The University *of* Western Ontario
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 2011
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Electronically completed forms are to be submitted to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190 or to jstanle2@uwo.ca) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety.

Please ensure that all questions are fully and clearly answered. Failure to do so will lead to the form being returned, which will cause delays in your approval and frustration for you and your colleagues on the Committee.

If you are re-submitting this form as requested by the Biohazards Subcommittee, please make modifications to the form in bold print, highlighted in yellow. Please re-submit forms electronically.

PRINCIPAL INVESTIGATOR:	**Timothy Scholl**
DEPARTMENT:	**Medical Biophysics**
ADDRESS:	**Robarts Research Institute, Rm 2241B**
PHONE NUMBER:	**519-931-5777 X20019**
EMERGENCY PHONE NUMBER(S):	**519-854-2234 (Cell)**
EMAIL:	**scholl@uwo.ca**

Location of experimental work to be carried out :

Building :	**Robarts Research Institute**	Room(s):	**RRI 0270**
Building :	**Robarts Research Institute**	Room(s):	**RRI 2276**
Building :	**Robarts Research Institute**	Room(s):	**RRI 2245**

***For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).**

FUNDING AGENCY/AGENCIES: **Cancer Imaging Network of Ontario, OICR**

GRANT TITLE(S): **Molecular Imaging with Hyperpolarized 13C-Enriched Pyruvate to Quantify Therapeutic Response for Glioblastoma in a Rat Model of Cancer**

UNDERGRADUATE COURSE NAME(IF APPLICABLE):

List all personnel working under Principal Investigators supervision in this location:

Name	UWO E-mail Address	Date of Biosafety Training
Heeseung (Patrick) Lim	**hlim62@uwo.ca**	**23/11/11**
Paula Pflugfelder	**pflugfe@imaging.robarts.ca**	**Scheduled Jan 2012**

Please explain how the biological agents are used in your project and how they are stored and disposed of. The BARF without this description will not be reviewed.

This research aims to use a new form of molecular imaging with MRI to gauge the therapeutic response for Glioblastoma using a rat model of cancer. Approximately one million Rat C6 Glioma cells (CCL-107) will be surgically implanted into the caudate nucleus of Wistar rats using a stereotactic frame under isoflurane anesthesia (RRI 2276). The burr hole will be filled with bone wax and the incision sutured. The animals will receive split-dose radiotherapy using CT guidance (RRI 0270) and chemotherapy (intra-peritoneal injection of Temozolomide). The animals will then be imaged using a hyperpolarized contrast agent with magnetic resonance imaging.

The cells are contained in a plastic vial and are stored in a freezer (RRI) until thawed and cultured (RRI 2276) for surgical implantation. After the experimental endpoint, all animals will be sacrificed and incinerated. Any remaining Glioma cells will also be disposed of using incineration.

The Glioma cells will be obtained from the research group led by Ting Lee at LHRI (BIO-LHRI-0064). They culture and test these cells. They will provide an aliquot of these cells which will be placed in a plastic vial inside a plastic bag. This bag will be placed inside a styrofoam box on dry ice for transport from LHRI to RRI where the vial of cells will be transferred to a freezer for storage until cell culture.

Please include a ONE page research summary or teaching protocol in lay terms.
Forms with summaries more than one page will not be reviewed.

This research activity will employ a new form of molecular imaging to quantify response to split-dose radiotherapy with and without chemotherapy for glioma using a rat model of disease. Metabolically active, 13C-enriched endogenous compounds can be highly magnetized (hyperpolarized) in vitro and then used as injectable probes of cellular metabolism in animal models of disease. As a result of this hyperpolarization process, the magnetic resonance signal derived from these compounds is enhanced by nearly five orders of magnitude and specially optimized magnetic resonance imaging spectroscopy can be used to map the spatial and temporal distributions of these probes and their resulting metabolic compounds. In particular, hyperpolarized 13C-enriched pyruvate is an excellent probe of metabolism, yielding important information specifically about glycolysis in the cytoplasm of cells. This information can be exploited for detection of cancer and quantification of its response to therapies.

Using an established protocol, Wistar rats will be randomized into four groups. All rats will receive tumour implantation consisting of approximately one million C6 glioma cells injected into the caudate nucleus using a stereotactic frame under isoflurane anesthesia. One group (Group 1) will be an untreated control. Treatment of the remaining cohort of rats will begin approximately on day 12 when the tumour diameter reaches about 5 mm. Group 2 will receive radiotherapy (15 Gy in two fractions, administered using image guided radiotherapy) Group 3 will receive chemotherapy treatment (daily intra-peritoneal injection of 7.5 mg/kg Temozolomide over 5 days). The final group (group 4) will be treated with both radio- and chemotherapy (15 Gy in two image-guided fractions and daily 7.5 mg/kg TMZ over 5 days). Split dose radiotherapy is given in separate days to allow an MR imaging session in between to enable us to monitor response from each dose fraction by comparing with the baseline and post-treatment imaging data. We deliver a total dose of 15 Gy to the whole rat brain but in two fractions. An MR compatible fixture will used to immobilize and setup the rat brain in a reproducible position for MR imaging and for the radiation delivery in the micro-CT scanner.

Rats will be anesthetized with isoflurane for the duration of the magnetic resonance imaging experiment with hyperpolarized pyruvate. The tail vein will be catheterized and periodically flushed with heparinized saline until imaging time when it will be used as a port to introduce hyperpolarized 13C-pyruvate contrast medium. This medium will be buffered to a pH between 7 and 8 at a temperature of ~35°C. The concentration of buffered pyruvate is 100mM and the volume of each injection will be 2.5ml, which will be injected over 12s. A dual-tuned 1H – 13C RF coil system will be used for high resolution proton imaging (~100μm) followed by lower resolution 13C spectroscopic imaging of pyruvate metabolism. Each animal will be scanned weekly with this protocol until it shows signs of neurological deficit (e.g. motor abnormalities) suggesting tumour progression or a maximum of 4 weeks post-tumour implantation. Finally, the animals will be injected with Pimonidazole (a hypoxia marker) at 1 hour prior to sacrifice. Animals will be incinerated after sacrifice.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ☐ YES ☒ NO
(non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? ☐ YES ☐ NO
If YES, please give the name of the species ____________________
What is the origin of the microorganism(s)? ____________________

Please describe the risk (if any) of escape and how this will be mitigated:

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Full Scientific Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3

****Please attach a Material Safety Data Sheet or equivalent from the supplier if the bacterium used is not on this link:***
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

Additional Comments: ____________________

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ☒ YES ☐ NO
(If NO, please proceed to Section 3.0)

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	☐ Yes ☐ No		Not applicable
Rodent	☒ Yes ☐ No	**Cell cultures from the Lee Lab at LHRI**	**2010-273 (Scholl)**
Non-human primate	☐ Yes ☐ No		
Other (specify)	☐ Yes ☐ No		

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	☐ Yes ☐ No			
Rodent	☒ Yes ☐ No	**C6 Glioblastoma (ATCC# CCL-107)**	**Level 1**	**Cedarlane through Lee Lab at LHRI**
Non-human primate	☐ Yes ☐ No			
Other (specify)	☐ Yes ☐ No			

Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required ☒ 1 ☐ 2 ☐ 2+ ☐ 3

Additional Comments: **This cell line is maintained by the Lee group (Hoffman) at LHRI (BIO-LHRI-0064). It will be shared with our research group. The Lee group obtained these cells from Cedarlane Laboratory (ATCC# CCL 109)**

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? ☐ YES ☒ NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Blood (fraction) or other Body Fluid		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (unpreserved)		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3

Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

Additional Comments: _____

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? ☐ YES ☒ NO If **NO**, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? ☐ YES, complete table below ☐ NO

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?

**** Please attach a Material Safety Data Sheet or equivalent if available.***
***** Please attach a plasmid map.***
******No Material Safety Data Sheet is required for the following strains of E. coli:***
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?
☐ YES, complete table below ☐ NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction

**** Please attach a Material Safety Data Sheet or equivalent.***

4.3.1 Will virus be replication defective? ☐ YES ☐ NO

4.3.2 Will virus be infectious to humans or animals? ☐ YES ☐ NO

4.3.3 Will this be expected to increase the containment level required? ☐ YES ☐ NO

5.0 Will genetic sequences from the following be involved?

- HIV ☒ NO ☐ YES, specify
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens ☒ NO ☐ YES, specify
- SV 40 Large T antigen ☒ NO ☐ YES
- E1A oncogene ☒ NO ☐ YES
- Known oncogenes ☒ NO ☐ YES, specify
- Other human or animal pathogen and or their toxins ☒ NO ☐ YES, specify

5.1 Is any work being conducted with prions or prion sequences? ☒ NO ☐ YES

Additional Comments: _____

6.0 Human Gene Therapy Trials

6.1 Will human clinical trials be conducted involving a biological agent? ☐ YES ☒ NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 7.0

6.2 If YES, please specify which biological agent will be used:
Please attach a full description of the biological agent.

6.3 Will the biological agent be able to replicate in the host? ☐ YES ☐ NO

6.4 How will the biological agent be administered?

6.5 Please give the Health Care Facility where the clinical trial will be conducted:

6.6 Has human ethics approval been obtained? ☐ YES, number: ☐ NO ☐ PENDING

7.0 Animal Experiments

7.1 Will live animals be used? ☒ YES ☐ NO If **NO**, please proceed to section 8.0

7.2 Name of animal species to be used **Rat**

7.3 AUS protocol # **2010-273**

7.4 List the location(s) for the animal experimentation and housing. **HSACF, RRI 0270, RRI 2276, RRI 2245**

7.5 Will any of the agents listed in section 4.0 be used in live animals
☒ NO ☐ YES, specify:

7.6 Will the agent(s) be shed by the animal:
☐ YES ☒ NO, please justify: **Cells are implanted into the rat brain and cannot be shed after surgery.**

8.0 Use of Animal species with Zoonotic Hazards

8.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? ☐ YES ☒ NO - If **NO**, please proceed to section 9.0

8.2 Will live animals be used? ☐ YES ☐ NO

8.3 If **YES**, please specify the animal(s) used:

♦ Pound source dogs	☐ YES	☐ NO
♦ Pound source cats	☐ YES	☐ NO
♦ Cattle, sheep or goats	☐ YES, species	☐ NO
♦ Non-human primates	☐ YES, species	☐ NO
♦ Wild caught animals	☐ YES, species & colony #	☐ NO
♦ Birds	☐ YES, species	☐ NO
♦ Others (wild or domestic)	☐ YES, specify	☐ NO

8.4 If no live animals are used, please specify the source of the specimens:

9.0 Biological Toxins and Hormones

9.1 Will toxins or hormones of biological origin be used? ☒ YES ☐ NO If **NO**, please proceed to Section 10.0

9.2 If YES, please name the toxin(s) or hormones(s) **Temozolomide**
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

9.3 What is the LD_{50} (specify species) of the toxin or hormone **Oral (Rat) 1937 mg/kg, IP (Rat) 1414 mg/kg**

9.4 How much of the toxin or hormone is handled at one time*? **100 mg**

9.5 How much of the toxin or hormone is stored*? **200 mg**

9.6 Will any biological toxins or hormones be used in live animals? ☒ YES ☐ NO
If **YES**, Please provide details: **Temozolomide will be used for chemotherapy for rat gliomas in conjunction with radiotherapy. Dosage: 7.5 mg/kg of Temozolomide is dissolved in 1 ml/kg of DMSO (dimethyl sulfoxide) for i.p. injection Ex: 200 g rat: 1.5 mg TMZ in 0.2 ml DMSO**

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

Additional Comments: **Temozolomide is used as a chemotherapeutic agent for glioma. This therapy will be repeated daily for 5 consecutive days. Agent will be kept under locked storage and used in approved lab.**

10.0 Insects

10.1 Do you use insects? ☐ YES ☒ NO - If **NO**, please proceed to Section 11.0

10.2 If YES, please give the name of the species.

10.3 What is the origin of the insect?

10.4 What is the life stage of the insect?

10.5 What is your intention? ☐ Initiate and maintain colony, give location:
☐ "One-time" use, give location:

10.6 Please describe the risk (if any) of escape and how this will be mitigated:

10.7 Do you use insects that require a permit from the CFIA permit? ☐ YES ☐ NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

11.0 Plants

11.1 Do you use plants? ☐ YES ☒ NO - If **NO**, please proceed to Section 12.0

11.2 If YES, please give the name of the species.

11.3 What is the origin of the plant?

11.4 What is the form of the plant (seed, seedling, plant, tree...)?

11.5 What is your intention? ☐ Grow and maintain a crop ☐ "One-time" use

11.6 Do you do any modifications to the plant? ☐ YES ☐ NO
If yes, please describe:

11.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:

11.8 Is the CFIA permit attached? ☐ YES ☐ NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

12.0 Import Requirements

12.1 Will any of the above agents be imported? ☐ YES, country of origin ☒ NO
If **NO**, please proceed to Section 13.0

12.2 Has an Import Permit been obtained from HC for human pathogens? ☐ YES ☐ NO

12.3 Has an import permit been obtained from CFIA for animal or plant pathogens? ☐ YES ☐ NO

12.4 Has the import permit been sent to OHS? ☐ YES, please provide permit # ☐ NO

13.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biological agents in Sections 1.0 to 9.0 have been trained.

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name Timothy J. Scholl ***Date:*** January 9, 2012

14.0 Containment Levels

14.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. ☒ 1 ☐ 2 ☐ 2+ ☐ 3

14.2 Has the facility been certified by OHS for this level of containment?
- ☒ YES, location and date of most recent biosafety inspection:
- ☐ NO, please certify
- ☐ NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants):

15.0 Procedures to be Followed

15.1 Are additional risk reduction measures necessary beyond containment level 1, 2, 2+ or 3 measures that are unique to these agents? ☐ YES ☒ NO

If **YES** please describe:

15.2 Please outline what will be done if there is an exposure to the biological agents listed such as a needlestick injury or an accidental splash:

This research involves a Rat Glioma cell line which is not a biological threat to humans. In the event of an accidental splash, the liquid containing these cells will be washed off with soap and water. In the case of an needlestick injury, the wound will be expressed to bleed, washed with soap and water and bandaged. The individual will be directed to go to UWO staff health.

15.3 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.shs.uwo.ca/workplace/workplacehealth.html

An X in the check box indicates you agree with the above statement... ☒

Enter Your Name Timothy J. Scholl ***Date:*** January 9, 2012

15.4 Additional Comments: **On the day of transport the Glioblastoma cells are frozen in a plastic vile and placed in a bag in dry ice in a styrofoam box. Box will be transferred from LHRI to RRI and contents placed in freezer.**

16.0 Approvals

1) UWO Biohazards Subcommittee:
SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: Ronald Worthington
Date: January 11, 2012

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

Cell Biology

ATCC® Number: CCL-107™ Order this Item Price: $279.00

Designations:	C6
Depositors:	G Sato
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Rattus norvegicus* deposited as *Rattus* sp.
Morphology:	fibroblast
Source:	**Organ:** brain **Disease:** glioma **Cell Type:** glial cell;
Cellular Products:	S-100 protein; produce glyceryl phosphate dehydrogenase in response to glucocorticoids; somatotrophin
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host
Receptors:	glucocorticoid
Virus Resistance:	poliovirus 3
Cytogenetic Analysis:	Stemline number is diploid. Karyotype is stable within the stemline number and is that of a normal male. Three cells with breaks; one with a secondary constriction, one with a dicentric, one with a rearrangement and four with terminal or centromere associations.
Comments:	The glial cell strain, C6, was cloned from a rat glial tumor induced by N-nitrosomethylurea by Benda et al. after a series of alternate culture and animal passages [PubMed: 4873531]. S-100 production increases ten fold as cells grow from low density to confluency.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 2.5%; horse serum to a final concentration of 15%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C

Related Links

NCBI Entrez Search
Make a Deposit
Frequently Asked Questions
Material Transfer Agreement
Technical Support
Related Cell Culture Products

Login Required

Product Information Sheet

BioProducts

Cell, microbial and molecular genomics products for the life sciences

BioServices

Bio-materials management; basic repository to complex partnership-level services

BioStandards

Biological Reference Material and Consensus Standards for the life science community

Protocol:

Subculturing:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37C.

Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:3 is recommended
Medium Renewal: 2 to 3 times per week

Preservation:

Freeze medium: culture medium, 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2004
recommended serum:ATCC 30-2020
recommended serum:ATCC 30-2040
0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101
Cell culture tested DMSO:ATCC 4-X

References:

1022: Benda P, et al. Differentiated rat glial cell strain in tissue culture. Science 161: 370-371, 1968. PubMed: 4873531
25965: Lightbody JJ, et al. Establishment of differentiated clonal strains of glial brain cells in culture. Fed. Proc. 27: 720, 1968.
32720: Chen Y, et al. Demonstration of binding of dengue virus envelope protein to target cells. J. Virol. 70: 8765-8772, 1996. PubMed: 8971005

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Material Safety Data Sheet

Version 3.5
Revision Date 11/05/2011
Print Date 01/11/2012

1. PRODUCT AND COMPANY IDENTIFICATION

Product name	:	Temozolomide
Product Number	:	76899
Brand	:	Fluka
Product Use	:	For laboratory research purposes.

Supplier	:	Sigma-Aldrich Canada, Ltd 2149 Winston Park Drive OAKVILLE ON L6H 6J8 CANADA	Manufacturer	: Sigma-Aldrich Corporation 3050 Spruce St. St. Louis, Missouri 63103 USA
Telephone	:	+1 9058299500		
Fax	:	+1 9058299292		
Emergency Phone # (For both supplier and manufacturer)	:	1-800-424-9300		
Preparation Information	:	Sigma-Aldrich Corporation Product Safety - Americas Region 1-800-521-8956		

2. HAZARDS IDENTIFICATION

Emergency Overview

WHMIS Classification

D1B	Toxic Material Causing Immediate and Serious Toxic Effects	Toxic by ingestion
D2A		Teratogen
D2B		Carcinogen
		Moderate skin irritant
		Moderate eye irritant
		Mutagen

GHS Classification

Acute toxicity, Oral (Category 4)
Skin irritation (Category 2)
Eye irritation (Category 2A)
Germ cell mutagenicity (Category 1B)
Carcinogenicity (Category 1B)
Reproductive toxicity (Category 1B)
Specific target organ toxicity - single exposure (Category 3)

GHS Label elements, including precautionary statements

Pictogram

Signal word: Danger

Hazard statement(s)

H302	Harmful if swallowed.
H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.
H340	May cause genetic defects.
H350	May cause cancer.
H360	May damage fertility or the unborn child.

Precautionary statement(s)

P201	Obtain special instructions before use.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P308 + P313	IF exposed or concerned: Get medical advice/ attention.

HMIS Classification

Health hazard:	2
Chronic Health Hazard:	*
Flammability:	0
Physical hazards:	0

Potential Health Effects

Inhalation	May be harmful if inhaled. Causes respiratory tract irritation.
Skin	May be harmful if absorbed through skin. Causes skin irritation.
Eyes	Causes eye irritation.
Ingestion	Toxic if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : 4-Methyl-5-oxo-2,3,4,6,8-pentazabicyclo[4.3.0]nona-2,7,9-triene-9-carboxamide
8-Carbamoyl-3-methylimidazo[5,1-d]-1,2,3,5-tetrazin-4(3*H*)-one
3-Methyl-4-oxo-8-imidazolo[5,1-d][1,2,3,5]tetrazinecarboxamide

Formula : $C_6H_6N_6O_2$

Molecular Weight : 194.15 g/mol

CAS-No.	EC-No.	Index-No.	Concentration
Temozolomide			
85622-93-1	-	-	-

4. FIRST AID MEASURES

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.

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

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

5. FIREFIGHTING MEASURES

Conditions of flammability
Not flammable or combustible.

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

Special protective equipment for firefighters
Wear self contained breathing apparatus for fire fighting if necessary.

Hazardous combustion products
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx)

Explosion data - sensitivity to mechanical impact
no data available

Explosion data - sensitivity to static discharge
no data available

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Use personal protective equipment. Avoid dust formation. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Avoid breathing dust.

Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

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

7. HANDLING AND STORAGE

Precautions for safe handling
Avoid contact with skin and eyes. Avoid formation of dust and aerosols.
Provide appropriate exhaust ventilation at places where dust is formed.

Conditions for safe storage
Keep container tightly closed in a dry and well-ventilated place.

Recommended storage temperature: 2 - 8 °C

Keep in a dry place.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N100 (US) or type P3 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

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

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

Skin and body protection
Complete suit protecting against chemicals, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

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

Specific engineering controls
Use mechanical exhaust or laboratory fumehood to avoid exposure.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	powder
Colour	off-white

Safety data

pH	no data available
Melting point/freezing point	no data available
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Autoignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Vapour pressure	no data available
Density	no data available
Water solubility	no data available
Partition coefficient: n-octanol/water	log Pow: -1.283
Relative vapour density	no data available
Odour	no data available
Odour Threshold	no data available
Evaporation rate	no data available

10. STABILITY AND REACTIVITY

Chemical stability
Stable under recommended storage conditions.

Possibility of hazardous reactions
no data available

Conditions to avoid
no data available

Materials to avoid
Strong oxidizing agents

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx)
Other decomposition products - no data available

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Oral LD50
no data available

Inhalation LC50
Dermal LD50
no data available

Other information on acute toxicity
no data available

Skin corrosion/irritation
no data available

Serious eye damage/eye irritation

no data available

Respiratory or skin sensitization
Prolonged or repeated exposure may cause allergic reactions in certain sensitive individuals. The preceding data, or interpretation of data, was determined using Quantitative Structure Activity Relationship (QSAR) modeling.

Germ cell mutagenicity
In vivo tests showed mutagenic effects

Genotoxicity in vitro - mouse - leukocyte
DNA damage

Genotoxicity in vitro - Human - leukocyte
DNA damage

Carcinogenicity

Possible human carcinogen

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.

Reproductive toxicity

no data available

Teratogenicity

Presumed human reproductive toxicant

Specific target organ toxicity - single exposure (Globally Harmonized System)
Inhalation - May cause respiratory irritation.

Specific target organ toxicity - repeated exposure (Globally Harmonized System)
no data available

Aspiration hazard
no data available

Potential health effects

Inhalation	May be harmful if inhaled. Causes respiratory tract irritation.
Ingestion	Toxic if swallowed.
Skin	May be harmful if absorbed through skin. Causes skin irritation.
Eyes	Causes eye irritation.

Synergistic effects
no data available

Additional Information
RTECS: NJ5927050

12. ECOLOGICAL INFORMATION

Toxicity

no data available

Persistence and degradability
no data available

Bioaccumulative potential
no data available

Mobility in soil
no data available

PBT and vPvB assessment
no data available

Other adverse effects

no data available

13. DISPOSAL CONSIDERATIONS

Product
Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
Not dangerous goods

IMDG
Not dangerous goods

IATA
Not dangerous goods

15. REGULATORY INFORMATION

WHMIS Classification

D1B	Toxic Material Causing Immediate and	Toxic by ingestion
D2A	Serious Toxic Effects	Teratogen
D2B		Carcinogen
		Moderate skin irritant
		Moderate eye irritant
		Mutagen

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all the information required by the Controlled Products Regulations.

16. OTHER INFORMATION

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Co., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Temozolomide

sc-203292

Material Safety Data Sheet

The Power to Question

Hazard Alert Code Key:	EXTREME	HIGH	MODERATE	LOW

Section 1 - CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

PRODUCT NAME
Temozolomide

STATEMENT OF HAZARDOUS NATURE
CONSIDERED A HAZARDOUS SUBSTANCE ACCORDING TO OSHA 29 CFR 1910.1200.

NFPA

SUPPLIER
Company: Santa Cruz Biotechnology, Inc.
Address:
2145 Delaware Ave
Santa Cruz, CA 95060
Telephone: 800.457.3801 or 831.457.3800
Emergency Tel: CHEMWATCH: From within the US and Canada: 877-715-9305
Emergency Tel: From outside the US and Canada: +800 2436 2255 (1-800-CHEMCALL) or call +613 9573 3112

PRODUCT USE
Antneopastic/ cytotoxic. Pro-drug of th alkylating agent MTIC Medicine

SYNONYMS
C6-H6-N6-O2, "8-carbamoyl-3-methylimidazo[5, 1-d]-1, 2, 3, 5-tetrazi", "8-carbamoyl-3-methylimidazo[5, 1-d]-1, 2, 3, 5-tetrazi", n-4(3H)-one, n-4(3H)-one, CCRG-8104, "3, 4-dihydro-3-methyl-4-oxoimidazo[5, 1-d]-1, 2, 3, 5-t", "3, 4-dihydro-3-methyl-4-oxoimidazo[5, 1-d]-1, 2, 3, 5-t", etrazine-8-carboxamide, etrazine-8-carboxamide, MB-39831, "M&B 39831", methazolastone, "3-methyl-4-oxo-3, 4-dihydroimidazo[5, 1-d](1, 2, 3, 5)tetrazine-8-", carboxamide, "3-methyl-4-oxo-3, 4-dihydroimidazo[5, 1-d](1, 2, 3, 5)tetrazine-8-", carboxamide, NSC-362856, Temador, Temodal, "antitumour imidazoltetrazine", "antineoplastic/ cytotoxic"

Section 2 - HAZARDS IDENTIFICATION

CANADIAN WHMIS SYMBOLS

EMERGENCY OVERVIEW
RISK
Risk of explosion by shock, friction, fire or other sources of ignition.
Harmful if swallowed.
May cause CANCER.
May impair fertility.
May cause harm to the unborn child.
Irritating to eyes and skin.

ACUTE HEALTH EFFECTS

SWALLOWED

■ Accidental ingestion of the material may be harmful; animal experiments indicate that ingestion of less than 150 gram may be fatal or may produce serious damage to the health of the individual.
■ The killing action of antineoplastic drugs used for cancer chemotherapy is not selective for cancerous cells alone but affect all dividing cells. Acute side effects include loss of appetite, nausea and vomiting, allergic reaction (skin rash, itch, redness, low blood pressure, unwellness and anaphylactic shock) and local irritation. Gout and renal failure can occur.

EYE

■ This material can cause eye irritation and damage in some persons.

SKIN

■ This material can cause inflammation of the skin oncontact in some persons.
■ The material may accentuate any pre-existing dermatitis condition.
■ Skin contact is not thought to produce harmful health effects (as classified using animal models). Systemic harm, however, has been identified following exposure of animals by at least one other route and the material may still produce health damage following entry through wounds, lesions or abrasions. Good hygiene practice requires that exposure be kept to a minimum and that suitable gloves be used in an occupational setting.
■ Entry into the blood-stream, through, for example, cuts, abrasions or lesions, may produce systemic injury with harmful effects. Examine the skin prior to the use of the material and ensure that any external damage is suitably protected.

INHALED

■ The material is not thought to produce either adverse health effects or irritation of the respiratory tract following inhalation (as classified using animal models). Nevertheless, adverse effects have been produced following exposure of animals by at least one other route and good hygiene practice requires that exposure be kept to a minimum and that suitable control measures be used in an occupational setting.

CHRONIC HEALTH EFFECTS

■ There is ample evidence that this material can be regarded as being able to cause cancer in humans based on experiments and other information.
Based on experiments and other information, there is ample evidence to presume that exposure to this material can cause genetic defects that can be inherited.
Ample evidence exists from experimentation that reduced human fertility is directly caused by exposure to the material.
Ample evidence exists, from results in experimentation, that developmental disorders are directly caused by human exposure to the material.
Anti-cancer drugs used for chemotherapy can depress the bone marrow with reduction in the number of white blood cells and platelets and bleeding. Susceptibility to infections and bleeding is increased, which can be life- threatening. Digestive system effects may include inflammation of the mouth cavity, mouth ulcers, esophagus inflammation, abdominal pain and bleeds, diarrhea, bowel ulcers and perforation. Reversible hair loss can result and wound healing may be delayed. Long-term effects on the gonads may cause periods to stop and inhibit sperm production. Most anti-cancer drugs can potentially cause mutations and birth defects, and coupled with the effects of the suppression of the immune system, may also cause cancer.
Long term exposure to high dust concentrations may cause changes in lung function i.e. pneumoconiosis; caused by particles less than 0.5 micron penetrating and remaining in the lung. Prime symptom is breathlessness; lung shadows show on X-ray.
Sub-Chronic (Target Organ Effects)
Multi-cycle studies were conducted in rats and dogs. Each cycle consisted of five consecutive daily doses followed by a 23-day non-treatment period. Dosage levels ranged from 25 mg/m3 to a high dose of 10000 mg/m3. Target organs identified during these studies including the blood forming
systems, lymphoreticular, alimentary, male reproductive systems, and the mammary glands in females. The blood forming lymophoreticular and alimentary systems recovered after cessation of dosing. Oncogenicity studies have not been conducted. However the results of the six-day
cycle in rats can be used to evaluate the carcinogenic potential. In this study, mammary tumours occurred within a relatively short time span at all dose levels (25, 125, 200 mg/m2). Considering that temozolomide is a prodrug of an alkylating agent MTIC, its carcinogenic potential is not unexpected.
Teratogenicity (Birth Defects):
Testing fro reproductive toxicity was performed in dose ranging studies with rats and rabbits and a developmental study in rats. At the higher dose levels, the percentage of viable and live foetuses decreased. Resorption and post implantation losses were also increased in high dose groups in both species. Temozolomide, like other alkylating agents, has the potential to produce embryo
lethality and malformation in rats and rabbits.
Reproductive effects:
Contraindicated for use during pregnancy. Woman of child-bearing potential should be advised to avoid pregnancy while they are receiving treatment and for six months after discontinuation of therapy. It is not known whether the drug is excreted in human milk thus it should not be used by nursing women.
Testicular toxicity has been observed in multiple-cycle tests in dogs and rats. The reversibility of the testicular changes has not been assessed. Reduced absolute testis weights occurred in rats and dogs. These effects on the testes suggest a strong possibility for additional potential reproductive effects.
Human Experience:
Temozolomide has been well characterised and possesses an acceptable clinical safety profile as demonstrated in 1017 patients with malignant glioma, melanoma, or other advanced cancers. The majority of patients received Temodal, once daily for five days, repeating every 28 days up to the high dose of 200 mg/m2/day. The most common treatment related adverse effects include nausea (50%), vomiting (42%), headache (37%), fatigue (30%) and constipation (28%). Nausea and vomiting were usually mild to moderate in severity and were resolved spontaneously or were controlled readily with standard antiemetics.
Haematological toxicities were reported as adverse effects in all Phase II and the majority of all Phase I studies only if it lead to transfusion, hospitalisation, or discontinuation of treatment. Haematological adverse effects include thrombocytopenia (9%), anaemia (7%), neutropenia (4%) and leukopenia (2%).
Dose limiting toxicity (DLT) was haematologic, consisting of decreased platelets and neutrophils and to a lesser extent, haemoglobin.. The DLT was 1000 mg/m2 as a single dose.
Out of 400 glioma patients treated with Temodal, 11 discontinued treatment due to adverse events possibly or definitely related to treatment. Two deaths were also judged to related to the treatment.
In a melanoma treatment study with 151 patients, five discontinued treatment due to an adverse effect judged related or possibly related to treatment.. Three patients died as a result of an adverse effect not clearly related to disease progression or complications.
Medical Conditions Aggravated by Exposure.
Temodal is contraindicated in patients who have a history of hypersensitivity reactions to its components. Use cautiously in

vomiting or partial bowel obstruction. Contraindicated for use during pregnancy. There is no clinical experience with use of Temodal in children under the age of 3 years. Elderly patients (>70 years
of age) appear to be at increased risk of neutropenia and thrombocytopenia, compared to younger patients

Section 3 - COMPOSITION / INFORMATION ON INGREDIENTS

HAZARD RATINGS

		Min	Max
Flammability:	1		
Toxicity:	2		
Body Contact:	2		
Reactivity:	3		
Chronic:	3		

Min/Nil=0
Low=1
Moderate=2
High=3
Extreme=4

NAME	CAS RN	%
temozolomide	85622-93-1	>98

Section 4 - FIRST AID MEASURES

SWALLOWED

■
- IF SWALLOWED, REFER FOR MEDICAL ATTENTION, WHERE POSSIBLE, WITHOUT DELAY.
- Where Medical attention is not immediately available or where the patient is more than 15 minutes from a hospital or unless instructed otherwise:
- For advice, contact a Poisons Information Center or a doctor.
- Urgent hospital treatment is likely to be needed.
- If conscious, give water to drink.
- INDUCE vomiting with fingers down the back of the throat, ONLY IF CONSCIOUS. Lean patient forward or place on left side (head-down position, if possible) to maintain open airway and prevent aspiration.

NOTE: Wear a protective glove when inducing vomiting by mechanical means.
- In the mean time, qualified first-aid personnel should treat the patient following observation and employing supportive measures as indicated by the patient's condition.
- If the services of a medical officer or medical doctor are readily available, the patient should be placed in his/her care and a copy of the MSDS should be provided. Further action will be the responsibility of the medical specialist.
- If medical attention is not available on the worksite or surroundings send the patient to a hospital together with a copy of the MSDS.

EYE

■ If this product comes in contact with the eyes:
- Wash out immediately with fresh running water.
- Ensure complete irrigation of the eye by keeping eyelids apart and away from eye and moving the eyelids by occasionally lifting the upper and lower lids.
- If pain persists or recurs seek medical attention.
- Removal of contact lenses after an eye injury should only be undertaken by skilled personnel.

SKIN

■ If skin contact occurs:
- Immediately remove all contaminated clothing, including footwear
- Flush skin and hair with running water (and soap if available).
- Seek medical attention in event of irritation.

INHALED

■
- If fumes or combustion products are inhaled remove from contaminated area.
- Other measures are usually unnecessary.

NOTES TO PHYSICIAN

■ For employees potentially exposed to antineoplastic and/ or cytotoxic agents on a regular basis, a preplacement physical examination and history (noting risk factors) is recommended. Periodic follow-up examinations should also be undertaken and should be overseen by a physician familiar with the toxic effects of the substance and full details of the nature of work undertaken by the employee.Following administration of antineoplastics, control of nausea and vomiting may be attempted by giving phenothiazines such as perphenazine, prochlorperazine, promethazine or thiethylperazine before antineoplastic agents are administered. In bone-marrow depression, transfusion of blood or platelets reduces the risk of life-threatening hemorrhage. Granulocyte transfusions and injection of antibiotics may be necessary to combat infection in the neutropenic patient. Hyperuricemia is avoided by the addition of allopurinol to treatment schedules and measures such as alkalization of the urine and hydration may be adopted. MARTINDALE: The Extra Pharmacopoeia, 28th Edition.

Section 5 - FIRE FIGHTING MEASURES

Vapour Pressure (mmHG):	Negligible
Upper Explosive Limit (%):	Not Available
Specific Gravity (water=1):	Not Available

Lower Explosive Limit (%): Not Available

EXTINGUISHING MEDIA

- Foam.
- Dry chemical powder.
- BCF (where regulations permit).
- Carbon dioxide.
- Water spray or fog - Large fires only.

FIRE FIGHTING

- Alert Emergency Responders and tell them location and nature of hazard.
- Wear breathing apparatus plus protective gloves.
- Prevent, by any means available, spillage from entering drains or water course.
- Use water delivered as a fine spray to control fire and cool adjacent area.
- DO NOT approach containers suspected to be hot.
- Cool fire exposed containers with water spray from a protected location.
- If safe to do so, remove containers from path of fire.
- Equipment should be thoroughly decontaminated after use.

GENERAL FIRE HAZARDS/HAZARDOUS COMBUSTIBLE PRODUCTS

- Combustible solid which burns but propagates flame with difficulty.
- Avoid generating dust, particularly clouds of dust in a confined or unventilated space as dusts may form an explosive mixture with air, and any source of ignition, i.e. flame or spark, will cause fire or explosion. Dust clouds generated by the fine grinding of the solid are a particular hazard; accumulations of fine dust may burn rapidly and fiercely if ignited.
- Dry dust can be charged electrostatically by turbulence, pneumatic transport, pouring, in exhaust ducts and during transport.
- Build-up of electrostatic charge may be prevented by bonding and grounding.
- Powder handling equipment such as dust collectors, dryers and mills may require additional protection measures such as explosion venting.

Combustion products include: carbon monoxide (CO), carbon dioxide (CO_2), nitrogen oxides (NOx), other pyrolysis products typical of burning organic material.
May emit poisonous fumes.
May emit corrosive fumes.
Assess operations based upon available dust explosion information to determine the suitability of preventative or protective systems as precautionary measures against possible dust explosions. If prevention is not possible, consider protection by use of containment, venting or suppression of dust handling equipment. Where explosion venting is considered to be the most appropriate method of protection, vent areas should preferably be calculated based on Kst rather than an St value. If nitrogen purging is considered as the protective system, it must operate with an oxygen level below the limiting oxygen concentration. The system should include an oxygen monitoring and shut-down facility in the event of excessive oxygen being detected.
The maximum surface temperature of enclosures potentially exposed to this material should be based on values obtained by taking 2/3 of the minimum ignition temperature (MIE) of the dust cloud. The effect of dust layers should be reviewed.
An isolated (insulated) human body can readily produce electrostatic discharges in excess of 50 mJ, but have been recorded up to 100 mJ.
Dust Explosion Hazard Class 3
Dusts fall into one of three Kst* classes. Class 1 dusts; Kst 1-200 m3/sec; Class 2 dusts; 201-299 m3/sec. Class 3 dusts; Kst 300 or more. Most agricultural dusts (grains, flour etc.) are Class 1; pharmaceuticals and other speciality chemicals are typically Class 1 or 2; most unoxidized metallic dusts are Class 3. The higher the Kst, the more energetically the dust will burn and the greater is the explosion risk.* Kst - a normalized expression of the burning dust pressure rise rate over time.

FIRE INCOMPATIBILITY

■ Avoid contamination with oxidizing agents i.e. nitrates, oxidizing acids,chlorine bleaches, pool chlorine etc. as ignition may result.

PERSONAL PROTECTION

Glasses:
Gloves:
Respirator:
Particulate

Section 6 - ACCIDENTAL RELEASE MEASURES

MINOR SPILLS

- Clean up waste regularly and abnormal spills immediately.
- Avoid breathing dust and contact with skin and eyes.
- Wear protective clothing, gloves, safety glasses and dust respirator.
- Use dry clean up procedures and avoid generating dust.
- Vacuum up or sweep up. NOTE: Vacuum cleaner must be fitted with an exhaust micro filter (HEPA type) (consider explosion-proof machines designed to be grounded during storage and use).
- Dampen with water to prevent dusting before sweeping.
- Place in suitable containers for disposal.

It is recommended that areas handling final finished product have cytotoxic spill kits available.
Spill kits should include:

- impermeable body covering,
- shoe covers,
- latex and utility latex gloves,
- goggles,
- approved HEPA respirator,
- disposable dust pan and scoop,
- absorbent towels,

- disposable sponges,
- sharps container,
- disposable garbage bag and
- hazardous waste label

To avoid accidental exposure due to waste handling of cytotoxics:

- Place waste residue in a segregated sealed plastic container.
- Used syringes, needles and sharps should not be crushed, clipped, recapped, but placed directly into an approved sharps container.
- Dispose of any cleanup materials and waste residue according to all applicable laws and regulations e.g, secure chemical landfill disposal.

All personnel likely to involved in a antineoplastic (cytotoxic) spill must receive practical training in:

- the correct procedures for handling cytotoxic drugs or waste in order to prevent and minimize the risk of spills
- the location of the skill kit in the area
- the arrangements for medical treatment of any affected personnel
- the procedure for containment of the spill, and decontamination of personnel and the environment, including the different procedures for major and minor spills
- the procedure for waste disposal according to the nature and extent of the spill

MAJOR SPILLS

■

- Clear area of personnel and move upwind.
- Alert Emergency Responders and tell them location and nature of hazard.
- Wear full body protective clothing with breathing apparatus.
- Prevent, by all means available, spillage from entering drains or water courses.
- Consider evacuation (or protect in place).
- No smoking, naked lights or ignition sources.
- Increase ventilation.
- Stop leak if safe to do so.
- Water spray or fog may be used to disperse / absorb vapour.
- Contain or absorb spill with sand, earth or vermiculite.
- Collect recoverable product into labelled containers for recycling.
- Collect solid residues and seal in labelled drums for disposal.
- Wash area and prevent runoff into drains.
- After clean up operations, decontaminate and launder all protective clothing and equipment before storing and re-using.
- If contamination of drains or waterways occurs, advise emergency services.

ACUTE EXPOSURE GUIDELINE LEVELS (AEGL) (in ppm)

AEGL 1: The airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic nonsensory effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.
AEGL 2: The airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape.
AEGL 3: The airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

Section 7 - HANDLING AND STORAGE

PROCEDURE FOR HANDLING

■ The National Institute of Health (USA) recommends that the preparation of injectable antineoplastic drugs should be performed in a Class II laminar flow biological safety cabinet and that personnel preparing drugs of this class should wear appropriate personal protective gear. Emphasise controls on containment.

- Avoid all personal contact, including inhalation.
- Wear protective clothing when risk of exposure occurs.
- Use in a well-ventilated area.
- Prevent concentration in hollows and sumps.
- DO NOT enter confined spaces until atmosphere has been checked.
- DO NOT allow material to contact humans, exposed food or food utensils.
- Avoid contact with incompatible materials.
- When handling, DO NOT eat, drink or smoke.
- Keep containers securely sealed when not in use.
- Avoid physical damage to containers.
- Always wash hands with soap and water after handling.
- Work clothes should be laundered separately.
- Launder contaminated clothing before re-use.
- Use good occupational work practice.
- Observe manufacturer's storing and handling recommendations.
- Atmosphere should be regularly checked against established exposure standards to ensure safe working conditions are maintained.

RECOMMENDED STORAGE METHODS

■ Glass container.

- Polyethylene or polypropylene container.
- Check all containers are clearly labelled and free from leaks.

■ Antineoplastics (cytotoxics):
- should be clearly identifiable to all personnel involved in their handling
- should be stored in impervious break-resistant containers
- should be stored in separate, clearly marked storage areas to minimize the risk of breakage, and to limit contamination in the event of leakage.

Spill kits should be available in storage areas.
- Store in original containers.
- Keep containers securely sealed.
- Store in a cool, dry, well-ventilated area.
- Store away from incompatible materials and foodstuff containers.
- Protect containers against physical damage and check regularly for leaks.
- Observe manufacturer's storing and handling recommendations.

SAFE STORAGE WITH OTHER CLASSIFIED CHEMICALS

X X + X X +

X: Must not be stored together
O: May be stored together with specific preventions
+: May be stored together

Section 8 - EXPOSURE CONTROLS / PERSONAL PROTECTION

EXPOSURE CONTROLS

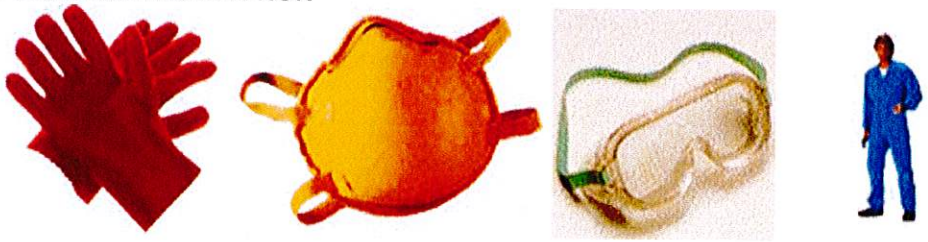
The following materials had no OELs on our records
• temozolomide: CAS:85622-93-1

MATERIAL DATA

TEMOZOLOMIDE:
■ Sensory irritants are chemicals that produce temporary and undesirable side-effects on the eyes, nose or throat. Historically occupational exposure standards for these irritants have been based on observation of workers' responses to various airborne concentrations. Present day expectations require that nearly every individual should be protected against even minor sensory irritation and exposure standards are established using uncertainty factors or safety factors of 5 to 10 or more. On occasion animal no-observable-effect-levels (NOEL) are used to determine these limits where human results are unavailable. An additional approach, typically used by the TLV committee (USA) in determining respiratory standards for this group of chemicals, has been to assign ceiling values (TLV C) to rapidly acting irritants and to assign short-term exposure limits (TLV STELs) when the weight of evidence from irritation, bioaccumulation and other endpoints combine to warrant such a limit. In contrast the MAK Commission (Germany) uses a five-category system based on intensive odour, local irritation, and elimination half-life. However this system is being replaced to be consistent with the European Union (EU) Scientific Committee for Occupational Exposure Limits (SCOEL); this is more closely allied to that of the USA.
OSHA (USA) concluded that exposure to sensory irritants can:
- cause inflammation
- cause increased susceptibility to other irritants and infectious agents
- lead to permanent injury or dysfunction
- permit greater absorption of hazardous substances and
- acclimate the worker to the irritant warning properties of these substances thus increasing the risk of overexposure.

It is the goal of the ACGIH (and other Agencies) to recommend TLVs (or their equivalent) for all substances for which there is evidence of health effects at airborne concentrations encountered in the workplace.
At this time no TLV has been established, even though this material may produce adverse health effects (as evidenced in animal experiments or clinical experience). Airborne concentrations must be maintained as low as is practically possible and occupational exposure must be kept to a minimum.
NOTE: The ACGIH occupational exposure standard for Particles Not Otherwise Specified (P.N.O.S) does NOT apply.
Airborne particulate or vapor must be kept to levels as low as is practicably achievable given access to modern engineering controls and monitoring hardware. Biologically active compounds may produce idiosyncratic effects which are entirely unpredictable on the basis of literature searches and prior clinical experience (both recent and past).
CEL TWA: 0.002 mg/m3 (cf Schering Plough OEG)

PERSONAL PROTECTION



Consult your EHS staff for recommendations

EYE

■
- Chemical protective goggles with full seal

- Contact lenses may pose a special hazard; soft contact lenses may absorb and concentrate irritants. A written policy document, describing the wearing of lens or restrictions on use, should be created for each workplace or task. This should include a review of lens absorption and adsorption for the class of chemicals in use and an account of injury experience. Medical and first-aid personnel should be trained in their removal and suitable equipment should be readily available. In the event of chemical exposure, begin eye irrigation immediately and remove contact lens as soon as practicable. Lens should be removed at the first signs of eye redness or irritation - lens should be removed in a clean environment only after workers have washed hands thoroughly. [CDC NIOSH Current Intelligence Bulletin 59]

HANDS/FEET

■ Suitability and durability of glove type is dependent on usage. Important factors in the selection of gloves include: such as:

- frequency and duration of contact,
- chemical resistance of glove material,
- glove thickness and
- dexterity

Select gloves tested to a relevant standard (e.g. Europe EN 374, US F739).

- When prolonged or frequently repeated contact may occur, a glove with a protection class of 5 or higher (breakthrough time greater than 240 minutes according to EN 374) is recommended.
- When only brief contact is expected, a glove with a protection class of 3 or higher (breakthrough time greater than 60 minutes according to EN 374) is recommended.
- Contaminated gloves should be replaced.

Gloves must only be worn on clean hands. After using gloves, hands should be washed and dried thoroughly. Application of a non-perfumed moisturiser is recommended.

- Rubber gloves (nitrile or low-protein, powder-free latex). Employees allergic to latex gloves should use nitrile gloves in preference.
- Double gloving should be considered.
- PVC gloves.
- Protective shoe covers.
- Head covering.

Experience indicates that the following polymers are suitable as glove materials for protection against undissolved, dry solids, where abrasive particles are not present.

- polychloroprene
- nitrile rubber
- butyl rubber
- fluorocaoutchouc
- polyvinyl chloride

Gloves should be examined for wear and/ or degradation constantly.

OTHER

■

- For quantities up to 500 grams a laboratory coat may be suitable.
- For quantities up to 1 kilogram a disposable laboratory coat or coverall of low permeability is recommended. Coveralls should be buttoned at collar and cuffs.
- For quantities over 1 kilogram and manufacturing operations, wear disposable coverall of low permeability and disposable shoe covers.
- For manufacturing operations, air-supplied full body suits may be required for the provision of advanced respiratory protection.
- Eye wash unit.
- Ensure there is ready access to an emergency shower.
- For Emergencies: Vinyl suit
- When handling antineoplastic materials, it is recommended that a disposal work-uniform (such as Tyvek or closed front surgical-type gown with knit cuffs) is worn.

RESPIRATOR

■

Protection Factor	Half-Face Respirator	Full-Face Respirator	Powered Air Respirator
10 x PEL	P1	-	PAPR-P1
	Air-line*	-	-
50 x PEL	Air-line**	P2	PAPR-P2
100 x PEL	-	P3	-
		Air-line*	-
100+ x PEL	-	Air-line**	PAPR-P3

* - Negative pressure demand ** - Continuous flow

Explanation of Respirator Codes:

Class 1 low to medium absorption capacity filters.

Class 2 medium absorption capacity filters.

Class 3 high absorption capacity filters.

PAPR Powered Air Purifying Respirator (positive pressure) cartridge.

Type A for use against certain organic gases and vapors.

Type AX for use against low boiling point organic compounds (less than 65°C).

Type B for use against certain inorganic gases and other acid gases and vapors.

Type E for use against sulfur dioxide and other acid gases and vapors.

Type K for use against ammonia and organic ammonia derivatives

Class P1 intended for use against mechanically generated particulates of sizes most commonly encountered in industry, e.g. asbestos, silica.

Class P2 intended for use against both mechanically and thermally generated particulates, e.g. metal fume.

Class P3 intended for use against all particulates containing highly toxic materials, e.g. beryllium.

The local concentration of material, quantity and conditions of use determine the type of personal protective equipment required.

Use appropriate NIOSH-certified respirator based on informed professional judgement. In conditions where no reasonable estimate of exposure can be made, assume the exposure is in a concentration IDLH and use NIOSH-certified full face pressure demand SCBA with a minimum service life of 30 minutes, or a combination full facepiece pressure demand SAR with auxiliary self-contained air supply. Respirators provided only for escape from IDLH atmospheres shall be NIOSH-certified for escape from the atmosphere in which they will be used.

■

- Employees exposed to confirmed human carcinogens should be authorized to do so by the employer, and work in a regulated area.
- Work should be undertaken in an isolated system such as a "glove-box" . Employees should wash their hands and arms upon completion of the assigned task and before engaging in other activities not associated with the isolated system.
- Within regulated areas, the carcinogen should be stored in sealed containers, or enclosed in a closed system, including piping systems, with any sample ports or openings closed while the carcinogens are contained within.
- Open-vessel systems are prohibited.
- Each operation should be provided with continuous local exhaust ventilation so that air movement is always from ordinary work areas to the operation.
- Exhaust air should not be discharged to regulated areas, non-regulated areas or the external environment unless decontaminated. Clean make-up air should be introduced in sufficient volume to maintain correct operation of the local exhaust system.
- For maintenance and decontamination activities, authorized employees entering the area should be provided with and required to wear clean, impervious garments, including gloves, boots and continuous-air supplied hood. Prior to removing protective garments the employee should undergo decontamination and be required to shower upon removal of the garments and hood.
- Except for outdoor systems, regulated areas should be maintained under negative pressure (with respect to non-regulated areas).
- Local exhaust ventilation requires make-up air be supplied in equal volumes to replaced air.
- Laboratory hoods must be designed and maintained so as to draw air inward at an average linear face velocity of 150 feet/ min. with a minimum of 125 feet/ min. Design and construction of the fume hood requires that insertion of any portion of the employees body, other than hands and arms, be disallowed.

Section 9 - PHYSICAL AND CHEMICAL PROPERTIES

PHYSICAL PROPERTIES

State	Divided Solid	Molecular Weight	194.15
Melting Range (°F)	413.6 (decomposes)	Viscosity	Not Applicable
Boiling Range (°F)	Not Applicable	Solubility in water (g/L)	Partly Miscible
Flash Point (°F)	>199.4	pH (1% solution)	Not Applicable
Decomposition Temp (°F)	413.6	pH (as supplied)	Not Applicable
Autoignition Temp (°F)	Not Available	Vapour Pressure (mmHG)	Negligible
Upper Explosive Limit (%)	Not Available	Specific Gravity (water=1)	Not Available
Lower Explosive Limit (%)	Not Available	Relative Vapor Density (air=1)	Not Applicable
Volatile Component (%vol)	Negligible	Evaporation Rate	Not Applicable

APPEARANCE

White crystalline solid; does not mix well with water. Flammability Color Physical State Odor Miscibility with water - White Solid Crystalline Partly Miscible

Section 10 - CHEMICAL STABILITY

CONDITIONS CONTRIBUTING TO INSTABILITY

■

- Presence of incompatible materials.
- Product is considered stable.
- Hazardous polymerization will not occur.

STORAGE INCOMPATIBILITY

■

- Avoid strong acids, bases.

Avoid reaction with oxidizing agents.

For incompatible materials - refer to Section 7 - Handling and Storage.

Section 11 - TOXICOLOGICAL INFORMATION

temozolomide

TOXICITY AND IRRITATION

■ unless otherwise specified data extracted from RTECS - Register of Toxic Effects of Chemical Substances.

TOXICITY	IRRITATION
Oral (Rat) LD50: 1937 mg/kg *	
Intraperitoneal (Rat) LD50: 1414 mg/kg *	
Oral (Mouse) (male:) LD50 891 mg/kg	
Oral (Mouse) (female:) LD50 1072 mg/kg *	

Tumourigenic agent, nausea, vomiting, leukopenia, thrombocytopenia, aplastic anaemia, granulocytopenia, diarrhoea recorded

closed patch technique.
* Schering Plough MSDS

Section 12 - ECOLOGICAL INFORMATION

Refer to data for ingredients, which follows:
TEMOZOLOMIDE:
■ DO NOT discharge into sewer or waterways.

Ecotoxicity

Ingredient	Persistence: Water/Soil	Persistence: Air	Bioaccumulation	Mobility
temozolomide	HIGH		LOW	HIGH

Section 13 - DISPOSAL CONSIDERATIONS

Disposal Instructions

All waste must be handled in accordance with local, state and federal regulations.
¦ Legislation addressing waste disposal requirements may differ by country, state and/ or territory. Each user must refer to laws operating in their area. In some areas, certain wastes must be tracked.
A Hierarchy of Controls seems to be common - the user should investigate:

- Reduction
- Reuse
- Recycling
- Disposal (if all else fails)

This material may be recycled if unused, or if it has not been contaminated so as to make it unsuitable for its intended use. Shelf life considerations should also be applied in making decisions of this type. Note that properties of a material may change in use, and recycling or reuse may not always be appropriate.
DO NOT allow wash water from cleaning equipment to enter drains. Collect all wash water for treatment before disposal.

- Antineoplastic (cytotoxic) wastes must be packed directly, ready for incineration, into color-coded, secure, labelled, leak-proof containers sufficiently robust to withstand handling without breaking, bursting or leaking.
- Containers of special design are available for particular needs (such as disposal of sharps) and should be used.
- Once filled and closed, such containers must never be re-opened.
- Immediate containers must bear a nationally accepted symbol or device depicting cytotoxic substances and be labelled with the words: CYTOTOXIC WASTE - INCINERATE in a style of lettering approved by the national/ state authority.
- Where policies and procedures permit the merging of cytotoxic wastes with medical waste in an outer container used for medical waste, cytotoxic waste must first be placed in identifiable color-coded/ labelled cytotoxic containers prior to merging.
- Management procedures must ensure that merged medical and cytotoxic waste is subjected to the incineration requirements appropriate for the total destruction of the cytotoxic waste.

WASTE STORAGE OF CYTOTOXIC WASTES For the storage of cytotoxic waste, segregated or merged with medical waste, provide:

- special storage areas with adequate lighting.
- waste security and restriction of access to authorized persons.
- storage areas designed to facilitate easy routine cleaning and maintenance to hygienic standards, or post-spill decontamination.
- storage of cytotoxic waste in standard, identifying bins or other appropriate containers.

COLLECTION OF CYTOTOXIC WASTES

- Procedures for the collection of cytotoxic wastes, which are compatible with existing operational needs, and which protect workers, other people and the environment, must be developed.
- Waste must be removed from the site by contractors whose workers have been instructed in the protective methods to be used against the hazards involved, and who comply with the safe work practices established by internal and/or national/ state policies. Contractors must instruct, train and direct their personnel in the safe and legal handling of cytotoxic wastes. Contractor's personnel should observe the operating procedures of the waste-generator.
- Transport of cytotoxic wastes, through the community, must comply with the appropriate national/ state codes.

DESTRUCTION OF CYTOTOXIC WASTES

- Destruction of cytotoxic wastes should be carried out in multi-chambered incinerators, licenced for this purpose, operating at 1100 deg. C. or more, with a residence time of at least 1 second.
- Operators must be trained in handling procedures and hazards involved with handling the waste.
- Waste which arrives at the incinerator inappropriately packaged should NOT be returned to the waste generator. An authorized representative of the waste generator must attend the incinerator site to rectify the situation.

Section 14 - TRANSPORTATION INFORMATION

NOT REGULATED FOR TRANSPORT OF DANGEROUS GOODS: DOT, IATA, IMDG

Section 15 - REGULATORY INFORMATION

No data for temozolomide (CAS: , 85622-93-1)

Section 16 - OTHER INFORMATION

Germany Hazard classification and labelling of medicines with antineoplastic effects (ATC Code L01

INN	CAS	Danger	CMR effects Cat 1&2	CMR effects Cat 3	Other
Temozolomid	85622- 93- 1	T	R 45 R 46 R 60 R 61		

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■ Classification of the mixture and its individual components has drawn on official and authoritative sources as well as independent review by the Chemwatch Classification committee using available literature references.
A list of reference resources used to assist the committee may be found at:
www.chemwatch.net/references.

■ The (M)SDS is a Hazard Communication tool and should be used to assist in the Risk Assessment. Many factors determine whether the reported Hazards are Risks in the workplace or other settings. Risks may be determined by reference to Exposures Scenarios. Scale of use, frequency of use and current or available engineering controls must be considered.

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