

SCHEME OF WORK

Serial No. PC006

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S.R.F. – Science Research Facility (1A43 & 1A41), H.L. - Histology Lab (2B108)

Due to potential risk to health from exposure to animal allergens the work covered by this risk assessment should not commence before reading the following three SRF risk assessments:-

Entering the Unit

Handling Animals

Scientific Procedures

Initial training in laboratory procedures will be given by Dr P. Cahusac, and all work must be approved by him. He should be consulted for further information or guidance on safety matters.

General precautions when handling all these chemicals includes wearing a lab coat and safety spectacles. Contact with skin should be avoided, and should be washed off with plenty of water as soon as possible. Spillages should be washed thoroughly with water. Eye wash bottles are available in rooms 1A41 and H.L., the buffered solution bottle should be used for acid or alkali.

General Purpose Chemicals.

Chemical	Stock Conc.	Use Conc.	Uses	Rooms
HCl	1.0M	1.0M	pH adjust	S.R.F.
NaOH	pellets	1.0M	pH adjust	S.R.F.
"	"	0.1M	ionto solns	S.R.F.
H ₂ SO ₄	0.1N	0.05N	etching	S.R.F.
KOH	pellets	34% soln	etching	S.R.F.
Isopropanol	100%	100%	cleaning	H.L., S.R.F.
Phosphate buffer	Tablets	150mM	physiol. saline	H.L., S.R.F.
Synthetic Interstitial Fluid	150mM	150mM	Perfuse Tissue bath	H.L.

The main hazard in using acids and alkalis is due to the extremely destructive effects on tissue of mucous membranes, eyes and skin. Isopropanol (isopropyl alcohol) is an irritant and has been shown to increase the risk of cancer in workers of production factories. It is also inflammable.

Stock solutions and pellets are kept in chemical cupboards in the animal house.

The pH adjusting solutions are kept in glass bottles on benchcote covered tops. The stoppers incorporate a pipette so that single drops can be added to solutions.

34% KOH (equivalent to 0.6M) is used to make Levick's aqueous solution for etching tungsten microelectrodes. Levick's also contains 71g NaNO₂ per 100ml water, see below. A 100ml pyrex beaker filled with Levick's solution in which a carbon electrode is immersed is used on one of the benches. In use, a 5cm length of 0.1mm tungsten wire is dipped vertically into the solution to etch the wire to a fine point. When out of use the beaker has parafilm sealing the top. A syringe (with needle) is also filled with Levick's for injection into the end of a glass capillary tube (less than 0.1ml) with a meniscus that is used to etch the tip of tungsten microelectrodes under the microscope. The syringe needle is covered with a top to prevent spillage.

H₂SO₄ is used in dilute solution (less than 0.05N) with chromic acid for etching carbon fibres. This procedure is carried out under the microscope in a similar way to that described above.

Isopropanol is used as a general cleaning agent, for example removing permanent marker writing. It should be used with adequate ventilation, and minimal quantities employed. It should not be used in the presence of a naked flame.

Phosphate buffer tablets are used for making up physiological saline. Possible hazard in their use is that particles can be irritant.

Microelectrode Making & Use.

Chemical	Stock Conc.	Use Conc.	Uses	Rooms
NaNO ₂	Pure powder	71% soln	Etching	S.R.F.
CrO ₃	10% solution	1% soln	Etching	S.R.F.
Cr ₂ O ₃	Pure powder	1% soln	Etching	S.R.F.
PtCl ₂	Pure powder	3% acidic soln	Plating	S.R.F.
KCN	1.25% solution	1.25% soln	Plating	S.R.F.
Pb Acetate	Pure powder	0.025% soln	Plating	S.R.F.
Pont.Sky Bl.	Pure powder	2% soln	Dye marking	S.R.F.
Phloxin B	Pure powder	2% soln	Dye marking	H.L., S.R.F.
Na acetate	1M solution	0.5M soln	Dye marking	S.R.F.
Acetone	100%	100%	carbon fibre	H.L., S.R.F.

NaNO₂ is the other ingredient for making Levick's solution (see above). The hazards of exposure to NaNO₂ include irritation to mucous membranes, skin and eyes. Acute toxic effects of ingestion include the induction of cyanosis. Chronic exposure has been shown to have mutagenic effects. Its use in the laboratory is described above.

CrO₃ and Cr₂O₃ are used in dilute acidic solutions to etch carbon fibres as

described above. Cr compounds are toxic and are highly irritating to the eyes, skin and respiratory system. Sensitization can occur. On chronic exposure Cr compounds are carcinogenic, mutagenic, induce perforating ulcers, and damage kidneys and lungs. As for Cr compounds **special precautions** should be adopted when making up solutions from the powder, and should include wearing a face mask. Special care should also be taken when handling the diluted solutions to avoid spillage.

PtCl₂ is used in a 3% acidic solution for plating the tips of microelectrodes with platinum black under the microscope. The solution is known as Kohlrausch solution: 3g PtCl₂, 25mg PbAc in 100ml 0.025N HCl. A syringe with needle is filled with the solution and is injected (less than 0.1ml) into a glass capillary tube to form a meniscus in which the tip of the electrode is immersed and 300nA current passed. PtCl₂ is harmful particularly in the form of fine dust (as stored). However the solution may be irritating to skin and eyes. The effects of chronic exposure are not known. **Special precautions** should be adopted when making up solutions from the powder, and should include wearing a face mask. Special care should also be taken when handling the diluted solutions to avoid spillage and to avoid contact with the KCN solution - see below.

KCN is stored and used as a 1.25% solution and is an ingredient in the gold chloride plating solution: 1.4g gold chloride, 1.25g KCN in 100ml water. This is used in the same way as described above for platinum plating except that gold plating is the result (50nA current used). Again small volumes (0.1ml) of the solution are used on any one occasion. KCN has alarming and potentially acute effects on inhalation, skin absorption or oral ingestion. Exposure can cause nausea, dizziness, headache, lung irritation, cyanosis, CNS depression. Repeated exposure may lead to an accumulation of cyanide in the body. Major acute effects are unlikely in our laboratory since such small amounts are used: less than 2mg/0.1ml quantity, and LD50 = 3mg/kg body weight. Repeated exposure is a danger, as is the production of HCN gas if the PtCl₂ and KCN solutions are inadvertently mixed, since the PtCl₂ solution is acidic. **Special precautions** should include the wearing of spectacles, and should be carried out in a well-ventilated room (room 1A41 is normally well-ventilated). Special care should be taken not to mix the two plating solutions. The different capillary tubes should be marked clearly with G and P for gold and platinum solutions respectively. Any person using cyanide compounds should undertake a University training course in the use of cyanide and its antidote.

Pb acetate is an ingredient in Kohlrausch solution - see above. In use this exists as 0.025% solution. Pb acetate is toxic though the dangers appear to be most obvious for chronic exposure where carcinogenic and mutagenic effects are reported. The special precautions have been mentioned above for the Kohlrausch solution.

Pontamine sky blue and Phloxin are used made up in 0.5M Na acetate solution. The dyes in their powder form are harmful. Pontamine sky blue in particular has been shown to be a carcinogen and mutagen. Na acetate may be harmful if ingested in quantity. These dye marking solutions are used in small quantities, e.g. less than 0.5ml per experiment. However **special precautions** should be taken during the preparation of the dye solutions, such as wearing safety spectacles and a face mask.

Acetone is used to lubricate the passage of a carbon fibre down a glass capillary tube. Acetone, apart from being highly inflammable, can be harmful on contact with the skin and eyes. Care should be taken to use the minimum volume necessary (e.g. less than 0.1ml per capillary), and should not be used in the presence of a naked flame.

Other Chemicals

Chemical	Stock Conc.	Use Conc.	Uses	Rooms
Urethane	Pure crystals	25% aqueous	Anaesthetic	S.R.F.
Formaldehyde	40% aq.	10% aqueous	Perfusion	S.R.F., H.L.
Paraformaldehyde	Pure powder	1% aqueous	histology	S.R.F.
Glutaraldehyde	25% aq.	2% aqueous	histology	S.R.F.
Diaminobenzidine	Pure powder	0.05% aqueous	histology	H.L.
Ethanol	100%	10% - 100% aq.	histology	H.L.
Xylene	100%	100%	histology	H.L.
Neutral red	Pure powder	21% aq. soln	Hist. stain	H.L.
Acetic acid	Glacial	1% in 90% ethanol	Hist. stain	H.L.
D.P.X.	-	as supplied	Histology	H.L.
Isoflurane	gas	3% in oxygen	Anaesthetic	S.R.F.

Urethane is used in a concentrated solution as an anaesthetic, where up to 5ml may be injected into an animal. Studies in animals have shown it to be carcinogenic, and it may be mutagenic. It can be harmful if ingested or absorbed through the skin. It can cause irritation of the skin and eyes. Spillage should be avoided and any contact with the skin should be washed immediately.

Forane (isoflurane) is used as a short-lasting anaesthetic (e.g. prior to urethane anaesthesia induction). This is the safest gaseous anaesthetic currently available. Forane is used by passing oxygen gas through a vapourizer, and the vapour is transmitted to a special scavenged anaesthetizing chamber. Ensure that the clamps on the feed pipes are correctly located and the Fluovac scavenger must be switched on (the cannister should be checked regularly that it's weight is less than 1400g). Loosen the inner chamber securing knob and push towards the rear of the unit - this moves the inner chamber to the rear and allows the anaesthetic gases to enter the inner chamber. Open the air bleed ring so that the holes line up. Put the animal in the inner chamber and close both doors. The oxygen flow used should be 2 litres/min and the vapourizer set to produce 3% forane vapour. Once the animal is anaesthetized switch the vapourizer to 0. Close the air bleed ring and move the inner chamber fully forward (this

flushes out the chamber). After 20 seconds switch off the Fluovac and remove the animal.

Formaldehyde is used to perfuse animals and so fix brain tissue. Formaldehyde is toxic and is an irritant to skin, eyes and mucous membranes. It is a carcinogen. **Special precautions** consist of carrying out all operations (e.g. perfusions, solution dilution etc) in a fume cupboard wearing gloves and spectacles. Formaldehyde vapour should not be allowed to mix with hydrochloric acid, since a potent carcinogen bis (chloromethyl) ether is formed. About 30ml of 10% solution is used to fix the brain in a small jar. This jar is taken to the H.L. where the brain is sectioned. In removing the brain from the jar it should be rinsed under the tap and the remaining formaldehyde solution flushed down the sink with plenty of water. Similar dangers attend the use of paraformaldehyde and glutaraldehyde (for cytochrome oxidase histology), and the same precautions taken.

Diaminobenzidine (for cytochrome oxidase histology) is believed to be a carcinogen, is harmful and a strong irritant. Special care must be taken in weighing out the small quantities used, especially since it is in a fine powder form. Safety glasses, face mask and gloves should be worn. Care should also be taken in its subsequent use in dilute solution for the tissue reaction.

Ethanol and xylene are used in histology for the preparation of sections for mounting on slides. They should be used in a room with good ventilation. They both have effects on the CNS as a narcotic, and can be irritating to mucous membranes and eyes. Ethanol is moderately toxic and irritant, while xylene has a low toxicity, but is a moderate irritant. Gloves and glasses may need to be worn, especially when using xylene which is absorbed through the skin. 1 litre of ethanol may be used on any one occasion for histology, while about 250 ml of xylene is used. These quantities are not large enough to warrant concern over fumes that might be created. These substances are however a fire hazard, so should not be used with naked flames present. Similar precautions should be enforced for D.P.X., which is a proprietary resin used for mounting sections. Only small quantities (5 ml) are used on any one occasion. Histo-mount can be used to replace D.P.X., although it too should be used with care - avoid contact with skin and eyes, and avoid prolonged breathing of vapour. Non-toxic Histo-Clear can be used more safely in place of xylene.

Neutral red is a stain used for histological processing of sections. It can be irritating to eyes, and may cause contact dermatitis. As with the other stains used, the dangers are only evident with the stock bottle containing the fine powder, and not in the 1% solution used for histology. Care should therefore be taken when weighing out powder, through use of spectacles and a face mask.

Pharmacological Agents

A great number of chemicals are used for their pharmacological actions. These chemicals are sometimes made up to 0.5M in water. Most of these chemicals are research chemicals and have not been tested for harmful effects, and therefore the hazards should be regarded as unknown. Great care should be taken while preparing solutions and in using these solutions. **Many of the compounds are neuroactive amino acids and are potentially neurotoxic in the central nervous system. Special precautions** should include the wearing of safety spectacles and a face mask during the preparation of drug solutions. An incomplete list of the compounds is given:

GABA, glutamate, AMPA, 1S,3R/1S,3S/1R,3S/1R,3R-ACPD, L-CCG, MCCG, MCPG, kainate, kynurenate, CPP, CNQX, NMDA, L-AP4, MAP4 quinolinic acid, L-quisqualic acid, 1-1,2diphenylethylpiperidine, Ro 25-6981, DCG-IV

OTHER HAZARDS

For emergency procedures see Dept Safety Regulations. Emergency tel no: 2222

General: Each S.R.F. laboratory has a carbon dioxide type fire extinguisher. The H.L. has water type extinguishers in the corridor and reception areas of the Neuropsychology Unit. A "sharps" bin must be kept in each lab for disposing of glass, scalpels, needles etc.

1 Use of liquid and gas cylinders

All cylinders when in use should be chained to the wall to prevent them toppling over, which could cause direct injury or result in the valve being damaged and the gas being released. Those cylinders not in use should be kept in a safe place out of the way. Cylinders that are not in use must be turned off at the valve on the cylinder.

Liquid carbon dioxide cylinders are used in the H.L. for freezing the microtome stage. 2 cylinders are kept, one in use the other spare. A spanner is kept in the H.L. for attaching the freezing stage hose. Ensure that ventilation is adequate during normal use (and this is usually the case for the H.L.). If larger amounts of gas are released (e.g. if a valve fails) the window in the teaching lab should be opened and all other people in the Neuropsychology Unit should be notified and the rooms vacated.

Industrial air cylinders are used in 1A43 of the S.R.F. Both cylinders in use (one for the Neurophore other for the floating table) are fitted with a regulator and are subject to the following procedure when turning off:

The supply valve should be shut off at the cylinder, the outlet vented to atmospheric pressure and then the pressure adjusting screw wound back in an anti-clockwise direction, so that zero output pressure will be registered when the gas from the cylinder is again turned on. The adjusting screw should be reset to give the required pressure each time the gas is turned on.

Pure oxygen cylinders are used in the S.R.F. room 1A69, for the isoflurane anaesthetic. 95% Oxygen/5% Carbon Dioxide cylinders are used in the S.R.F. room 1A43, for oxygenizing solutions for the isolated whisker bath.

2 Use of gas burner for preparing glass pipettes

There is a hazard of skin burns from the flame and heated glass, and also the hazard of starting a fire. A microtorch, a propane gas torch and a laboratory Bunsen burner are used in the S.R.F. (usually room 1A41) for the preparation of glass micropipettes. All procedures should be used over a non-combustible surface (sheet of steel on the bench). A fire blanket should be in easy reach (one is kept in room 1A41). Some work may also be carried out in the Mechanical Workshop. This must be done during office hours when the extractor fan can be switched on.

3 Electrical shock

The S.R.F. labs are all fitted with a trip switch in case of electrical short circuit occurring on any of the sockets, and therefore dangerous effects of shock avoided. Equipment should be checked for adequate earthing, for correct connection polarity and for good order of power connection cables. In the event of a mains failure disconnect the apparatus and contact the Maintenance Dept (ext 2444, or outside normal working hours ext 7001).

4 Handling animals

Microbial or viral disease may be transmitted from an infected animal to humans. Protective clothing (lab coats) should be worn, and personal hygiene ensured by washing with antiseptic soap. Any injuries inflicted by animals must be treated with first aid (in S.R.F. Office) and reported immediately. Staff are subjected to a health check once a year, and should have been vaccinated for tetanus.

5 Histology knives in H.L.

These knives are extremely sharp and should be treated with utmost respect, especially since there is no mechanical guard to protect against injury. The microtome should not be cleaned or adjusted with the knife in place. The knife must be removed after use and replaced into its storage box.