THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: July 9, 2010
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).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) 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

PRINCIPAL INVESTIGATOR	John Richard (Jack) Bend
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EMAIL	Jack.Bend@schulich.uwo.ca

Location of experimental work to be carried out: Building(s) _SDMRI_____ Room(s)_101; 108B_____

*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:1. CIHR; 2. Hospital for Sick Children Foundation_____
GRANT TITLE(S): 1. Modulation of gene expression and protein structure and function/activity by the antioxidant and pro-oxidant bilirubin. 2. Attenuation of oxidative stress-mediated adverse drug reactions by antioxidant constituents of TCM *in vitro* and *in vivo*

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

Name	UWO E-mail Address	Date of Biosafety Training
Lei Zhang, MD	Lei.Zhang@schulich.uwo.ca	October 29, 2008

Hepa 1c1c7 cells:

These cells are used to study various aspects of the toxicology of unconjugated bilirubin (UCB), particularly those related to oxidative stress and the activation of pro-apoptotic caspases. Changes to the disulfide proteome are evaluated by redox 2D-gel electrophoresis of cell lysate after exposure to various concentrations of UCB (5-100 µMolar).

Storage: Hepa 1c1c7 cells are stored in liquid nitrogen vapor phase in 90% Delbecco's modified minimum essential medium (without nucleosides) containing 10% fetal bovine serum, supplemented with 5% (v/v) DMSO.

Use: Hepa 1c1c7 cells are maintained in a Delbecco's modified minimal essential medium supplemented with 10% fetal bovine serum (FBS; final concentration of 40 µM), 20 µM L-glutamine, 50 µg/ml gentamycin sulfate, 100 IU/ml penicillin, 10 µg/ml streptomycin and 25 ng/ml amphotericin B. Cells are routinely grown in 75 cm^2 tissue culture flasks at 37°C in a 5% CO_2 humidified environment. For experiments, cells are seeded from the stock and cultured for 24 h before being treated with UCB.

Disposal: All liquid wastes are treated with bleach for at least 1 hour prior to disposal. All solids used during cell culture are autoclaved prior to disposal.

Jurkat, Clone E6-1 cells:

These cells are used to evaluate the cytoprotective effect of purified constituents of traditional Chinese medicines, alone and in combination, against oxidative stress caused by exposure to various concentrations of *tertiary*-butyl hydroperoxide (t-BHP) for different periods of time or to various concentrations of the toxic drug metabolite, sulfamethoxazole N-hydroxylamine, a product known to cause an adverse drug reaction in susceptible individuals. Cell damage and cell death, lipid peroxidation, protein oxidation and changes to the disulfide proteome are analyzed in cells exposed to t-BHP with and without pre-exposure to plant constituents at low µMolar concentrations.

Storage: Jurkat E6-1cells are stored in liquid nitrogen vapor phase in 90% RPMI-1640 medium, containing 10% fetal bovine serum, and supplemented with 5% (v/v) DMSO.

Use: The Jurkat E6.1 cells are maintained in a humidified atmosphere in BD Falcon™ 75 cm^2 or 150 cm^2 tissue culture flasks. Cell concentration are maintained between 1 x 10^5 and 1 x 10^6 viable cells/ml in RPMI-1640 medium modified to contain 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 4500 mg/L glucose, and 1500 mg/L sodium bicarbonate (RPMI-1640) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin G sodium and 100 µg/mL streptomycin sulphate (P/S) in a humidified atmosphere at 37°C and 5% CO. Cells are routinely treated at 1 x 10^5 cells per well in quadruplicate with final concentrations of plant in the 1-100 µM range in a flat-bottom 96-well plate for 6 or 24 h at 37°C and 5% CO_2 humidified environment.

Disposal: All liquid wastes are treated with bleach for at least 1 hour prior to disposal. All solids used during cell culture are autoclaved prior to disposal.

1. CIHR - Unconjugated Bilirubin Project

Unconjugated bilirubin (UCB) at elevated concentrations is responsible for the clinical manifestation of neonatal jaundice, which can lead to kernicterus and even death in newborn infants. The mechanism responsible for UCB toxicity is not completely understood, however, oxidative stress is known to play a role. The purpose of this project is to use a combination of cell biology, proteomic and genomic approaches to determine the mechanism(s) of toxicity in Hepa 1c1c7 cells.

We have shown that 50 μM UCB first causes elicited reversible cytosolic oxidation at 0.5 – 1 h in Hepa 1c1c7 cells followed by recovery of redox status t homeostasis and then enhanced, irreversible oxidation at later time points (>3 h). We also found that 70 nM UCB reduced intracellular redox status of these cells. UCB at 50 μM oxidizes several reactive thiol containing proteins in the cytosol (including peroxiredoxin 1 and peroxiredoxin 2 which have been identified by mass spectrometric analysis of tryptic peptides) and the mitochondria but not in the endoplasmic reticulum.

We have also employed microarray analysis to determine changes in gene regulation mediated by UCB at both pro- (50 μM) and antioxidant (70 nM) concentrations in Hepa 1c1c7 cells at 1 and 6 h. The changes observed in select genes were validated with qPCR. Using immunoblot analysis we validated these changes at the protein level for select genes and documented the activation of two proteins involved in the ER stress pathway, eIF2α and PERK. Following treatment with 50 μM UCB microarray analysis revealed the upregulation of many genes involved in ER stress (ATF3, BiP, CHOP, Dnajb1, and Herp). We demonstrate that upregulation of the pro-apoptotic transcription factor CHOP results in increased intracellular protein content. It was determined that activation of proteins involved in ER stress was an early event in UCB toxicity as eIF2α and PERK were both phosphorylated and activated by 1 h post-treatment. We also demonstrate that procaspase-12 content, a proposed initiator caspase in ER stress-mediated apoptosis is decreased by 4 h post-treatment. In conclusion this study demonstrates that elevated concentrations of UCB (50 μM) are able to activate select components of the ER stress pathway in Hepa 1c1c7 cells which may contribute to UCB-mediated apoptosis.

2. Hospital for Sick Children Foundation Project

Our hypothesis is that purified antioxidants that are common constituents of traditional Chinese Medicine (TCM) can attenuate the toxicity of electrophilic metabolites of sulfamethoxazole (SMX-NHOH in Jurkat E6.1 cells by decreasing the amount of reactive oxygen species (ROS) formed and maintaining the redox status of these cells. The positive control for oxidative stress in these experiments is treatment with t-BHP.

Some recent results from these studies have shown that relatively low concentrations of baicalein (BE), crocetin (Cro), resveratrol (Res), or schishenol (Sal) and 2 synthetic mixtures tested (5 μM) markedly attenuate cytotoxicity due to SMX-NHOH in Jurkat E6.1 cells. In addition, low concentration (5 μM) treatment with BE, Cro, Res or Sal singly and in combination attenuate SMX-NHOH-induced lipid hydroperoxide formation and protein oxidation (measured as protein carbonylation), however Res was not effective at decreasing protein carbonylation at this concentration. Low concentrations (1 or 5 μM) of the synthetic mixtures can attenuate SMX-NHOH-induced formation of disulfide mixed proteins, a change that correlates with cytotoxicity. Sal was a significant contributor to this cytoprotective effect. We are completing complementary experiments at the present time.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? O YES X 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? O YES X 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:

Name of Biological agent(s)*	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
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? X YES O 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	O Yes ⊗ No		Not applicable
Rodent	O Yes ⊗ No		
Non-human primate	O Yes ⊗ No		
Other (specify)	O 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)*	Supplier / Source
Human	X Yes O No	Jurkat, Clone E6-1	ATCC; TIB-152
Rodent (Mouse)	X Yes O No	Hepa 1c1c7	ATCC; CRL-2026
Non-human primate	O Yes O No		
Other (specify)	O Yes O 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 X 1 O 2 O 2+ O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES X 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/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

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? O YES X NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection

* Please attach a Material Data Sheet or equivalent if available.
** Please attach a plasmid map.

4.3 Will genetic modification(s) involving viral vectors be made? O YES, complete table below N 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.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? O YES X NO

4.6 Will virus be infectious to humans or animals? O YES X NO

4.7 Will this be expected to increase the containment level required? O YES X NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES X NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

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

5.2 Will the biological agent be able to replicate in the host? O YES O NO

5.3 How will the biological agent be administered? _____

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

5.5 Has human ethics approval been obtained? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? O YES XNO If no, please proceed to section 7.0

6.2 Name of animal species to be used_____

6.3 AUS protocol # _____

6.4 Will any of the agents listed in section 4.0 be used in live animals O YES, specify: _____ O NO

6.5 Will the agent(s) be shed by the animal: O YES O NO, please justify:

_____

_____

10.0 Plants

10.1 Do you use plants? O YES X 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 plant? _____

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

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

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

10.8 Is the CFIA permit attached? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? O YES, please give country of origin_____ X NO
If no, please proceed to Section 12.0

11.2 Has an Import Permit been obtained from HC for human pathogens? O YES O NO

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

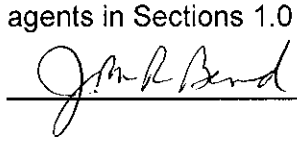
11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

12.0 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.

SIGNATURE 

13.0 Containment Levels

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

13.2 Has the facility been certified by OHS for this level of containment?

X YES, permit # if on-campus B10-UWO-0061

O NO, please certify

O NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

14.1 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.wph.uwo.ca/

SIGNATURE John R Bend Date: August 30, 2010

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.

_None

14.3 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury:

N/A - only cultured cells are being used.

15.0 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: _____
Date: _____

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

Special Conditions of Approval:

Cell Biology

ATCC® Number: **TIB-152™** Order this Item Price: **$272.00**

Designations:	Jurkat, Clone E6-1
Depositors:	A Weiss
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	suspension
Organism:	*Homo sapiens* (human)
Morphology:	lymphoblast 
Source:	**Disease:** acute T cell leukemia **Cell Type:** T lymphocyte;
Cellular Products:	interleukin-2 (interleukin 2, IL-2) [1609]
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 (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Receptors:	T cell antigen receptor, expressed
Antigen Expression:	CD3; Homo sapiens, expressed
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,12 D13S317: 8,12 D16S539: 11 D5S818: 9 D7S820: 8,12 THO1: 6,9.3 TPOX: 8,10 vWA: 18
Cytogenetic Analysis:	This is a pseudodiploid human cell line. The modal chromosome number is 46, occurring in 74% with polyploidy at 5.3%. The karyotype is 46,XY,-2,-18,del(2) (p21p23),del(18) (p11.2). Most cells had normal X and Y chromosomes.
Gender:	male
Comments:	

Related Links

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Product Information Sheet

BioProducts

Cell, microbial and molecular genomics products for the life sciences

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

BioStandards

Biological Reference Material and Consensus Standards for the life science community

	This is a clone of the Jurkat-FHCRC cell line, a derivative of the Jurkat cell line. [1609] The Jurkat cell line was established from the peripheral blood of a 14 year old boy by Schneider et al., and was originally designated JM. [50685] [112530] Clone E6-1 cells produce large amounts of IL-2 after stimulation with phorbol esters and either lectins or monoclonal antibodies against the T3 antigen (both types of stimulants are needed to induce IL-2 production. [1609] The line was cloned from cells obtained from Dr. Kendall Smith and are mycoplasma free. [1609]
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:** Cultures can be maintained by the addition of fresh medium or replacement of medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension at 1 X 10(5) viable cells/ml. Do not allow the cell density to exceed 3 X 10(6) cells/ml. **Interval:** Maintain cultures at a cell concentraion between between 1 X 10(5) and 1 X 10(6) viable cells/ml. **Medium Renewal:** Add fresh medium every 2 to 3 days (depending on cell density)
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Doubling Time:	48 hrs
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2001 recommended serum:ATCC 30-2020 derivative:ATCC CRL-1990 derivative:ATCC CRL-2063 derivative:ATCC TIB-153

Cell Biology

ATCC® Number:	**CRL-2026™**	Order this Item	Price:	**$349.00**

Designations:	Hepa-1c1c7
Depositors:	O Hankinson
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus* (mouse)
Morphology:	epithelial
Source:	**Strain:** C57L **Organ:** liver **Disease:** hepatoma
Cellular Products:	cytochrome P450IA1
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 (Roche FuGENE® Transfection Reagents)
Receptors:	aryl hydrocarbon (Ah)
Comments:	This is a derivative of the BW7756 mouse hepatoma that arose in a C57/L mouse. The cells are highly inducible for cytochrome P450IA1. This and mutant derivatives of it are useful for studying the aryl hydrocarbon (Ah) receptor and P450IA1 regulation.
Propagation:	**ATCC complete growth medium:** Alpha minimum essential medium without nucleosides, 90%; fetal bovine serum, 10% **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	

Related Links

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Protocol:

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 that 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 37°C 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 37°C.

Preservation:	**Subcultivation Ratio:** A subcultivation ratio of 1:4 to 1:10 is recommended **Medium Renewal:** Every 2 to 3 days **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Doubling Time:	18 hrs
Related Products:	recommended serum:ATCC 30-2020
References:	22320: Bernhard HP, et al. Expression of liver phenotypes in cultured mouse hepatoma cells: synthesis and secretion of serum albumin. Dev. Biol. 35: 83-96, 1973. PubMed: 4362668 22342: Hankinson O, et al. Genetic and molecular analysis of the Ah receptor and of Cyp1a1 gene expression. Biochimie 73: 61-66, 1991. PubMed: 1851644 22593: Hankinson O. Single-step selection of clones of a mouse hepatoma line deficient in aryl hydrocarbon hydroxylase. Proc. Natl. Acad. Sci. USA 76: 373-376, 1979. PubMed: 106390

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