# Safety Documentation

Please select the forms you require by selecting the check boxes below.
You can select more than one.

☑ **Risk Assessment** ☑ **Method Statement** ☑ **Chemicals COSHH**

Once you have made your selections, scroll down and complete the forms.

**Buttons**: [+] will add a row to a list [X] will delete a row from a list

You may save this file to a local drive at any time.
When you have finished, save the file to a local drive and email it to your supervisor for authorisation.

**Supervisors** - There is a sign-off section at the end of the document set that must be completed.

**Staff may "self authorise", (as a supervisor), but the forms must still be submitted to the DSO for approval.**

**IMPORTANT:**

YOU ***MUST NOT*** START ANY PRACTICAL WORK UNTIL THESE FORMS HAVE BEEN RETURNED TO YOU WITH **BOTH** YOUR SUPERVISOR's AND DSO's APPROVAL SIGNATURES ATTACHED.

## Please complete these fields

| | |
|---|---|
| School or Service | Wolfson School of Mechanical, Electrical and Manufacturing Engineering |
| Department | Centre for Biological Engineering |
| Originator name | Sotiria Toumpaniari |
| email address | s.toumpaniari@lboro.ac.uk |
| Location | H34, H25 |
| Project / Activity / Task | Fixation and dehydration of biological samples for scanning electron microscopy imaging |
| Supervisor Name | Prof Sotiris Korossis |

Version : 2.19

# Loughborough University
## Centre for Biological Engineering

# Risk Assessment

Reference: SAF/MEME 6513

Location: H34, H25

Originator: Sotiria Toumpaniari

Project / Activity / Task: Fixation and dehydration of biological samples for scanning electron microscopy imaging

Is this process risk assessment for a : (✓) Laboratory / Workshop ( ) General use

| Category 1: Machinery & work equipment: | | | | + |
|---|---|---|---|---|
| Design and Construction | Mechanical hazards | Electrical hazards | Radiation hazards | |
| N/A | N/A | Electrical test lables current | Heat(Inc. IR) | x |
| Category 2: Workplace | | | | + |
| N/A | | | | x |
| Category 3: Hazardous and/or Harmful substances | | | | + |
| Flammable substances | | | | x |
| Irritant substances | | | | x |
| Toxic substances | | | | x |
| Corrosive substances | | | | x |
| Sensitising substances | | | | x |
| Cancer causing substances | | | | x |
| Category 4: Work activity | | | | + |
| Lone working out of hours | | | | x |
| Category 5: Work organisation | | | | + |
| N/A | | | | x |

## Explain the risks associated with these hazards

People / Groups at risk: Everyone in the room — x

| Enter risk details here:- | Impact | Probability | Risk Score |
|---|---|---|---|
| Flammable substances | Harmful | Likely | High |

| What are the control measures? | Lowers Impact | Lowers Probability | + |
|---|---|---|---|
| Keep the chemicals in special cupboard for flammables | Significantly | Significantly | x |
| Do not have sources of ignition around the chemicals | Significantly | Significantly | x |
| Keep/store the pots with chemicals on spill trays | Significantly | Significantly | x |

## Process Risk Assessment Form (Continued)

| | | | |
|---|---|---|---|
| Appropriately label the pots with the chemicals | Significantly | Significantly | x |

Residual Risk: Low

| People / Groups at risk | Operator only | |
|---|---|---|
| | | x |

| Enter risk details here:- | Impact | Probability | Risk Score |
|---|---|---|---|
| Irritant,sensitising,toxic and cancer causing chemicals | Very Harmful | Likely | Unacceptable |

| What are the control measures? | Lowers Impact | Lowers Probability | + |
|---|---|---|---|
| Use only in fume hood | Significantly | Significantly | x |
| Wear appropriate PPE- lab coat and gloves | Significantly | Significantly | x |
| Keep/store the pots with chemicals on spill trays | Significantly | Significantly | x |
| Appropriately label the pots with the chemicals | Significantly | Significantly | x |

Residual Risk: Low

+ Add another Risk

### Who may be at risk as a result of this activity?

| Personnel Group | Maximum (Task setup/ Re-configuration) | High (Performing the task) | Medium (Observing the task) | Low (Present, but not involved) | Lone Working (Out of hours) | No Exposure Permitted | Total |
|---|---|---|---|---|---|---|---|
| Academic Staff | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Technical Staff | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Research Staff (PDRA) | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
| Research Students (PhD) | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
| Students (Undergraduate / MSc) | 0 | 0 | 8 | 0 | 0 | 0 | 8 |
| Visitors | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Others - Over-type as needed | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 0 | 5 | 9 | 0 | 0 | 0 | 14 |

### With these controls in place, the risk is:

Process Risk Assessment Form (Continued)

**The activity is LOW RISK  - and is effectively controlled**

# Loughborough University
## Centre for Biological Engineering

# Safety Method Statement

Reference: SAF/MEME 6513

Location: H34, H25

Originator: Sotiria Toumpaniari

Project / Activity / Task: Fixation and dehydration of biological samples for scanning electron microscopy imaging

| What equipment will be used in this activity? | + |
|---|---|
| Pipette gun | x |
| Stripettes | x |
| Duran bottles | x |
| pH meter | x |
| Falcon tubes | x |
| Fume hood | x |
| Well plates or petri dish | x |
| Plastic working tray | x |
| Parafilm | x |
| Biosafety cabinet | x |
| Aspirator | x |
| Spill tray | x |
| 150ml Sterillin pots | x |

| What training must be completed to do this activity? | + |
|---|---|
| Use of chemical substances | x |
| Use of biological samples | x |

| What chemicals are being used? (These must be included in the COSHH Form) | + |
|---|---|
| Sodium hydroxide | x |
| Glutaraldehyde | x |
| Paraformaldehyde solution | x |

| Spill and accident procedures. | + |
|---|---|
| Dispose contaminated gloves. Leave note with a name of the operator and sate mentioning not to move anything from the area. Store pots with the chemicals on a spill tray. | x |

| Procedure in the event of an emergency. (How to leave the process in a safe condition in such an event) | + |
|---|---|
| CBE code of practice, SOP004, SOP037, SOP038, SOP039 | x |

| References. | + |
|---|---|
| | x |

## Detailed sequential description of the process

| Process step | Precautionary measures and comments | + |
|---|---|---|

## Safety Method Statement (Continued)

| Process step | Precautionary measures and comments | + |
|---|---|---|
| Wear PPE mentioned above and place a plastic tray on working surface. | Check if PPE is damaged and replace if it is. | x |
| Prepare NaOH solution using pipette gun and stripettes . | Work in fume hood and add first water and NaOH solution. Avoid spillages. | x |
| In a fume hood, prepare 2.5% glutaraldehyde solution or 2% glutaraldehyde and 2% formaldehyde in 1M NaOH solution using pipette gun and stripettes. | Work only in hood. Add NaOH solution, then glutaraldehyde and finally formaldehyde, if required. | x |
| In a biosafety cabinet, aspirate the medium from samples using aspirator and stripette. | Carefully decontaminate the biosafety cabinet and aspirator before use. | x |
| Wash samples x3 with PBS using a pipette gun and use aspirator in between to remove PBS. | Avoid spillages by using pipette gun and aspirator. | x |
| In a fume hood, immerse samples in appropriately labeled sterilin pots filled with 2.5% glutaraldehyde solution or 2% glutaraldehyde and 2% formaldehyde in 1M NaOH solution using pipette gun and stripettes. | Avoid spillages by using pipette gun | x |
| Transfer the labeled Sterilin pots with immersed samples to a spill tray or safety rack and leave overnight in the fridge or on the bench. | Seal pots very well using parafilm and place them on spill tray. | x |
| Maintain sterilin pots with the samples sealed and place them in a box and continue the process in S building. | Check integrity of parafilm and change it if it has been compromised. | x |

# Loughborough University
# Centre for Biological Engineering

## COSHH Form

Reference: MEME 698, 699, 695.

Location: H34, H25

Originator: Sotiria Toumpaniari

Project / Activity / Task: Fixation and dehydration of biological samples for scanning electron microscopy imaging

**CHEMICAL NAME**

**Glutaraldehyde (Glutaral (>= 25 - < 30%)/ Methanol (>=**

CAS No. 111-30-8/ 67-56-1

W.E.L. (Itel / stel)

Hazard Rating: High.

OVERALL RISK: Medium

| Amount used | | Period of use (hrs) | The process is: | Physical State | | Exposure Potential |
|---|---|---|---|---|---|---|
| 10 | ml | 18 | Closed | Volatile Liquid | ☑ Eyes ☑ Skin ☑ Inhaled ☑ Ingested | Low |

This chemical has a high health risk associated with it.

| Hazard Statement and Description | Precaution Statement and Description |
|---|---|
| H225 Highly flammable liquid and vapour. | P261 Avoid breathing dust/fume/gas/mist/vapours/spray. |
| H301 Toxic if swallowed. | P273 Avoid release to the environment. |
| H302 Harmful if swallowed. | P280 Wear protective gloves/protective clothing/eye protection/face protection. |
| H311 Toxic in contact with skin | P303 + P361 + P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminate |
| H314 Causes severe skin burns and eye damage. | P304 + P340 IF INHALED: Remove victim to fresh air and keep at rest in a position com |
| H317 May cause an allergic skin reaction. | P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remov |
| H318 Causes serious eye damage. | P310 Immediately call a POISON CENTER or doctor/physician. |
| H330 Fatal if inhaled. | P342 + P311 If experiencing respiratory symptoms: Call a POISON CENTER or doctor/p |
| H331 Toxic if inhaled. | P391 Collect spillage. |
| H332 Harmful if inhaled. | |
| H334 May cause allergy or asthma symptoms or breathing difficulties i | |
| H335 May cause respiratory irritation. | |
| H370 Causes damage to organs. | |
| H371 May cause damage to organs. | |
| H400 Very toxic to aquatic life. | |
| H411 Toxic to aquatic life with long lasting effects. | |
| EUH071 Corrosive to the respiratory tract. Contains lead. Should not be | |

| | |
|---|---|
| Justify the use of this chemical: | Glutaral is one of the chemicals that consist glutaraldehyde that is the main fixative for scanning electron microscopy, because paraformaldehyde makes the samples brittle. It is a better fixative for SEM for samples with thickness <=1mm than paraformaldehyde. |

**How will the precautions listed above be implemented?**

Use personal protective equipment (gloves and lab coat). Avoid inhalation of vapour or mist by opening bottle in fume hood in CTMF. Containers which are opened must be carefully resealed and kept upright to prevent leakage. Do not let product enter drains.

# COSHH Form (Continued)

| Special Storage and Containment Measures | Disposal Method |
|---|---|
| Keep container tightly closed in a dry and well-ventilated place. Recommended storage temperature -20°C. Store under inert gas. Air sensitive. | Collect waste in a Duran-type bottle and when full, place it in Pod 2 to be safely discarded. |
| How will spillages be dealt with? | *Please note: any material used to clean up a spill of hazardous material must also be disposed of as hazardous material.* Click here to see spill procedures |
| Spill kit | |

**CHEMICAL NAME**

**4% Paraformaldehyde solution (Paraformaldehyde**

CAS No. 30525-89-4/ 7647-14-

W.E.L. (Itel / stel)

Hazard Rating: High

OVERALL RISK: Medium

| Amount used | | Period of use (hrs) | The process is: | Physical State | | Exposure Potential |
|---|---|---|---|---|---|---|
| 50 | ml | 18 | Open | Non-Volatile Liquid | ☑ Eyes ☑ Skin ☐ Inhaled ☐ Ingested | Low |

This chemical has a high health risk associated with it.

| Hazard Statement and Description | Precaution Statement and Description |
|---|---|
| H317 May cause an allergic skin reaction. | P280 Wear protective gloves/protective clothing/eye protection/face protection. |
| H318 Causes serious eye damage. | P302 + P352 IF ON SKIN: Wash with plenty of soap and water. |
| H351 Suspected of causing cancer. | P338 Remove contact lenses, if present and easy to do. Continue rinsing. |
| | P310 Immediately call a POISON CENTER or doctor/physician. |
| Justify the use of this chemical: | The fixation reagent is very important for how the samples is going to be processed. Proper tissue fixation is essential for accurate histopathologic evaluation. Formaldehyde fixation is thought to form cross links between the aldehydes and the proteins, creating a gel, thus retaining cellular constituents in their in vivo relationship. Once properly fixed, the tissue should be able to withstand the subsequent stages of tissue processing or staining. |
| How will the precautions listed above be implemented? | |
| Containers with 4% paraformaldehyde solution will be opened only in fume hood. Wear PPE- nitrile gloves, lab coat and goggles. | |

| Special Storage and Containment Measures | Disposal Method |
|---|---|
| Keep container tightly closed in a dry and well-ventilated place. Store at 2-4 °C. | Keep waste of formalin and formaldehyde in a single container and when full, transfer it in pod 2. |
| How will spillages be dealt with? | *Please note: any material used to clean up a spill of hazardous material must also be disposed of as hazardous material.* Click here to see spill procedures |
| Absorbent cloth / tissue | |

**CHEMICAL NAME**

**Sodium hydroxide**

CAS No. 1310-73-2

W.E.L. (Itel / stel)

Hazard Rating: High

OVERALL RISK: Medium

| Amount used | | Period of use (hrs) | The process is: | Physical State | | Exposure Potential |
|---|---|---|---|---|---|---|
| 5 | ml | | Semi Closed | Non-Volatile Liquid | ☑ Eyes ☑ Skin ☐ Inhaled ☐ Ingested | Low |

| Hazard Statement and Description | Precaution Statement and Description |
|---|---|
| H290 May be corrosive to metals. | P280 Wear protective gloves/protective clothing/eye protection/face protection. |
| H314 Causes severe skin burns and eye damage. | P301 + P330 + P331 IF SWALLOWED: rinse mouth. Do NOT induce vomiting. |

# COSHH Form (Continued)

| | | |
|---|---|---|
| H315 Causes skin irritation. | P303 + P361 + P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminate | x |
| H318 Causes serious eye damage. | P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remov | x |
| H319 Causes serious eye irritation. | P310 Immediately call a POISON CENTER or doctor/physician. | x |
| How will the precautions listed above be implemented? | | |
| Use only in hood and preferably on a plastic tray to prevent contact with metals. Wear gloves and labcoat. If it is not used in hood, then wear goggles. | | |
| Special Storage and Containment Measures | Disposal Method | + |
| Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage. Store in cool place. | Add hydrochloric acid to neutralise it and pour in the drain. | x |
| How will spillages be dealt with? | *Please note: any material used to clean up a spill of hazardous material must also be disposed of as hazardous material.* *Click here to see spill procedures* | |
| Absorbent cloth / tissue | | |

+ Add another chemical

Statement of work (Process to be undertaken)

Prepare 0.1M buffer using 50% sodium hydroxide (NaOH) solution in H2O.
For immersion fixation, use 2.5% glutaraldehyde in 0.1M buffer. The time of fixation is dependent upon the dimensions of the sample to be fixed. The largest recommended size is 1 mm^3, when there is optimal penetration. Fixation overnight in fridge.
For perfusion fixation, use 2% glutaraldehyde in 2% formaldehyde 0.1M buffer. The conditions depend upon the animal, its age and the organ required.

Show image

Personal protection requirements not covered in the precaution statements above.

Always wear overshoes in CBE. Appropriate clothing (long trousers and skirts), closed shoes.
Wear self-contained breathing apparatus for firefighting if necessary, because carbon oxides arise from decomposing glutarldehyde from fire. Seal properly containers with glutaraldehyde.

Sources of information and references

https://www.sigmaaldrich.com/chemistry/solvents/sureseal.html
https://www.sigmaaldrich.com/catalog/product/sial/g5882?lang=en&region=GB
https://biotech.unl.edu/tem-fixation-protocols-microscopy

Reference to **existing approved** Risk Assessment

With the current controls, the risk of using these chemicals is: Medium

Supervisor to check that the process involving the safe use of these chemicals has been satisfactorily evaluated

# Supervisor and Departmental Safety Office (DSO) Sign-off.

## Supervisors

Please check the documents above and if you want to approve them:

1) Electronically sign this document
2) Save it to a local drive (You will be prompted to do this)
3) eMail the signed document to the DSO.

## DSO

Please review the documents above and if you want to approve them:

1) Enter the reference numbers as appropriate
2) Electronically sign this document
3) Save it to a local drive (You will be prompted to do this)
3) eMail the signed document to the originator

**IF YOU DO NOT WANT TO AUTHORISE THE FORMS,**

Please do not sign the form, but click the "Not Approved" check-box and return it to the originator by email stating why and what you expect them to do to put it right in the comments box below.

Not Approved ☐

Supervisors Signature: Professor Sotiris Korossis — Digitally signed by Professor Sotiris Korossis
Date: 2020.02.24 18:09:33 Z

Form Reference Numbers

| Risk Assessment | Method Statement | COSHH Assessment |
|---|---|---|
| SAF/MEME 6513 | SAF/MEME 6513 | MEME 698, 699, 695. |

DSO Signature: Digitally signed by David Green
Date: 2020.08.03 12:31:43 +01'00'
Adobe Acrobat version: 2020.009.20074

**This document set must be reviewed and re-approved at the following times:**

1) After the first occurrence of the activity described above (Review only)
2) After any change to the procedure or reagents used
3) After any incident resulting from this activity
4) At least annually from the date of approval

Next Review: 23/02/2021

Review comments