

SOP# BC1010
Liquid Diet Administration – A Rat Model of FASD

Last revised: 20 March 2015

REVISION HISTORY

	<i>Revision Date</i>	<i>Author</i>	<i>Position</i>
1.	20 March 2015	Christine Fontaine	Graduate Student
	19 May 2016	Christine Fontaine	Graduate Student

PURPOSE

To describe the process of making and administering a liquid diet to rats in order to generate an animal model of FASD in offspring.

TRAINING

You must have completed the online tutorial, a facility orientation and hands-on training for the procedure listed.

You must be trained in the following SOPs;

- a. [AC2007: Rodent-Handling and Injection Techniques](#)

POLICY

The University of Victoria will follow the current guidelines of the CCAC (Canadian Council on Animal Care) and the University of Victoria Occupational Health, Safety & Environment. All staff members will be trained in the Workplace Hazardous Materials Information System (WHMIS).

DESCRIPTION

A liquid diet is a method by which researchers can most accurately generate rodent models of fetal alcohol spectrum disorders (FASDs). The liquid diet is designed to deliver all daily requirements of vitamins, minerals and calories to the animal and is mixed with water and ethanol for ethanol dams or with water only for pair-fed control dams. In this model, 35.5% of calories provided to ethanol dams are derived from ethanol, which achieves a blood alcohol concentration (BAC) between 80-180mg/dl. See Patten et al., 2014 for a review of animal models of FASD.

RESPONSIBILITY

Researchers in the Christie laboratory.

MATERIALS

- Liquid diet powders (Ethanol & Control; obtained from Dyets Inc.; Weinberg-Keiver diet)
- 95% Ethanol
- Distilled water
- Graduated Cylinder
- Blender
- 2 x 1L clean glass bottles
- Scale
- Feeding Data Sheets (see appendix)

PROCEDURE

1. Making Liquid Diet

- a. Find diet of choice in freezer (Either Ethanol or Pair-fed control)
- b. For 1L of Ethanol Diet:
 - a. Weigh 156.1 g of frozen ethanol diet
 - b. Add 66.4ml of 95% EtOH via a clean graduated cylinder to the diet in a blender

- c. Fill to 1L with dH2O via a second graduated cylinder for specific use with dH2O
 - d. Blend thoroughly
 - e. Pour into clean glass diet bottle and label bottle with type of diet, contents, date of preparation and your initials as per WHIMS regulations
 - f. Store diet in fridge for up to one week before replacing with fresh diet
- c. For 1L of Pair-Fed Control Diet:
- a. Weigh 245.76g of frozen control diet
 - b. Add diet to blender and fill to 1L with dH2O using a graduated cylinder for specific use with dH2O
 - c. Blend thoroughly
 - d. Pour into clean glass diet bottle and label bottle with type of diet, contents, date of preparation and your initials as per WHIMS regulations
 - e. Store diet in fridge for up to one week before replacing with fresh diet

Note: For feeding of a single rat (or two), prepare only 500mL of each diet

2. Administering Liquid Diet to Pregnant Dams

Note: Feeding should take place between **2:30 and 5:00pm** every day of pregnancy

- a. Follow SOP# AC2030 to confirm the presence of sperm via a vaginal lavage using the eye dropper technique as early as possible in the morning
- b. Immediately following confirmation of presence of sperm:
 - a. Remove all solid chow
 - b. Assign to either the EtOH or Pair-fed group
 - c. Write current date as GD1 on cage card & label with group and write 'PREGNANT' on top of cage card
 - d. Add "DO NOT FEED" sign in front of cage card
 - e. Weigh dam & record weight on liquid diet sheet for that dam (see Figure 1 and Appendix)
- c. EtOH dam feeding:
 - a. Collect a clean water bottle and stopper
 - b. Place on scale & tare
 - c. On first day (**GD1**), add ~100g of pair-fed diet and ~50g of EtOH diet
 - d. Remove bottle & stopper from scale and tare
 - e. Record total weight of diet, bottle and stopper on the liquid diet sheet for that dam (see Figure 1 of Appendix) under "Food Supplied"
 - f. Place on cage top of that dam such that the rat can access the spout

- g. On the subsequent day (**GD2**), weigh the total weight of the diet, bottle and stopper remaining after first day of diet administration under “Food Remaining” column
 - h. Empty bottle and clean thoroughly
 - i. Place bottle & stopper on scale & tare
 - j. Add ~50g of pair-fed diet and ~100g of EtOH diet
 - k. Remove bottle & stopper from scale and tare
 - l. Record total weight of diet, bottle and stopper on the liquid diet sheet for that dam (See Figure 1 of Appendix) under “Food Supplied”
 - m. Place on cage top of that dam such that the rat can access the spout
 - n. On the following day (GD3), weigh the total weight of the diet, bottle and stopper remaining after second day of diet administration under “Food Remaining” column
 - o. Empty bottle and clean thoroughly
 - p. Place bottle & stopper on scale DO NOT TARE
 - q. Add ~150g of the EtOH diet. You can match to approximate total weights from previous two days
 - r. Record this total weight of the diet, bottle and stopper on the liquid diet sheet for that dam (See Figure 1 and Appendix) under “Food Supplied”
 - s. For all subsequent days, follow steps **n-r**, providing ~150g of EtOH diet each day. Record weight of dam when specified by liquid diet sheet (See Figure 1 of Appendix; GD 1, 7, 14, 21)
 - t. On final day (GD22) weigh food remaining from GD21 and replace diet bottle with solid rat chow. Rat will remain on solid rat chow for the remainder of its life.
- d. Pair-fed dam feeding:
- a. Match rat to a rat previously administered EtOH diet based on weight and age.
 - b. Enter food consumption and weight information for that paired EtOH dam in online spreadsheet (See Figure 2 of Appendix)
 - c. Enter weight of pair-fed dam
 - d. The spreadsheet will calculate the weight of food to be provided to pair-fed rat based on food consumption of paired EtOH dam.
 - e. Copy these values in top left-hand corners of “Food Supplied” column of specified days on the liquid diet sheet for the pair-fed dam
 - f. After each weighing of Pair-fed dam, enter weights into spreadsheet in order to calculate the amount of food to provide for subsequent days
 - g. For feeding:
 - i. Place clean water bottle and stopper on scale & tare

- ii. Add specified weight of pair-fed diet calculated by spreadsheet
- iii. Remove bottle & stopper from scale and tare
- iv. Weigh total weight of diet, bottle and stopper and record on liquid diet sheet (see Figure 1 of Appendix) under “Food Supplied”
- v. Place bottle on cage such that the rat can reach the spout
- vi. On the following day, record the total weight of the diet, bottle and stopper under “Food Remaining” on liquid diet sheet
- vii. Repeat i-vi for all days of gestation. Record weight of dam when specified by liquid diet sheet (See Figure 1 of Appendix; GD 1, 7, 14, 21).
- viii. On final day (GD22) weigh food remaining from GD21 and replace diet bottle with solid rat chow. Rat will remain on solid rat chow for the remainder of its life.

Notes:

- Check bottle stoppers to ensure that diet has not dried onto the ball and sealed the spout.
- All animals have *ad libitum* access to water
- Monitor food consumed and animal weights as per animal use protocol and report to your observations to your direct supervisor in the laboratory.
- If you see pups with the dam or that the dam is giving birth, do not disturb and report your observations to your direct supervisor in the laboratory.

REFERENCES

http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/GUIDES/ENGLISH/toc_v1.htm

Patten, AR, Fontaine, CJ and Christie, BR. (2014). A comparison of the different animal models of fetal alcohol spectrum disorders and their use in studying complex behaviours. *Frontiers in Pediatrics*. 2: 93.

Appendix

Weigh on specified days during pregnancy (Days 1,4,14,21)

For first two days of gestation, mix EtOH diet with Pair-fed diet as specified for a total of ~ 150g of diet

Record total weight remaining of diet, bottle and stopper combined

Ignore. This is calculated electronically

Write your initials after feeding

Add ~150g of EtOH diet and record the total weight of the diet, bottle and stopper in this column

DAM#: _____ DOB of Dam: _____ Litter ID: _____
 Condition (circle): ETHANOL PAIR_FED CONTROL
 Matched w/: _____
 (Weigh on 1st Breeding Day and GD 1, 7, 14, and 21)

Date: dd/mm/yy	Gestation Day	Dam Weight (g)	Food Supplied (g) ^{***}	Food Remaining (24 hr later) (g) [*]	Food Consumed (g)	Notes:
	N/A	NA	NA	NA	NA	First Breeding Day Male #:
	GD1		1/3 EtOH			Plug Found: Start Controlled Diet
	GD2	NA	2/3 EtOH			
	GD3	NA	3/3 EtOH			
	GD4	NA				
	GD5	NA				
	GD6	NA				
	GD7	NA				
	GD8	NA				

Figure 1. Layout of Liquid Diet Feeding Sheets. Each dam is accompanied by a liquid diet feeding sheet to measure and monitor food consumption throughout gestation. Follow instructions specified in this figure in order to fill out forms correctly. These diet sheets are kept in a binder in the colony room and are permanently stored in the laboratory once animal is euthanized. On the final day, as specified by this sheet (GD22), all animals (EtOH and Pair-fed) are switched from the liquid diet to regular solid rat chow.