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## SPONSOR

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## NV REPORT NUMBER

X5L337

## STUDY COMPLETION DATE

February 6, 1996

## TEST ARTICLE IDENTIFICATION

Etherium Gold  
Lot Number: 109845

## TEST PERFORMED

FDA Acute Oral Toxicity Screen of  
Food and Color Additives used in Food

## STUDY DIRECTOR

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**STUDY DATES**

Study Initiation Date: January 15, 1996  
Study Completion Date: February 6, 1996  
Date On Test: January 15, 1996  
Date Off Test: January 29, 1996  
Date Sample Received: December 27, 1995

**TEST ARTICLE IDENTIFICATION**

Etherium Gold  
1 amber glass bottle containing 56.7 g of powder  
Lot Number: 109845  
Storage Condition: Room Temperature

**SUMMARY OF RESULTS**

A dose of 5000 mg/kg of the test material, administered orally, resulted in no mortality to five male and five female Sprague-Dawley rats.



## ACUTE ORAL TOXICITY

### INTRODUCTION

*This procedure is designed to determine the acute oral toxicity of the substance under test.*

*Ten Sprague-Dawley rats, five males and five females, receive a 5,000 mg/kg oral dose of the test material. Two additional rats, one male and one female, are dosed with the vehicle as a control. The animals are weighed prior to dosing, on Day 7 and at the conclusion of the study. They are observed twice daily during the study and any toxic signs are noted. On Day 14, the animals are euthanized and necropsied. The final report contains records of mortality and any toxic signs observed during either the clinical observations or the necropsy.*

### MATERIALS AND METHODS

#### TEST SYSTEM

|                   |                                                                                                                                                 |
|-------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|
| Species           | Rat                                                                                                                                             |
| Strain            | Albino Sprague-Dawley                                                                                                                           |
| Source            | Simonsen Laboratories, Gilroy, CA                                                                                                               |
| Sex               | 5 males and 5 females                                                                                                                           |
| Weight            | Males: 222.0 - 234.0 grams<br>Females: 185.1 - 200.0 grams                                                                                      |
| Age               | 7-10 weeks                                                                                                                                      |
| Housing           | In polycarbonate plastic cages, by sex in groups of no more than five; maintained at 18 - 26° C and 55 ± 15% relative humidity.                 |
| Feed              | Purina Laboratory Rodent Diet #5001 water <i>ad libitum</i> . Food was withheld 22 hours prior to dosing and was restored 3 hours after dosing. |
| Identification    | By cage cards identifying dose group and sex, and by tail marking within dose groups.                                                           |
| Photoperiod       | Diurnal (12 hours on - 12 hours off)                                                                                                            |
| Quarantine Period | Five days                                                                                                                                       |

#### JUSTIFICATION FOR TEST SYSTEM

Rats are the recommended species for acute oral testing by the USFDA Center for Food Safety and Applied Nutrition, Redbook I NTIS Document PB83-170896





**Table 1  
Supplies**

| Item                                                              | Lot Number | Manufacturer         | Expiration Date |
|-------------------------------------------------------------------|------------|----------------------|-----------------|
| Sodium Pentobarbital                                              | F4A006     | Anpro Pharmaceutical | 1/1/96          |
| Perfectum® Stainless Steel Animal Feeding Needle (3 in, 16 guage) | NA         | Popper & Sons        | NA              |
| Sterile Water for Injection, USP (WFI)                            | C303735    | Baxter               | 9/1/96          |
| Carboxymethylcellulose                                            | 32H0921    | Sigma                | 10/1/97         |

**Table 2  
Study Design**

| Group     | Number | Sex | Route of Administration | Dose (mg/kg) | Vehicle          | Dose Volume |
|-----------|--------|-----|-------------------------|--------------|------------------|-------------|
| Test 1    | 5      | M   | Oral                    | 5000         | WFI <sup>1</sup> | 10 mL/kg    |
| Test 2    | 5      | F   | Oral                    | 5000         | WFI              | 10 mL/kg    |
| Control 1 | 1      | M   | Oral                    | NA           | WFI              | 10 mL/kg    |
| Control 2 | 1      | F   | Oral                    | NA           | WFI              | 10 mL/kg    |

1. Sterile Water for Injection, USP.

### SAMPLE PREPARATION AND DOSING PROCEDURE

*Animal Preparation* - Ten healthy rats, five males and five females, were used to determine the oral toxicity of the test material. Twenty two hours prior to dosing, food was withheld. Food was restored three hours after dosing.

*Sample Preparation* - The test material was suspended at a concentration of 0.5 g/ml in a solution of 0.5 percent Carboxymethylcellulose (CMC) prepared in WFI.

*Dosing Procedure* - The dose was administered by means of a 3 inch metal intubation needle attached to a 3 mL plastic hypodermic syringe. The test solution was drawn into the syringe and administered orally at a volume of 10 mL/kg.

Two rats one male and one female, were dosed with deionized water at a dose of 10 mL/kg as controls.

*Euthanasia* - At the end of the study, the animals were euthanized with an intraperitoneal dose of Sodium Pentobarbital, Injection (1 mL).



## OBSERVATIONS

*Clinical Observations* - All of the animals were observed several times in the three hours after dosing and at least twice a day for fourteen days after that. The animals were observed for clinical signs of toxicity such as unkempt appearance, altered feeding habits, weight loss, and other signs of distress or physical depression, and for any signs of recovery from these signs. These signs were recorded for each animal exhibiting them. Observations included onset, description, and duration. If animals were found moribund they were isolated and if necessary, euthanized.

*Weights* - All of the animals were weighed on Day 0 (prior to test material administration), Day 7, Day 14 and at death.

*Necropsy* - Gross necropsies were performed on all animals that died during the study. Animals that survived the fourteen day observation period were euthanized and necropsied on Day 14. All gross abnormalities were recorded.

**Table 3**  
**Study Schedule**

| Time                              | Procedure                                                                          |
|-----------------------------------|------------------------------------------------------------------------------------|
| Day -1                            | Food withheld for approximately twenty-two hours                                   |
| Day 0                             | Weighing and Dosing                                                                |
| Day 0 (0 to 3 Hours after dosing) | Observation of animals (necropsy if needed)                                        |
| Day 0 (3 Hours after dosing)      | Food provided <i>ad libitum</i>                                                    |
| Days 1 to 6                       | Daily observation (necropsy if needed)                                             |
| Day 7                             | Daily observation, weighing and necropsy as needed                                 |
| Days 8 to 13                      | Daily observation (necropsy if needed)                                             |
| Day 14                            | Daily observation, weighing, euthanization and necropsies of any surviving animals |

## RESULTS AND DISCUSSION

*Clinical Observations* - The clinical observations are summarized in Table 4. No toxic signs were observed during the 14 day observation period.

*Weights* - All of the animals gained weight and remained healthy during the test period (see Table 5).

*Necropsy* - Upon gross necropsy, no abnormalities were observed in the test or control animals (see Table 6).

## CONCLUSION

At an oral dose of 5000 mg/kg of body weight in male and female Sprague-Dawley rats, this test material produced no mortalities.



**Table 4**  
**Summary of Clinical Observations**

| Day After Dosing | Male      |                | Female    |                |
|------------------|-----------|----------------|-----------|----------------|
|                  | No. Alive | Clinical Signs | No. Alive | Clinical Signs |
| 0                | 5         | 5 NT           | 5         | 5 NT           |
| 1                | 5         | 5 NT           | 5         | 5 NT           |
| 2                | 5         | 5 NT           | 5         | 5 NT           |
| 3                | 5         | 5 NT           | 5         | 5 NT           |
| 4                | 5         | 5 NT           | 5         | 5 NT           |
| 5                | 5         | 5 NT           | 5         | 5 NT           |
| 6                | 5         | 5 NT           | 5         | 5 NT           |
| 7                | 5         | 5 NT           | 5         | 5 NT           |
| 8                | 5         | 5 NT           | 5         | 5 NT           |
| 9                | 5         | 5 NT           | 5         | 5 NT           |
| 10               | 5         | 5 NT           | 5         | 5 NT           |
| 11               | 5         | 5 NT           | 5         | 5 NT           |
| 12               | 5         | 5 NT           | 5         | 5 NT           |
| 13               | 5         | 5 NT           | 5         | 5 NT           |
| 14               | 5         | 5 NT           | 5         | 5 NT           |

NT = No toxic signs



**Table 5**  
**Animal Weights**

| <b>Animal Number</b> | <b>Sex</b> | <b>Dose Volume (mL)</b> | <b>Day 0 Wt(g)</b> | <b>Day 7 Wt(g)</b> | <b>Day 14 Wt(g)</b> | <b>Weight Gain Wt(g)</b> |
|----------------------|------------|-------------------------|--------------------|--------------------|---------------------|--------------------------|
| 1                    | F          | 1.9                     | 190.7              | 234.8              | 235.8               | 45.1                     |
| 2                    | F          | 1.9                     | 193.7              | 223.5              | 243.5               | 49.8                     |
| 3                    | F          | 1.9                     | 194.9              | 218.2              | 248.2               | 53.3                     |
| 4                    | F          | 1.9                     | 185.1              | 214.9              | 240.6               | 55.5                     |
| 5                    | F          | 1.9                     | 189.8              | 216.1              | 235.4               | 45.6                     |
| Mean:                |            |                         | 190.8              | 221.5              | 240.7               | 49.9                     |
| Standard Deviation:  |            |                         | 3.8                | 8.1                | 5.4                 | 4.6                      |
| 1                    | M          | 2.3                     | 234.0              | 269.3              | 315.7               | 81.7                     |
| 2                    | M          | 2.2                     | 222.0              | 247.4              | 284.8               | 62.8                     |
| 3                    | M          | 2.3                     | 228.6              | 263.7              | 311.5               | 82.9                     |
| 4                    | M          | 2.3                     | 233.4              | 274.1              | 323.8               | 90.4                     |
| 5                    | M          | 2.3                     | 227.4              | 256.1              | 291.1               | 63.7                     |
| Mean:                |            |                         | 229.1              | 262.1              | 305.4               | 76.3                     |
| Standard Deviation:  |            |                         | 4.9                | 10.6               | 16.7                | 12.4                     |
| <b>Controls</b>      |            |                         |                    |                    |                     |                          |
| 1                    | F          | 2.0                     | 200.0              | 223.5              | 236.3               | 36.3                     |
| 2                    | M          | 2.3                     | 233.2              | 285.0              | 316.1               | 82.9                     |





**Table 6**  
**Observations at Necropsy**

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| <b>Animal No.</b> | <b>Sex</b> | <b>Observations</b>       |
|-------------------|------------|---------------------------|
| 1                 | M          | No abnormalities observed |
| 2                 | M          | No abnormalities observed |
| 3                 | M          | No abnormalities observed |
| 4                 | M          | No abnormalities observed |
| 5                 | M          | No abnormalities observed |
| <br>              |            |                           |
| 1                 | F          | No abnormalities observed |
| 2                 | F          | No abnormalities observed |
| 3                 | F          | No abnormalities observed |
| 4                 | F          | No abnormalities observed |
| 5                 | F          | No abnormalities observed |
| <br>              |            |                           |
| <b>Controls</b>   |            |                           |
| 1                 | M          | No abnormalities observed |
| 2                 | F          | No abnormalities observed |

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**PROCEDURE REFERENCE**

NV SOP 16G-38

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**NORTHVIEW PACIFIC LABORATORIES, INC.**

*Robert A. Noonan* 2/9/96

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