Background on the Freeze-dried Whole Grape Powder

1. The grape powder provided by the California Table Grape Commission (CTGC) is made solely from whole California grapes; it is not an extract.

2. Any results observed in feeding studies using grape powder should be directly attributed to grape consumption. The grapes have been provided in freeze-dried, powdered form. The powder is not and will not be made available for commercial use or sale. It was created solely for the purpose of providing researchers with access to California grapes year-round, to ensure solid, reproducible data.

3. The freeze-dried grape powder is made from a composite of fresh red, green and black California grapes, based on actual consumption patterns of consumers. It is a mix of seeded and seedless varieties that have been frozen, ground with food-quality dry ice, freeze-dried, and re-ground using Good Manufacturing Practices for food products throughout. The powder was processed and stored to preserve the integrity of the biologically active compounds found in fresh grapes. As with fresh grapes, the powder is known to contain polyphenols, including resveratrol, flavans (including catechin), flavonols (including quercetin), anthocyanins and simple phenolics.

4. One serving of fresh grapes (3/4 cup or 126 g) is equivalent to 23 g of the CTGC freeze-dried whole grape powder.

GRAPE POWDER USE IN CELL STUDIES

For cell studies, the grape powder must be made into an extract. The commission will provide the grape powder extract needed for funded proposals conducting in vitro work.
GRAPE POWDER USE IN ANIMAL FEEDING STUDIES

For animal studies, the grape powder should be incorporated into the animal diet to ensure control over the amount fed and consumed. This should be done in a manner that will minimize exposure to heat processes and oxidation. One recommended approach is to add it into the powder diet being fed to the animals (such as a semi-purified AIN diet powder). Another recommended approach is to incorporate the grape powder into the animal diet pellets.

Grape powder should NOT be provided in the animal’s drinking water. In water it doesn’t dissolve, but creates a fairly thick suspension which may not be accepted well by mice, and could inhibit their ability to get the full dose of grape powder. Grape powder can be administered as a suspension by gavage to ensure the entire dose of grapes is consumed, however this typically requires special expertise and care to avoid animal loss.

Whenever adding grape powder to the diet, it is important to make sure that the control diets are normalized to the experimental diets so that the percentage of calories from protein, carbohydrate and fat are matched. Additionally, the control for animal diets containing grapes should be matched for sugar type: ripe grapes contain about half glucose and half fructose (not sucrose) and the grape preparation contains about 90% sugar.

Grape powder should always be stored in the freezer at or below – 20° C. It is hygroscopic in nature, and tends to absorb moisture and clump if exposed to humidity.

Recommended dosage range for animal studies: 3 to 5% grape powder in the diet. (Where 100 g of the final diet contains between 3 and 5 g of grape powder.)

GRAPE POWDER USE IN HUMAN CLINICAL STUDIES

In human studies, the grape powder should be mixed with water, and then consumed all at once as a drink. The following protocol (Addendum A) suggests 6 oz. of water to 46 g of powder, but the amount of water may be adapted to subject’s tolerance level, as long as the full dose of grape powder is consumed within 30 minutes.

The grape powder will be packaged in vacuum-sealed pouches according to the dose approved for the study. The grape powder is very hygroscopic, so must be protected from moisture and water until reconstituted. Subjects should keep their powder pouches stored in the freezer at all times.

A grape powder placebo is available for use in control diets.

Recommended dosage range for human studies: between 46 g – 115 g per day (to provide between 2 to 5 servings of grapes per day).
## ADDENDUM A

**Suggested Dosing Protocol for Grape Powder – 46 g Dose**

### Important Information:
- Material should be stored in moisture impermeable packaging at -70°C until weighing.
- Hygroscopic material: protect from water until reconstituted.
- Dose subject within 30 minutes of reconstitution.
- Re-shake material just prior to dosing.

### Purpose:
To disperse 46 g of grape powder in 180 mL (6 fl. oz.) of water. *A larger dose may require more water.

### Equipment:
- Ziploc Snap ‘n Seal XS container (1 cup) or equivalent
- Volumetric measuring device
- Off-the-shelf distilled water (for reconstitution and rinse)

### Procedure:

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<thead>
<tr>
<th>Step</th>
<th>Instructions</th>
<th>Signature/Date</th>
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<tbody>
<tr>
<td>1.</td>
<td>Weigh 46 g of grape powder into Ziploc Snap ‘n Seal XS container and record actual weight. Actual Weight of Powder  g</td>
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<tr>
<td>2.</td>
<td>Add approximately 180 mL (6 fl. oz.) of water to container with grape powder. Record time of reconstitution. Time of Reconstitution</td>
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<tr>
<td>3.</td>
<td>Close lid tightly and shake for a minimum of 30 seconds.</td>
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<td>4.</td>
<td>Visually confirm that no un-wetted powder remains. Continue shaking if needed.</td>
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<td>5.</td>
<td>Just prior to dosing subject, shake material for a minimum of 30 seconds.</td>
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<tr>
<td>6.</td>
<td>Dose reconstituted grape powder to subject. Note: must be dosed within 30 minutes of reconstitution. Time of Dosing</td>
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<tr>
<td>7.</td>
<td>Rinse container with at least 30 mL (1 fl. oz.) of water.</td>
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<td>8.</td>
<td>Dose rinse water to subject.</td>
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