

# Chemical Hygiene Plan

## Important Telephone Numbers:

1. 911 for All Emergencies
2. (435) 797-1939 Utah State University Police Department (Non-Emergency Line)
3. (435) 716-9500 Logan Fire Department (Non-Emergency Line)

## 1. Purpose

This chemical hygiene plan establishes a formal written program for protecting users against health and safety hazards associated with exposure to hazardous chemicals or field conditions that may lead to accident or injury. This chemical hygiene plan (CHP) describes the proper use and handling procedures to be followed by faculty, staff, and all other personnel working with hazardous chemicals in laboratory settings (NR 309) or in the field (Green Canyon Ecology Center and Irrigated Pasture Project).

## 2. Employee Rights and Responsibilities

Employees and other personnel who work at the aforementioned locations have the right to be informed about the potential hazards of the chemicals in their work areas and to be properly trained to work safely with these substances. All personnel, including principal investigators, laboratory supervisors, laboratory technicians, student workers, and support staff have a responsibility to maintain a safe work environment. All personnel working with chemicals are responsible for staying informed on the chemicals in their work areas, safe work practices and SOPs, and proper personal protective equipment (PPE) required for the safe performance of their laboratory work.

### A. Laboratory Supervisor Responsibilities

The Laboratory Supervisor is the individual that is ultimately responsible for the overall laboratory and field operations, ensuring that the requirements of the CHP are followed by all staff members that work in the lab and in the field. The Principal Investigator (PI), Juan Villalba, is the Laboratory and Field Supervisor. The Laboratory and Field Supervisor may delegate some safety duties to a qualified individual, but ultimately remains responsible for the safety of all personnel working in the laboratory. Specifically, the Supervisor must:

1. Understand applicable environmental health and safety rules, including the contents of the CHP;
2. Identify hazardous conditions or operations in the laboratory and field and establish SOPs and hazard assessments to effectively control or reduce hazards;
3. Ensure that all laboratory personnel that work with hazardous chemicals receive appropriate training (see <https://rgs.usu.edu/ehs/laboratory-safety-initial/> for laboratory safety training at USU and lab training registration)

4. Maintain written records of lab-specific training
5. Ensure that appropriate Personal Protective Equipment (PPE) (e.g., laboratory coats, gloves, eye protection, etc.) and engineering control equipment (e.g., chemical fume hood) are made available, in good working order, and being used properly.
6. Conduct periodic lab inspections and immediately take steps to abate hazards that may pose a risk to life or safety upon discovery of such hazards; and

### **B. Laboratory Employee Responsibilities**

All employees (e.g., graduate research assistants, graduate students, undergraduate students, lab technicians, post-doctoral researchers, and visiting scientists) in laboratories that use, handle, or store hazardous chemicals must:

1. Review and follow the requirements of the CHP.
2. Follow all verbal and written laboratory safety rules, regulations, and SOPs required for the tasks assigned.
3. Develop and practice good personal chemical hygiene habits such keeping work areas clean and uncluttered.
4. Plan, review, and understand the hazards of materials and processes in the laboratory prior to conducting work.
5. Utilize appropriate measures to control hazards, including consistent and proper use of engineering controls, administrative controls, and PPE.
6. Understand the capabilities and limitations of PPE.
7. Immediately report all accidents, near misses, and unsafe conditions to the Laboratory Supervisor.
8. Complete all required mandatory safety training and provide written documentation to the Laboratory Supervisor;
9. Inform the Laboratory Supervisor of any work modifications ordered by a physician as a result of medical surveillance, occupational injury, or chemical exposure.

### **3. Hazardous Chemical Classification Systems**

Chemical classification systems are designed to communicate hazards. The three most widely used classification systems are the OSHA Globally Harmonized System for Classifying and Labeling Chemicals (implemented under the OSHA Hazard Communication Standard), the National Fire Protection Association (NFPA) system of classifying the severity of hazards, and the Department of Transportation (DOT) hazard classes. These classification systems are used by chemical manufacturers when creating safety data sheets and chemical labels, therefore it is important that lab employees understand the basic elements of each classification system.

## A. Globally Harmonized System for Classifying Chemicals

The Globally Harmonized System (GHS) is a world-wide system adopted by OSHA for standardizing and harmonizing the classification and labeling of chemicals. The objectives of the GHS are to:

- Define health, physical, and environmental hazards of chemicals;
- Create classification processes that use available data on chemicals for comparison with the defined hazard criteria (numerical hazard classification is based on a 1 – 5 scale, 1 being the most hazardous and 5 being the least hazardous); and
- Communicate hazard information, as well as protective measures, on labels and Safety Data Sheet (SDS), formerly known as Material Safety Data Sheets (MSDS).

### Safety Data Sheets

The SDS provides comprehensive information that is imperative for the safe handling of hazardous chemicals. Laboratory personnel should use the SDS as a resource to obtain information about hazards and safety precautions. SDSs cannot provide information for hazards in all circumstances. However, the SDS information enables the employer to develop an active program of worker protection measures such as training on hazard mitigation. Chemical manufacturers are required to use a standard format when developing SDSs. The SDS will contain 16 headings which are illustrated in Figure 1: GHS Required sections of a safety data

1.	Identification of the substance or mixture and of supplier	9.	Physical and chemical properties
2.	Hazards identification	10.	Stability and reactivity
3.	Composition/information on ingredients	11.	Toxicological information
4.	First aid measures	12.	Ecological information
5.	Firefighting measures	13.	Disposal considerations
6.	Accidental release measures	14.	Transport considerations
7.	Handling and storage	15.	Regulatory information
8.	Exposure controls/personal protection	16.	Other information

sheet.

### Chemical Labeling

The GHS standardized label elements, which are not subject to variation and must appear on the chemical label, contain the following elements:

- 1) Symbols (hazard pictograms) are used to convey health, physical and environmental hazard information, assigned to a GHS hazard class and category;
- 2) Signal Words such as "Danger" (for more severe hazards) or "Warning" (for less severe hazards), are used to emphasize hazards and indicate the relative level of severity of the hazard assigned to a GHS hazard class and category;
- 3) Hazard statements (e.g., "Danger! Extremely Flammable Liquid and Vapor") are standard phrases assigned to a hazard class and category that describe the nature of the hazard; and
- 4) Precautionary statements are recommended measures that should be taken to minimize or prevent adverse effects resulting from exposure to the hazardous chemical.

Figure 2. GHS Hazard Pictograms


Figure 3. GHS Hazard classification system for flammable

Category	Criteria	Pictogram	Signal Word	Hazard Statement
1	Flash point < 23 °C Boiling point ≤ 35 °C		Danger	Extremely flammable liquid and vapor
2	Flash point < 23 °C Boiling point > 35 °C		Danger	Highly flammable liquid and vapor
3	Flash point ≥ 23 °C and < 60 °C		Warning	Flammable liquid and vapor
4	Flash point ≥ 60 °C and ≤ 93 °C		Warning	Combustible liquid
5	There is no Category 5 for flammable liquids			

liquids

## B. National Fire Protection Association Rating System

The NFPA system uses a diamond-shaped diagram of symbols and numbers to indicate the degree of hazard associated with a particular chemical. This system was created to easily and quickly communicate hazards to first responders in the event of an emergency situation. These diamond-shaped symbols are placed on chemical containers to identify the degree of hazard associated with the specific chemical or chemical mixture. The NFPA system is a common way to identify chemical hazards and should be understood by laboratory employees. The NFPA 704 numerical rating system is based on a 0 – 4 system; 0 meaning no hazard and 4 meaning the most hazardous (note: this in contrast to the GHS system of 1 –5 where 1 is the most hazardous and 5 is the least hazardous). Figure 2.4 illustrates the NFPA hazard rating system and identifies both the hazard categories and hazard rating system.

<b>Health Hazard</b> 4 - Deadly 3 - Extreme Danger 2 - Hazardous 1 - Slightly Hazardous 0 - Normal Material	<b>Fire Hazard</b> 4 - FP* < 73° F 3 - FP < 100° F 2 - 100° F < FP < 200° F 1 - FP > 200° F 0 - Will Not Burn	<b>Reactivity Hazard</b> 4 - May Detonate 3 - Shock/Heat May Detonate 2 - Violent Chemical Change	<b>Specific Hazard</b> ACID - Acid ALK - Alkali COR - Corrosive W - No Water R - Radioactive

Figure 4. NFPA Hazard Rating System

## C. Department of Transportation (DOT) Hazard Classes

The DOT regulates the transportation of all hazardous materials in the United States, and defines a hazardous material as any substance that has been determined to be capable of posing an unreasonable risk to health, safety, or property when transported in commerce.

Chemical Stores (435-797-1616;

[http://www.chem.usu.edu/facilities/chem\\_stores](http://www.chem.usu.edu/facilities/chem_stores)

will help with transportation of hazardous materials on campus.

#### 4. RECOMMENDED CHEMICAL SPILL CLEANUP PROCEDURES

YOU **SHOULD NOT** CLEAN UP A SPILL IF:

- You don't know what the spilled material is
- You lack the necessary protection or equipment to do the job safely
- The spill is too large to contain
- The spilled material is highly toxic
- You feel any symptoms of exposure

Instead contact: (<https://rgs.usu.edu/ehs/ehs-tools/>) Under accident reporting. OR, if immediately health-threatening call **911** (campus phone)

#### SPILL RESPONSE SCHEME

##### **Evaluate and Notify**

- Assess the toxicity, flammability, or other properties of material (see label & MSDS)
- For flammables, remove or turn off ignition sources such as motors, pumps, fridges.
- Determine if there is an immediate health threat to you or your neighbors. If so, alert neighbors, isolate the area and call for help using the phone numbers above.
- If spill is minor, begin cleanup following steps below

##### **Containment/Cleanup**

- Don appropriate gloves, eye protection, lab coat, etc.
- Per SDS use absorbents\* (e.g., "spill pillows" for solvents), or neutralizers appropriate for the material\*, e.g. sodium bicarbonate for acids, citric for bases.
- Protect floor drains with absorbents or barriers around them
- Package and label waste. Include contaminated clothes, rags, equipment, etc.
- Store temporarily in a fume hood if material is volatile

##### **Followup**

- Send a Haz. Pickup request (<https://rgs.usu.edu/ehs/ehs-tools/>)
- Reorder and restock cleanup materials used
- Inform EHS if there were any personnel exposures, or release to the environment

## **SELECTING THE PROPER GLOVES**

All gloves are permeable, only the permeation rate varies, depending on the chemical, the glove material and thickness, temperature, concentration gradient, etc. However, once a material begins to permeate the glove, it will continue until an equilibrium is reached. You must, therefore, decide when it is appropriate to discard dirty gloves.

Check gloves before use for signs of wear or penetration. Disposable gloves can be inflated to check for pinholes. When removing gloves, be careful to avoid touching the outside of the gloves with your bare hands. Always remove gloves before leaving lab.

Disposable gloves provide minimal protection and should be used accordingly. If using concentrated solvents, corrosives or toxics, more heavy-duty gloves should be worn. These provide more protection, but have the drawback of being more cumbersome. Note also that about 15% of the population is allergic to latex to some degree.

This website suggests an appropriate glove as a function of the chemical in use:

<https://www.coleparmer.com/safety-glove-chemical-compatibility?PubID=WU&persist=True&gclid=CKG0xKyJp9ECFQ90fgod1YEPGA>

## **Safety Data Sheets (Formerly known as MSDS)**

<http://www.sigmaaldrich.com/safety-center.html>

<https://www.fishersci.com/us/en/catalog/search/sdshome.html>



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# Standard Operating Procedures – Lab 309

## Standard Operating Procedure – Use of Freeze Dryer

### Section 1 - Personal Protective Equipment

1. Lab Coat or Lab Gown
2. Hair tied back if long
3. Proper enclosed footwear
4. Safety glasses or goggles
5. Nitrile or latex gloves

### Section 2 – Potential Hazards + Safety precautions

1. Freeze dryer generates a high vacuum which can cause implosion of glass ampoules – this poses a threat especially to eyes – **wear safety glasses at all times when using the freeze dryer.**
2. Glass ampoules can break if mishandled, and the sharps generated pose a risk of cuts – such cuts are especially dangerous if hazardous microbes are present in the ampoules. Handle ampoules with care at all times, and know the risks and properties of the microbes you are working on.
3. Freeze dryer generates low temperatures. Ampoules and other parts of the machine may cause cold burns if touched to exposed skin. Wear gloves when handling these cold parts, or avoid contact.
4. Freeze dryer is an electrical device and thus poses electrical shock hazard if misused or if faulty. Inspect machine for any problems before use (e.g. problems with power supply, sparks, burning smell etc). If any problems present, do not use machine, notify service centre and/or manufacturer. Do not allow water to enter the internal parts of the machine. Keep flammables away from the machine.
5. **Do not use freeze drier for evaporating samples containing organic solvents!**
6. Do not use the freeze dryer unless an experienced user has shown you in detail how to use it.

## **Standard Operating Procedure – Digestibility Using the Gas Production Technique**

### **Section 1 - Personal Protective Equipment**

1. Lab Coat or Lab Gown
2. Hair tied back if long
3. Proper enclosed footwear
4. Safety glasses or goggles
5. Nitrile or latex gloves
6. Palpation gloves

### **Section 2 – Potential Hazards + Safety precautions**

**Rumen fluid extraction:** Restrain animal in a cattle chute. Stay always behind a fence before the animal is restrained. Open the canula, use palpation gloves and introduce a ½ L cup in the ventral area of the rumen environment to collect rumen fluid. Fill a 2 L thermos with rumen fluid and transport immediately to the lab.

#### **Incubation:**

1. Set temperature for the incubator at 38°C.
2. Check the reactor bottles for cracks and other damages. Be careful to prevent cuts if bottles are broken. Discard broken bottles.
3. Fill inoculum, substrate/cellulose and buffer into the glass reactors according to the experimental set-up. Shake the bottle gently to prevent clumping.
4. Control the pH-value, recommended values are between 7.5 and 8.
5. Close the bottle using the rubber plug (with attached cannula) and the screw cap. Do it gently to prevent breaking the glass.
6. Glass reactors can break if mishandled, and the sharps generated pose a risk of cuts. Handle reactors with care at all times.
7. The reactor flasks are put under vacuum app. 0.7 bar. Close the cock and control the stability of the vacuum (pressure tightness) using the manometer.
8. The reactor will receive a small over pressure of CO<sub>2</sub>, which will be discharged just connecting a needle to the manometer. Be careful when handling the needle.
9. Measure the pressure on the reactors every day or as often as possible (wear safety goggles!) by connecting the manometer on top of the reactor and introducing the needle. Set the manometer to zero before (atmospheric). Also make sure to conduct a fast measurement, so the gas inside the reactor cannot cool down to lose volume.

10. Pressures inside the reactors should not exceed 1 bar over atmospheric due to security reasons. If the measured pressure approaches this value the gas has to be discharged.

11. Discharge the gas left inside after the experiment period, remove the screw lid and the rubber plug and rinse everything with water.

## Standard Operating Procedure – weigh of Cr2O3 for intake estimates

### Section 1 - Personal Protective Equipment

1. Lab Coat or Lab Gown
2. Hair tied back if long
3. Proper enclosed footwear
4. Safety glasses or goggles
5. Nitrile or latex gloves

### Section 2 – Potential Hazards + Safety precautions

## MSDS-Cr2O3

### Hazards Identification

**Potential Acute Health Effects:** Hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion, of inhalation.

**Potential Chronic Health Effects:** Hazardous in case of inhalation. Slightly hazardous in case of skin contact (sensitizer). CARCINOGENIC EFFECTS: A4

(Not classifiable for human) by ACGIH, 3 (Not classifiable for human.) by IARC. MUTAGENIC EFFECTS: Mutagenic for mammalian somatic cells. Mutagenic for bacteria and/or yeast.

TERATOGENIC EFFECTS: Not available.

DEVELOPMENTAL TOXICITY: Not available. The substance may be toxic to upper respiratory tract, skin. Repeated or prolonged exposure to the substance can produce target organs damage.

### First Aid Measures

#### General advice

Consult a physician. Show this safety data sheet to the doctor in attendance.

#### If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

#### In case of skin contact

Wash off with soap and plenty of water. Consult a physician.

#### Eye Contact:

Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention.

#### Skin Contact:

In case of contact, immediately flush skin with plenty of water. Cover the irritated skin with an emollient. Remove contaminated clothing and shoes. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention.

#### Serious Skin Contact:

Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek medical attention.

#### Inhalation:

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.

**Serious Inhalation:** Not available.

**Ingestion:**

Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. If large quantities of this material are swallowed, call a physician immediately. Loosen tight clothing such as a collar, tie, belt or waistband.

**Serious Ingestion:** Not available.

**Most important symptoms and effects, both acute and delayed**

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

**Indication of any immediate medical attention and special treatment needed**

No data available

**Firefighting Measures**

**Suitable extinguishing media**

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

**5.2 Special hazards arising from the substance or mixture**

No data available

**Advice for firefighters**

Wear self-contained breathing apparatus for firefighting if necessary.

**Accidental Release Measures**

**Personal precautions, protective equipment and emergency procedures**

Use personal protective equipment. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Avoid breathing dust.

**6.2 Environmental precautions**

Do not let product enter drains.

**Methods and materials for containment and cleaning up**

Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

**Handling and Storage**

Further processing of solid materials may result in the formation of combustible dusts. The potential for combustible dust formation should be taken into consideration before additional processing occurs. Provide appropriate exhaust ventilation at places where dust is formed. For precautions see section 2.2.

**Conditions for safe storage, including any incompatibilities**

Keep container tightly closed in a dry and well-ventilated place

Hygroscopic Handle and store under inert gas. Keep in a dry place.

Storage class (TRGS 510): Non Combustible Solids

**Exposure controls**

**Appropriate engineering controls**

Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

**Personal protective equipment**

**Eye/face protection**

Safety glasses with side-shields conforming to EN166 Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU)

### **Skin protection**

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Material: Nitrile rubber

Minimum layer thickness: 0.11 mm

Break through time: 480 min

Material tested: Dermatril® (KCL 740 / Aldrich Z677272, Size M)

Splash contact

Material: Nitrile rubber

Minimum layer thickness: 0.11 mm

Break through time: 480 min

Material tested: Dermatril® (KCL 740 / Aldrich Z677272, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de,

test method:

EN374

If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

### **Body Protection**

Choose body protection in relation to its type, to the concentration and amount of dangerous substances, and to the specific work-place., The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

### **Respiratory protection**

Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

### **Control of environmental exposure**

Do not let product enter drains.

## **Standard Operating Procedure – Condensed Tannin Analyses**

### **Section 1 - Personal Protective Equipment**

1. Lab Coat or Lab Gown
2. Hair tied back if long
3. Proper enclosed footwear
4. Safety glasses or goggles
5. Nitrile or latex gloves

### **Determination of Total Condensed Tannins**

This procedure is an adaptation of method for determining total condensed tannins developed by Terrill et al. (1992). The method quantifies both tannins that can be extracted by organic solvents such as acetone (extractable condensed tannins) as well as those that remain bound to the plant tissue after treatment with organic solvents (bound condensed tannins). This procedure uses 70% acetone as a solvent which has been shown to give more complete and repeatable tannin extraction than other solvents (Terrill et al. 1990).

This procedure is a colorimetric method based on butanol-HCl procedure. The primary classes of condensed tannins found in forage legumes are Procyanidins and Prodelphinidins which are composed of chains of cyanidin and delphinidin subunits respectively. When placed in a heated butanol-HCl solution the condensed tannins break down into cyanidin and delphinidin subunits (Porter et al. 1986). These cyanidin and delphinidin subunits show up as red, pink, or purple colorants in the butanol-HCl solution, with the exact color varying depending upon the relative percentage of cyanidin and delphinidin subunits present. Therefore the concentration of these cyanidin and prodelphinidin subunits can be measured by a spectrophotometer.

Purified tannin from the plant species being analyzed is used as the standard for determining tannin content. There are two reasons for doing so. First, using purified tannin is considered to give results that are more accurate and “biologically relevant” than those obtained using catechin (Terrill et al. 1990). Second, using commercial preparations such as tannic acid, gallic acid or catechin introduces repeatability problems associated with product variation between manufacturers. Makkar and Becker (1994) found variations between different suppliers of tannic acid could result in a 20%-30% difference in calculated tannin content of a given plant sample, thus making it difficult to make meaningful comparisons between results obtained by different labs using tannic acid from different suppliers.

#### *1. Sample Preparation*

When samples are collected in the field they should be placed immediately on dry ice and transported to a deep freeze or directly to a freeze drier. Samples should not be allowed to thaw between the time they are collected and freeze-dried. Allowing samples to thaw reduces

tannin extractability as does air-drying or oven-drying (Terrill et al. 1990). After samples are freeze-dried they should be ground in Wiley mill to pass through a 1mm screen and stored in a cool, dry place.

## *II. Isolating the Purified Tannin for Standards*

To obtain the purified tannin it is necessary to “sacrifice” 100-300 grams of ground fresh or freeze-dried plant material. For high tannin species like sericea lespedeza 100 grams will be sufficient, but 300 grams may be needed for low-tannin species such as birdsfoot trefoil. Final yield of pure tannin will be about 20% of the extractable tannin content of the forage. For example 100 grams of sericea lespedeza with 10% extractable tannin content will yield about 2 grams of purified tannin. The procedure needs advanced planning. The sephadex LH-20 beads need to be soaked overnight prior to the procedure. The procedure not counting freeze-drying will take 1-2 people approximately 16 hours. The procedure will require 100 grams of sephadex LH-20 beads, 1-2 liters of diethyl ether, 2-3 liters of methanol and 4-6 liters of acetone.

### A.) Extraction of tannin from plant material.

1) Mix the ground plant material in a beaker with a solution of 70% acetone, 30% water. Stir thoroughly and pour mixture into 50 ml or larger centrifuge tubes and centrifuge until the solids settle out. Pour off the supernatant (liquid) and save.

2) Pour additional 70% acetone over the pellet (solid) from the above step, vortex and re-centrifuge. Save the supernatant and combine with that from the first extraction. Two extractions will extract the majority of tannins but a third extraction will slightly increase the total tannin yield.

### B.) Removal of Plant Pigments

1) Pour the acetone extract obtained above into a large separation funnel. Add 1 ml of diethyl ether for every 2 ml of acetone extract. Mix until a phase separation occurs. Draw off and save the lower aqueous layer (light brown or yellow) which contains the tannin and discard the upper organic layer containing plant oils, chlorophyll and other pigments.

2) Repeat 3-4 times to remove all plant pigments and oils.

### C.) Removal of Organic Solvents

Take aqueous layer obtained above and place in a rotary evaporator. Start out at a relatively low temperature until all of the diethyl ether is removed then increase heat to remove acetone residues. If any solids remain in liquid they should here be removed by centrifugation. Measure and record the volume of aqueous solution.

### D.) Removal of Non-tannin Phenolics

In this step you will “adhere” the tannin and non-tannin phenolics to the sephadex beads and then remove the non-tannin phenolics with 50% methanol.



1) Pour 100 gram of sephadex LH-20 beads into a beaker and add 550 ml distilled water and soak overnight. The next day pour off the excess water into a graduated cylinder and record volume. The difference between the volume added and the volume removed will be the volume absorbed by the beads.

2) Combine swollen sephadex beads with the aqueous solution from section C in a large beaker and add an amount of methanol equal to the amount of aqueous solution plus the amount of water absorbed by the sephadex beads. Stir this solution for 30 minutes and then let set for 2-3 hours to allow the tannin to adhere to the beads.

3) Mix up 2 liters of 50% methanol 50% distilled water solution.

4) Set up a large Buchner funnel on a vacuum filtration apparatus, cover bottom with filter paper and moisten with 50% methanol and begin vacuum. Pour the sephadex/water/methanol solution from step #2 into the Buchner funnel. Wash the beads until the liquid coming off them is completely clear. Try to avoid letting the beads get sucked dry during this process. This process will take 1-3 hours and will require 1-2 liters of 50% methanol. Discard the liquid.

#### E.) Recovery of Tannin from Beads

1) After discarding the methanol rinse above, rinse the vacuum bottle well with acetone and place the Buchner funnel with beads back on the vacuum bottle.

2) Rinse the sephadex beads with 70% acetone, 30% water until the beads are snowy white. This should take about 1-1.5 liters of the 70% acetone. SAVE the liquid.

#### F.) Removal of Acetone and Water and Recovery of Pure Tannin

1) Take liquid rinse above and place in rotary evaporator to remove all traces acetone and methanol.

2) After organic solvents have been removed, pour the liquid out in a shallow layer in metal freeze-drier pans. Place pans in deep freeze overnight. The next day place pans into freeze drier. It should take about a week for removal of all the water leaving the purified tannin which looks like cotton candy and will be anywhere from off-white to reddish-brown depending upon the plant species. Store tannin in a cold, dry place until it is needed.

### *III. Determining Extractable Condensed Tannins*

#### A.) Preparation of 70% acetone solution.

1) Measure out 740 ml of HPLC grade acetone and 310 ml of distilled water. This will yield one liter of mix due to 5% loss in volume that occurs when acetone and water are mixed.

2) Weigh out 1 gram of ascorbic acid and dissolve in the liter of acetone/water mix.

#### B.) Preparation of the butanol-HCl solution.

Measure out 950 ml of 1-butanol, and add 50 ml of concentrated (37%) HCl. Due the hazards involved with handling concentrated acids this step should be performed under a fume hood and appropriate eye and skin protection equipment should be worn. Always add acid to butanol, not butanol to acid.

#### C.) Weighing sub-samples

Weigh and record triplicate 500 mg sub-samples of each plant sample

#### D.) Extracting samples

- 1) Pipette 20 ml of the 70% acetone mix into each centrifuge tube, cap and vortex mix each tube twice for 10 seconds each time.
- 2) Place into heated shaking bath (32 degrees Celsius, 95 RPM) for 15 minutes.
- 3) Remove tubes from bath, briefly hand mix and centrifuge for 15 minutes at 900 G's. This corresponds to the 40 setting on the international model K centrifuge in our lab. RCF in G's is calculated by the formula  $G = (11.17 \times 10^{-7}) RN^2$  where R is radius in mm from center of centrifuge spindle the far tip of centrifuge tube and N is the speed of the spindle in RPM.
- 4) Decant liquid into labeled beakers and cover with parafilm.
- 5) Repeat steps 1-4 twice for a total of three extractions.
- 6) Combine the three extracts and pour into a 100 ml graduated cylinder. Use an eyedropper to add 70% acetone solution to bring volume to nearest whole ml. Record volume in notebook and pour into new 50 ml screwtop centrifuge tubes. (Final volume will be 55-60 ml so a small amount of extract will be discarded).
- 7) Place extracts in freezer until you are ready to perform the butanol-HCl or Folin-Ciocalteu analyses. Put the residues in the freezer as well to save for the bound condensed tannin analysis. Extracts will keep up to 30 days in freezer.

#### E.) Determining Extractable Condensed tannins using the butanol-HCl procedure

- 1) To prepare the 1mg/ml stock solution used in the standard curve, weigh out 100 mg of pure tannin, dissolve in 50ml of 70% acetone and carefully pour into a 100 ml volumetric flask. Then bring volume up to exactly 100 ml. Allow to "equilibrate" in refrigerator for 3 hours before using. The stock solution will keep for up to a month as long as it is kept refrigerated and capped.
- 2) To construct the standard curve pipette 20 ul (microliters) of the stock solution into each of 16 ml screwcap test tubes and do the same likewise for the 50, 100, 200, and 250 ul concentrations.

- 3) Pipette 100 ul of each extraction obtained in section D into triplicate 16 ml screwtop test tubes. This will give a 3 X 3 subsampling regime--three subsamples taken from each sample and 3 absorbance values for each subsample.
- 4) Pipette 5 ml of butanol-HCl into each tube containing the stock solution and plant sample extracts and mix with a vortex mixer. Also prepare a blank tube with 5 ml of butanol-HCl solution.
- 5) Place in a 95 degree Celsius water bath and cap each tube with a marble.
- 6) After 1 hour and 15 minutes remove tubes from bath and cool them in ice water.
- 7) Set the spectrophotometer at 550 nm, with filter appropriate for 550nm wavelength and "blank" the machine with the blank tube.
- 8) Record the absorbance of each of the standard curve and sample tubes.
- 9) Calculate the percent extractable condensed tannins by calculating mg/ml based on the standard curve equation, multiplying by ten (100 ul is 0.1 ml) then multiplying by ml of extract to total mg grams of extractable tannin. Then divide mg extractable tannin by sample weight and multiply by 100 to get percent extractable tannin. Once the dry matter of the freeze-dried sample is determined, the numbers can be corrected to a 100% dry matter basis.

#### *IV. Determining Bound Condensed Tannins*

##### *A.) Recovery of residues from the acetone extraction.*

- 1.) Weigh a 150 mm Whatman #1 filter paper to establish a tare weight and write sample number on it with a lead pencil. Due to variation in filter weights it is necessary to pre-weigh each filter.
- 2) Fold and place inside a funnel on a vacuum apparatus. Wet the filter down with 100% acetone. Dump the residue from procedure III.-D above, onto the filter and thoroughly rinse out residue centrifuge tube onto the filter with 100% acetone.
- 3) Thoroughly wash the residue in the filter with 100% acetone. Maintain vacuum until sample and filter are both dry.
- 4) Allow filter and sample to air dry for an additional two hours.
- 5) Weigh the dried filter with residue and calculate the net weight of residue.
- 6) Place residues back in the centrifuge tubes they came from, making sure that the tubes are thoroughly dried.

##### *B.) Measuring bound condensed tannins with the butanol-HCl procedure.*

- 1.) Weigh triplicate 10 mg samples of residue and place in 16 ml screw-top test tubes. This again is a 3 X 3 subsampling regime, three subsamples taken from each sample and 3 absorbance values recorded for each subsample.
- 2) Construct a standard curve with the 1 mg/ml stock solution and a blank butanol-HCl tube as in section III.-E.
- 3) Pipette 5 ml of butanol-HCl into each residue tube and mix with vortex mixer.
- 4) Place all tubes into 95 degree Celsius water bath for one hour and 15 minutes.
- 5) Cool tubes in ice water.
- 6) Remix the residue tubes with vortex mixer. For high-tannin samples such as sericea lespedeza it may be necessary to add another 5 ml of butanol-HCl to keep absorbance values within the range that can be read by the spectrophotometer. Make appropriate adjustments in calculations.
- 7) Centrifuge the residue tubes for 15 minutes at 350 G's using special inserts to hold the tubes in place.
- 8) Record absorbance values for each tube on spectrophotometer at 550 nm.
- 9) Calculate bound condensed tannins by calculating grams of tannin via standard curve equation then dividing by proportion of residue sampled to get mg of bound condensed tannin in samples, and dividing by sample weight to get percent bound condensed tannins. When dry matter of samples is determined, convert to a dry matter basis.

#### *Determining Total Extractable Phenolics*

##### *Folin-Ciocalteu Method*

This method quantifies the total amount of phenolic compounds--both tannins and lower molecular weight compounds--that can be extracted by organic solvents. For this procedure, use the same acetone-extracts used to measure extractable condensed tannins in the total condensed tannin procedure. This procedure uses the Folin-Ciocalteu reagent. It is considered superior to the older Folin-Dennis reagent in that it is less likely to react with non-phenolic reducing agents such as ascorbic acid (Julkunen-Titto 1985).

The Folin-Ciocalteu reagent contains sodium molybdate and sodium tungstate. These compounds react with the hydroxyl group (OH<sup>-</sup>) in phenolic compounds. The products of these reactions form a blue color which can be measured by spectrophotometer. The exact shade of blue that is produced depends upon both the concentration of phenolics in an extract and the position of the hydroxyl groups on the phenolic molecules (Julkunen-Titto 1985).

##### *I. Preparation of Folin-Ciocalteu Reagent and Sodium Carbonate Buffer Solutions*

A.) To prepare the Folin-Ciocalteu reagent and make mixture of 10% purchased Folin-Ciocalteu concentrate (Sigma F-9252) and 90% distilled water. It is important to mix a new batch each day. If multiple “runs” are to be made throughout the day, try to minimize the amount of light exposure the Folin-Ciocalteu reagent receives.

B.) To prepare the sodium carbonate buffer dissolve 75 grams of  $\text{Na}_2\text{CO}_3 \cdot \text{H}_2\text{O}$  (not the anhydrous sodium carbonate) in one liter of distilled water. To minimize the time needed to dissolve the sodium carbonate use a heated magnetic stirring apparatus at low heat. It may take over an hour for sodium carbonate to completely dissolve. Once mixed the sodium carbonate buffer solution is quite stable and be kept for several weeks.

## *II. Performing the Procedure*

A.) The standard curve can be constructed with the condensed tannin stock solution used in the Total Condensed Tannin procedure. Alternatively the curve can be constructed using a stock solution made by dissolving 100 mg of gallic acid (Sigma G-7384) in 100 ml of distilled water. It will take about 45 minutes on a magnetic stirring apparatus to dissolve the gallic acid. A new gallic acid stock solution needs to be made each day. My research showed that the standard curve obtained with purified sericea lespedeza tannin was very similar to that obtained with gallic acid.

B.) Construct the standard curve by pipetting 20, 50, 100, 150 and 200  $\mu\text{l}$  aliquots into 25 x 150 ml test tubes.

C.) Pipette 100  $\mu\text{l}$  of acetone extract into the 25 x 150 ml test tubes. Produce 3 tubes for each subsample, a 3x3 subsampling regime, 3 subsamples per sample and 3 tubes read from each subsample.

D.) Pipette 5 ml of Folin-Ciocalteu reagent into each test tube, including one blank tube. Vortex mix each tube, then pipette 4 ml of sodium carbonate buffer solution into each tube. There are a few important notes on this step. First, the Folin reagent must be added 2-3 minutes before the sodium carbonate solution or an incomplete reaction will result. Second, it is important to run similar sized batches so that the time interval between the time the Folin reagent is added and the buffer is added is relatively consistent across samples.

E.) Let the tubes react at room temperature for 2 hours.

F.) Set the spectrophotometer at 675 nm with filter appropriate for 675 nm wavelength. Blank spectrophotometer with the blank tube and read the standards and samples. It is important to run small enough batches that all of the tubes can be read within an hour time frame.

G.) Calculate percent total extractable phenolics as with the extractable condensed tannin procedure.

# MSDS-TANNIN ANALYSES

## 1. Acetone

### Hazards Identification

Potential Acute Health Effects: Hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion, of inhalation. Slightly hazardous in case of skin contact (permeator).

### Potential Chronic Health Effects:

CARCINOGENIC EFFECTS: A4 (Not classifiable for human or animal.) by ACGIH. MUTAGENIC EFFECTS: Not available.

TERATOGENIC EFFECTS: Not available. DEVELOPMENTAL TOXICITY: Classified Reproductive system/toxin/female, Reproductive system/toxin/male [SUSPECTED]. The substance is toxic to central nervous system (CNS). The substance may be toxic to kidneys, the reproductive system, liver, skin. Repeated or prolonged exposure to the substance can produce target organs damage.

### First Aid Measures

#### Eye Contact:

Check for and remove any contact lenses. Immediately flush eyes with running water for at least 15 minutes, keeping eyelids open. Cold water may be used. Get medical attention.

#### Skin Contact:

In case of contact, immediately flush skin with plenty of water. Cover the irritated skin with an emollient. Remove contaminated clothing and shoes. Cold water may be used. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention.

#### Serious Skin Contact:

Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek medical attention.

#### Inhalation:

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention if symptoms appear.

#### Serious Inhalation:

Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband. If breathing is difficult, administer oxygen. If the victim is not breathing, perform mouth-to-mouth resuscitation. Seek medical attention.

#### Ingestion:

Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention if symptoms appear.

**Serious Ingestion:** Not available.

**Flammability of the Product:** Flammable.

**Auto-Ignition Temperature:** 465°C (869°F)

**Flash Points:** CLOSED CUP: -20°C (-4°F). OPEN CUP: -9°C (15.8°F) (Cleveland).

**Flammable Limits:** LOWER: 2.6% UPPER: 12.8%

**Products of Combustion:** These products are carbon oxides (CO, CO<sub>2</sub>).

**Fire Hazards in Presence of Various Substances:** Highly flammable in presence of open flames and sparks, of heat.

**Explosion Hazards in Presence of Various Substances:**

Risks of explosion of the product in presence of mechanical impact: Not available. Slightly explosive in presence of open flames and sparks, of oxidizing materials, of acids.

**Fire Fighting Media and Instructions:**

Flammable liquid, soluble or dispersed in water. SMALL FIRE: Use DRY chemical powder. LARGE FIRE: Use alcohol foam, water spray or fog.

**Special Remarks on Fire Hazards:** Vapor may travel considerable distance to source of ignition and flash back.

**Special Remarks on Explosion Hazards:**

Forms explosive mixtures with hydrogen peroxide, acetic acid, nitric acid, nitric acid + sulfuric acid, chromic anhydride, chromyl chloride, nitrosyl chloride, hexachloromelamine, nitrosyl perchlorate, nitryl perchlorate, permonosulfuric acid, thiodiglycol + hydrogen peroxide, potassium ter-butoxide, sulfur dichloride, 1-methyl-1,3-butadiene, bromoform, carbon, air, chloroform, thitriazyperchlorate.

**Spills**

**Small Spill:**

Dilute with water and mop up, or absorb with an inert dry material and place in an appropriate waste disposal container.

**Large Spill:**

Flammable liquid. Keep away from heat. Keep away from sources of ignition. Stop leak if without risk. Absorb with DRY earth, sand or other non-combustible material. Do not touch spilled material. Prevent entry into sewers, basements or confined areas; dike if needed. Be careful that the product is not present at a concentration level above TLV. Check TLV on the MSDS and with local authorities.

**Precautions:**

Keep locked up. Keep away from heat. Keep away from sources of ignition. Ground all equipment containing material. Do not ingest. Do not breathe gas/fumes/ vapor/spray. Wear suitable protective clothing. In case of insufficient ventilation, wear suitable respiratory equipment. If ingested, seek medical advice immediately and show the container or the label. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents, reducing agents, acids, alkalis.

**Storage:**

Store in a segregated and approved area (flammables area) . Keep container in a cool, well-ventilated area. Keep container tightly closed and sealed until ready for use. Keep away from direct sunlight and heat and avoid all possible sources of ignition (spark or flame).

**Engineering Controls:**

Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapors below their respective threshold limit value. Ensure that eyewash stations and safety showers are proximal to the work-station location.

**Personal Protection:**

Splash goggles. Lab coat. Vapor respirator. Be sure to use an approved/certified respirator or equivalent. Gloves.

**Personal Protection in Case of a Large Spill:**

Splash goggles. Full suit. Vapor respirator. Boots. Gloves. A self contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

**Exposure Limits:**

TWA: 500 STEL: 750 (ppm) from ACGIH (TLV) [United States] TWA: 750 STEL: 1000 (ppm) from OSHA (PEL) [United States] TWA: 500 STEL: 1000 [Australia] TWA: 1185 STEL: 2375 (mg/m<sup>3</sup>) [Australia] TWA: 750 STEL: 1500 (ppm) [United Kingdom (UK)] TWA: 1810 STEL: 3620 (mg/m<sup>3</sup>) [United Kingdom (UK)] TWA: 1800 STEL: 2400 from OSHA (PEL) [United States] Consult local authorities for acceptable exposure limits.

## 2. Methanol

**Potential Acute Health Effects:**

Hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion, of inhalation. Slightly hazardous in case of skin contact (permeator). Severe over-exposure can result in death.

**Potential Chronic Health Effects:**

Slightly hazardous in case of skin contact (sensitizer). CARCINOGENIC EFFECTS: Not available. MUTAGENIC EFFECTS: Mutagenic for mammalian somatic cells. Mutagenic for bacteria and/or yeast. TERATOGENIC EFFECTS: Classified POSSIBLE for human.

DEVELOPMENTAL TOXICITY: Not available. The substance is toxic to eyes. The substance may be toxic to blood, kidneys, liver, brain, peripheral nervous system, upper respiratory tract, skin, central nervous system (CNS), optic nerve. Repeated or prolonged exposure to the substance can produce target organs damage. Repeated exposure to a highly toxic material may produce general deterioration of health by an accumulation in one or many human organs.

**First Aid Measures****Eye Contact:**

Check for and remove any contact lenses. Immediately flush eyes with running water for at least 15 minutes, keeping eyelids open. Cold water may be used. Get medical attention.

**Skin Contact:**

In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Cover the irritated skin with an emollient. Cold water may be used. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention immediately.

**Serious Skin Contact:**

Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek immediate medical attention.

**Inhalation:**

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention immediately.



**Serious Inhalation:**

Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband. If breathing is difficult, administer oxygen. If the victim is not breathing, perform mouth-to-mouth resuscitation. **WARNING:** It may be hazardous to the person providing aid to give mouth-to-mouth resuscitation when the inhaled material is toxic, infectious or corrosive. Seek immediate medical attention.

**Ingestion:**

If swallowed, do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention immediately.

**Serious Ingestion:** Not available.

**Fire and Explosion Data**

**Flammability of the Product:** Flammable.

**Auto-Ignition Temperature:** 464°C (867.2°F)

**Flash Points:** CLOSED CUP: 12°C (53.6°F). OPEN CUP: 16°C (60.8°F).

**Flammable Limits:** LOWER: 6% UPPER: 36.5%

**Products of Combustion:** These products are carbon oxides (CO, CO<sub>2</sub>).

**Fire Hazards in Presence of Various Substances:**

Highly flammable in presence of open flames and sparks, of heat. Non-flammable in presence of shocks.

**Explosion Hazards in Presence of Various Substances:**

Risks of explosion of the product in presence of mechanical impact: Not available. Explosive in presence of open flames and sparks, of heat.

**Fire Fighting Media and Instructions:**

Flammable liquid, soluble or dispersed in water. **SMALL FIRE:** Use DRY chemical powder. **LARGE FIRE:** Use alcohol foam, water spray or fog.

**Special Remarks on Fire Hazards:**

Explosive in the form of vapor when exposed to heat or flame. Vapor may travel considerable distance to source of ignition and flash back. When heated to decomposition, it emits acrid smoke and irritating fumes. **CAUTION: MAY BURN WITH NEAR INVISIBLE FLAME**

**Special Remarks on Explosion Hazards:**

Forms an explosive mixture with air due to its low flash point. Explosive when mixed with Chloroform + sodium methoxide and diethyl zinc. It boils violently and explodes.

**Spills****Small Spill:**

Dilute with water and mop up, or absorb with an inert dry material and place in an appropriate waste disposal container.

**Large Spill:**

Flammable liquid. Poisonous liquid. Keep away from heat. Keep away from sources of ignition. Stop leak if without risk.

Absorb with DRY earth, sand or other non-combustible material. Do not get water inside container. Do not touch spilled material. Use water spray to reduce vapors. Prevent entry into sewers, basements or confined areas; dike if needed. Call for assistance on disposal. Be careful that the product is not present at a concentration level above TLV. Check TLV on the

MSDS and with local authorities.

### **Handling and Storage**

#### **Precautions:**

Keep locked up. Keep away from heat. Keep away from sources of ignition. Ground all equipment containing material. Do not ingest. Do not breathe gas/fumes/ vapor/spray. Wear suitable protective clothing. In case of insufficient ventilation, wear suitable respiratory equipment. If ingested, seek medical advice immediately and show the container or the label. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents, metals, acids.

#### **Storage:**

Store in a segregated and approved area. Keep container in a cool, well-ventilated area. Keep container tightly closed and sealed until ready for use. Avoid all possible sources of ignition (spark or flame).

#### **Exposure**

##### **Engineering Controls:**

Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapors below their respective threshold limit value. Ensure that eyewash stations and safety showers are proximal to the work-station location.

##### **Personal Protection:**

Splash goggles. Lab coat. Vapor respirator. Be sure to use an approved/certified respirator or equivalent. Gloves.

##### **Personal Protection in Case of a Large Spill:**

Splash goggles. Full suit. Vapor respirator. Boots. Gloves. A self-contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

##### **Exposure Limits:**

TWA: 200 from OSHA (PEL) [United States] TWA: 200 STEL: 250 (ppm) from ACGIH (TLV) [United States] [1999] STEL: 250 from NIOSH [United States] TWA: 200 STEL: 250 (ppm) from NIOSH SKIN TWA: 200 STEL: 250 (ppm) [Canada] Consult local authorities for acceptable exposure limits.

## **3-Butanol**

### **Hazards Identification**

#### **Potential Acute Health Effects:**

Very hazardous in case of skin contact (irritant, permeator), of eye contact (irritant), of ingestion, of inhalation. Slightly hazardous in case of skin contact (sensitizer). Inflammation of the eye is characterized by redness, watering, and itching. Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering.

#### **Potential Chronic Health Effects:**

CARCINOGENIC EFFECTS: Not available. MUTAGENIC EFFECTS: Not available. TERATOGENIC EFFECTS: Not available. DEVELOPMENTAL TOXICITY: Not available. Repeated or prolonged exposure is not known to aggravate medical condition.

## **First Aid Measures**

### **Eye Contact:**

Check for and remove any contact lenses. Immediately flush eyes with running water for at least 15 minutes, keeping eyelids open. Cold water may be used. Get medical attention immediately.

### **Skin Contact:**

In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Cover the irritated skin with an emollient. Cold water may be used. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention immediately.

### **Serious Skin Contact:**

Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek immediate medical attention.

### **Inhalation:**

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention if symptoms appear.

### **Serious Inhalation:**

Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband. If breathing is difficult, administer oxygen. If the victim is not breathing, perform mouth-to-mouth resuscitation. Seek medical attention.

### **Ingestion:**

Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. If large quantities of this material are swallowed, call a physician immediately. Loosen tight clothing such as a collar, tie, belt or waistband.

**Serious Ingestion:** Not available.

## **Flammability**

**Flammability of the Product:** Flammable.

**Auto-Ignition Temperature:** 343°C (649.4°F)

**Flash Points:** CLOSED CUP: 28.9°C (84°F). OPEN CUP: 36.1°C (97°F) (Cleveland).

**Flammable Limits:** LOWER: 1.4% UPPER: 11.2%

**Products of Combustion:** These products are carbon oxides (CO, CO<sub>2</sub>).

### **Fire Hazards in Presence of Various Substances:**

Highly flammable in presence of open flames and sparks. Flammable in presence of heat, of oxidizing materials, of reducing materials, of combustible materials.

### **Explosion Hazards in Presence of Various Substances:**

Risks of explosion of the product in presence of mechanical impact: Not available. Risks of explosion of the product in presence of static discharge: Not available.

### **Fire Fighting Media and Instructions:**

Flammable liquid, soluble or dispersed in water. SMALL FIRE: Use DRY chemical powder. LARGE FIRE: Use alcohol foam, water spray or fog. Cool containing vessels with water jet in order to prevent pressure build-up, autoignition or explosion.

**Special Remarks on Fire Hazards:** May form explosive mixtures with air. CAUTION: MAY BURN WITH NEAR INVISIBLE FLAME

**Small Spill:**

Dilute with water and mop up, or absorb with an inert dry material and place in an appropriate waste disposal container.

**Large Spill:**

Flammable liquid. Keep away from heat. Keep away from sources of ignition. Stop leak if without risk. Absorb with DRY earth, sand or other non-combustible material. Do not touch spilled material. Prevent entry into sewers, basements or confined areas; dike if needed. Be careful that the product is not present at a concentration level above TLV. Check TLV on the MSDS and with local authorities.

**Handling and Storage****Precautions:**

Keep away from heat. Keep away from sources of ignition. Ground all equipment containing material. Do not ingest. Do not breathe gas/fumes/ vapor/spray. In case of insufficient ventilation, wear suitable respiratory equipment. If ingested, seek medical advice immediately and show the container or the label. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents, reducing agents.

**Storage:**

Store in a segregated and approved area. Keep container in a cool, well-ventilated area. Keep container tightly closed and sealed until ready for use. Avoid all possible sources of ignition (spark or flame).

**Exposure Controls****Engineering Controls:**

Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapors below their respective threshold limit value. Ensure that eyewash stations and safety showers are proximal to the work-station location.

**Personal Protection:**

Splash goggles. Lab coat. Vapor respirator. Be sure to use an approved/certified respirator or equivalent. Gloves.

**Personal Protection in Case of a Large Spill:**

Splash goggles. Full suit. Vapor respirator. Boots. Gloves. A self contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

**Exposure Limits:**

CEIL: 152 (mg/m<sup>3</sup>) Consult local authorities for acceptable exposure limits.

## 4. Hydrochloric Acid

**Hazards Identification****Potential Acute Health Effects:**

Very hazardous in case of skin contact (corrosive, irritant, permeator), of eye contact (irritant, corrosive), of ingestion. Slightly hazardous in case of inhalation (lung sensitizer). Non-corrosive for lungs. Liquid or spray mist may produce tissue damage particularly on mucous membranes of eyes, mouth and respiratory tract. Skin contact may produce burns. Inhalation of the spray

mist may produce severe irritation of respiratory tract, characterized by coughing, choking, or shortness of breath.

Severe over-exposure can result in death. Inflammation of the eye is characterized by redness, watering, and itching. Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering.

**Potential Chronic Health Effects:**

Slightly hazardous in case of skin contact (sensitizer). CARCINOGENIC EFFECTS: Classified 3 (Not classifiable for human.) by IARC [Hydrochloric acid]. MUTAGENIC EFFECTS: Not available.

TERATOGENIC EFFECTS: Not available.

DEVELOPMENTAL TOXICITY: Not available. The substance may be toxic to kidneys, liver, mucous membranes, upper respiratory tract, skin, eyes, Circulatory System, teeth. Repeated or prolonged exposure to the substance can produce target organs damage. Repeated or prolonged contact with spray mist may produce chronic eye irritation and severe skin irritation. Repeated or prolonged exposure to spray mist may produce respiratory tract irritation leading to frequent attacks of bronchial infection. Repeated exposure to a highly toxic material may produce general deterioration of health by an accumulation in one or many human organs.

**First Aid Measures**

**Eye Contact:**

Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Cold water may be used. Get medical attention immediately.

**Skin Contact:**

In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Cover the irritated skin with an emollient. Cold water may be used. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention immediately.

**Serious Skin Contact:**

Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek immediate medical attention.

**Inhalation:**

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention immediately.

**Serious Inhalation:**

Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband. If breathing is difficult, administer oxygen. If the victim is not breathing, perform mouth-to-mouth resuscitation. WARNING: It may be hazardous to the person providing aid to give mouth-to-mouth resuscitation when the inhaled material is toxic, infectious or corrosive. Seek immediate medical attention.

**Ingestion:**

If swallowed, do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention immediately.

**Serious Ingestion:** Not available.

**Fire and Explosion Data**

**Flammability of the Product:** Non-flammable.

**Auto-Ignition Temperature:** Not applicable.

**Flash Points:** Not applicable.

**Flammable Limits:** Not applicable.

**Products of Combustion:** Not available.

**Fire Hazards in Presence of Various Substances:** of metals

**Explosion Hazards in Presence of Various Substances:** Non-explosive in presence of open flames and sparks, of shocks.

**Fire Fighting Media and Instructions:** Not applicable.

**Special Remarks on Fire Hazards:**

Non-combustible. Calcium carbide reacts with hydrogen chloride gas with incandescence. Uranium phosphide reacts with hydrochloric acid to release spontaneously flammable phosphine. Rubidium acetylene carbides burns with slightly warm hydrochloric acid. Lithium silicide in contact with hydrogen chloride becomes incandescent. When dilute hydrochloric acid is used, gas spontaneously flammable in air is evolved. Magnesium boride treated with concentrated hydrochloric acid produces spontaneously flammable gas. Cesium acetylene carbide burns hydrogen chloride gas. Cesium carbide ignites in contact with hydrochloric acid unless acid is dilute. Reacts with most metals to produce flammable Hydrogen gas.

**Special Remarks on Explosion Hazards:**

Hydrogen chloride in contact with the following can cause an explosion, ignition on contact, or other violent/vigorous reaction:

Acetic anhydride AgClO + CCl<sub>4</sub> Alcohols + hydrogen cyanide, Aluminum Aluminum-titanium alloys (with HCl vapor), 2-Amino ethanol, Ammonium hydroxide, Calcium carbide Ca<sub>3</sub>P<sub>2</sub> Chlorine + dinitroanilines (evolves gas), Chlorosulfonic acid Cesium carbide Cesium acetylene carbide, 1,1-Difluoroethylene Ethylene diamine Ethylene imine, Fluorine, HClO<sub>4</sub> Hexalithium disilicide H<sub>2</sub>SO<sub>4</sub> Metal acetylides or carbides, Magnesium boride, Mercuric sulfate, Oleum, Potassium permanganate, beta-Propiolactone Propylene oxide Rubidium carbide, Rubidium, acetylene carbide Sodium (with aqueous HCl), Sodium hydroxide Sodium tetraselenium, Sulfonic acid, Tetraselenium tetranitride, U<sub>3</sub>P<sub>4</sub>, Vinyl acetate. Silver perchlorate with carbon tetrachloride in the presence of hydrochloric acid produces trichloromethyl perchlorate which detonates at 40 deg. C.

**Spills**

**Small Spill:**

Dilute with water and mop up, or absorb with an inert dry material and place in an appropriate waste disposal container. If necessary: Neutralize the residue with a dilute solution of sodium carbonate.

**Large Spill:**

Corrosive liquid. Poisonous liquid. Stop leak if without risk. Absorb with DRY earth, sand or other non-combustible material. Do not get water inside container. Do not touch spilled material. Use water spray curtain to divert vapor drift. Use water spray to reduce vapors. Prevent entry into sewers, basements or confined areas; dike if needed. Call for assistance on disposal. Neutralize the residue with a dilute solution of sodium carbonate. Be careful that the product is not present at a concentration level above TLV. Check TLV on the MSDS and with local authorities.

**Handling and Storage**

**Precautions:**

Keep locked up. Keep container dry. Do not ingest. Do not breathe gas/fumes/ vapor/spray. Never add water to this product. In case of insufficient ventilation, wear suitable respiratory equipment. If ingested, seek medical advice immediately and show the container or the label. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents, organic materials, metals, alkalis, moisture. May corrode metallic surfaces. Store in a metallic or coated fiberboard drum using a strong polyethylene inner package.

**Storage:** Keep container tightly closed. Keep container in a cool, well-ventilated area.

**Exposure Controls****Engineering Controls:**

Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapors below their respective threshold limit value. Ensure that eyewash stations and safety showers are proximal to the work-station location.

**Personal Protection:**

Face shield. Full suit. Vapor respirator. Be sure to use an approved/certified respirator or equivalent. Gloves. Boots.

**Personal Protection in Case of a Large Spill:**

Splash goggles. Full suit. Vapor respirator. Boots. Gloves. A self-contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

**Exposure Limits:**

CEIL: 5 (ppm) from OSHA (PEL) [United States] CEIL: 7 (mg/m<sup>3</sup>) from OSHA (PEL) [United States] CEIL: 5 from NIOSH

CEIL: 7 (mg/m<sup>3</sup>) from NIOSH TWA: 1 STEL: 5 (ppm) [United Kingdom (UK)] TWA: 2 STEL: 8 (mg/m<sup>3</sup>) [United Kingdom

(UK)] Consult local authorities for acceptable exposure limits.

## 5. MSDS Folin-Ciocalteu Reagent

**Hazards Identification****Potential Acute Health Effects:**

Very hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion, . Hazardous in case of skin contact (corrosive, permeator), of eye contact (corrosive). Liquid or spray mist may produce tissue damage particularly on mucous membranes of eyes, mouth and respiratory tract. Skin contact may produce burns. Inhalation of the spray mist may produce severe irritation of respiratory tract, characterized by coughing, choking, or shortness of breath. Severe over-exposure can result in death. Inflammation of the eye is characterized by redness, watering, and itching. Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering.

**Potential Chronic Health Effects:**

CARCINOGENIC EFFECTS: Classified 4 (No evidence.) by NTP, None. by OSHA, None. by NIOSH [Bromine]. Classified 3 (Not classifiable for human.) by IARC [Hydrogen chloride]. MUTAGENIC EFFECTS: Mutagenic for bacteria and/or yeast.

[Lithium sulfate monohydrate]. TERATOGENIC EFFECTS: Not available. DEVELOPMENTAL TOXICITY: Not available. The substance is toxic to mucous membranes. The substance may be toxic to kidneys, the nervous system, liver, cardiovascular system, upper respiratory tract, skin, eyes, central nervous system (CNS), teeth, thyroid. Repeated or prolonged exposure to the substance can produce target organs damage. Repeated or prolonged contact with spray mist may produce chronic eye irritation and severe skin irritation. Repeated or prolonged exposure to spray mist may produce respiratory tract irritation leading to frequent attacks of bronchial infection. Repeated exposure to a highly toxic material may produce general deterioration of health by an accumulation in one or many human organs.

### **First Aid Measures**

#### **Eye Contact:**

Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Cold water may be used. Get medical attention immediately.

#### **Skin Contact:**

In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Cover the irritated skin with an emollient. Cold water may be used. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention immediately.

#### **Serious Skin Contact:**

Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek immediate medical attention.

#### **Inhalation:**

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention immediately.

#### **Serious Inhalation:**

Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband.

WARNING: It may be hazardous to the person providing aid to give mouth-to-mouth resuscitation when the inhaled material is toxic, infectious or corrosive. Seek immediate medical attention.

#### **Ingestion:**

Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. If large quantities of this material are swallowed, call a physician immediately. Loosen tight clothing such as a collar, tie, belt or waistband.

**Serious Ingestion:** Not available.

### **Fire and Explosion data**

**Flammability of the Product:** Non-flammable.

**Auto-Ignition Temperature:** Not applicable.

**Flash Points:** Not applicable.

**Flammable Limits:** Not applicable.

**Products of Combustion:** Not available.

**Fire Hazards in Presence of Various Substances:** Not applicable.

**Explosion Hazards in Presence of Various Substances:** Non-explosive in presence of open flames and sparks, of shocks.



**Fire Fighting Media and Instructions:** Not applicable.

**Special Remarks on Fire Hazards:** Not available.

**Special Remarks on Explosion Hazards:** Mixtures with nitromethane are explosive (Phosphoric Acid)

## **Spills**

### **Small Spill:**

Dilute with water and mop up, or absorb with an inert dry material and place in an appropriate waste disposal container. If necessary: Neutralize the residue with a dilute solution of sodium carbonate.

### **Large Spill:**

Corrosive liquid. Poisonous liquid. Stop leak if without risk. Absorb with DRY earth, sand or other non-combustible material. Do not get water inside container. Do not touch spilled material. Use water spray curtain to divert vapor drift. Use water spray to reduce vapors. Prevent entry into sewers, basements or confined areas; dike if needed. Call for assistance on disposal. Neutralize the residue with a dilute solution of sodium carbonate. Be careful that the product is not present at a concentration level above TLV. Check TLV on the MSDS and with local authorities.

## **Handling and Storage**

### **Precautions:**

Keep locked up. Keep container dry. Do not ingest. Do not breathe gas/fumes/ vapor/spray. Never add water to this product.

In case of insufficient ventilation, wear suitable respiratory equipment. If ingested, seek medical advice immediately and show the container or the label. Avoid contact with skin and eyes. May corrode metallic surfaces. Store in a metallic or coated fiberboard drum using a strong polyethylene inner package.

**Storage:** Keep container tightly closed. Keep container in a cool, well-ventilated area.

### **Engineering Controls:**

Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapors below their respective threshold limit value. Ensure that eyewash stations and safety showers are proximal to the work-station location.

### **Personal Protection:**

Face shield. Full suit. Vapor respirator. Be sure to use an approved/certified respirator or equivalent. Gloves. Boots.

### **Personal Protection in Case of a Large Spill:**

Splash goggles. Full suit. Vapor respirator. Boots. Gloves. A self contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

## **Exposure**

### **Exposure Limits:**

Sodium molybdate (VI) dihydrate TWA: 5 (mg/m<sup>3</sup>) from ACGIH (TLV) [United States] Inhalation Respirable. TWA: 5 (mg/m<sup>3</sup>) from OSHA (PEL) [United States] Inhalation Respirable. TWA: 15 (mg/m<sup>3</sup>) from OSHA (PEL) [United States] Inhalation Total.

Bromine TWA: 0.66 STEL: 1.3 (mg/m<sup>3</sup>) from ACGIH (TLV) [United States] TWA: 0.1 STEL: 0.2 (ppm) from ACGIH (TLV) [United States] TWA: 0.1 from OSHA (PEL) [United States] TWA: 0.7 (mg/m<sup>3</sup>) from OSHA (PEL) [United States] TWA: 0.66 STEL: 2 (mg/m<sup>3</sup>) [United Kingdom (UK)] TWA: 1 STEL: 0.3 (ppm) [United Kingdom (UK)] Hydrogen chloride STEL: 7.5 (mg/ m<sup>3</sup>) from ACGIH (TLV) [United States] STEL: 5 (ppm) from ACGIH (TLV) [United States] CEIL: 5 (ppm) from NIOSH CEIL: 7.5 (mg/m<sup>3</sup>) from NIOSH CEIL: 5 (ppm) from OSHA (PEL) [United States] CEIL: 7 (mg/m<sup>3</sup>) from OSHA (PEL) [United States] Phosphoric Acid TWA: 1 STEL: 3 (mg/m<sup>3</sup>) from ACGIH (TLV) [United States] TWA: 1 STEL: 3 (mg/m<sup>3</sup>) from OSHA (PEL) [United States] TWA: 1 STEL: 3 (mg/m<sup>3</sup>) from NIOSH TWA: 1 STEL: 3 (mg/m<sup>3</sup>) [Mexico] Sodium tungstate dihydrate TWA: 5 STEL: 10 (mg/m<sup>3</sup>) from ACGIH (TLV) [United States] Bromine<sup>33</sup>

## Standard Operating Procedure – Saponin Analyses

### Section 1 - Personal Protective Equipment

1. Lab Coat or Lab Gown
2. Hair tied back if long
3. Proper enclosed footwear
4. Safety glasses or goggles
5. Nitrile or latex gloves

### SOP for Saponin Analysis in Alfalfa

Collecting & preparing plant samples:

1. Clip about 50-100 grams of vegetative material and place in cooler with dry or regular ice until they can be placed in freezer.
2. Freeze-dry plant samples.
3. Grind them in a Wiley mill to pass through a 1mm screen.
- 4.

Extracting Saponins to create standard:

\*note: extraction of saponins for the standard requires 25-50 grams of dried ground material, depending on how much pure saponin desired (~25 grams plant material = 0.2111 grams saponin).

STEP A: Soxhlet Extraction

1. Measure 25 grams of ground alfalfa and place in paper thimble.
2. Extract with HPLC grade hexane (~ 700mL) using soxhlet method for ~ 30 hours. Discard hexane.
3. Remove thimble and set out to dry overnight.
4. Continue soxhlet extraction using methanol (700-750mL) for ~ 30 hours. Keep extraction with methanol solvent.

STEP B: Chloroform Extraction

1. Use a rotary evaporator (roto-vap) to remove excess methanol solvent (~1.5 hours).
2. Add 600mL of 50/50 chloroform/distilled water to extract and decant into a separation funnel.
3. Drain lower chloroform layer to discard.
4. Add 300 mL of 50/50 chloroform/water, shake and let settle. Drain lower chloroform layer to discard.
5. Repeat step 4 for a total of 4 chloroform extractions.
- 6.

STEP C: Butanol Extraction

1. Combine 300mL N-butanol with 100mL distilled water in separation funnel. Shake and let settle. Drain lower layer of water do discard.
2. Mix 100mL of water-saturated N-butanol from step C1 with aqueous solution from step B and 1.5 grams sodium chloride in separation funnel.
3. Drain lower polar layer to discard.
4. Add 100mL water-saturated N-butanol to separation funnel with non-polar layer, mix, let settle and drain lower polar layer to discard.
5. Repeat step 4 for a total of 3 butanol extractions.
6. Roto-vap extract to dryness.

#### STEP D: Purifying Saponin Extract

1. Add ~ 4mL methanol to flask and sonicate to dissolve all dry residue.
2. Introduce drop-wise into acetone (~ 900mL).
3. Butchner funnel acetone to isolate precipitate. Add precipitate back into flask with ~ 2mL methanol, sonicate and drip into fresh acetone (~ 900mL).
4. Repeat step 3 (2X) and after the fourth and final time, allow the precipitate to stand in acetone for 16 hours.
5. Collect the precipitate by using a butchner funnel and allow it to dry overnight at room temperature. Yield = .2111 grams purified saponin.

#### STEP E: Creating Standard Curve

\*Note: Any glassware, especially the test tubes and caps, used for the entire procedure must be washed and rinsed properly to remove all residual soap or it will confound results.

1. Measure 10mg saponin extract and add to 10mL distilled water in 16mL glass centrifuge test tube.
2. Using a serial dilution create a 1mg/ml, 0.5mg/ml, 0.25mg/ml, 0.125mg/ml, etc... in 7 (exact brand as the first) test tubes.
3. Shake for 2 minutes and let settle for 15-20 minutes. Measure and record foam.
4. Using excel obtain an  $R^2$  value for the curve. Should be at least 0.97.

Determining saponin % using small-scale extractions:

Method developed by Steve, Dale, and Andrea

#### STEP A: Methanol Extraction

1. Weigh out 50 mg of dried and ground plant material in triplicates and put into 16ml screw-cap test tubes.
2. Add 5 mL methanol and vortex for 2 minutes or put on roto-gei for 30 minutes.

3. Centrifuge for 5 minutes at 2500 rpm.
4. Pipette or decant supernatant into 20 mL vials.
5. Repeat steps 2-3 for a total of 2 methanol extractions.
6. Dry down methanol extractions.

#### STEP B: Chloroform Extraction

\*Note: use teflon caps and glass pipettes and vials for chloroform extractions.

1. Add 5 mL distilled water and 5 mL chloroform to each sample from step A. Shake until all dried residue is dissolved.
2. Centrifuge 5 minutes at 2500rpm.
3. Pipette lower chloroform layer out to discard.
4. Add 5ml chloroform to each vial.
5. Repeat steps 2-4 for a total of 4 chloroform extractions.

#### STEP C: N-Butanol Extraction

1. Combine 150mL N-butanol with 50mL distilled water and 0.75 grams sodium chloride in separation funnel. Shake and let settle. Drain lower layer of water to discard.
2. After discarding the lower chloroform layer from the 4<sup>th</sup> chloroform extraction in step B, add 5ml water-saturated N-butanol (from step C1) to each sample.
3. Shake then centrifuge 5 minutes at 2500rpm.
4. Pipette upper non-polar layer out and put into new and labeled 16mL test tubes.

\*Note: these test tubes will be used for the foam test so they must be the same brand used in creating the standard curve. Also, if one doesn't have a nitrogen dryer to dry samples down, this step may use 20mL vials, put in sand bath and improvise blowers with glass pipettes and tubing. The dry residue will dissolve in water and can then be transferred to a 16mL centrifuge tube for the foam test.

5. Add 5ml water-saturated N-butanol to each sample from step C2. Shake and centrifuge 5 minutes at 2500rpm.
6. Repeat step C4 so there is an accumulation of ~ 15ml of non-polar supernatant in the new and labeled test tubes.
7. Dry down N-butanol extractions.

#### STEP D: Foam Test

1. Add 5mL distilled water to each sample. Shake for 2 minutes, let stand for 15-20 minutes then measure and record foam.

2. Calculate the percent of saponins by calculating mg/ml based on the standard curve equation (for instance, if the 0.25mg/ml from the standard rendered 2.8 cm of foam, use the number 0.089 by dividing 0.25 by 2.8), multiply by the cm of foam the sample rendered, multiply by 5, divide by the weight of the sample used in the extraction, lastly multiply by 100 for a percent by weight saponin content.

\*Note: foam may be reduced (if a re-measurement is required) by centrifuging.

## MSDS-SAPONIN ANALYSES

### 1. Hexane

#### Hazards Identification

##### Potential Acute Health Effects:

Hazardous in case of skin contact (permeator), of ingestion, of inhalation. Slightly hazardous in case of skin contact (irritant), of eye contact (irritant).

##### Potential Chronic Health Effects:

CARCINOGENIC EFFECTS: Not available. MUTAGENIC EFFECTS: Mutagenic for bacteria and/or yeast. TERATOGENIC EFFECTS: Not available. DEVELOPMENTAL TOXICITY: Not available. The substance may be toxic to peripheral nervous system, skin, central nervous system (CNS). Repeated or prolonged exposure to the substance can produce target organs damage.

#### First Aid Measures

##### Eye Contact:

Check for and remove any contact lenses. Immediately flush eyes with running water for at least 15 minutes, keeping eyelids open. Get medical attention if irritation occurs.

**Skin Contact:** Wash with soap and water. Cover the irritated skin with an emollient. Get medical attention if irritation develops.

##### Serious Skin Contact:

Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek medical attention.

##### Inhalation:

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention if symptoms appear.

##### Serious Inhalation:

Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband. If breathing is difficult, administer oxygen. If the victim is not breathing, perform mouth-to-mouth resuscitation. Seek medical attention.

##### Ingestion:

Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention if symptoms appear.

**Serious Ingestion:** Not available.

**Flammability of the Product:** Flammable.

**Auto-Ignition Temperature:** 225°C (437°F)

**Flash Points:** CLOSED CUP: -22.5°C (-8.5°F). (TAG)

**Flammable Limits:** LOWER: 1.15% UPPER: 7.5%

**Products of Combustion:** These products are carbon oxides (CO, CO<sub>2</sub>).

**Fire Hazards in Presence of Various Substances:**

Highly flammable in presence of open flames and sparks, of heat. Non-flammable in presence of shocks.

**Explosion Hazards in Presence of Various Substances:**

Risks of explosion of the product in presence of mechanical impact: Not available.

Risks of explosion of the product in presence of static discharge: Not available.

**Fire and Explosion Data**

**Fire Fighting Media and Instructions:**

Flammable liquid, insoluble in water. SMALL FIRE: Use DRY chemical powder. LARGE FIRE: Use water spray or fog.

**Special Remarks on Fire Hazards:**

Extremely flammable liquid and vapor. Vapor may cause flash fire.

**Special Remarks on Explosion Hazards:** Not available.

**Spills**

**Small Spill:** Absorb with an inert material and put the spilled material in an appropriate waste disposal.

**Large Spill:**

Flammable liquid, insoluble in water. Keep away from heat. Keep away from sources of ignition. Stop leak if without risk.

Absorb with DRY earth, sand or other non-combustible material. Do not get water inside container. Do not touch spilled material. Prevent entry into sewers, basements or confined areas; dike if needed. Call for assistance on disposal. Be careful that the product is not present at a concentration level above TLV. Check TLV on the MSDS and with local authorities.

**Handling and Storage**

**Precautions:**

Keep locked up. Keep away from heat. Keep away from sources of ignition. Ground all equipment containing material.

Do not ingest. Do not breathe gas/fumes/ vapor/spray. Avoid contact with skin. Wear suitable protective clothing. In case of insufficient ventilation, wear suitable respiratory equipment. If ingested, seek medical advice immediately and show the container or the label. Keep away from incompatibles such as oxidizing agents.

**Storage:**

Store in a segregated and approved area. Keep container in a cool, well-ventilated area. Keep container tightly closed and sealed until ready for use. Avoid all possible sources of ignition (spark or flame).

**Exposure Controls/Personal Protection**

**Engineering Controls:**

Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapors below their respective threshold limit value. Ensure that eyewash stations and safety showers are proximal to the work-station location.

**Personal Protection:**

Safety glasses. Lab coat. Vapor respirator. Be sure to use an approved/certified respirator or equivalent. Gloves (impervious).

**Personal Protection in Case of a Large Spill:**

Splash goggles. Full suit. Vapor respirator. Boots. Gloves. A self-contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

**Exposure Limits:**

TWA: 500 (ppm) from OSHA (PEL) [United States] Inhalation TWA: 1800 (mg/m<sup>3</sup>) from OSHA (PEL) [United States] Inhalation TWA: 176 (mg/m<sup>3</sup>) from ACGIH (TLV) [United States] SKIN TWA: 50 (ppm) from ACGIH (TLV) [United States] SKIN TWA: 500 STEL: 1000 (ppm) from ACGIH (TLV) [United States] Inhalation TWA: 1760 STEL: 3500 (mg/m<sup>3</sup>) from ACGIH (TLV) [United States] Inhalation Consult local authorities for acceptable exposure limits.

## 2. Butanol

**Hazards Identification**

**Potential Acute Health Effects:**

Very hazardous in case of skin contact (irritant, permeator), of eye contact (irritant), of ingestion, of inhalation. Slightly hazardous in case of skin contact (sensitizer). Inflammation of the eye is characterized by redness, watering, and itching. Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering.

**Potential Chronic Health Effects:**

CARCINOGENIC EFFECTS: Not available. MUTAGENIC EFFECTS: Not available. TERATOGENIC EFFECTS: Not available.

DEVELOPMENTAL TOXICITY: Not available. Repeated or prolonged exposure is not known to aggravate medical condition.

**First Aid Measures**

**Eye Contact:**

Check for and remove any contact lenses. Immediately flush eyes with running water for at least 15 minutes, keeping eyelids open. Cold water may be used. Get medical attention immediately.

**Skin Contact:**

In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Cover the irritated skin with an emollient. Cold water may be used. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention immediately.

**Serious Skin Contact:**

Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek immediate medical attention.



**Inhalation:**

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention if symptoms appear.

**Serious Inhalation:**

Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband. If breathing is difficult, administer oxygen. If the victim is not breathing, perform mouth-to-mouth resuscitation. Seek medical attention.

**Ingestion:**

Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. If large quantities of this material are swallowed, call a physician immediately. Loosen tight clothing such as a collar, tie, belt or waistband.

**Serious Ingestion:** Not available.

**Fire and Explosion Data**

**Flammability of the Product:** Flammable.

**Auto-Ignition Temperature:** 343°C (649.4°F)

**Flash Points:** CLOSED CUP: 28.9°C (84°F). OPEN CUP: 36.1°C (97°F) (Cleveland).

**Flammable Limits:** LOWER: 1.4% UPPER: 11.2%

**Products of Combustion:** These products are carbon oxides (CO, CO<sub>2</sub>).

**Fire Hazards in Presence of Various Substances:**

Highly flammable in presence of open flames and sparks. Flammable in presence of heat, of oxidizing materials, of reducing materials, of combustible materials.

**Explosion Hazards in Presence of Various Substances:**

Risks of explosion of the product in presence of mechanical impact: Not available. Risks of explosion of the product in presence of static discharge: Not available.

**Fire Fighting Media and Instructions:**

Flammable liquid, soluble or dispersed in water. SMALL FIRE: Use DRY chemical powder. LARGE FIRE: Use alcohol foam, water spray or fog. Cool containing vessels with water jet in order to prevent pressure build-up, autoignition or explosion.

**Special Remarks on Fire Hazards:** May form explosive mixtures with air. CAUTION: MAY BURN WITH NEAR INVISIBLE FLAME

**Special Remarks on Explosion Hazards:** Not available.

**Spills****Small Spill:**

Dilute with water and mop up, or absorb with an inert dry material and place in an appropriate waste disposal container.

**Large Spill:**

p. 3

Flammable liquid. Keep away from heat. Keep away from sources of ignition. Stop leak if without risk. Absorb with DRY earth, sand or other non-combustible material. Do not touch spilled material. Prevent entry into sewers, basements or confined areas; dike if needed. Be careful that the product is not present at a concentration level above TLV. Check TLV on the MSDS and with local authorities.

**Handling and storage****Precautions:**

Keep away from heat. Keep away from sources of ignition. Ground all equipment containing material. Do not ingest. Do not breathe gas/fumes/ vapor/spray. In case of insufficient ventilation, wear suitable respiratory equipment. If ingested, seek medical advice immediately and show the container or the label. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents, reducing agents.

**Storage:**

Store in a segregated and approved area. Keep container in a cool, well-ventilated area. Keep container tightly closed and sealed until ready for use. Avoid all possible sources of ignition (spark or flame).

**Exposure Controls**

**Engineering Controls:**

Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapors below their respective threshold limit value. Ensure that eyewash stations and safety showers are proximal to the work-station location.

**Personal Protection:**

Splash goggles. Lab coat. Vapor respirator. Be sure to use an approved/certified respirator or equivalent. Gloves.

**Personal Protection in Case of a Large Spill:**

Splash goggles. Full suit. Vapor respirator. Boots. Gloves. A self contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

**Exposure Limits:**

CEIL: 152 (mg/m<sup>3</sup>) Consult local authorities for acceptable exposure limits.

### 3. Chloroform

**Hazards Identification**

**Potential Acute Health Effects:** Hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion, of inhalation.

Slightly hazardous in case of skin contact (permeator).

**Potential Chronic Health Effects:** CARCINOGENIC EFFECTS: Classified + (Proven.) by NIOSH.

Classified A3 (Proven for animal.) by ACGIH, 2B (Possible for human.) by IARC. Classified 2

(Some evidence.) by NTP. MUTAGENIC EFFECTS: Mutagenic for mammalian somatic cells.

Mutagenic for bacteria and/or yeast. TERATOGENIC EFFECTS: Not available.

DEVELOPMENTAL TOXICITY: Not available. The substance may be toxic to kidneys, liver, heart.

Repeated or prolonged exposure to the substance can produce target organs damage.

**First Aid Measures**

**Eye Contact:** Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Cold water may be used. WARM water MUST be used. Get medical attention.

**Skin Contact:** In case of contact, immediately flush skin with plenty of water. Cover the irritated skin with an emollient. Remove contaminated clothing and shoes. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention.

**Serious Skin Contact:** Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek immediate medical attention.

**Inhalation:** If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.

**Serious Inhalation:** Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband. If breathing is difficult, administer oxygen. If the victim is not breathing, perform mouth-to-mouth resuscitation.

**WARNING:** It may be hazardous to the person providing aid to give mouth-to-mouth resuscitation when the inhaled material is toxic, infectious or corrosive. Seek medical attention.

**Ingestion:** Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. If large quantities of this material are swallowed, call a physician immediately. Loosen tight clothing such as a collar, tie, belt or waistband.

**Serious Ingestion:** Not available.

#### **Fire and Explosion Data**

**Flammability of the Product:** Non-flammable.

**Auto-Ignition Temperature:** Not applicable.

**Flash Points:** Not applicable.

**Flammable Limits:** Not applicable.

**Products of Combustion:** Not available.

**Fire Hazards in Presence of Various Substances:** Not applicable.

**Explosion Hazards in Presence of Various Substances:** Risks of explosion of the product in presence of mechanical impact: Not available. Risks of explosion of the product in presence of static discharge: Not available.

**Fire Fighting Media and Instructions:** Not applicable.

**Special Remarks on Fire Hazards:** Not available.

**Special Remarks on Explosion Hazards:** May explode if it comes in contact with aluminum powder, lithium, perchlorate, pentoxide, bis(dimethylamino)dimethylstannane, potassium, potassium-sodium alloy, sodium (or sodium hydroxide or sodium methoxide), and methanol

#### **Spills**

**Small Spill:** Absorb with an inert material and put the spilled material in an appropriate waste disposal.

**Large Spill:** Absorb with an inert material and put the spilled material in an appropriate waste disposal. Be careful that the product is not present at a concentration level above TLV. Check TLV on the MSDS and with local authorities.

#### **Handling and Storage**

**Precautions:** Do not ingest. Do not breathe gas/fumes/ vapor/spray. Wear suitable protective clothing. In case of insufficient ventilation, wear suitable respiratory equipment. If ingested, seek medical advice immediately and show the container or the label. Avoid contact with skin and eyes. Keep away from incompatibles such as metals, alkalis.

**Storage:** Keep container tightly closed. Keep container in a cool, well-ventilated area. Sensitive to light. Store in light-resistant containers.

#### **Exposure Controls/Personal Protection**

**Engineering Controls:** Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapors below their respective threshold limit value. Ensure that eyewash stations and safety showers are proximal to the workstation location.

**Personal Protection:** Splash goggles. Lab coat. Vapor respirator. Be sure to use an approved/certified respirator or equivalent. Gloves.

**Personal Protection in Case of a Large Spill:** Splash goggles. Full suit. Vapor respirator. Boots. Gloves. A self-contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

**Exposure Limits:** TWA: 10 (ppm) [Australia] Inhalation TWA: 2 (ppm) from OSHA (PEL) [United States] Inhalation STEL: 9.78 (mg/m<sup>3</sup>) from NIOSH Inhalation STEL: 2 (ppm) from NIOSH Inhalation TWA: 9.78 (mg/m<sup>3</sup>) from OSHA (PEL) [United States] Inhalation TWA: 10 (ppm) from ACGIH (TLV) [United States] [1999] Inhalation TWA: 2 (ppm) [United Kingdom (UK)] Inhalation TWA: 9.9 (mg/m<sup>3</sup>) [United Kingdom (UK)] Inhalation Consult local authorities for acceptable exposure limits.



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# Standard Operating Procedures – Green Canyon Ecology Center

## Standard Operating Procedure – General Care of Livestock

1. Make sure sheep have an ample supply of clean water at all times. Small water troughs should be cleaned frequently and large troughs should be cleaned at least once a week.

Feed should be clean and mold-free. Feeders should be kept free from fecal material.

Don't change the type of feed you are feeding abruptly. Abrupt changes in feed can cause acidosis and/or bloat.

Animals should have access to trace mineralized salt blocks at all times.

Vaccinations. Lambs should be given vaccinated against *Clostridium* (enterotoxemia) and tetanus at 30 and 60 days of age.

Lambs should have their feet trimmed and they should receive a drench of antiparasitic drugs in the spring.

## Standard Operating Procedure – Mixing Feed for Rations and Use of Mixer

Operator should be able to demonstrate competent safe operation to supervisor prior to authorization of use.

### Section 1 - Personal Protective Equipment

- Eye protection required
- Long of loose hair must be tied back or contained
- Hearing protection required
- Approved dust mask required
- Work gloves required
- Protective clothing required
- No jewelry, watches, ring, necklaces
- No loose fitting clothing

### Section 2 - Potential Hazards + Safety precautions

Crushing hazards when operating the machine. Worker entanglement hazards on moving parts. Bystander or operator run over. Projectile debris hazards from rotating parts and feed. Noise. Confined space. No worker should enter the mixer when is not properly checked that the machine is turned off.

## **Procedure**

- Inspect required personnel protective equipment and replace if required
- Put on all required personnel protective equipment
- Start the mixer by pulling the rope attached to the engine
- Fill and move the lever for mixing operation
- After 15-20 minutes (depending on the type of feed) begin discharging the feed material from mixer to a container
- Never climb on mixer during operation.

If an emergency occurs while conducting this task, or there is an equipment malfunction, shut the equipment off immediately.

Report any hazardous situation to your supervisor immediately.

## **Avoid Being Caught in Moving Parts**

Feeds should be fed into the mixer safely, to avoid contact with the equipment's moving parts. Follow these feeding procedures to keep you safe.

- Insert feeds only when the mixer is at full operating speed.
- Keep hands and feet outside of the feed chute.
- Let go of feed at least ½ m from the intake and walk away to avoid being hit or dragged into the mixer by its moving parts.

## **Safe Fueling Procedures**

- Gasoline and other fuels are flammable. Following safe fueling procedures will help reduce the risk of fuel ignition.
- Always shut off the engine and wait at least 5 minutes for the engine to cool before refueling.
- Use only approved fuel containers and store in a well ventilated area, away from direct sunlight.
- Never smoke or have an open flame near fuel.
- Touch the fuel nozzle to the chipper/shredder before opening the fuel cap to reduce the chance that a static spark will ignite the fuel.
- Keep the nozzle in contact with the filler neck while fueling.
- Replace the cap as soon as you are finished.
- Use a funnel or non-spill nozzle when fueling to reduce spillage.
- Wipe up all spills immediately, before starting the engine.
- Never clean your hands or tools with gasoline. Use a nonflammable solvent instead.



## **Standard Operating Procedure – Grinding Feed for Rations**

Operator should be able to demonstrate competent safe operation to supervisor prior to authorization of use.

### **Section 1 - Personal Protective Equipment**

- Eye protection required
- Long of loose hair must be tied back or contained
- Hearing protection required
- Approved dust mask required
- Work gloves required
- Protective clothing required
- No jewelry, watches, ring, necklaces
- No loose fitting clothing
- Close-fitting clothes
- Long pants and sturdy, non-slip boots

### **Section 2 - Potential Hazards + Safety precautions**

You can be seriously injured or killed if you incorrectly operate a chipper/shredder. The most commonly reported causes of serious accidents are:

1. Being Caught in Moving Parts
2. Struck by Chipper/Shredder's Hood

Sometimes you may be tempted to take risky shortcuts. Remember that an accident can leave you permanently injured or cut your life short. For your safety and the safety of those around you, do not take unnecessary risks. No deadline is so pressing you can't take the time to do your work safely.

Do not operate machinery if you are tired or have taken drugs or alcohol. If you are on medication, discuss with your doctor or pharmacist if you are capable of safely operating machinery

### **Procedure**

Place the machine on a level surface that is not concrete, paved or gravel. Operating on these surfaces can cause thrown material to ricochet and injure or kill the operator or bystanders. Also keep other workers from falling into the chipper/shredder by ensuring the location of the machine is not directly in front of or below where other employees are working.

Make sure that there is a tarp below the grinder so you catch all the ground material in the tarp.

Ensure the hood, enclosing the chipper/shredder's knives, is closed and latched before operating. Also, check for loose or missing pins in the hood latch as well as cracked or worn hinges. If the hood is not properly secured, it could fly off and seriously injure or kill you. The chipper/shredder should never be operated with the hood open.

Ensure the cutting chamber is free of foreign objects or other debris such as accumulated woody plant material. Woody plant material and foreign objects can be thrown from the chipper at high rates of speed.

Fluid leaks can cause fires and breakdowns. Report signs of leaking fluid to your supervisor immediately.

Move the lever to the "run" position and pull the rope by the engine until the engine starts. Start the chipper/shredder at the lowest possible speed and listen for any noise or vibration that could indicate loose or broken parts. If heard, turn off the machine.

Be aware at all times of the location of coworkers. Keep all others out of the area. Check the operator's manual for additional procedures.

Begin delivering the feed you want to grind to the intake of the grinder.

You can push the machine in the flat area once there is an accumulation of ground feed underneath the grinder.

Once you finished grinding, disconnect the power supply by moving the power lever to the off position. You can tell the machine has stopped when no noise or vibration is heard. Check the operator's manual for additional procedures.

### **Avoid Being Caught in Moving Parts**

Materials should be fed into the chipper/shredder safely, to avoid contact with the equipment's moving parts. Follow these feeding procedures to keep you safe.

- Feed material only when the chipper/shredder is at full operating speed.
- Feed branches from the side of the chute, not in front of it. This will reduce the risk of you being caught and dragged into the machine. Standing to the side of the equipment will also make it easier for you to reach the emergency shut-off switch in the event of an accident.
- Keep hands and feet outside of the feed chute.
- Use a push stick to help feed small pieces through the chipper/shredder to keep you at a distance from the machine's moving parts. Do not push materials into the chute with your hands or feet, pitch forks, shovels, etc.
- Let go of material as soon as it begins to be pulled into the machine and walk away to avoid being hit or dragged into the chipper by limbs you are feeding.

- Feed the branches butt end first to keep the chipper from being jammed and to reduce the kickback of material.
- Lay shorter pieces of material on top of longer pieces and feed through the machine.
- Collect small materials such as leaves and twigs and put them with the chipped material instead of feeding them through the chipper/shredder.

### **Safe Fueling Procedures**

- Gasoline and other fuels are flammable. Following safe fueling procedures will help reduce the risk of fuel ignition.
- Always shut off the engine and wait at least 5 minutes for the engine to cool before refueling.
- Use only approved fuel containers and store in a well ventilated area, away from direct sunlight.
- Never smoke or have an open flame near fuel.
- Touch the fuel nozzle to the chipper/shredder before opening the fuel cap to reduce the chance that a static spark will ignite the fuel.
- Keep the nozzle in contact with the filler neck while fueling.
- Replace the cap as soon as you are finished.
- Use a funnel or non-spill nozzle when fueling to reduce spillage.
- Wipe up all spills immediately, before starting the engine.
- Never clean your hands or tools with gasoline. Use a nonflammable solvent instead.

### **Transportation & Traffic**

Never move, load or transport a chipper/shredder while the engine is running. First, shut off the power supply, wait for all moving parts to come to a complete stop and then disconnect the spark plug wire.

### **Standard Operating Procedure – Grinding Feed for Chemical Analyses using a Wiley Mill**

Laboratory mills are devices used for grinding of plant tissue or other similar materials. Use of laboratory mills may present a variety of potential safety hazards.

#### **Mill Set-up**

- Use the right tool for the job. Laboratory mills such as the Thomas Scientific Wiley Mill are intended for grinding plant samples; they should not be used to grind soil or other hard materials.
- Before using, always inspect the laboratory mill for damage or disrepair.

- If a laboratory mill fails the pre-use inspection, notify your supervisor and remove the mill from service by attaching a red tag that states “DO NOT USE.” Complete red tag with appropriate information.
- Make sure the mill is unplugged before making any adjustments or cleaning.
- With the mill unplugged, remove the cover and inspect the feed chute, grinding blades, screen and delivery chute.
- Confirm that the proper screen and delivery chute are attached and change as necessary.
- If parts of the mill need cleaning, use a small brush, a vacuum, and/or compressed air (See Safety Note #153). Ethanol may be used if necessary to prevent contamination of samples.

### **Operating Tips**

- Make sure the sample is dry before milling; samples containing excessive moisture or oils will stick to the walls of the mill chamber.
- Use in a well ventilated area. If samples are excessively dusty, use the mill under a ventilated hood, or use a fan to exhaust the dust outside the work area.
- In general, respiratory protection should not be necessary when using a laboratory mill. However, a paper dust mask (not an N-95 respirator) may be used voluntarily for nuisance dust. If samples are suspected of containing molds or other disease-causing agents, contact ANR Environmental Health & Safety (<http://ucanr.org/askehs>) to conduct an exposure assessment to determine the appropriate personnel protective equipment.
- Wear eye protection (safety glasses or goggles) when using a laboratory mill.
- Do not put your hands or fingers inside the feed chute when feeding samples into the mill. If necessary, use a feeder stick to push material into the grinding chamber.
- If the feed chute becomes clogged or material becomes jammed, immediately shut off the mill.

### **Cleaning and Shutdown**

- Clean the mill as described above in Mill Set-up.
- It is a good practice to unplug the mill when not in use.
- Consult the operator’s manual for maintenance procedures such as lubrication or blade replacement.

## **Standard Operating Procedure – Building Pens for housing sheep**

### **Section 1 - Personal Protective Equipment**

- Long of loose hair must be tied back or contained
- Work gloves required
- Protective clothing required
- No jewelry, watches, ring, necklaces
- No loose fitting clothing
- Close-fitting clothes
- Long pants and sturdy, non-slip boots

### **Section 2 - Potential Hazards + Safety precautions**

You can be cut with the panels or the wire used to tie the panels. Work gloves are required as well as non-slip boots, long pants and long-sleeve shirts to avoid skin cuts.

## **Standard Operating Procedure – Safe Sheep Handling**

### **Catching sheep**

It is easier to catch a sheep if it is part of a group, rather than by itself. Catching sheep is also easier in a pen. The smaller the pen, the better. If you can, use gates or hurdles to make the pen as small as possible. If you are in the paddock, bunch the sheep into a corner for catching.

Get the sheep into a corner, extending your arms to make a visual barrier. Approach the sheep slowly and calmly. The sheep may try to escape but will probably not move away from the wall or fence, so its moves can be anticipated.

If you can, come up quickly through the 'blind spot' directly behind the sheep. Grab it under the chin. Do not grab the wool as this causes pain, distress and bruising.

Do not chase sheep around the pen or paddock. It's tiring and potentially dangerous for both the sheep and the catcher. Sheep that are repeatedly chased become flighty, stressed and difficult to work with.

You can use a neck or leg crook, which gives you a longer reach, for catching sheep. You can also use it to catch a sheep by the hind leg, but sometimes this can damage the legs and udder.

### **Controlled holding and walking**

The easiest way to keep a sheep still is to stand it against rails or a fence and hold it with your knees and a hand under the chin.

To walk a sheep, stand over the sheep's shoulders with a leg either side of the sheep and your hand under the chin. You can control the sheep with pressure from your knees. Walk the sheep forward by squeezing it with your knees, and/or squeezing the top of the tail with one hand, keeping your other hand under the sheep's chin. It can be difficult for a short person to walk a tall sheep in this way, so make sure it is within your capabilities.

### **Sitting a sheep up**

The best way to sit a sheep up is to turn the sheep's head on to its shoulder.

In a clear area, hold the sheep against your braced knees with one hand under its chin and one on its rump. Turn the sheep's head to the rear with one hand while forcing the rump down against your leg with the other hand. When the sheep is no longer standing, lift the front leg and sit the sheep securely on its rump.

### **Manually handling sheep**

Manually handling sheep is a physically demanding job. Due to the size and strength of sheep, you face problems like back strain and knee injuries when handling and restraining them.

Injuries also happen when sheep run into you and/or knock you over. Sheep are not usually aggressive but, if they are frightened, they can hurt people when trying to get away. For example, they may jump when stressed. They can jump with enough force to break a handler's leg, or high enough to strike a handler in the chest or face and knock them over.

### **Lifting sheep**

Sheep are large and heavy animals and can fight against being lifted. This creates the potential for strains and back twisting injuries.

Avoid lifting sheep if possible. Use gates and ramps where available. If you have to lift a sheep, use your legs, not your back.

Only physically fit and strong people should lift sheep. No one should lift a sheep if it is too heavy for them.

## **Standard Operating Procedures – Lewiston Irrigated Pasture Facility**

### **Standard Operating Procedure – Cattle Managing Procedures**

#### **Section 1 - Personal Protective Equipment**

- Long of loose hair must be tied back or contained
- Work gloves required
- Protective clothing required
- No jewelry, watches, ring, necklaces
- No loose fitting clothing
- Close-fitting clothes
- Long pants and sturdy, non-slip boots

#### **Loading, unloading and transportation- Potential Hazards + Safety precautions**

- Cattle sorting and holding pens should allow handling without undue stress, and be located near the loading/unloading facility.
- All vehicles used to transport cattle should provide for the safety of personnel and cattle during loading, transporting and unloading.
- Strictly adhere to safe load levels with regard to animal weight and space allocation.

- Personnel hauling cattle in farm and ranch trailers must ensure that adequate space is provided so that cattle have sufficient room to stand with little risk of being forced down because of overcrowding.
- When the vehicle is not full, safely partition cattle into smaller areas to provide load stability for the cattle and the vehicle.
- No gap which would allow injury to an animal should exist between the ramp, its sides, flooring, and the vehicle.
- Vehicle doors and internal gates should be sufficiently wide to permit cattle to pass through easily without bruising or injury.
- Cattle should be loaded, unloaded, and moved through facilities with patience and as quietly as possible to reduce stress and injury.

### **Animal Weighing- Potential Hazards + Safety precautions**

- Measurement of live weight gain requires the fasting of animals to minimize the variation caused by 'gut-fill' from digesta.
- Any cattle fasting for 12-24 hours must have water available in the holding pen.
- Weights of cattle are obtained on an individual basis to determine their daily gain. One other purpose for weighing cattle individually is to use the weights to block the cattle into weight groups or to sort animals into treatment groups.
- On weigh days, cattle are brought to the group pen by the scale located at the animal working facility. The scale is zeroed, and it is checked periodically as animals within a group are weighed to ensure that the indicator returns to zero when no animal is on the scale. After all animals from a group have been weighed, the scale is checked for zero, cattle are returned to their respective pastures, and the next group is weighed.
- Cattle should be handled quietly before, during and after the procedure.
- Adjust hydraulic chute to the appropriate size of cattle to be handled. Regular cleaning and maintenance of working parts is imperative to ensure the system functions properly and is safe for the cattle and handlers.
- Floors should be properly drained and barns and handling alleys should provide adequate traction to prevent injuries to animals and handlers.
- Handling alleys and housing pens should be free of sharp edges and protrusions to prevent injury to animals and handlers.
- Design and operate alleys and gates to avoid impeding cattle movement.
- When operating gates and catches, reduce excessive noise, which may cause distress to the animals.
- Avoid slippery surfaces, especially where cattle enter a single file alley leading to a chute or where they exit the chute. Grooved concrete, metal grating (not sharp), rubber mats or deep sand can be used to minimize slipping and falling.
- Quiet handling is essential to minimize slipping.
- Under most conditions, no more than 2% of the animals should fall outside the chute.



- Take advantage of cattle's flight zone and point of balance to move them. For safety and welfare reasons, minimize the use of electric prods. Non-electric driving aids, such as plastic paddles, sorting sticks, flags or streamers (affixed to long handles) should be used to quietly guide and turn animals. When cattle continuously balk, cattle handlers should investigate and correct the reason rather than resort to overuse of electric prods.

#### **Tips for better cattle handling**

- Cattle have wide angle vision in excess of 300°.
- Position yourself adjacent to the head of the lead animal and at about 90° to the direction the group is to be moved.
- Be at a distance appropriate for the particular group (i.e., where your presence is recognized but the lead animal does not attempt to move away from you).
- Be on the side of the lead animal that you wish the group to turn away from (cattle are uncomfortable losing eye contact with you and are reluctant to turn with their back towards you).
- Moving forward 90° will cause the lead animal to slow down, and moving behind 90° will either speed the animal or encourage it to turn towards you.