Standard Operating Procedures Carr Group June 2012

Personal protection

There will almost invariably be a senior person assigned to be in charge of a chemical laboratory, irrespective of the nature of the work to be done there. However, it must be emphasized that the exercise of care and the adoption of safe working procedures is the responsibility of each and every person in that laboratory. If there is any doubt as to the safety of a proposed experiment, advice should be sought from an experienced person rather than just hoping for the best.

All workers must adopt a responsible attitude to their work and avoid any thoughtless, ignorant or hurried behavior which may lead to an accident and possible harm to themselves or to others. They should always pay attention to what is going on around them and be aware of the possible dangers arising from the work of others as well as from their own experiments.

No worker should be allowed in a laboratory without a full-length protective coat, preferably white, since spillages and stains are then more readily detected. *Furthermore, all personnel, including visitors, must wear safety spectacles or goggles at all times.*

Eating, drinking and smoking in the laboratory should also be forbidden; these constitute a further, avoidable, risk of the ingestion of toxic substances, and in the case of smoking an obvious fire hazard.

Tidiness and cleanliness

Tidiness is a major factor in laboratory safety; the laboratory must be kept clean and tidy at all times. Passageways between and around the benches and near exits must not be blocked with equipment or furniture. Floors must be kept in good condition to prevent slipping or tripping, i.e. they must be kept free from oil or water, and from any protrusion. Any spillage on a floor or bench should be cleaned up immediately.

Some indication of a chemist's practical ability is apparent from the appearance of the working bench. This should always be kept clean and dry; this is easily done if suitable wet and dry rags are kept at hand. Apparatus not immediately required should be kept as far as possible in a cupboard beneath the bench; if it must be placed on the bench, it should be arranged in a neat and orderly manner. Dirty apparatus can be placed in a plastic bowl away from the working area until it can be cleaned and put away. *Solid waste and filter papers must not be thrown in the sink.*

All glassware should be scrupulously clean and, for most purposes, dry before being employed in preparative work in the laboratory. It is advisable to develop the habit of cleaning all glass apparatus immediately after use as the nature of the contaminant will, in general, be known. Furthermore, the cleaning process becomes more difficult if the dirty apparatus is allowed to stand for a considerable period, particularly if volatile solvents have evaporated in the meantime.

Accident procedure

Every person working in a laboratory should ensure that he or she knows where the exits and fire escapes are situated and that there is free access to them. All workers should also note the positions of fire extinguishers, fire blankets and drench showers, and make sure that they know how to use them. The checking of such equipment should be carried out by the proper authorities (LSO) at regular intervals. The worker should also be familiar with the location of the first-aid equipment provided for emergency use, and the position of the nearest telephone; the numbers of the appropriate medical teams, hospitals and fire department should be displayed in a prominent position.

Eyewash

Eyewash stations provide an effective means of treatment when chemicals come in contact with the eyes. Eyewash stations should be readily available and accessible to all laboratory personnel. An eye injury usually accompanies a skin injury. For this reason, eye wash stations should be located near the safety shower and/or drench hose so that eyes and body can be washed.

Water/eye solutions should not be directly aimed onto the eyeball, but rather, aimed at the base of the nose. This increases the chance of effectively rinsing the eyes free of chemicals (harsh streams of water may drive particles further into the eyes).

- Eyelids may have to be **forcibly** opened to attempt eye rinse.
- Flood eyes and eyelids with water/eye solution for a minimum of 15 minutes.
- Remove contact lenses as soon as possible to rinse eyes of any harmful chemicals.
- Eye wash stations should be drained and tested weekly by laboratory personnel and inspected every six months.

Safety showers

Safety showers provide an effective means of treatment in the event that chemicals are spilled or splashed onto the skin or clothing. Safety shower facilities should be installed wherever corrosive chemicals are used (e.g. acids or alkalis) and must be readily available to all personnel.

- Safety showers should be in a **clearly marked** location.
- Laboratory workers should be able to locate the shower with their **eyes closed** (emergency situations may leave victims temporarily blind).
- Safety showers are operated by a triangular rod.
- Safety showers should supply a continuous stream of water to cover the entire body.
- Individuals should remove contaminated clothing, including shoes and jewelry, while under an operating shower.
- Safety showers should be located **away from electrical panels or outlets**.
- If at all possible, safety shower facilities should be installed near appropriate drainage systems.

After-hours working

No person should ever work alone in a laboratory.

Experiments which must be left running overnight the apparatus labeled clearly as to the nature of the reaction and the likely hazards. Clear instructions must be left so that an unqualified person can terminate the experiment in an emergency. 'Please leave on' notices should be left alongside any service which is to be left running (water, electricity).

Storage of chemicals in laboratory areas

Chemicals should never be allowed to accumulate on benches or in fume cupboards, but should always be returned to their proper places on robust storage shelves; incompatible chemicals should be separated from one another. Heavy containers and bottles of dangerous chemicals should be returned as soon as possible to the main chemical store where specific regulations for safe storage apply. Strict regulations also apply to the quantity of solvents that may be stored in a specific laboratory; furthermore such solvents must be stored in a fire-proof steel cabinet fitted with a vapor-seal door, and an area must be assigned and properly equipped for the safe dispensing of flammable and toxic solvents.

All containers (bottles, ampoules, vials, etc.) of chemicals purchased commercially bear a clear label indicating the nature of the contents, and a hazard symbol, together with risk and safety summaries specifying the possible danger associated with the contents.

Refrigerator

- Each refrigerator, freezer or other cooling unit should be prominently labeled with appropriate hazard signs to indicate whether it is suitable for storing hazardous chemicals. Label chemical hazard refrigerators with the sign ‘For Chemical Storage Only. No Foof or Drink Allowed.’
- Chemicals stored in refrigerators should be placed on a spill tray with edges sufficiently high to contain the spilled contents of any containers that are placed in the tray.
- The containers placed in the refrigerator should be completely sealed or capped, securely placed, and labeled. Avoid capping materials with aluminum foil, parafilm, corks, and glass stoppers.
- Refrigerator should be frost free to prevent water drainage.

Labeling reagents

Label all storage areas, refrigerators, etc., appropriately, and keep all chemicals in properly labeled containers.

- Date all bottles when received and when opened.
- Note expiration dates on chemicals.
- Note special storage conditions.

Be familiar with the appropriate protective measures to take when exposed to the following classes of hazardous materials. Information is available from your lab supervisor, DES and the chemistry library:

- Flammables
- Radioactive Compounds
- Corrosives
- Biohazards
- Toxics
- Carcinogens
- Reactives
- Compressed Gases

Segregate chemicals by compatibility groups for storage.

Be aware of the potential interactions of lab furniture and equipment with chemicals used or stored in the lab. (e.g., are oxidizers stored directly on wooden shelving?)
Post warning signs for unusual hazards such as flammable materials, biohazards or other special problems.

Fume hood

Chemical fume hoods capture, contain, and expel emissions generated by hazardous chemicals. In general, it is a good idea to conduct all laboratory chemical experiments in a fume hood. While you may be able to predict the release of undesirable or hazardous effluents in some laboratory operations, "surprises" can always happen. Therefore, the fume hood offers an extra measure of protection.

- All laboratory workers with access to a laboratory chemical fume hood should be familiar with its use.
- Maintain the sash at or below the optimum operating height as designated by the label with an arrow.
- Never block, even partially, exhaust ports or slots in the rear wall and ceiling of the hood.
- Work back in the hood, at least 6 in. (15 cm) from the front edge of the hood.
- Keep your face outside the plane of the hood sash.

- Keep the inside of the hood clean and uncluttered.
- The hood should always be in good condition and capable of routine use. Any hood or component of ventilation not properly functioning must be taken out of service and clearly tagged.
- The lab worker should not be able to detect strong odors released from materials in the hood. If odors are detected, check to make sure that the ventilation fan is turned on.
- All protective clothing should be worn when working with chemicals in the hood. In addition to gloves, safety glasses, and lab coats, a face shield or explosion shield will provide an extra measure of safety from reactive chemicals.
- Solid objects or materials should not be allowed to enter the exhaust ducts at the rear of the hood, as they can become lodged in the duct or fan.

Glassware

Glass apparatus should be carefully examined before use and any which is cracked, chipped, flawed or dirty should be rejected. Minute cracks in glassware for use in evacuated systems are particularly dangerous.

Waste disposal

Waste material must *never* be allowed to accumulate in the laboratory; it should be removed regularly from the laboratory area for storage in suitable containers so that it can be disposed of appropriately. There should be separate bins with properly fitting lids for broken glassware and for flammable materials such as paper or cloths which may have been used to mop up flammable liquids.

HPLC Solvent Waste Disposal

Secondary containment for all bottles collecting HPLC solvent waste.

Perchloric acid: low concentrations used (10 mM in aqueous), suitable for disposal in HPLC solvent waste container.

Record all HPLC waste information on the manifested waste record (concentration and volume) before disposing of in the correct container designated for HPLC waste.

Levels of responsibility in experiment planning

1. Junior technical staff and students in sub-degree and first-year undergraduate courses need considerable guidance, for their own safety, and for that of other users of the laboratory. Experiments are selected in a sequence by supervisory staff to ensure increasing experimental competence and confidence. Frequently a short list of questions relating to the experiment is provided to reinforce the understanding of the practical and theoretical aspects of the experiment. In these circumstances experiment planning requires that the worker should carefully study the complete details and advice provided, and form a clear idea of what is to be done and precisely how it is proposed to do it.
2. At a second stage the student or technician accepts more responsibility in the experiment planning. Thus although instruction sheets (or book references) are suitably detailed, a level of technical competence in routine operations acquired from earlier work is now expected.

3. The third (but not of course the last) stage of increasing expertise, usually requires the planning of experiments which involve the use of advanced techniques or equipment, and the planning of 'open-ended' experiments and undergraduate projects leading to postgraduate-type work.

Recording of results

In addition to a written account of the work done, including notes on any special apparatus used, details of all volumes, weights, temperatures, times, chromatographic procedures (e.g. TLC, column chromatography) and conditions and results, etc., must all be recorded.

During a reaction, all unexpected happenings and anything not understood should be carefully recorded at the time. In such a case, the worker should always try to unravel the reason for failure of the reaction and to make suitable changes in the procedure, rather than just hurrying to repeat the experiment without modification.

HAZARDS

All workers in a laboratory must act responsibly in the interests of their own safety and that of their colleagues.

Members of a laboratory should have ready access to appropriate books dealing with commonly encountered hazards.

Explosion and fire hazards

The following guidelines are followed:

1. The use of a substance known to be explosive should be avoided if a safer alternative can be used.
2. If an explosive or dangerously reactive substance has to be used, then it should be used in the smallest possible quantity and with all the appropriate precautions which are indicated below.
3. Workers should try to foresee and avoid the situation where a dangerously reactive chemical is likely to come into contact with combustible material, or where an explosive substance is likely to be subjected to the stimulus of shock or excess heat.
4. Reactions known or likely to involve explosion or fire hazards should always be tried out on a small scale first, and only then carefully scaled up in stages if no warning signs of danger are apparent (e.g. no undue rise in temperature or evolution of gas, etc.).

5. For notably exothermic reactions involving dangerously active reagents, the safest procedure is to add the reagent dropwise, with rapid stirring, at the same rate as it is used up.

Explosive compounds

They may explode under the stimulus of heat, impact or friction, or apparently spontaneously.

1. Acetylene gas and the acetylide salts of heavy metals; silver and copper acetylides are extremely shock-sensitive.
2. Perchlorate salts of organic chemicals and any water free perchlorate are very shock sensitive and potentially explosive. Never evaporate a solution containing perchlorates or perchloric acid.
3. Polyacetylenes and some halogenated acetylenes.
4. Hydrazoic acid and all azides, both organic and inorganic (only sodium azide is safe); aryl azides and silver azide may be inadvertently formed during some reactions.
5. Diazonium salts (when solid) and diazo compounds.
6. Inorganic nitrates, especially ammonium nitrate. The nitrate esters of polyhydric alcohols.
7. Polynitro compounds, e.g. picric acid (and heavy metal picrates), trinitrobenzene (TNB), trinitrotoluene (TNT); all these substances are safe when damp with water.
8. Metal salts of nitrophenols.
9. Peroxides; these are a common cause of explosions due to their formation in ether solvents. Concentrated aqueous hydrogen peroxide solution.
10. Nitrogen tribromide, trichloride and triiodide; these are all highly sensitive and violently explosive, and should never be prepared or used unless absolutely necessary.

Potentially dangerous mixtures

Powerful oxidants are particularly dangerous when mixed with easily oxidized organic substances such as simple alcohols, poly hydric alcohols, carbohydrates and cellulose-containing materials such as paper, cloth or wood. They are also dangerous when mixed with elements such as sulphur and phosphorus, and with finely divided metals such as magnesium powder. The following are common examples:

1. Perchloric acid, chlorates and perchlorates.
2. Chromium trioxide ('chromic anhydride'), chromates and dichromates. Concentrated nitric acid and nitrates.

3. Permanganates.
4. Concentrated hydrogen peroxide.
5. Liquid oxygen and liquid air.

Some specific dangers of explosion

Peroxides in ether solvents. This is one of the commonest causes of explosions in organic chemistry laboratories. Simple dialkyl ethers such as diethyl ether and di-isopropyl ether, and cyclic ethers such as 1,4-dioxane and tetrahydrofuran, form less volatile peroxides on exposure to air and light. If therefore one of these solvents is purified by distillation, the peroxide content in the residue is progressively increased and eventually a violent explosion may occur. In view of this:

1. such solvents should not be stored for long periods or in half empty bottles; containers should be of dark glass;
2. before the solvents are distilled a peroxide test should be carried out, and, if positive, the peroxide must be remove
3. since purified ethers in contact with air rapidly peroxidise again (10 minutes in the case of tetrahydrofuran) they should be retested for peroxides and purified if necessary immediately before use.

Solid sodamide and potassium metal. Both of these substances undergo surface oxidation to give oxide films which may initiate explosions when the samples are handled. In the case of potassium, surface oxidation occurs even when the metal is stored under oil, and the act of paring off the oxide film with a knife may initiate an explosion. Samples of potassium which are heavily encrusted with oxide should not be used but should be carefully destroyed by adding the lumps to a large excess of propan-2-ol. Similarly, old or obviously encrusted (yellow) lumps of sodamide should not be ground in a pestle and mortar, but should be destroyed by mixing with solid ammonium chloride.

Alkali metals with chlorinated solvents. The alkali metals sodium, potassium and lithium (and also other metals, e.g. aluminium and magnesium, especially when finely divided), are all violently reactive towards halogenated organic compounds, notably the common chlorinated solvents such as carbon tetrachloride. Lumps or chips of these metals should *never* be washed with halogenated solvents - a violent explosion can result.

Azides. Explosive aryl azides may be formed inadvertently during the Sandmeyer and other diazonium reactions. Explosive silver azide forms when solutions of ammoniacal silver nitrate (Tollen's reagent) are allowed to stand before use. This is extremely dangerous; Tollen's reagent should always be freshly prepared, taking care not to exceed the recommended concentrations. Unused reagent should be destroyed by the addition of aqueous sodium chloride.

Liquid nitrogen. Liquid nitrogen (b.p. – 196 °C) contains some liquid oxygen (b.p. -183 °C) as an impurity and therefore evaporation leads to an increasing proportion of liquid oxygen, so that before complete evaporation occurs the residual liquid may contain *up to* 80 *per cent of liquid oxygen.* Contact of this residue with organic or combustible material of almost any sort is likely to cause an explosion. If Dewar flasks containing liquid nitrogen have been used as cooling baths, great care must be taken to ensure that all liquid nitrogen and oxygen has evaporated *completely* before the Dewar flask is used for another purpose (e.g. as an acetone-solid carbon dioxide cooling bath).

Glass vacuum assemblies. Before using any glass apparatus for vacuum distillation or sublimation it should be examined to ensure that, it is of the correct thickness and type (thin-walled glassware and conical shaped flasks are not suitable), and that it is free from cracks and flaws. Vacuum desiccators should always be used in the smallest suitable size and should be encased in wire safety cages. Dewar flasks can cause considerable damage since they may collapse violently ('implode') if they are maltreated. All Dewar flasks should therefore be bound, over their entire length, with adhesive tape to contain flying fragments of glass in the event of an implosion.

Opening glass ampoules. Ampoules of volatile chemicals must be thoroughly cooled before opening. Cooling must be effected with care, particularly if the contents are highly reactive (e.g. boron trichloride). If cooling is mishandled the glass may crack and the release of the contents into the cooling bath may lead to a violent explosion. Ampoules should not be cooled to a low temperature *too quickly.* Cooling in ice-water initially, followed by ice-salt, will usually be satisfactory; cooling to solid carbon dioxide temperatures is not necessary.
If the contents have a tendency to decompose, considerable pressure may develop in the sealed ampoule on storage, and great care should be taken during opening. The cooled ampoule should be removed from the cooling bath and wrapped in strong cloth behind a safety

screen. A clean scratch should be made in the neck of the ampoule with a sharp file or glass knife and the neck cracked off by touching the scratch with the molten end of a thin red-hot glass rod.

Ampoules should be well cooled before resealing. Resealing should be avoided if possible however; it is best to obtain a smaller size of ampoule and use the whole of the contents for one experiment.

Compressed gas cylinders. In view of the high pressures involved, any possibility of slow leakage from a cylinder of a flammable or toxic gas should be carefully guarded against. Thus, gas should never be drawn from a cylinder unless the appropriate reduction valve has been correctly fitted. The main cylinder valve should never be opened more than is necessary to provide the required gas flow (two full turns of the spindle at the most *); when the cylinder is not in use the gas should be shut off at the main valve and not at the regulator, which should then be bled of surplus pressure and closed. A suspected leak may be tested for by brushing with the approved leak detection solution, usually 1 per cent aqueous Teepol.

The valves and screw threads of cylinders and regulators should *never* be greased since this may lead to an explosion. If a cylinder has a very stiff spindle valve or if the screw threads are damaged, it should be returned to the suppliers for replacement. Similarly, defective regulators and pressure gauges should never be used.

Fire hazards

Fire hazards in organic chemistry laboratories are often considerable due to the quantities of volatile and flammable chemicals, particularly solvents, which are commonly used.

Flammable solvents. Particular care should be taken when handling flammable solvents (and other chemicals) which are also *highly volatile.* The vapor may drift to a distant ignition source and burn back to ignite the main bulk of the liquid. An important rule is never to allow any vapor of a volatile chemical to escape into the open laboratory. A measure of the flammability of a compound is given by the *flash point* (the temperature at which the liquid gives rise to ignitable vapor). Any liquid with a flash point of less than 15 QC should be regarded as dangerously flammable and treated accordingly. If a solvent also has a low *auto ignition temperature* (the temperature at which the vapor will spontaneously ignite in air), it should be treated with particular care.

Ignition sources. Naked flames should rarely be used in organic chemistry laboratories. The heating of reaction mixtures is much more safely accomplished by means of a steam bath, an electric heating mantle or an oil bath heated by means of a small electric immersion heater (or, less safely, by a hot plate). If Bunsen flames have to be used, they should be lit only after a careful survey of neighboring apparatus and chemicals has revealed no fire hazard. The flame should be turned out whenever it is not actually in use; a gas-air Bunsen flame may be invisible in bright sunlight and thus the cause of a fire or burning accident.

If flammable vapor is allowed to accumulate in the vicinity of electrical devices such as thermostats, stirrer motors, vacuum pumps, drying ovens, etc., it may be ignited by sparking from electrical contacts; this may be minimized by good laboratory ventilation and the prevention of the local build-up of solvent vapors.

Sodium residues. Bottles containing sodium wire previously used for solvent drying constitute a fire and explosion hazard. The sodium, sometimes heavily coated with hydroxide or oxide film, should be covered with propan-2-ol and set aside with occasional swirling until all the sodium particles are destroyed (at least 2 hours). The contents of the bottle should then be poured into a large excess of water (water should *not* be added to the bottle) and the bottle washed out several times with industrial spirit. Only then can the bottle be safely rinsed with water.

Fires involving sodium metal are very hot and localized and are best dealt with by smothering with sand or by using a dry powder extinguisher, *not* a carbon tetrachloride or carbon dioxide extinguisher.

Metal hydrides. Lithium hydride, sodium hydride, potassium hydride and lithium aluminium hydride all react violently with water liberating hydrogen; the heat of reaction may cause explosive ignition. Excess metal hydride from a reaction must be destroyed by the careful addition of ethyl acetate or acetone.

THE DANGEROUS OPERATIONS LABORATORY

The conduct of explosive or violent reactions

All potentially violent reactions should therefore be conducted on an open bench, with the apparatus surrounded by safety shielding on all sides but open at the top.

Reactive inorganic reagents

Suitable protective clothing including gloves should be worn. Adequate protection of the eyes is absolutely essential and safety spectacles, or preferably goggles or a visor, must always be worn. When there is any possibility of inhalation of reactive vapors or dusts, all operations should normally be conducted in a fume cupboard. Additional protection may be provided by a gas mask or well-fitting dust mask.

If any corrosive liquid or solid is spilled on to the skin it should be immediately washed off with copious quantities of water; in cases of splashes in the eyes, every second counts. Any spillages should be cleaned up without delay, preferably with the aid of sand. Flooding a spillage on a floor or bench with water is not always advisable if this is likely to spread the corrosive material and cause it to lodge in crevices and between floorboards.

Strong acids and bases

All strong acids and bases and some weak acids and slightly soluble bases (e. g. glacial acetic acid, hydrofluoric acid, hydrobromic acid and calcium hydroxyde) are corrosive. When in contact with the eyes or the skin, they irreversibly destroy living tissues. The more concentrated the acid or base and/or the longer the contact, the greater the destruction.

Strong acids. All of the following react violently with bases and most give off very harmful vapors.

Hydrobromic acid and hydrogen bromide.

Hydrochloric acid and hydrogen chloride.

Hydrofluoric acid and hydrogen fluoride - both react readily with glass and quickly destroy organic tissue. New thick rubber or plastic gloves should be worn after carefully checking that no holes are present. Skin burns must receive immediate and specialized medical attention.

Nitric acid (concentrated and fuming).

Perchloric acid (explosion danger).

Sulphuric acid (concentrated and 'oleum') - should always be mixed with water very carefully, by pouring into cold water as a thin stream to prevent acid splashes or spray. 'Chromic acid' cleaning mixtures have the corrosive properties of concentrated sulphuric acid as well as the dangerous oxidising properties of the chromic acid.

Chlorosulphonic acid - this is a highly corrosive liquid which reacts violently with water.

Strong bases. Calcium oxide, potassium hydroxide and sodium hydroxide - these react violently with acids, generate heat on contact with water, and have a powerful corrosive action on the skin, particularly the corneal tissue of the eye.

Halogens. All are toxic and corrosive. Great care should be exercised when working with fluorine, which is violently reactive towards a wide range of substances. The interhalogen compounds are also powerfully reactive.

Reactive halides. All of the following are highly reactive, particularly towards water; ampoules Of liquids should be opened in a fume cupboard after cooling, observing the precautions.
Boron trichloride; phosphorus tribromide; trichloride and pentachloride; silicon tetrachloride.
Aluminium chloride and titanium (v) chloride are rather less reactive.

Hazards due to toxic chemicals
Nearly all substances are toxic to some extent and the adoption of safe and careful working procedures which prevent the entry of foreign substances into the body is therefore of paramount importance, and should become second nature to all laboratory workers.

Ingestion (through the mouth). It is strongly recommended that no one should ever eat, drink or smoke in a laboratory. Workers should always wash their hands thoroughly on leaving a laboratory and before eating.
Inhalation (into the lungs). All toxic powders, volatile liquids and gases should only be handled in efficient fume cupboards. The practice of sniffing the vapors of unknown compounds for identification purposes should be conducted with caution.
Direct absorption (through the skin into the bloodstream). The danger may be reduced by wearing rubber or plastic gloves, in addition to the usual laboratory white coat. Protective gloves are often permeable to organic solvents and are easily punctured; they should therefore be frequently inspected and replaced when necessary.

Mercury and mercury compounds. Generally, mercury(II) salts are more toxic than mercury(I) salts. Liquid organic mercury compounds are highly poisonous and dangerous by inhalation and absorption through the skin, whereas solid organomercurials are less toxic. However, all mercury compounds should be treated with caution and any long-term exposure avoided.

Elemental mercury readily evolves the vapor which constitutes a severe cumulative and chronic hazard. A severe mercury vapor hazard may occur through misuse of mercury-containing vacuum gauges attached to oil vacuum pumps. If the gauge is turned about its axis too quickly, mercury may be sucked into the pump and circulated with the hot oil to release large quantities of mercury vapor into the atmosphere. If there is any possibility of this having happened, the pump must not be used and should be stripped down and cleaned as soon as possible.

Vacuum pumps

Mechanical vacuum pumps used in laboratories pose many hazards. There are mechanical hazards associated with any moving parts and there are also chemical hazards associated with contaminating the pump oil with volatile substances and subsequently releasing them into the laboratory. Safety glasses, chemical protective gloves and a laboratory coat must be worn at all times when operating a vacuum apparatus. Additional personal protective equipment, such as splash goggles, face shields, and/or an explosion shield can be used to further protect against the hazards of vacuum procedures.

- Ensure that pumps have belt guards in place during operation.
- Ensure that power cords and switches are free from defects.
- Do not place pumps in an enclosed, unventilated cabinet.
- Do not operate pumps near containers of flammable chemicals.
- Do not use solvents which might damage the pump.
- Always close the valve between the vacuum vessel and the pump before shutting off the pump to avoid sucking vacuum oil into the system.
- Place a pan under pumps to catch oil drips.
- Check oil levels and change oil when necessary. Replace and properly dispose of vacuum pump oil. Used pump oil must be disposed as hazardous waste.
- Ensure that all associated equipment and apparatus to be used are rated for the vacuum pressures that will be achieved.
- Conduct vacuum operations behind a shield or in a fume hood and always wear safety glasses.

- Always use a trap on vacuum lines to prevent liquids from being drawn into the pump, house vacuum line, or water drain.
- Do not allow water, solvents and corrosive gases to be drawn into vacuum systems.
- Assemble vacuum apparatus in a manner that avoids strain, particularly to the neck of the flask.
- Inspect all stopcocks on the vacuum system for visible signs of scratches or other damage to help ensure that they do not leak.
- Avoid putting pressure on a vacuum line to prevent stopcocks from popping out or glass apparatus from exploding.
- Position vacuum apparatus in such a way that the possibility of being accidentally knocked or bumped is minimized. If necessary, place transparent plastic shielding around it to prevent injury from flying glass in case of an explosion.
- Monitor vacuum line pressure with the appropriate sensors and gauges to ensure that you do not over-charge the system.
- Never carry or move evacuated vacuum line components.
- Protects the pump and the piping from the potentially damaging effects of the material,
- Protects people who must work on the vacuum lines or system, and
- Prevents vapors and related odors from being emitted back into the laboratory or system exhaust.
- Locate the cold trap between the system and vacuum pump.
- Ensure that the cold trap is of sufficient size and cold enough to condense vapors present in the system.
- Check frequently for blockages in the cold trap.
- Use isopropanol/dry ice or ethanol/dry ice instead of acetone/dry ice to create a cold trap. Isopropanol and ethanol are less expensive, less toxic, and less prone to foam.
- Use appropriate cryogenic gloves when handling the dry ice.
- Do not use dry ice or liquefied gas refrigerant bath within a closed system. These can create uncontrolled and dangerously high pressures.
- Maintain a cold trap between a vacuum pump and the apparatus - do not use liquid nitrogen as trap coolant when pumping organic compounds (liquid oxygen may condense in the trap, leading to explosive oxidation).

- Do not apply a vacuum to a flat-bottomed flask. Use only containers that can withstand vacuum operations – heavy-walled round-bottomed glassware or specifically-designed glassware (e.g., Erlenmeyer filtration flasks).
- Carefully inspect vacuum glassware before and after each use. Dispose of any glass that is chipped, scratched, broken, or otherwise stressed.
- Although glass vessels are frequently used in pressure and vacuum systems, they can explode or implode violently, either spontaneously from stress failure or from an accidental blow. The following precautions must be adhered to when using glass vessels.
- Ensure the glass vessel is designed for the intended operation.
- Inspect the stopcocks of glass vessels for visible signs of scratches or other damage to help ensure that they do not leak.
- Never rely on corks, rubber stoppers or plastic tubing as pressure-relief devices.
- All large volume glass components should be made of Pyrex or similar glass and wrapped partially with friction tape to guard against flying glass. Plastic components are a good alternative to glass, but still require shielding.

1. Check that the cold trap is clean and dry before attaching to the vacuum line.
2. With the Dewar lowered and the system closed to air, turn the vacuum pump on and ensure vacuum performance is leak free (check gauge display).
3. Never immerse a cold trap in liquid nitrogen unless under vacuum as liquid oxygen (blue in color) may condense. The result of this can be a violent explosion caused by re-vaporization or by oxidation of organic solvents etc.
4. Attach the apparatus to the manifold via thick walled rubber tubing. Evacuate the apparatus.

Vacuum desiccators

Vacuum desiccators should be enclosed in approved shielding device or protected with a framework of wire, nylon or other suitable material.

Glass desiccators often have a slight vacuum due to contents cooling. When possible, use molded plastic desiccators with high tensile strength. For glass desiccators, use a perforated metal desiccator guard.

Air admittance should be carried out gradually. When opening a desiccator, make sure atmospheric pressure has been restored.
Never carry desiccator that is under high vacuum. Implosion may occur if the vessel is dropped or handled roughly.

Dry ice

Unless precautions are taken, dry ice will damage fingers or hands, for example, by freezing them. Do not handle dry ice with bare hands; if your skin is even slightly moist, severe freezing can result. Wear cryo-gloves. Wear googles while chipping dry ice. Do not lower your head into a dry ice chest; no oxygen is present, and suffocation can result.

Using high-pressure air

If air under pressure is directed toward intact skin, it can penetrate without making any visible opening and cause the nearly skin area to expand like a balloon. The pain from such an event can be severe, and the damage to the issues can require hospitalization. Never direct air under pressure toward yourself or any other person.

Electrical safety

Working with chemicals and apparatus

- Plan your work before starting a laboratory procedure. Be sure you know what to do if you or another laboratory worker has an accident.
- Keep your workspace free of clutter.
- Set up clean, dry apparatus, firmly claimed and away from the edge of the lab bench. Choose sizes that can properly accommodate the operation to be performed, allowing at least 35% free space.
- Examine your glassware closely for flaws such as cracks and chips. Damaged glassware should be repaired or discarded in a waste container labeled for broken glass.
- A properly placed pan under a reaction vessel or container will act as a secondary containment to confine spilled liquids in the event of glass breakage.
- When working with flammable compounds do not allow burners or other ignition sources in the vicinity.
- Whenever is possible, use electric heater in place of a gas burner.

- Support and orient large separatory funnels so that the stopcock will not be loosened by gravity. Use retainer rings on stopcock plugs.
- Use securely positioned clamps to support condensers; secure attached water hoses with wire or clamps.
- Position apparatus that is attached to a ring stand so that the center of gravity of the system is over the base. Arrange the apparatus so, that heaters and baths can be removed quickly.
- Never place any apparatus, equipment, boxes, containers of chemicals, or any objects on the floor.
- Never heat a closed container. Use an appropriate gas trap whenever evolution of hazardous gases or fumes is possible.

PURIFICATION OF COMMONLY USED SOLVENTS

Commercially available grades of organic solvents are of adequate purity for use in many reactions provided that the presence of small quantities of water (the most widespread impurity in all organic solvents) is not harmful to the course of the reaction, and also that the presence of other impurities (e.g. ethanol in diethyl ether) is unlikely to cause undesirable side reactions. The commercially available grades for general use are often accompanied by specifications indicating the amount and nature of any impurities present.

While the drying agent selected for preliminary and final drying must of course have no chemical action on the organic solvent, with some solvents a specific chemical treatment is necessary to remove impurities other than water before drying is attempted. Apart from impurities arising during the manufacturing processes, many organic solvents undergo autoxidation on standing with the formation of dangerously explosive peroxides. Such solvents should always be tested for the presence of peroxides, and if present these should be removed according to the methods detailed below under individual examples, before other purification processes are attempted.

Rigorously dried organic solvents are frequently markedly hygroscopic. The distillation assembly may be protected by a suitable drying tube, but the distillation is better carried out under an atmosphere of nitrogen and the distilled solvent similarly stored under nitrogen. If the solvent is stored in a multi-necked flask fitted with a nitrogen inlet and outlet tube, a measured amount of solvent can be removed by syringe with little or no contamination of the solvent with air or moisture. It is often convenient to remove final traces of water with the aid of a molecular sieve and to store the dried solvent in the presence of the sieve.

Dichloromethane (Methylene chloride.) The commercial grade is purified by washing with portions of concentrated sulphuric acid until the acid layer remains colorless, and then with water, sodium carbonate solution and water again. It is dried initially over calcium chloride and then distilled from calcium hydride before use. The fraction b.p. 40 – 41° C is collected. Dichloromethane should be stored in a brown bottle away from light over Type 3A molecular sieve.

Methanol. Most of the water may be removed from commercial methanol by distillation through an efficient fractionating column; no constant boiling point mixture is formed as is the case with ethanol. Anhydrous methanol can be obtained from the fractionally distilled solvent by standing over a Type 3A molecular sieve or by treatment with magnesium metal using the procedure given for super-dry ethanol described below. It should be stored over Type 3A molecular sieve beads. Pure methanol has b.p. 65 °C/760 mmHg.

Propan-1-ol. The purest available commercial propan-l-ol *(propyl alcohol)* should be dried with anhydrous potassium carbonate or with anhydrous calcium sulphate, and distilled through an efficient fractionating column. The fraction, b.p. 96.5 - 97.5 °C/760mmHg, is collected.

Propan-2-ol. Test for peroxide by adding 0.5 ml of propan-2-ol to 1ml of 10 per cent potassium iodide solution acidified with 0.5 ml of dilute (l :5) hydrochloric acid and mixed with a fewdrops of starch solution just prior to the test: if a blue (or blue-black) coloration appears in one minute, the test is positive. To remove peroxide heat under reflux 1litre of propan-2-ol with 10-15 g of solid tin(II) chloride for half an hour. Test a portion of the cooled solution for peroxide: if iodine is liberated, add further 5 g portions of tinII) chloride and heat under reflux for half-hour periods until the test is negative. Add about 200 g of calcium oxide and heat under reflux for 4 hours, and then distil, discarding the first portion of distillate. The water content may be further reduced by allowing the distillate to stand over calcium metal or a Type 5A molecular sieve for several days, followed by further fractionation. Anhydrous propan-2-ol has b.p. 82 – 83℃/760 mmHg.

Diethyl ether. The chief impurities in commercial ether are water and ethanol. If present, the peroxide may be removed by shaking 1 liter of ether with 10-20 ml of a concentrated solution of an iron(n) salt prepared either by dissolving 60 g of iron(II) sulphate in a mixture of 6ml of concentrated sulphuric acid and 110ml of water, or by dissolving 100g of iron(II) chloride in a mixture of 42 ml of concentrated hydrochloric acid and 85 ml of water.]

Peroxide may also be removed by shaking with an aqueous solution of sodium sulphite or with solid tin(II) chloride or by passage through a column of alumina. It is worthy of note that all dialkyl ethers have a tendency to form explosive peroxides and they should be routinely tested before further purification leading to a final distillation process is attempted.

Tetrahydrofuran. Water and peroxides are the major impurities. Peroxide, if present, *must* be removed by passage through a column of alumina, or by shaking with iron(II) sulphate solution as described under diethyl ether before drying and further purification is attempted. If the latter method is employed the solvent should then be dried initially over calcium sulphate or solid potassium hydroxide, before being heated under reflux over calcium hydride or lithium aluminium hydride. The solvent is finally fractionally distilled. Pure tetrahydrofuran has b.p. 65 - 66 °C/760 mmHg; it should be stored over calcium hydride under nitrogen, and labeled with the date of purification.

Handling organolithium reagents

Organolithium reagents (methyl lithium, *n*-butyl lithium, *sec*-butyl lithium, and *t*-butyl lithium) are highly moisture sensitive and react violently with water. Upon reaction with water, they can ignite and/or liberate highly toxic gases.

Organolithium reagents are extremely flammable upon exposure to water, and as such are generally sold as solutions in an organic solvent. They must be handled under an inert atmosphere to minimize exposure to both air and moisture. They can cause severe burns and are highly corrosive. In addition to the high flammability, they can be harmful to the liver, kidneys and nervous system.

Use of organolithium reagents requires training by a senior lab member and the approval by Phil.

Organolithium reagents should only be used in the fume hood.

Clear all areas where the reagent will be opened prior to use. Do not use any organolithium reagent while working alone.

There are two options for the handling of organolithium reagents. The first option is the use of a syringe and needle, the second is to transfer using a cannula. For smaller amounts of reagent, the preferred technique is to use a syringe and needle, while a cannula transfer is safer and more practical for amounts >15 mL.

Wear a laboratory coat made of flame-retardant material or cotton. Wear flame-resistant gloves over the top of chemically resistant gloves, and safety glasses.

Work inside the hood. Keep the hood sash as low as possible to prevent spills/splashes outside the hood.

Proper syringe/needle transfer is as follows:

- All glassware and solvents should be dried prior to the treatment with any organolithium reagent. Flame-dried glassware which has been cooled under an inert atmosphere just before use is ideal.
- Upon charging a dry flask with dry reagents/solvents and reaching the desired temperature, the appropriate organolithium reagent can be added.
- Some organolithium reagents are stored at room temperature (i.e. methyllithium, *n*-butyllithium) and can be used as such, but some (i.e. *t*-butyllithium) are stored under refrigeration and must be warmed to room temperature prior to use.
- Organolithium reagents are contained in a Sure-Seal bottle, the septum of which can be pierced with a clean, dry needle.
- A clean, dry reagent needle (<16 gauge) with the appropriate size syringe is used to pierce the septum and inert gas is drawn in to the syringe, then the needle is removed and the gas inside is expelled to the atmosphere. This process is repeated several times, piercing the same hole in the septum, to remove most air from the needle and syringe. Upon purging the needle appropriately, the tip is submerged below the level of reagent and the required amount is drawn up into the syringe. Be careful to hold the end of the plunger as well as the joint where the needle and syringe meet. If either of these comes apart the reagent will come out and a fire will most likely ensue, potentially leading to injury and damage.
- Once the desired amount of organolithium reagent has been drawn into the syringe, the tip of the needle is pulled above the level of reagent, and the plunger is pulled out slightly to draw a blanket of inert gas into the needle. The needle is then removed from the reagent bottle and quickly pierced into the septum of the reaction flask. At this point, the organolithium is dispensed, usually slowly to control heat evolution. Be careful to hold the needle and syringe together, as applying force to dispense the reagent can cause them to separate and expose the reagent to moisture.
- Once the addition of reagent is complete, the needle can be removed from the reaction flask. The needle can be rinsed with hexanes multiple times, then water. Clean the needle immediately after use to prevent clogging. To close the reagent bottle, simultaneously place a piece of tape over the hole formed by the needle while removing it from the bottle. This should seal the bottle and keep air and/or moisture from entering. Replace the cap and wrap the outside with parafilm to further ensure safe storage.

Do not use water or a CO_2 extinguisher on the fire. In case of contact with your person, get to the nearest safety shower immediately. Remove all contaminated clothing and get underneath the running water quickly. A large amount of water should put out the fire.

Fully quench all reagents before disposal. For quenching an excess organolithium, slowly add to a pre-cooled flask of isopropanol or acetone at 0 °C in an ice bath in a dry flask. Control heat evolution by slow addition of the reagent. Alternatively, the reagent can be quenched by the slow addition to dry CO_2. Rinse all flasks three times with hexanes and quench all washes.

General rules for usage of rotary evaporators

1. The solvent collection flask of the unit should always be emptied prior use to prevent accidentally mixing of incompatible chemicals.

2. The flask with the solution is placed on the rotary evaporator. The use of a bump trap prevents the solution from accidentally splashing into the condenser (and being contaminated). It is highly advisable to start with a clean bump bulb in case something bumps over after all! This would allow the experimenter to recover the solution or solid.

3. A metal or Keck clip is used to secure the flask and the bump trap.

4. The dial on the motor is used for speed control of the flask rotation. A typical rotavap uses a variable speed sparkless induction motor that spins at 0-220 rpm and provides high constant torque.

5. The aspirator vacuum is turned on. On most models, the vacuum on/off control is managed by turning a stopcock at the top of the condenser. This stopcock is later also used to vent the setup after the solvent is removed.

6. The flask is lowered into the water bath. The water bath temperature should not exceed the boiling point of the solvent!! For small amounts of common solvents the bath heater is not needed.

7. The solvent should start collecting on the condenser and drip into the receiving flask. Some solvents (such as diethyl ether or dichloromethane) are so volatile that they will also evaporate

from the receiving flask and be discharged down the drain. An additional trap (with dry-ice or liquid nitrogen) can be placed between the vacuum source and the condenser unit. This is particularly important of a membrane pump is used as vacuum source.

8. Once all the solvent evaporated (or whatever is desired at this point), the vacuum is released. The flask is raised out of the water bath and the spinning is discontinued.

9. The bump trap has to be cleaned and the receiving flask is emptied upon completion of the evaporation.

General rules for Old Equipment

Old equipment and boxes should be placed in storage areas that reduce clutter for a safe working environment.

Colum packing safety procedure

The Haskel pump used for packing chromatography columns is an air driven HIGH PRESSURE liquid pump. Therefore, improper usage of the pump could result in serious injury or the possibility of injecting into the flesh. The following precautions should be taken during the packing process.

1- Always wear appropriate safety appareal, safety glasses, head gear, lab coat, and gloves.
2- Provide guards or shields around the equipment to protect personnel.
3- As an extra precaution, place a bucket containing stacks of paper towels beneath the column being pack to absorb the kinetic energy and reduce damage in case of a flying object.
4- Obtain a material safety data sheet and take appropriate safety measures for the liquid being handled.
5- All fittings must be tight to avoid leakage or damage from flying objects.
6- While packing a column, pressure should be increased slowly and should never exceed the maximum working pressure.
7- Before you disassemble a chromatography column, make sure that the pressure drops to zero to prevent particles from getting sprayed.