FREEDOM OF INFORMATION SUMMARY
Combined Use of COBAN® and BMD® in Turkey Feeds

## I. GENERAL INFORMATION:

**NADA Number:** 140-937

**Sponsor:** Elanco Animal Health
A Division of Eli Lilly and Company
Lilly Corporate Center
Indianapolis, IN 46285

**Generic Names:** Monensin Sodium
Bacitracin Methylene Disalicylate

**Trade Name:** COBAN® and BMD®
No Trade Name Proposed for Combination

**Pharmaceutical Classification:** Anticoccidial (Polyether Ionophore)
Growth Promotant (Polypeptide Antibiotic)

**Marketing Status:** OTC

## II. INDICATIONS FOR USE:

For the prevention of coccidiosis caused by Eimeria adenoeides, E. meleagrimitis, and E. gallopavonis, and for increased rate of weight gain and improved feed efficiency in growing turkeys.

## III. DOSAGE FORMS:

This NADA provided for the combined use of these two approved Type A medicated articles, BMD® as per 21 CFR § 558.76 and COBAN® as per 21 CFR § 558.355 into Type C medicated feed. COBAN® is supplied as a Type A Medicated Article in 50-pound bags in two different concentrations; 45 and 60 grams of monensin activity per pound. BMD® is supplied as a Type A Medicated Article in 50-pound bags in six different concentrations; 10, 25, 30, 40, 50 and 60 grams of bacitracin methylene disalicylate activity per pound.

**ROUTE OF ADMINISTRATION:** Orally, in the feed.

**RECOMMENDED DOSAGE:**

The recommended dosage of bacitracin methylene disalicylate is 4 to 50 grams per ton in combination with 54 to 90 grams per ton of monensin sodium in Type C medicated feeds for growing turkeys.

| | |
|---|---|
| Bacitracin methylene disalicylate: | 4 to 50 grams per ton |
| Monensin sodium: | 54 to 90 grams per ton |

## IV. EFFECTIVENESS:

Battery Challenge Study:

A series of four well-controlled battery challenge studies using ten-day-old Large White, Nicholas strain turkey poults, raised under environmentally-controlled conditions, were conducted to determine the anticoccidial effectiveness of COBAN® in the presence of BMD®. The turkeys were challenged with three species of Eimeria as individual infections and as a mixed infection of all three species. These studies were conducted at Lilly Research Laboratories, P.O. Box 708, Greenfield, IN 46140. Data from the single and mixed infection studies are provided in this summary.

Experiment Nos. T1S8C8886, T1S8C86C4, T1S8C86C2 and T1S8C8875

Investigators: K.W. Bafundo, Ph.D. and D.J. Donovan
Greenfield Laboratories
P.O. Box 708
Greenfield, IN 46140

Five treatments (Tables 1 to 4) were replicated four times using four poults per replicate in ten-day-old turkeys in an environment free of coccidia prior to the initiation of the experiment. Water and medicated feed were supplied ad libitum, and proper ambient temperature and a constant lighting schedule were maintained for the duration of each experiment. A typical medicated turkey starter ration was provided for two days prior to the coccidial exposure and fed thereafter. Rations were assayed for BMD® and COBAN®. Assayed levels were found to contain appropriate levels of each drug. Forty-eight hours after the initiation of each experiment, each bird was inoculated with sporulated oocysts of one of the following Eimeria adenoeides; E. meleagrimitis; E. gallopavonis; or a combination of all the aforementioned species.

In each experiment, all treatment groups (with the exception of the noninfected, nonmedicated control group) were infected with coccidia. Parameters measured were mortality, weight gain, feed efficiency and lesion scores at six days following inoculation. Data were statistically evaluated using Analysis-of Variance. Means were compared using mean separation procedures. Means and replications are presented in Tables 1 to 4.

TABLE 1
Experiment No. T1S8C8886
Means of Percent Mortality, Weight Gain, Feed/Gain,
and Intestinal Lesion Scores of Turkeys Inoculated with E. meleagrimitis (1)

| Treatment (PPM) | Percent Mortality (2) | | Wt. Gain (3) | | Feed/Gain (4) | | Intestinal Lesion Scores | |
|---|---|---|---|---|---|---|---|---|
| | No. | Mean | No. | Mean | No. | Mean | No. | Mean |
| Noninfected, Nonmedicated Controls | 4 | 0.0 | 4 | 232.2 | 4 | 1.534 | 4 | 0.00 |
| Infected, Nonmedicated Controls | 4 | 0.0 | 4 | 220.7 | 4 | 1.549 | 4 | 3.75 |
| COBAN®, 60 | 4 | 0.0 | 4 | 230.2 | 3 | 1.532 | 4 | 1.02 |
| BMD®, 220 | 4 | 0.0 | 4 | 215.3 | 4 | 1.508 | 4 | 3.25 |
| COBAN®+BMD®, 60+220 | 4 | 0.0 | 4 | 220.6 | 4 | 1.548 | 4 | 0.88 |

(1) E. meleagrimitis STERWIN (80,000 oocysts/bird)
(2) Due to coccidiosis
(3) Per survivor
(4) Reps. without mortality

TABLE 2
Experiment No T1S8C86C4
Means of Percent Mortality, Weight Gain, Feed/Gain,
Intestinal and Cecal Lesion Scores of Turkeys Inoculated with E. gallopavonis (1)

| Treatment (PPM) | | | | | | | Lesion Scores | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Percent Mortality (2) | | Wt. Gain (3) | | Feed/Gain (4) | | Intestinal | | Cecal | |
| | No. | Mean | No. | Mean | No. | Mean | No. | Mean | No. | Mean |
| Noninfected, Nonmedicated Controls | 4 | 0.0 | 4 | 186.7 | 3 | 1.680 | 4 | 0.00 | 4 | 0.00 |
| Infected, Nonmedicated Controls | 4 | 0.0 | 4 | 146.6 | 4 | 2.115 | 4 | 2.44 | 4 | 2.25 |
| COBAN®, 60 | 4 | 0.0 | 4 | 196.4 | 3 | 1.601 | 4 | 0.00 | 4 | 0.00 |
| BMD®, 220 | 4 | 0.0 | 4 | 139.8 | 2 | 2.067 | 4 | 1.50 | 4 | 1.02 |
| COBAN®+BMD®, 60+220 | 4 | 0.0 | 4 | 215.8 | 3 | 1.598 | 4 | 0.00 | 4 | 0.00 |

(1) E. gallopavonis F.S. 646 (20,000 oocysts/bird)
(2) Due to coccidiosis
(3) Per survivor
(4) Reps. without mortality

TABLE 3
Experiment No. T1S8C86C2
Means of Percent Mortality, Weight Gain,
Feed/Gain, Cecal Lesion Scores of Turkeys Inoculated with E. adenoeides (1)

| Treatment (PPM) | Percent Mortality (2) | | Wt. Gain (3) | | Feed/Gain (4) | | Cecal Lesion Scores | |
|---|---|---|---|---|---|---|---|---|
| | No. | Mean | No. | Mean | No. | Mean | No. | Mean |
| Noninfected, Nonmedicated Controls | 4 | 0.0 | 4 | 206.9 | 4 | 1.576 | 4 | 0.00 |
| Infected, Nonmedicated Controls | 4 | 0.0 | 4 | 172.8 | 4 | 1.846 | 4 | 2.06 |
| COBAN®, 60 | 4 | 0.0 | 4 | 213.6 | 3 | 1.557 | 4 | 0.00 |
| BMD®, 220 | 4 | 0.0 | 4 | 184.7 | 4 | 1.717 | 4 | 2.06 |
| COBAN®+BMD®, 60+220 | 4 | 0.0 | 4 | 211.3 | 4 | 1.540 | 4 | 0.00 |

(1) E. adenoeides F.S. 232 (20,000 oocysts/bird)
(2) Due to coccidiosis
(3) Per survivor
(4) Reps. without mortality

TABLE 4
Experiment No T1S8C8875
Means of Percent Mortality, Weight Gain, Feed/Gain,
Intestinal and Cecal Lesion Scores of Turkeys
Inoculated with E. meleagrimitis, E. gallopavonis and E. adenoeides (1)

| Treatment (PPM) | Percent Mortality (2) | | Wt. Gain (3) | | Feed/Gain (4) | | Lesion Scores: Intestinal | | Lesion Scores: Cecal | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. | Mean | No. | Mean | No. | Mean | No. | Mean | No. | Mean |
| Noninfected, Nonmedicated Controls | 4 | 0.0 | 4 | 209.6 | 2 | 1.516 | 4 | 0.38 | 4 | 0.25 |
| Infected, Nonmedicated Controls | 4 | 0.0 | 4 | 179.2 | 2 | 1.563 | 4 | 6.63 | 4 | 1.44 |
| COBAN®, 60 | 4 | 0.0 | 4 | 240.7 | 4 | 1.354 | 4 | 2.46 | 4 | 0.75 |
| BMD®, 220 | 4 | 0.0 | 4 | 208.9 | 4 | 1.616 | 4 | 3.85 | 4 | 2.29 |
| COBAN®+BMD®, 60+220 | 4 | 0.0 | 4 | 240.6 | 3 | 1.389 | 4 | 2.94 | 4 | 0.63 |

(1) E. meleagrimitis STERWIN (20,000 oocysts/bird)
E. gallopavonis STERWIN (10,000 oocysts/bird)
E. adenoeides STERWIN (20,000 oocysts/bird)
(2) Due to coccidiosis
(3) Per survivor
(4) Reps. without mortality

The results of these tests indicate that COBAN®, in the presence of BMD®, effectively controlled coccidial infections when performance, lesion scores and mortality were evaluated. The addition of BMD® had no adverse effect on the ability of COBAN® to control coccidial infections.

Floor-Pen Non-Challenge Studies

Three floor pen studies were conducted using 1840 birds. BMD® was used at 50 grams per ton in combination with COBAN® at 90 grams per ton of feed. The experiments were designed to evaluate the effects of BMD® in the presence of COBAN® on body weight gain and feed efficiency.

Experiment No. AR-T-48-90

Investigator: Dr. Park W. Waldroup
Department of Animal Sciences
University of Arkansas
Fayetteville, AR 72701

Monitor: Ralph V. Fell, Ph.D.
A.L. Laboratories, Inc.
Route 9, Box 42
Pine Bluff, AR 71603

Poults were housed in a steel truss building with insulated roof, sidewalls, and a three foot curtain along each side. Temperature and ventilation were regulated using thermostatically-controlled fans and an automatic curtain. Evaporative cooling was employed as needed to maintain normal temperature. A thermostatically-controlled gas brooder was used to maintain brooding temperature of 90°F for the first seven days of the study with a gradual 5°F per week decrease in temperature until a 70°F temperature was maintained. Lighting was provided 23 hours per day. Pens were arranged in four rows; each pen measured 7 ft x 8 ft. Two hanging tube feeders and an automatic waterer were used in each pen. Plastic feeder lids and water jars were used for the first seven days to provide supplemental feed and water. Feed and water were provided ad libitum. The basal diets were formulated to meet or exceed all NRC nutrient recommendations. Medicated diets were prepared by mixing the indicated amount of each premix, supplied by the sponsor, with the appropriate amount of basal feed. Samples of each batch of medicated feed were assayed for drug content. Poults of the Large White, Nicholas strain were obtained from a commercial hatchery and distributed randomly by sex into 32 pens, so that 16 pens contained 18 males each and 16 pens contained 22 females each. The 32 pens used in this study permitted eight replications of males and females for each treatment group. Treatments (COBAN®, 90 grams per ton; BMD® 50 grams per ton) were assigned to pens in a randomized block design. Birds were weighed by pens at study termination (101 days-of-age for females and 115 days-of-age for males). All pens of turkeys were checked daily during the study.

Experiment No. MO-T-49-90

Investigator: Randall A. Primo
Ponderosa Research Company
French Village, MO 63036

Monitor: Ralph V. Fell, Ph.D.
A.L. Laboratories, Inc.
Route 9, Box 42
Pine Bluff, AR 71603

Poults were housed in a conventional insulated, curtain-sided house with dirt floor. Temperature and ventilation were regulated using thermostatically-controlled curtains, overhead fans equally spaced throughout the building, and fan jets and air tubes located above each alleyway extending the length of the building. Thermostatically-controlled infrared heat lamps and propane heaters were used for supplemental heating. Lighting was provided continuously throughout the study. Pens were arranged in four rows of 12 pens; each pen measured 8 ft x 8 ft. Each pen contained used shavings for litter. Fresh wood shavings were added to cover the used litter. One hanging tube feeders and an automatic waterer were used in each pen. Feed and water were provided ad libitum. The basal diets were formulated to meet or exceed all NRC nutrient recommendations. Medicated diets were prepared by mixing the indicated amount of each premix, supplied by the sponsor, with the appropriate amount of basal feed. Samples of each batch of medicated feed were assayed for drug content. Poults of the Large White, Nicholas strain were obtained from a commercial hatchery and distributed randomly by sex into 32 pens, so that 16 pens contained 19 males each and 16 pens contained 23 females each. The 32 pens used in this study permitted eight replications of males and females for each treatment group. Treatments (COBAN®, 90 grams per ton; BMD® 50 grams per ton) were assigned to pens in a randomized block design. Birds were weighed by pens at study termination (106 days-of-age for females and 125 days-of-age for males). All pens of turkeys were checked daily during the study.

Experiment No. CO-T-59-92

Investigator: Dr. Carey Quarles
Colorado Quality Research
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Monitor: James T. Skinner, Ph.D.
A.L. Laboratories, Inc.
One Executive Dr., PO Box 1399
Fort Lee, NJ 07024

Poults were housed in a controlled environment building with a concrete floor. Ventilation was accomplished by positive pressure with overhead inlet tubes. Lighting was provided 23 hours per day. Pens were arranged in two rows of 24 pens; each pen measured 4 ft x 10 ft. Each pen contained used pine shavings for litter. Litter was uniform over all pens and fresh shavings were added to cover the used litter. Two hanging tube feeders and an automatic waterer were used in each pen. Feed and water were provided ad libitum. The basal diets were formulated to meet or exceed all NRC nutrient recommendations. Medicated diets were prepared by mixing the indicated amount of each premix, supplied by the sponsor, with the appropriate amount of basal feed. Samples of each batch of medicated feed were assayed for drug content. Poults of the Large White, Nicholas strain were obtained from a commercial hatchery and distributed randomly by sex into 32 pens, so that 16 pens contained 15 males each and 16 pens contained 18 females each. The 32 pens used in this study permitted eight replications of males and females for each treatment group. Treatments (COBAN®, 90 grams per ton; BMD® 50 grams per ton) were assigned to pens in a randomized block design. Birds were weighed by pens at study termination (93 days-of-age for females and 112 days-of-age for males). All pens of turkeys were checked a minimum of twice daily during the study.

Summary of Floor-Pen Non-Challenge Studies

The three described floor-pen studies, using 1840 turkey poults (832 males and 1008 females), were conducted under conditions simulating actual field use to determine the growth promoting effects of BMD® in the presence of COBAN®. The series of studies was designed and conducted to simulate varying climatic and geographical conditions, weather, and management practices.

The General Linear Model (GLM) Procedure of the Statistical Analysis System (SAS Institute Inc., Cary, NC, 1988) was used to conduct the statistical analyses of these data. Data was analyzed separately for tom and hen turkeys. Analysis-of-Covariance was conducted for each study using a statistical model that included a covariate adjusting for differences in pen densities. Bartlett's test was performed on the mean square errors from each study. The data from all three studies was pooled for determination of treatment effects. The pooled analysis for feed efficiency data in tom turkeys consisted of a weighted Analysis-of-Covariance because Bartlett's test indicated heterogeneity of variances among studies. The weights used were the inverse of the

root mean square error for each study. The pooled analysis for average daily gain and mortality data (in hen and tom turkeys) and for feed efficiency data (in hen turkeys) was an unweighted Analysis-of-Covariance because Bartlett's test indicated that mean square errors were homogeneous. The Analysis-of-Covariance statistical model included a covariate adjusting for differences in pen density. Percent mortality was analyzed using an arcsine, square root transformation.

Results from these studies are summarized in Tables 5 and 6. The pooled study statistical analyses demonstrated that BMD® in the presence of COBAN® increases average daily gain ($P \leq 0.05$ and $P \leq 0.005$; for tom and hen turkeys, respectively) and improves feed efficiency ($P \leq 0.015$ and $P \leq 0.032$; for tom and hen turkeys, respectively). There was no effect of BMD® in the presence of COBAN® on mortality.

TABLE 5
Least-Squares Means for Average Daily Gain and Feed Efficiency for Hen Turkeys by Study and Pooled Analysis

| | COBAN® | | COBAN® + BMD® | |
|---|---|---|---|---|
| Study | Average Daily Gain (kg) | Feed Efficiency | Average Daily Gain (kg) | Feed Efficiency |
| AR-T-48-90 | 0.0744 | 2.408 | 0.0768 | 2.325 |
| MO-T-49-90 | 0.0697 | 2.515 | 0.0729 | 2.415 |
| CO-T-59-92 | 0.0835 | 2.257 | 0.0856 | 2.207 |
| Pooled Analysis | 0.0759 | 2.384 | 0.0784 | 2.326 |

TABLE 6
Least-Squares Means for Average Daily Gain and Feed Efficiency for Tom Turkeys by Study and Pooled Analysis

| | COBAN® | | COBAN® + BMD® | |
|---|---|---|---|---|
| Study | Average Daily Gain (kg) | Feed Efficiency | Average Daily Gain (kg) | Feed Efficiency |
| AR-T-48-90 | 0.1024 | 2.318 | 0.1059 | 2.271 |
| MO-T-49-90 | 0.0976 | 2.636 | 0.0996 | 2.600 |
| CO-T-59-92 | 0.1133 | 2.336 | 0.1153 | 2.310 |
| Pooled Analysis | 0.1042 | 2.428 | 0.1071 | 2.392 |

In accordance with CVM's guideline entitled "*Guideline for Drug Combinations for Use in Animals*" (October 1983), Elanco Animal Health is permitted the range of 4 to 50 grams per ton of BMD® (21 CFR § 558.76(d)(1)(i)), and 54 to 90 grams per ton of COBAN® (21 CFR § 558.355(f)(2)(i)), in Type C medicated feed. The data support range approval because both BMD® and COBAN® were previously approved for these ranges, the data demonstrate that BMD® and COBAN® in combination contributes in the manner claimed on the label at a dosage within the approved range. The data from the battery challenge studies and floor-pen non-

challenge studies demonstrate the effectiveness of COBAN® and BMD® for the prevention of coccidiosis, improved feed efficiency and increased rate of weight gain.

## V. ANIMAL SAFETY:

The basic animal safety data for the individual drugs may be found in NADA 46-592 for BMD®, and NADA 130-736 for COBAN®. The effectiveness studies described in Section IV demonstrate that no ill effects occurred when the drugs were combined indicating that they are as safe when fed in combination as when fed alone.

This application is in accord with the CVM's Target Animal Safety Guidelines for New Animal Drugs (June 1989). Additional safety studies were not required because: (1) the drugs have been approved singularly and (2) adequate documentation has been provided to show that these compounds are compatible in combination when used in turkey feeds. Therefore, based on the data in the original NADA's, the non-interference studies, the floor-pen efficacy studies and the drug residue elimination study, it is concluded that this combination of drugs may be safely fed to growing turkeys.

## VI. HUMAN FOOD SAFETY:

### A. Toxicity Tests

The basic toxicity data for the bacitracin methylene disalicylate may be found in NADA 46-592 sponsored by A.L. Laboratories, (approved 15 March 1976, 41 FR 10793; and approved 14 August 1981, 46 FR 41041); for monensin, the data may be found in NADA 38-878 sponsored by Elanco Products Co., (approved 20 May 1970, 35 FR 7734), and NADA 130-736 monensin for turkeys (approved 30 April 1987, 52 FR 15718).

### B. Tolerances and Safe Concentration of Residues

The tolerance for residues of bacitracin methylene disalicylate (BMD®) in uncooked edible tissues is established at 0.5 ppm as negligible residue, (21 CFR § 556.70). A Tolerance for monensin (COBAN®) residues is not needed (21 CFR § 556.420).

The safe concentrations for total monensin in uncooked edible turkey tissues are: 1.5 ppm in muscle, 3.0 ppm in skin with adhering fat, and 4.5 ppm in liver.

### C. Tissue Residue Depletion Studies

A tissue residue study was conducted to demonstrate that there is no change in the residue depletion pattern for each drug when bacitracin methylene disalicylate and monensin were fed to turkeys in a two-drug combination. There were two segments to the study: (1) validation of the assay by assaying spiked tissue samples with the cited drugs, and (2) assays for bacitracin methylene disalicylate and monensin conducted on tissue samples harvested from medicated birds raised in commercial production. Five male and five female turkeys were

medicated with monensin (90 g/ton) and BMD® (200 g/ton) for 27 days and slaughtered at zero withdrawal (6 hr). The tissues were assayed for monensin (skin fat) and BMD® (muscle). All tissues tested showed less than 0.04 ppm of monensin and less than 0.3 ppm of BMD®, which are the respective limits of detection for these assays. These results are comparable to those obtained when each drug is administered alone.

These data confirm that each drug in the presence of the other does not exceed its approved safe concentration or tolerance. Therefore, these data support a zero withdrawal period for human consumption of turkeys treated with bacitracin methylene disalicylate plus monensin in the feed under CVM's combination drug policy.

**D. Assay Non-interference Study**

1. Bacitracin Methylene Disalicylate Assay

   Tissue assay non-interference and method validation studies for bacitracin methylene disalicylate tissue assay were conducted by spiking control samples with bacitracin methylene disalicylate and monensin and then assaying for bacitracin methylene disalicylate residues. The assay results demonstrated that there is no interference by monensin for bacitracin methylene disalicylate. (AAC8722).

2. Monensin Assay

   Tissue assay non-interference and method validation studies for monensin tissue assay were conducted by spiking control samples with bacitracin methylene disalicylate and monensin and then assaying for monensin residues. The assay results demonstrated that there is no interference by bacitracin methylene disalicylate for monensin. (AAC8722).

**E. Regulatory Methods**

1. Bacitracin

   Antibiotic Residue in Milk, Dairy Products and Animal Tissues: Methods, Reports, Protocols. National Center for Antibiotic and Insulin Analyses. Dept. HEW Washington, DC 20204, Rev. October 1968. Modified Method for Determination of Bacitracin in Tissue, Test Procedure Code 9A, A.L. Laboratories Inc., One Executive Drive, P.O. Box 1399, Fort Lee, NJ 07024

2. Monensin

   Determination of Monensin in Tissues and Eggs. Method 5801654. Eli Lilly and Company, Box 708, Greenfield, IN 46140.

## VII. AGENCY CONCLUSIONS:

The data submitted in support of this NADA comply with the requirements of Section 512 of the Act and demonstrate that COBAN® (54-90 g/ton) plus BMD® (4-50 g/ton) are safe and effective for the claims indicated in Section II of this FOI summary.

Pursuant to 21 CFR § 514.106 (b)(2), this combination NADA approval is regarded as a Category II supplemental change which did not require a reevaluation of safety and efficacy data in the parent NADAs. The drugs are to be fed in Type C medicated feeds, in accordance with Section II and III of the FOI Summary and the Blue Bird labeling that is attached to this document.

Residue data show monensin is well within the established safe concentrations of 4.5 ppm in liver, 3.0 ppm skin/fat and 1.5 ppm muscle of the turkey at zero withdrawal. Residue data show bacitracin methylene disalicylate is well below tolerance of 0.5 ppm in edible turkey tissues at zero withdrawal.

The battery challenge studies demonstrated that COBAN® in the presence of BMD® prevented coccidiosis when the birds were exposed to the three major species of *Eimeria* infecting turkeys. The data from three floor-pen non-challenge studies demonstrate the effectiveness of BMD® (50 g/ton) in the presence of COBAN® (90 g/ton) for increased rate of weight gain and improved feed efficiency. In accordance with CVM's guideline entitled "*Guideline for Drug Combinations for Use in Animals*" (October 1983), Elanco Animal Health is permitted the range of 4 to 50 grams per ton of BMD®, and 54 to 90 grams per ton of COBAN® in the Type C medicated feed for prevention of coccidiosis caused by Eimeria adenoeides, E. meleagrimitis, and E. gallopavonis; and for increased rate of weight gain and improved feed efficiency in turkeys, as shown in Section II of this FOI summary.

Under section 512(c)(2)(F)(ii) of the FFDCA, this approval for food producing animals qualifies for three years of marketing exclusivity beginning on the date of approval because the application contains reports of new clinical or field investigations (other than bioequivalence or residue studies) essential to the approval of the application and conducted or sponsored by the applicant.