On Day 2 there were significant increases in $AUC_{0\text{-tlast}}$ for Zn, Mn, and Se in treated animals in comparison with the controls. Although Cu $AUC_{0\text{-tlast}}$ was increased by approximately 15% in treated groups on Day 2 versus controls, the difference was not statistically significant for the 1x dose, but a significant difference was noted between the 3x and 5x groups. The increase in $AUC_{0\text{-tlast}}$ was dose-related for both males and females on Day 0 and Day 2. Overall, a dose-related increase in Zn, Cu, Mn, and Se for C_{max} was observed on Days 0 and 2.

Conclusion: This study supports an acceptable safety profile for the use of MULTIMIN® 90 in cattle when used at the labeled dose and regimen. At the 1x dose [1 mL/100 lb. (45 kg) BW] for 3 consecutive days, MULTIMIN® 90 was well tolerated with the only significant treatment related effect being injection site swelling and inflammation. At the 3x dose [3 mL/100 lb. (45 kg) BW] for 3 consecutive days, MULTIMIN® 90 was well tolerated with the only significant treatment related effects being injection site swelling and inflammation; a decrease in feed consumption compared to controls; and a decrease in serum cholesterol compared to controls. At the 5x dose [5 mL/100 lb. (45 kg) BW] for 3 consecutive days, MULTIMIN® 90 resulted in treatment related effects of injection site swelling and inflammation; a decrease in feed consumption compared to controls; a decrease in serum cholesterol compared to controls; and hepatic centrilobular necrosis and associated serum chemistry changes caused by copper toxicity.

Based on the results of this study, product labeling states in the “Animal Safety Warnings and Precautions” subsection, “Selenium and copper are toxic if administered in excess. MULTIMIN® 90 may cause clinical signs associated with copper toxicity or selenium toxicity, including death, if overdosed or used in conjunction with excessive dietary levels of copper and selenium or other selenium or copper products. Additional zinc, copper, manganese, or selenium products should not be administered at the same time. Do not use concurrently with other injectable selenium and copper products. Do not use concurrently with selenium or copper boluses.” Furthermore, in the “Adverse Reactions” section, the labeling states, “Accidental overdose of copper or selenium through misdosing or the use of multiple sources, including the use of injectable products in addition to high dietary levels, can result in adverse events, including death, depression, weakness, ataxia, salivation, and drooling.”

C. Reproductive Safety Assessment

Reproductive safety in male and female cattle was evaluated in a weight of evidence approach using scientific literature and ten years of pharmacovigilance reports. Twenty-three studies within 20 peer-reviewed scientific papers were evaluated, the oldest of which was published in 2002. Of these papers, seven studies did not use the final approved dose or U.S. formulation of MULTIMIN® 90.

Twenty-one of these studies evaluated female cattle reproductive parameters. A wide range of ages and breeds of female cattle were represented across these studies, including both heifers and multiparous cows, with both beef and dairy breeds represented under various management conditions. MULTIMIN® 90 administration was evaluated during estrus and late first through third trimester of pregnancy across these studies, with some studies evaluating multiple timepoints prior to breeding and during gestation. Twelve studies evaluated MULTIMIN® 90 administration at approximately one month prior to artificial insemination; one study each evaluated MULTIMIN® 90 administered at Day 78 of gestation, Day 91, and Days 169 and 239 of gestation. Four studies evaluated MULTIMIN® 90 administration at Days 230 and 260 of gestation. Two studies evaluated MULTIMIN® 90 administration between two to four weeks prior to calving.

A total of 3507 female cattle were administered MULTIMIN® 90 across these studies. The most common variable evaluated was conception rate, using both artificial insemination (AI) and natural service. Some studies evaluated cow health issues such as mastitis/metritis/retained placentas and calf health. The majority of these studies demonstrated no difference in variables such as conception rate, BW, presence of various ovarian structures, and calf survival between treated and control animals. Occasional statistical differences were demonstrated, primarily in AI-conception rates, between treated and control animals. These differences were noted both in the positive and negative direction; however, because MULTIMIN® 90 appeared to either increase or decrease AI-conception rate in various studies, they were not considered treatment-related across all studies. Additionally, the differences were not considered clinically relevant.

Two studies evaluated bull reproductive safety, representing 289 treated young beef breed bulls, with repeated semen evaluations and breeding soundness examinations after administration either once or twice, 90 days apart. No treatment-related, clinically important differences were reported in either study.

Pharmacovigilance reports from 2010 to 2020 were also evaluated, representing over 98,000,000 animals treated in the U.S. over the ten-year period. Of these animals, 560 were reported to have adverse reactions which were primarily attributed to injection site reactions or suspected overdose toxicities. No adverse reactions reported involved reproductive issues such as male/female infertility, abortions, calf abnormalities or neonatal deaths.

Given the large number of animals that have received this product over the past nine years with lack of reproductive-specific adverse events reported, as well as the substantial number of cattle receiving this product in scientific studies with a lack of significant negative reproductive effects, it was concluded that reproductive safety in

both male and female cattle has been demonstrated with the exception of during first trimester of pregnancy because no studies that were evaluated included the administration of MULTIMIN® 90 during that time period. Product labeling advises against use during the first trimester in pregnant cows and heifers because reproductive safety testing has not been evaluated.

References used for the weight of evidence approach:

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IV. HUMAN FOOD SAFETY

A. Microbial Food Safety

Microbial food safety (antimicrobial resistance) information for MULTIMIN® 90 was evaluated using a qualitative antimicrobial resistance risk assessment. The hazard was identified as bacteria of human health concern that become resistant to antimicrobials important to human medicine as a direct result of exposure to the trace metals (zinc, copper, manganese, and selenium) present in MULTIMIN® 90. The *release* assessment, described as the probability that these trace elements administered in cattle will result in the emergence of antimicrobial-resistant bacteria in or on treated cattle under the proposed conditions of use, was determined to be medium. The *exposure* assessment, described as the likelihood of human exposure to antimicrobial-resistant bacteria through consumption of edible products from treated cattle, was determined to be low. The *consequence* assessment, described as any potential human health consequences arising from exposure to metal-resistant bacteria or metal resistance determinants, by considering the human medical importance of metals used in the treatment of human infectious diseases, was determined to be important. Heavy metals are not currently ranked as important drugs in human medicine; however, by default, the consequence assessment yielded a low ranking. The overall *risk estimation* was derived to be low. The proposed conditions of use for MULTIMIN® 90 in cattle are compatible with the Agency's risk management strategies associated with a product having an overall risk estimation of low.

Decision Statement:

The Agency's integration of the degree of risk derived from three individual risk assessments (medium, low, and important) resulted in an overall *risk estimation* of low. The conditions of use for MULTIMIN® 90 in cattle are compatible with the Agency's risk management strategies for a drug with an estimated low risk.

B. Toxicology

Zinc, copper, manganese, and selenium are essential micronutrients to humans and also are present in various food sources. Therefore, the Agency considered that it is more appropriate to determine the safety of the proposed use of MULTIMIN® 90 through a margin-of-exposure (MOE)/margin of safety (MOS) approach, which takes into consideration both the reference value and residue exposure. In addition, the Agency recognized that there are high background levels of copper or selenium in the liver of untreated beef cattle, in which case it is more appropriate to consider these background levels (see Section C).

The tolerable upper intake levels (ULs) for various age groups (including infants and children) set by the Institute of Medicine (IOM) range from 2-11, 1-10, and 4-40 mg/day for manganese, copper, and zinc respectively (Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc, Institute of Medicine, 2001). A UL represents the highest average daily nutrient intake level that was likely to pose no adverse health effects to the general population. The FDA set an acceptable daily intake (ADI) of 0.4 mg/day for selenium (68 FR 52339, September 3, 2003), which is the same value as the UL for adults set by IOM. The normal range for selenium in liver of beef cattle is 0.1 to 1.2 parts per million (ppm) (68 FR 52339, September 3, 2003).

The tissue residue exposure data (see Section C) demonstrated that the potential human exposure to residues of these micronutrients through consumption of edible tissues from treated cattle generally would be well below the referenced values. Consequently, the Agency considered that there are sufficient margins between the referenced values and human exposure to residues to ensure the safety of humans consuming edible tissues from treated cattle. This also factors in consumption of these micronutrients from other sources.

C. Residue Chemistry

1. Summary of Residue Chemistry Studies

   a. Study to Establish Withdrawal Period

      (1) Tissue Residue Depletion Study

      Title: Residue Depletion Study of Multimin Solution for Injection for Cattle (Copper Carbonate/Benzyl Alcohol Formulation) (Study No. V8167)

      Study Dates: September 30, 2017, to January 22, 2018

      Study Locations:
      In-Life Facility: Fondettes, France
      Analytical Facility: Evreux Cedex, France

Study Design:
Objective: To determine the tissue residues in cattle injected once subcutaneously (SC) with MULTIMIN® 90 solution at 1 mL/50 kg BW

Study Animals: Thirty-nine cattle of various breeds (Salers, Charolais, Blonde d'Aquitaine, Limousine and cross breeds of these breeds) were used. Twenty-four test and fifteen control animals were used (20 males and 19 females). The mean weight of the animals on Day -2 was 334 kg (range 270 to 421 kg).

Drug Administration: A single subcutaneous (SC) injection of MULTIMIN® 90 at 1 mL/50 kg BW was administered on Day 0.

Measurements and Observations: Three male and three female test cattle were slaughtered at 8 hours and at 8, 14 and 28 days after treatment. Five control cattle (2 or 3 males, 2 or 3 females) were slaughtered at 8, 14 and 28 days after treatment. Muscle and injection site tissue (500 g core and 300 g surrounding tissue), 150 g liver (cross section of right and left lobes), 150 g kidney (cross section of both kidneys) and 150 g perirenal fat were collected from each animal. Tissue samples were analyzed for zinc, copper, manganese, and selenium using inductively coupled plasma-mass spectrometry (ICP-MS) (Version No. 44739Vbb.MET_V03).

Results:

As shown in Tables IV.1-IV.4, manganese in any tissue from cattle treated with MULTIMIN® 90 does not exceed the UL for manganese (2-11 mg/day) at and after Day 8. BLQ means below the limit of quantitation (LOQ).

Table IV.1. Manganese concentration (ppm) in injection site tissue (core) of cattle injected SC a single time with 1 mL/50 kg bodyweight MULTIMIN® 90.

Sampling Timepoint (days)	Control Animals	Treated Animals	Maximum Residues Adjusted for Food Consumption Values (0.3 kg muscle)
0	Not used	0.837 to 8.26 (n=6)	2.5 mg/day
8	BLQ (n=4) 0.108 (n=1)	BLQ (n=4) 0.108 and 0.115 (n=2)	0.03 mg/day
14	BLQ (n=5)	BLQ (n=4) 0.1 and 0.124 (n=2)	0.04 mg/day
28	BLQ (n=4) 0.1 (n=1)	BLQ (n=3) 0.104-0.121 (n=3)	0.04 mg/day

LOQ for manganese = 0.0922 ppm

Table IV.2. Manganese concentration (ppm) in liver of cattle injected SC a single time with 1 mL/50 kg bodyweight MULTIMIN® 90.

Sampling Timepoint (days)	Control Animals	Treated Animals	Maximum Residues Adjusted for Food Consumption Values (0.1 kg liver)
0	Not used	3.13-4.81 (n=6)	0.48 mg/day
8	2.80-3.85 (n=5)	2.51-3.40 (n=6)	0.34 mg/day
14	3.05-3.59 (n=5)	3.07-4.10 (n=6)	0.41 mg/day
28	2.78-3.71 (n=5)	2.90-3.77 (n=6)	0.38 mg/day

Table IV.3. Manganese concentration (ppm) in kidney of cattle injected SC a single time with 1 mL/50 kg bodyweight MULTIMIN® 90.

Sampling Timepoint (days)	Control Animals	Treated Animals	Maximum Residues Adjusted for Food Consumption Values (0.05 kg kidney)
0	Not used	2.19-3.38 (n=6)	0.17 mg/day
8	0.982-1.39 (n=5)	1.10-1.26 (n=6)	0.06 mg/day
14	0.895-1.36 (n=5)	1.12-1.45 (n=6)	0.07 mg/day
28	0.955-1.30 (n=5)	0.996-1.38 (n=6)	0.07 mg/day

Table IV.4. Manganese concentration (ppm) in fat of cattle injected SC a single time with 1 mL/50 kg bodyweight MULTIMIN® 90.

Sampling Timepoint (days)	Control Animals	Treated Animals	Maximum Residues Adjusted for Food Consumption Values (0.05 kg fat)
0	Not used	BLQ (n=5) 0.157 (n=1)	0.01 mg/day
8	BLQ (n=5)	BLQ (n=5) 0.142 (n=1)	0.01 mg/day
14	BLQ (n=5)	BLQ (n=5) 0.175 (n=1)	0.01 mg/day
28	BLQ (n=4) 0.157 (n=1)	BLQ (n=6)	0.00 mg/day

LOQ for manganese = 0.0922 ppm

As shown in Tables IV.5-IV.8, copper in injection site tissue, kidney, and fat from cattle treated with MULTIMIN® 90 does not exceed the UL for copper (1-10 mg/day) at and after Day 8. Copper concentrations in core injection site tissue of treated animals were higher on Day 0 than on subsequent withdrawal days and were higher than concentrations in control animals. Copper concentrations in muscle surrounding injection site tissue, kidney, fat and liver on Day 0 were not different from those on subsequent withdrawal days nor were they different between control and treated animals on Days 8, 14, and 28. Two liver samples at 28 days withdrawal had residue concentrations that exceeded the UL (11.4 and 10.1 mg/day), but were comparable to the concentrations reported by U.S.

Department of Agriculture for copper in beef liver (Agricultural Research Service, FoodData Central, https://fdc.nal.usda.gov/).

Table IV.5. Copper concentration (ppm) in injection site tissue (core) of cattle injected SC a single time with 1 mL/50 kg bodyweight MULTIMIN® 90.

Sampling Timepoint (days)	Control Animals	Treated Animals	Maximum Residues Adjusted for Food Consumption Values (0.3 kg muscle)
0	Not used	2.7 to 19.9 (n=6)	6.0 mg/day
8	0.488-0.546 (n=5)	0.481-0.881 (n=6)	0.26 mg/day
14	0.424-0.734 (n=5)	0.471-1.115 (n=6)	0.33 mg/day
28	0.524-0.670 (n=5)	0.544-0.797 (n=6)	0.24 mg/day

Table IV.6. Copper concentration (ppm) in liver of cattle injected SC a single time with 1 mL/50 kg bodyweight MULTIMIN® 90.

Sampling Timepoint (days)	Control Animals	Treated Animals	Maximum Residues Adjusted for Food Consumption Values (0.1 kg liver)
0	Not used	43.6-86.1 (n=6)	8.6 mg/day
8	24.3-74.5 (n=5)	56.2-81.2 (n=6)	8.1 mg/day
14	28.7-88.2 (n=5)	43.2-94.3 (n=6)	9.4 mg/day
28	44.1-63.5 (n=5)	50.4-114 (n=6)	11.4 mg/day

Table IV.7. Copper concentration (ppm) in kidney of cattle injected SC a single time with 1 mL/50 kg bodyweight MULTIMIN® 90.

Sampling Timepoint (days)	Control Animals	Treated Animals	Maximum Residues Adjusted for Food Consumption Values (0.05 kg kidney)
0	Not used	3.85-5.38 (n=6)	0.27 mg/day
8	3.87-4.62 (n=5)	4.00-4.62 (n=6)	0.23 mg/day
14	2.94-5.12 (n=5)	3.93-4.76 (n=6)	0.24 mg/day
28	3.61-4.40 (n=5)	3.38-4.82 (n=6)	0.24 mg/day

Table IV.8. Copper concentration (ppm) in fat of cattle injected SC a single time with 1 mL/50 kg bodyweight MULTIMIN® 90.

Sampling Timepoint (days)	Control Animals	Treated Animals	Maximum Residues Adjusted for Food Consumption Values (0.05 kg fat)
0	Not used	BLQ (n=1) 0.189-0.385 (n=5)	0.02 mg/day
8	BLQ (n=2) 0.153-0.262 (n=3)	BLQ (n=4) 0.174 and 0.261 (n=2)	0.01 mg/day
14	BLQ (n=4) 0.27 (n=1)	BLQ (n=3) 0.153-0.242 (n=3)	0.01 mg/day
28	BLQ (n=3) 0.153 and 0.185 (n=2)	BLQ (n=2) 0.173-0.216 (n=4)	0.01 mg/day

LOQ for copper = 0.146 ppm

As shown in Tables IV.9-IV.12, zinc in injection site tissue, kidney, liver and fat from cattle treated with MULTIMIN® 90 does not exceed the UL for zinc (4-40 mg/day). Zinc concentrations in core injection site tissue and kidney of treated animals were higher on Day 0 than concentrations in control animals and on subsequent withdrawal days. Zinc concentrations in muscle surrounding injection site tissue, fat and liver on Day 0 were not different than on subsequent withdrawal days nor were they different between control and treated animals on Days 8, 14, and 28.

Table IV.9. Zinc concentration (ppm) in injection site tissue (core) of cattle injected SC a single time with 1 mL/50 kg bodyweight MULTIMIN® 90.

Sampling Timepoint (days)	Control Animals	Treated Animals	Maximum Residues Adjusted for Food Consumption Values (0.3 kg muscle)
0	Not used	34.9-71.2 (n=6)	21 mg/day
8	34.5-59.4 (n=5)	33.9-64.5 (n=6)	19 mg/day
14	30-50.2 (n=5)	33.1-45.7 (n=6)	14 mg/day
28	39.6-57.1 (n=5)	34.1-61.9 (n=6)	19 mg/day

Table IV.10. Zinc concentration (ppm) in liver of cattle injected SC a single time with 1 mL/50 kg bodyweight MULTIMIN® 90.

Sampling Timepoint (days)	Control Animals	Treated Animals	Maximum Residues Adjusted for Food Consumption Values (0.1 kg liver)
0	Not used	28.1-77.2 (n=6)	7.7 mg/day
8	37.4-46.5 (n=5)	32.5-79.1 (n=6)	7.9 mg/day
14	35.0-92.5 (n=5)	35.4-44.0 (n=6)	4.4 mg/day
28	34.9-42.2 (n=5)	36.7-46.5 (n=6)	4.7 mg/day

Table IV.11. Zinc concentration (ppm) in kidney of cattle injected SC a single time with 1 mL/50 kg bodyweight MULTIMIN® 90.

Sampling Timepoint (days)	Control Animals	Treated Animals	Maximum Residues Adjusted for Food Consumption Values (0.05 kg kidney)
0	Not used	22.4-27.5 (n=6)	1.4 mg/day
8	17.6-19.0 (n=5)	18.3-20.3 (n=6)	1.0 mg/day
14	15.2-19.9 (n=5)	17.5-20.1 (n=6)	1.0 mg/day
28	18.0-18.9 (n=5)	17.1-19.4 (n=6)	1.0 mg/day

Table IV.12. Zinc concentration (ppm) in fat of cattle injected SC a single time with 1 mL/50 kg bodyweight MULTIMIN® 90.

Sampling Timepoint (days)	Control Animals	Treated Animals	Maximum Residues Adjusted for Food Consumption Values (0.05 kg fat)
0	Not used	0.735-2.65 (n=6)	0.1 mg/day
8	0.71-2.71 (n=5)	0.481-2.59 (n=6)	0.1 mg/day
14	0.858-2.96 (n=5)	0.406-1.86 (n=6)	0.1 mg/day
28	1.13-2.75 (n=5)	1.02-5.0 (n=6)	0.3 mg/day

As shown in Tables IV.13-IV.16, selenium in kidney and fat from cattle treated with MULTIMIN® 90 does not exceed the ADI for selenium (0.4 mg/day). Selenium in liver is slightly less than the ADI at 0 days withdrawal and is much less than the ADI at 8 days withdrawal. Selenium in injection site muscle depletes to the ADI by 14 days withdrawal.

Table IV.13. Selenium concentration (ppm) in injection site tissue (core) of cattle injected SC a single time with 1 mL/50 kg bodyweight MULTIMIN® 90.

Sampling Timepoint (days)	Control Animals	Treated Animals	Maximum Residues Adjusted for Food Consumption Values (0.3 kg muscle)
0	Not used	0.297-3.12 (n=6)	0.9 mg/day
8	BLQ (n=1) 0.156-0.237 (n=4)	BLQ (n=2) 0.168-0.685 (n=4)	0.2 mg/day
14	BLQ (n=2) 0.150-0.178 (n=3)	0.155-1.40 (n=6)	0.4 mg/day
28	BLQ (n=2) 0.150-0.161 (n=3)	BLQ (n=2) 0.149-0.240 (n=4)	0.1 mg/day

LOQ for selenium = 0.145 ppm

Table IV.14. Selenium concentration (ppm) in liver of cattle injected SC a single time with 1 mL/50 kg bodyweight MULTIMIN® 90.

Sampling Timepoint (days)	Control Animals	Treated Animals	Maximum Residues Adjusted for Food Consumption Values (0.1 kg liver)
0	Not used	2.12-3.87 (n=6)	0.39 mg/day
8	0.598-1.16 (n=5)	1.05-2.63 (n=6)	0.26 mg/day
14	0.489-1.22 (n=5)	1.08-2.40 (n=6)	0.24 mg/day
28	0.372-0.566 (n=5)	0.796-1.11 (n=6)	0.11 mg/day

Table IV.15. Selenium concentration (ppm) in kidney of cattle injected SC a single time with 1 mL/50 kg bodyweight MULTIMIN® 90.

Sampling Timepoint (days)	Control Animals	Treated Animals	Maximum Residues Adjusted for Food Consumption Values (0.05 kg kidney)
0	Not used	2.10-2.48 (n=6)	0.12 mg/day
8	1.18-1.44 (n=5)	1.32-1.49 (n=6)	0.07 mg/day
14	0.808-1.41 (n=5)	1.33-1.50 (n=6)	0.08 mg/day
28	0.920-1.06 (n=5)	0.876-1.22 (n=6)	0.06 mg/day

Table IV.16. Selenium concentration (ppm) in fat of cattle injected SC a single time with 1 mL/50 kg bodyweight MULTIMIN® 90.

Sampling Timepoint (days)	Control Animals	Treated Animals	Maximum Residues Adjusted for Food Consumption Values (0.05 kg fat)
0	Not used	BLQ (n=5) 0.152 (n=1)	0.01 mg/day
8	BLQ (n=5)	BLQ (n=6)	0.00 mg/day
14	BLQ (n=5)	BLQ (n=6)	0.00 mg/day
28	BLQ (n=5)	BLQ (n=6)	0.00 mg/day

LOQ for selenium = 0.145 ppm

Conclusion: The data indicate that a withdrawal period is needed to address selenium residues in injection site muscle, which are above the ADI at 0 days withdrawal.

(2) Milk Residue Depletion Study

Title: Evaluate the effect of a single injection of MultiMin-90 on levels of selenium in milk and serum of lactating Holstein dairy cows. (Study No. IACUC #2013-0056)

Study Dates: August 1-31, 2015

Study Locations: Ithaca, NY

Study Design:
Objective: To evaluate the baseline levels of selenium in milk and serum of dairy cows and to evaluate the effect of a single injection of MULTIMIN® 90 on milk and serum selenium levels.

Study Animals: Twenty lactating Holstein dairy cows were used.

Drug Administration: A single SC injection of MULTIMIN® 90 at 1 mL/100 kg BW was administered.

Measurements and Observations: Blood and milk were sampled at 0 hour prior to treatment, at 8 hours, at 24 to 336 hours every 24 hours, and 672 hours post-treatment. Milk samples were analyzed for selenium using chromatographic separation coupled with inductively coupled plasma-mass spectrometry (ICP-MS).

Results: Selenium concentrations in milk from all treated cows were significantly higher at 8 hours after treatment but not at sampling timepoints after 8 hours. Selenium concentrations in primiparous treated cows (but not

multiparous cows) were significantly higher at 8 hours after treatment. The baseline measurement of selenium in milk before treatment was 28.7 ng/mL with the lower and upper 95% confidence interval of 26.6 and 30.8, respectively. At 8 hours after treatment, the mean concentration of selenium was 31.2 ng/mL (lower and upper 95% confidence interval was 29.1, 33.3) in control animals and 37.1 ng/mL (lower and upper 95% confidence interval was 35.0, 39.2) in treated animals. At 24 hours after treatment, the mean concentration of selenium was 27.4 ng/mL (lower and upper 95% confidence interval was 25.3, 29.5) in control animals and 30.2 ng/mL (lower and upper 95% confidence interval was 28.1, 32.2) in treated animals.

Conclusion: These data indicate that selenium concentrations in milk returned to baseline concentrations by 24 hours post-treatment. Selenium concentrations in milk from all animals are very low compared to the ADI for selenium (0.4 mg/day).

2. Target Tissue and Marker Residue

A target tissue is not identified. Residues of zinc, copper, and manganese do not accumulate above the ULs in edible tissues after treatment. Selenium accumulates at the injection site, but none of the tissues (injection site tissue and the edible tissues) functions as a target tissue, in which the absence of residues in that tissue are an indication of residues in other tissues. The marker residue for selenium is parent drug.

3. Tolerances

Tolerances for manganese, copper, zinc and selenium are not assigned because the tissue residues are evaluated in reference to the ULs for manganese (2-11 mg/day), zinc (4-40 mg/day) and copper (1-10 mg/day), and the published ADI (0.4 mg/day) for selenium. For purposes of monitoring selenium in muscle including injection site, we assign a target testing level of 1.3 ppm (0.4 mg/day ÷ 0.3 kg).

4. Withdrawal Period and Milk Discard Time

A 14-day withdrawal period allows selenium residues in all edible tissues, including injection site muscle, to deplete to the ADI prior to slaughter of the treated animal. A zero-milk discard time is assigned because selenium residues in milk do not increase above the ADI in cattle treated with MULTIMIN® 90.

D. Analytical Method for Residues

1. Description of Analytical Method

An official method is not needed because a tolerance is not being assigned for selenium. An ICP/MS analytical method, similar to that used in Study No. V8167, is available from USDA/FSIS.

2. Availability of the Method

   An analytical method for analysis of residues of selenium is available from USDA/FSIS at: https://www.fsis.usda.gov/wps/wcm/connect/b9a63ea1-cae9-423b-b200-36a47079ae49/CLG-TM3.pdf?MOD=AJPERES.

V. USER SAFETY

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to MULTIMIN® 90:

Not for use in humans. Keep out of reach of children. Do not allow children access to used or empty syringes. Wash hands after use.

This product is highly concentrated in zinc, copper, manganese, and selenium. Due to a potential risk of zinc, copper, manganese, and selenium toxicity, care should be taken when handling the product to avoid accidental self-injection. Symptoms of exposure to zinc, copper, manganese, and selenium include aches, chills, nausea, vomiting, diarrhea, tachycardia, epigastric pain, tremors, and irritability.

In case of accidental self-injection or ingestion, SEEK IMMEDIATE MEDICAL ATTENTION and take the vial with you.

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 MULTIMIN® 90, when used according to the label, is safe and effective for the conditions of use in the General Information Section above. Additionally, data demonstrate that residues in food products derived from species treated with MULTIMIN® 90 will not represent a public health concern when the product is used according to the label.

A. Marketing Status

This product may be dispensed only by or on the order of a licensed veterinarian (Rx marketing status). Adequate directions for lay use cannot be written because professional expertise is required to monitor the safe use of the product, including treatment of any adverse reactions.

B. Exclusivity

MULTIMIN® 90, as approved in our approval letter, qualifies for FIVE years of marketing exclusivity beginning as of the date of our approval letter. This drug qualifies for exclusivity under section 512(c)(2)(F)(i) of the FD&C Act because this is the first time we are approving at least one of the active moieties in a new animal drug application submitted under section 512(b)(1) of the FD&C Act.

C. Patent Information

For current information on patents, see the Green Book Reports in the Animal Drugs @ FDA database.