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	CBE
Originator name	Oliver Frost
email address	o.g.frost@lboro.ac.uk
Location	CBE, H25
Project / Activity / Task	'Proof of concept for separation of young from old (senescent) cells – improving efficacy and safety for clinical use'
Supervisor Name	Rob J Thomas

Version : 2.19

Loughborough University
CBE

Risk Assessment

Reference: SAF/MEME/7904 CBEBRA2

Location: CBE, H25

Originator: Oliver Frost

Project / Activity / Task: 'Proof of concept for separation of young from old (senescent) cells – improving efficacy and safety for clinical use'

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	N/A	N/A	x
Category 2: Workplace				+
Slips/Trips/Falls on the level				x
Category 3: Hazardous and/or Harmful substances				+
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: Operator and people in proximity			x
Enter risk details here:-	Impact	Probability	Risk Score
Slips/Trips/Falls on the level	Harmful	Highly Unlikely	Low
What are the control measures?	Lowers Impact	Lowers Probability	+
Organise room to have nothing on the floor that can be a trip hazard. Reduce movement between labs if possible.	Significantly	Significantly	x
		Residual Risk	Low
People / Groups at risk: Operator and people in proximity			x
Enter risk details here:-	Impact	Probability	Risk Score
Aerosols/splashes from irritant substances & sensitiser	Harmful	Highly Unlikely	Low
What are the control measures?	Lowers Impact	Lowers Probability	+
Work in fume hood or BSC	Significantly	Significantly	x
Wear PPE	Significantly	Significantly	x

Process Risk Assessment Form (Continued)

			Residual Risk	Low
People / Groups at risk	Operator only			x
Enter risk details here:-		Impact	Probability	Risk Score
Lone Working out of hours		Slightly Harmful	Unlikely	Low
What are the control measures?		Lowers Impact	Lowers Probability	+
Loughborough University Lone working policy to be followed, with the use of the lone working app and contacting security on occasions of lone working. Will send OOH 1st contact a text message on entry to the lab and another when leaving. Depending on the length of OOH work needed, further text updates will be used (hourly/2 hourly). permission to work out of hours must be obtained prior to work commencing. It is advised to inform security so that they are aware of your location on campus for the duration of your lone working/out of hours, and also inform Security when you leave the premises . Inform academic supervisor and a colleague of intention to lone work and state duration of stay. Ensure you have mobile phone on person at all times - security mobile number is 0800 526966 - security staff are also trained First Aiders Always remember to log out of lone working app when leaving building at completion of the work. Inform lab management of intention to work out of hours.		Significantly	Significantly	x
Will be aware of all safety procedures (including for emergency), and numbers		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	0	0	0	0	0
Technical Staff	0	0	0	1	0	0	1
Research Staff (PDRA)	0	0	0	3	0	0	3
Research Students (PhD)	0	1	0	0	1	0	2
Students (Undergraduate / MSc)	0	0	0	0	0	0	0
Visitors	0	0	0	0	0	0	0
Others - Over-type as needed	0	0	0	0	0	0	0

Process Risk Assessment Form (Continued)

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
Total	0	1	0	4	1	0	6

With these controls in place, the risk is:

The activity is LOW RISK - and is effectively controlled

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CBE

Safety Method Statement

Reference: SAF/MEME/7904

Location	CBE, H25	Originator	Oliver Frost
Project / Activity / Task	'Proof of concept for separation of young from old (senescent) cells – improving efficacy and safety for clinical use'		

What equipment will be used in this activity?	+
Centrifuge Tubes	x
BSC	x
Incubator	x

What training must be completed to do this activity?	+
CBE Training (Completed in November 2021)	x
CBE Training Refresher (Completed in November 2023)	x

What chemicals are being used? (These must be included in the COSHH Form)	+
InSolution Doxorubicin, Hydrochloride	x
5-Aza-2-deoxycytidine	x
Bafilomycin	x
DDAO galactosidase (9H-(1,3Dichloro-9,9-Dimethylacridin-2-One-7-yl) B-D-Galactopyranoside	x

Spill and accident procedures.	+
The spill kits have been identified in change rooms . Soak up with inert absorbent material and dispose of as hazardous waste. Keep in suitable closed container for disposal. As detailed in SOP039 on pages 25-28. Then the accident will be reported on the university online system.	x

Procedure in the event of an emergency. (How to leave the process in a safe condition in such an event)	+
Leave a note with details of the user and name of the chemical asking not to move anything from the area. If the fire alarm sounds continuously, make equipment safe then evacuate the building to assembly point. Only return when informed that it is safe to do so	x

References.	+
CBE code of practice, SOP003, SOP004, SOP037, SOP038, SOP048	x

Detailed sequential description of the process

Process step	Precautionary measures and comments	+

Safety Method Statement (Continued)

Process step	Precautionary measures and comments	+
Cells will be grown in T175 flasks for a period of 3 days in the incubator in H25. Once they reach confluency the media will be aspirated and replaced with media containing 200 nM of "InSolution Doxorubicin, Hydrochloride" or 200 nM "5-Aza-2-deoxycytidine". These will be prepared by adding 0.4 µL of the 10 mM "InSolution Doxorubicin, Hydrochloride" or 10 mM "5-Aza-2-deoxycytidine" stock solutions in 25 mL of DMEM cell culture media. Once the media is replaced the flasks will be placed back in the incubator for 48 hours. After 48 hours the media will be aspirated and replaced with normal DMEM media and cells will be cultured for another 10 days. After 10 days, the media will be aspirated and treated with 0.1 µM of Bafilomycin for 1 hour in the incubator. This will be prepared by diluting 6 µL of 400 µM Bafilomycin stock solution in 25 mL of DMEM culture media. After 1 hour the media will be aspirated and cells will be treated for 2 hours with 20 µM DDAO galactosidase in the incubator. This will be prepared by diluting 1 µL of 20 mM DDAO galactosidase stock solution in 10 mL PBS. After this, cells will be washed with PBS twice, detached using Trypsin and analyzed with a Flow cytometer. FACSCanto	All work will be done in BSCs in sterile conditions due to the work on cell cultures. Personal PPE equipment will be worn at all times and waste disposal will be done as described in this COSSH form.	x

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CBE

COSHH Form

Reference: SAF/MEME/2181-2184

Location: CBE, H25

Originator: Oliver Frost

Project / Activity / Task: 'Proof of concept for separation of young from old (senescent) cells – improving efficacy and safety for clinical use'

CHEMICAL NAME

InSolution Doxorubicin, Hydrochloride

CAS No. 25316-40-9

W.E.L. (Itel / stel)

Hazard Rating: High

OVERALL RISK: Medium

Amount used		Period of use (hrs)	The process is:	Physical State		Exposure Potential
0.004	ml	48	Semi Closed	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
H302 Harmful if swallowed.	P201 Obtain special instructions before use.
H340 May cause genetic defects.	P202 Do not handle until all safety precautions have been read and understood.
H350 May cause cancer.	P264 Wash … thoroughly after handling.
H360FD May damage fertility. May damage the unborn child.	P270 Do no eat, drink or smoke when using this product.
	P301 + P312 IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unw
	P308 + P313 IF exposed or concerned: Get medical advice/attention.

Justify the use of this chemical:	This chemical is essential for inducing stem cell senescence. It is widely used in other groups and research facilities and it is the gold standard for inducing cell aging. Currently there are no other safer alternatives in existence Chemical will be used to induce stem cell senescence. Once cells have been treated with 4 ul of Doxorubicin diluted in 20 ml of DMEM cell culture media (to achieve 20 nM of Doxorubicin) for 48 hours in the incubator, the solution will be aspirated and replaced with fresh DMEM cell culture media without Doxorubicin

How will the precautions listed above be implemented?

Following SOP037, all relevant PPE will be worn to ensure safe handing and avoid contact with skin. These include a standard side fastening white laboratory coat with elasticated sleeves, gloves, safety glasses. Gloves will be removed in accordance with good practice, without touching the outer surface, thereby avoiding skin contact with the substance. Once removed, used gloves will be disposed of as biohazardous waste (SOP003) and will be placed into the autoclave waste stream. The entire procedure will be undertaken within a BSC, thereby ensuring adequate ventilation and reducing the risk spillage or getting in contact with skin.
All solid waste such as tips will be disposed in the cytotoxic boxes (purple) while liquid waste will be placed in a single container and when full, it will be transfered to Gas pod 2.

Special Storage and Containment Measures	Disposal Method
-20C appropriately labelled	Aqueous waste - Check with Technician or Supervisor
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 - All waste will be treated as cytotoxic and will be disposed of through the cytotoxic waste route. All solids will be disposed of in purple cytotoxic sharps container while all liquid will be placed in carefully labeled glass bottles before placing in gas pod 2 when full. All spillages will be dealt with according to SOP038.

COSHH Form (Continued)

CHEMICAL NAME

5-Aza-2-deoxycytidine

CAS No. 2353-33-5

W.E.L. (Itel / stel)

Hazard Rating: High

OVERALL RISK: Medium

Amount used		Period of use (hrs)	The process is:	Physical State		Exposure Potential
0.0004	ml	48	Semi Closed	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
H302 Harmful if swallowed.	P201 Obtain special instructions before use.
H315 Causes skin irritation.	P301 + P312 IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unw
H319 Causes serious eye irritation.	P302 + P352 IF ON SKIN: Wash with plenty of soap and water.
H335 May cause respiratory irritation.	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remov
H341 Suspected of causing genetic defects.	P308 + P313 IF exposed or concerned: Get medical advice/attention.
H360 May damage fertility or the unborn child.	

Justify the use of this chemical:	The chemical will be used to induce stem cell senescence. It works differently from Doxorubicin and there are no other safer alternatives. Once cells have been treated with 5-Aza-2-deoxycytidine diluted in 20 ml of DMEM cell culture media (0.4 ul of 10 mM to achieve 200 nM) for 48 hours in the incubator, the solution will be aspirated and replaced with fresh DMEM cell culture media without 5-Aza-2-deoxycytidine

How will the precautions listed above be implemented?

Following SOP037, all relevant PPE will be worn to ensure safe handing and avoid contact with skin. These include a standard side fastening white laboratory coat with elasticated sleeves, gloves, safety glasses. Gloves will be removed in accordance with good practice, without touching the outer surface, thereby avoiding skin contact with the substance. Once removed, used gloves will be disposed of as biohazardous waste (SOP003) and will be placed into the autoclave waste stream. The entire procedure will be undertaken within a BSC, thereby ensuring adequate ventilation and reducing the risk spillage or getting in contact with skin.
All solid waste such as tips will be disposed in the cytotoxic boxes (purple) while liquid waste will be placed in a single container and when full, it will be transfered to Gas pod 2.

Special Storage and Containment Measures	Disposal Method
-20C appropriately labelled	Aqueous waste - Check with Technician or Supervisor
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 - all waste will be treated as cytotoxic and will be disposed of through the cytotoxic waste route. All solids will be disposed of in purple cytotoxic sharps container while all liquid will be placed in carefully labeled glass bottles before placing in gas pod 2 when full. All spillages will be dealt with according to SOP038.

CHEMICAL NAME

Balifomycin A1

CAS No. 88899-55-2

W.E.L. (Itel / stel)

Hazard Rating: High

OVERALL RISK: Medium

Amount used		Period of use (hrs)	The process is:	Physical State		Exposure Potential
0.00001	g		Semi Closed	Non-Volatile Liquid	☑ Eyes ☑ Skin ☑ Inhaled ☐ Ingested	Low

Hazard Statement and Description	Precaution Statement and Description

COSHH Form (Continued)

H319 Causes serious eye irritation.	P261 Avoid breathing dust/fume/gas/mist/vapours/spray.	x
H335 May cause respiratory irritation.	P280 Wear protective gloves/protective clothing/eye protection/face protection.	x
H315 Causes skin irritation.	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remov	x
	P302 + P352 IF ON SKIN: Wash with plenty of soap and water.	x

How will the precautions listed above be implemented?

Following SOP037, all relevant PPE will be worn to ensure safe handing and avoid contact with skin. These include a standard side fastening white laboratory coat with elasticated sleeves, gloves, safety glasses. Gloves will be removed in accordance with good practice, without touching the outer surface, thereby avoiding skin contact with the substance. Once removed, used gloves will be disposed of as biohazardous waste (SOP003) and will be placed into the autoclave waste stream. The entire procedure will be undertaken within a BSC, thereby ensuring adequate ventilation and reducing the risk spillage or getting in contact with skin.
All solid waste such as tips will be disposed in the cytotoxic boxes (purple) while liquid waste will be placed in a single container and when full, it will be transfered to Gas pod 2.

Special Storage and Containment Measures	Disposal Method	+
-20C appropriately labelled	Aqueous waste - Check with Technician or Supervisor	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*	

Spill kit - all waste will be treated as cytotoxic and will be disposed of through the cytotoxic waste route. All solids will be disposed of in purple cytotoxic sharps container while all liquid will be placed in carefully labeled glass bottles before placing in gas pod 2 when full. All spillages will be dealt with according to SOP038.

CHEMICAL NAME

DDAO galactosidase (9H-(1,3-Dichloro-9,9-

CAS No.

W.E.L. (Itel / stel)

Amount used		Period of use (hrs)	The process is:	Physical State		Hazard Rating	
0.001	ml	1	Semi Closed	Non-Volatile Liquid	Eyes, Skin, Inhaled, Ingested	Low	Low

x

OVERALL RISK:

Low

Hazard Statement and Description	Precaution Statement and Description	+
No Hazard Statements applicable	No Precaution statements applicable	x

How will the precautions listed above be implemented?

Following SOP037, all relevant PPE will be worn to ensure safe handing and avoid contact with skin. These include a standard side fastening white laboratory coat with elasticated sleeves, gloves, safety glasses. Gloves will be removed in accordance with good practice, without touching the outer surface, thereby avoiding skin contact with the substance. Once removed, used gloves will be disposed of as biohazardous waste (SOP003) and will be placed into the autoclave waste stream. The entire procedure will be undertaken within a BSC, thereby ensuring adequate ventilation and reducing the risk spillage or getting in contact with skin.

Special Storage and Containment Measures	Disposal Method	+
-20C appropriately labelled	Aqueous waste - Check with Technician or Supervisor	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)

Cultured cells will be treated with "In solution Doxorubicin" or "5-Aza-2-deoxycytidine" for 48 hours before removing it and replacing the cell culture media with media without Doxorubicin. After 7 days in culture, the media will be removed and

Show image

COSHH Form (Continued)

cells will be treated with Bafilomycin A1 for 1 hour in the incubator followed by a 2 hour treatment with DDAO galactosidase. After two hours the cells be detached using Trypsin and analysed with a flow cytometer.

Personal protection requirements not covered in the precaution statements above.

Closed shoes and overshoes (CBE).

Sources of information and references

https://www.sigmaaldrich.com/catalog/product/mm/504042?lang=en&region=GB
https://www.sigmaaldrich.com/catalog/product/sigma/a3656?lang=en&region=GB
https://www.fishersci.co.uk/shop/products/bafilomycin-a1-95-acrosorganics/10295441

Reference to **existing approved** Risk Assessment

CBE/BRA/096

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: Rob Thomas — Digitally signed by Rob Thomas Date: 2024.01.15 13:28:36 Z

Form Reference Numbers

Risk Assessment	Method Statement	COSHH Assessment
SAF/MEME/7904 CBEBR	SAF/MEME/7904	SAF/MEME/2181-2184

DSO Signature: Keven G Smith — Digitally signed by Keven G Smith Date: 2024.01.18 12:22:00 Z

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: 18 Jan 2025

Review comments