

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:	Brad Urquhart
DEPARTMENT:	Physiology and Pharmacology
ADDRESS:	DSB room 2011
PHONE NUMBER:	83756
EMERGENCY PHONE NUMBER(S):	226 663 4389
EMAIL:	Brad.Urquhart@schulich.uwo.ca

Location of experimental work to be carried out :

Building : Dental Sciences Building	Room(s): 2011 and 2011D
Building : _____	Room(s): _____
Building : _____	Room(s): _____

***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: **NSERC**

GRANT TITLE(S): **Regulation of Drug Transport Proteins and Drug Metabolizing Enzymes by Nuclear Receptors**

UNDERGRADUATE COURSE NAME(IF APPLICABLE): _____

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

<u>Name</u>	<u>UWO E-mail Address</u>	<u>Date of Biosafety Training</u>
Tom Velenosi	tvelenos@uwo.ca	Sept 17, 2010
Dave Feere	dfeere@uwo.ca	May 3, 2010
_____	_____	_____
_____	_____	_____
_____	_____	_____

Animal carcasses are incinerated as per standard procedures. Residual tissue fractions not used for experimentation are incubated in a 10% bleach solution for at least ten minutes prior to disposal.

3. CLINICAL STUDIES

USE

Human blood and/or tissue are obtained from human patients with CKD. The blood samples are obtained from patients who have been administered a drug as part of a pharmacokinetic study. Plasma is isolated from the blood by centrifugation. The resultant plasma is extracted across a C18 solid phase extraction cartridge and the concentration of drugs and/or metabolites determined by ultra-performance liquid chromatography.

For human intestine or liver experiments, microsomes are isolated by differential centrifugation and drug metabolism activity determined using probe substrates as described under the cell line section. Although patients with kidney disease are screened for infections such as hepatitis and HIV, universal precautions are taken when working with any human specimens.

STORAGE

All plasma samples are stored at either -20 degrees Celsius or -80 degrees Celsius depending on the stability of the drug/metabolite being investigated. All tissue specimens are stored at -80 degrees Celsius.

DISPOSAL

Residual blood samples from plasma isolation are autoclaved prior to disposal. Residual tissue fractions not used in experiments are treated in 10% bleach for at least 10 minutes prior to disposal.

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

Brief Description of Research - Biohazard Subcommittee

PI: Brad Urquhart, PhD

Assistant Professor

Department of Physiology and Pharmacology

Dental Sciences Building room 2011 C

Tel: 519 661 2111 ext 83756

E-mail: Brad.Urquhart@schulich.uwo.ca

Laboratory: Dental Sciences Building room 2011

The effect of chronic kidney disease on drug disposition

My laboratory investigates why people with kidney failure respond differently to medications than people with normal kidney function. There are 3 themes of research that my laboratory uses which include clinical studies, animal studies and in vitro studies.

Clinical Studies

These studies involve human subjects and evaluate tissue expression of proteins and mRNA for genes known to be involved in drug response. Further, these studies will evaluate the concentration of drugs in human blood. We will also isolate DNA from blood samples to evaluate epigenetic changes of target genes in patients with kidney failure. The majority of this work will involve patients with kidney failure who are screened and found negative for conditions such as HIV and Hepatitis. This screening is routinely performed as part of their renal care at LHSC. Although the patients are screened, work carried out in the laboratory will follow strict level 2 containment guidelines under the assumption that every specimen is potentially infectious.

Animal studies

Sprague-Dawley and Wistar rats are used in various models (i.e. surgery or diet induced) kidney failure. Tissues from the animals will be evaluated for protein and mRNA expression of genes known to be involved in drug response. Further, intestinal segments will be used in an everted sac model to evaluate drug transport across the intestine. Hepatocytes will also be isolated for determination of extra-renal drug metabolism and transport in the context of kidney failure.

In Vitro Studies

Mammalian cell lines such as Caco-2, HepaRG, HeLa or MDCKII will be used as model systems to evaluate the effect of compounds that accumulate in the plasma of patients with kidney disease (i.e. uremic toxins). These cells will be treated with uremic toxins and the expression and activity of drug transporter proteins and drug metabolizing enzymes will be evaluated by Western blotting, Chromatin Immunoprecipitation, RT-PCR and Ultra-Performance Liquid Chromatography (UPLC).

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
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 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	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		Not applicable
Rodent	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		
Non-human primate	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		
Other (specify)	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> 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	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Caco-2 HepaRG Hela	1 1 1 1	ATCC Millipore ATCC
Rodent	<input type="checkbox"/> Yes <input type="checkbox"/> No			
Non-human primate	<input type="checkbox"/> Yes <input type="checkbox"/> No			
Other (specify)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	MDCKII	1	Sigma

**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: _____

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	Patients	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> Unknown	N/A	<input type="checkbox"/> 1 <input checked="" type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Blood (fraction) or other Body Fluid	Patients	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> Unknown	N/A	<input type="checkbox"/> 1 <input checked="" type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Organs or Tissues (unpreserved)	Patients	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> Unknown	N/A	<input type="checkbox"/> 1 <input checked="" type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3

Human Organs or Tissues (preserved)		Not Applicable		Not Applicable
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Additional Comments: Human tissue used includes blood, liver and intestinal specimens.

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 **Sprague Dawley and Wistar Rats**

7.3 AUS protocol # **2009-058**

7.4 List the location(s) for the animal experimentation and housing. **Medical/Dental Sciences Animal Facility**

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:

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 | <input type="checkbox"/> YES | <input type="checkbox"/> NO |
| ◆ Pound source cats | <input type="checkbox"/> YES | <input type="checkbox"/> NO |
| ◆ Cattle, sheep or goats | <input type="checkbox"/> YES, species | <input type="checkbox"/> NO |
| ◆ Non-human primates | <input type="checkbox"/> YES, species | <input type="checkbox"/> NO |
| ◆ Wild caught animals | <input type="checkbox"/> YES, species & colony # | <input type="checkbox"/> NO |
| ◆ Birds | <input type="checkbox"/> YES, species | <input type="checkbox"/> NO |
| ◆ Others (wild or domestic) | <input type="checkbox"/> YES, specify | <input type="checkbox"/> 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)
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

9.3 What is the LD₅₀ (specify species) of the toxin or hormone

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

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

9.6 Will any biological toxins or hormones be used in live animals? YES NO
If **YES**, Please provide details:

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

Additional Comments: _____

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 Brad Urquhart **Date:** November 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: **August 2012**
 NO, please certify
 NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants): **Bio-UW)-245**

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:
Immediate washing of the affected area and the affected person will be taken to the emergency room.

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 Brad Urquhart **Date:** November 12, 2012

15.4 Additional Comments: _____

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: _____
Date: _____

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

Special Conditions of Approval:

Cell Biology

ATCC® Number:	HTB-37™	Order this Item	Price:	\$431.00 (for-profit list price) \$359.17 (non-profit list price) Log In with customer # to see your price
Designations:	Caco-2			Related Links ▶
Depositors:	J Fogh			NCBI Entrez Search
Biosafety Level:	1			Cell Micrograph
Shipped:	frozen			Make a Deposit
Medium & Serum:	See Propagation			Frequently Asked Questions
Growth Properties:	adherent			Material Transfer Agreement
Organism:	<i>Homo sapiens</i> epithelial			New!
Morphology:	 PHOTO			Technical Support
Source:	Organ: colon Disease: colorectal adenocarcinoma keratin			 Product Information Sheet
Cellular Products:	retinoic acid binding protein 1 retinol binding protein 2			
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.			BioProducts
Restrictions:	NaviCyte Scientific holds the exclusive commercial distribution rights to the Caco-2 cell line as deposited by the Memorial Sloan-Kettering Cancer Center (SK) with the American Type Culture Collection (ATCC). Note: All uses of ATCC® HTB-37™, other than for research by a non-commercial or academic entity, require a license and use authorization from NaviCyte Scientific under its exclusive arrangement with Memorial Sloan-Kettering.			Cell, microbial and molecular genomics products for the life sciences
Applications:	transfection host			BioServices
Receptors:	heat stable enterotoxin (Sta, E. coli), expressed epidermal growth factor (EGF), expressed			Bio-materials management; basic repository to complex partnership-level services
Virus Susceptibility:	Human immunodeficiency virus 1			
Tumorigenic:	Yes Amelogenin: X CSF1PO: 11 D13S317: 11,13,14 D16S520: 12,12			BioStandards
				Biological Reference Material and

Application Note

HepaRG™ cells and their application to diverse endpoints

Nicola J. Hewitt. SWS, Erzhausen, Germany.

Abstract

Reproducible measurements of drug uptake, metabolism and toxicity require robustly functioning hepatic cells with stable, high expression of transporters and metabolic enzymes. Fulfilling this need are HepaRG™ cells. These widely published, terminally differentiated hepatocellular carcinoma cells have facilitated numerous studies of uptake, metabolism, and disposition of drug candidates. In this review, we describe the current uses of HepaRG™ cells and their position on the market as a hepatic model used by the pharmaceutical, chemical and cosmetics industries.



Figure 1.

HepaRG™ cells are an easy, convenient, functionally relevant model for diverse applications traditionally requiring hepatocyte model systems.

Why are hepatocyte models needed?

Although it is crucial to assess the effects of preclinical drug candidates on the liver, efficient completion of this step is hindered by the limited, sporadic supply of primary human hepatocytes (PHHs), the traditionally used hepatic model system. PHHs have little, if any, proliferative capacity, a short lifespan and lose their differentiated functions when cultured even for a few days. There are also large donor-specific variations in initial and longer-term functions and enzyme activities

(especially CYPs). Therefore, a long-lived hepatic model that features good proliferation, stable phenotype, low variability and has the advantage of an abundant supply would be an extremely useful tool as an alternative to PHHs. There have been numerous reports of potential replacements for PHHs but until now no single cell model has exhibited a phenotype or functions close enough to PHHs to be sufficiently predictive¹. The origin and characterization of HepaRG™ cells is reviewed here, along with their application to metabolism and toxicology assays (summarized in Table 1).

Table 1.

Characteristics and applications of HepaRG™ cells and other cell lines (characteristics of HepaRG™ cells are referring to those in a differentiated state, unless otherwise specified).

Characteristic	HepaRG™ cells	Other cell lines
Hepatic-specific markers	<ul style="list-style-type: none"> • Good carbohydrate metabolism, produce albumin, and eliminate galactose and sorbitol at comparable rates to PHHs. • Express aldolase B (20% of PHH), cytokeratin 8 and 18 and hepatocyte-specific antigen, CD26, and E-cadherin (markers of apical and lateral polarized phenotype), ZO-1 (tight junctions marker), CD49a, and are p53 competent. • Poor urea production. • As with PHHs, HepaRG™ cells are negative for α-fetoprotein. 	<ul style="list-style-type: none"> • Lack a variable and substantial set of liver-specific functions, making them unsuitable as representative of <i>in vivo</i> PHHs. • No aldolase B mRNA in HepG2 cells and albumin and haptoglobin mRNA levels are much lower than those in HepaRG™ cells. • Poor urea production. • α-Fetoprotein is highly expressed.
Enzyme characteristics (See Table 2)	<ul style="list-style-type: none"> • Differentiated HepaRG™ cells retain many drug metabolizing enzymes (DMEs) at levels comparable to those in PHH cultures. • CYP2D6 and CYP2C9 polymorphic. 	<ul style="list-style-type: none"> • HepG2 cells, although retaining some DMEs, have low (CYP1A2, CYP2B6) or absent (CYP2C9, CYP2E1 and CYP3A4) CYP expression apart from foetal forms (CYP1A1 and CYP3A7). • CYP activities are also low or absent.
DME regulation, transcription factors and nuclear receptors	<ul style="list-style-type: none"> • The transcription factor, AhR, and nuclear receptors, CAR, PXR and PPAR, responsible for the regulation of many DMEs and endogenous compounds are all expressed in high levels in differentiated HepaRG™ cells. 	<ul style="list-style-type: none"> • AhR, PXR and PPAR are all expressed in HepG2 cells but (with the exception of PPAR) are present at much lower levels than differentiated HepaRG™ cells or PHHs. • CAR has never been reported to be expressed at high levels in any other hepatoma cell line.
Drug transporters	<ul style="list-style-type: none"> • Express functional sinusoidal and canalicular transporter functions and regulation. 	<ul style="list-style-type: none"> • Uptake transporters expression absent except for MDR1, MRP1, and breast cancer resistance protein, which were expressed similar levels to PHHs. • No detectable expression of OATP-C, NTCP, OCT1 or BSEP.
Induction of CYPs	<ul style="list-style-type: none"> • CYP activities are readily induced. • Can be used to classify compounds as inducers or non-inducers of CYP3A4 as well as to predict the extent of induction <i>in vivo</i> in humans. 	<ul style="list-style-type: none"> • CYP1A2 and CYP3A4 induced in Fa2N4 cells but not CYP2B6 because they lack CAR. EC₅₀ values for CYP3A4 mRNA induction in Fa2N4 cells by a number of compounds were ~3-fold lower than in PHHs but the EC₅₀ for rifampicin was 10-fold higher. • HepG2 cells are not suitable for CYP3A induction assays due to a lack of many DMEs, many liver-specific functions and CAR.
Cytotoxicity	<ul style="list-style-type: none"> • Sensitive to hepatotoxicants whose toxicity is metabolism- and/or transporter-dependent. • Suitable for higher throughput screening. • The only known hepatic model to predict steatosis. • Best model for phospholipidosis. 	<ul style="list-style-type: none"> • HepG2 cells fail to detect a number of known hepatotoxicants due to a lack of DMEs and transporters. • HepG2 cells are poor models for steatosis and phospholipidosis.
Genotoxicity and carcinogenicity	<ul style="list-style-type: none"> • Can be adapted to micronucleus and Comet assays to detect direct and bioactivated genotoxicants. • Gene expression analysis correlates well with known <i>in vivo</i> effects of carcinogens. 	<ul style="list-style-type: none"> • HepG2 cells are more relevant than rodent cell lines but they still lack bioactivating enzymes, resulting in the potential for false negative results. • Different HepG2 clones do not give reproducible results for dietary genotoxins.
Inflammation	<ul style="list-style-type: none"> • Responsive to lipopolysaccharide. • Express important inflammatory mediators. • Effects of IL-1β on transporter function similar to PHHs. Represent a good model for sepsis and cholestasis. 	<ul style="list-style-type: none"> • HepG2 cells have been used to model nitric oxide and IL-8 release in response to inflammatory stimuli; however, the responses may be different from those <i>in vivo</i> due to low DME activities.
Virus infection	<ul style="list-style-type: none"> • HepaRG™ cells retain functional and efficient signaling pathways involved in pro-inflammatory effects. • Uniquely susceptible to viral infection. 	<ul style="list-style-type: none"> • Hepatoma cells, such as HepG2, have a limited use in viral infection research since they have impaired antiviral responses. • Not susceptible to hepatitis B virus infection.
3D bioartificial livers	<ul style="list-style-type: none"> • Suitable for 3D culture and use in bioreactors. • Morphology and DME functions similar to that of PHHs in 3D. 	<ul style="list-style-type: none"> • Not suitable for bioreactors due to poor DME and transporter functions.

Characterization of HepaRG™ cells

Origin of HepaRG™ cells

The HepaRG™ cell line was established from a tumor of a female patient suffering from chronic hepatitis C infection and hepatocarcinoma. Although they are likely to have originated from ductular structures (rather than mature hepatocytes or bile ducts) associated with long-term HCV infection, HepaRG™ cells do not contain any part of the HCV genome or express any HCV protein². These cells are different from HepG2 cells in that the latter are derived from hepatoblastoma cells, whereas HepaRG™ cells are derived from a hepatocarcinoma. When passaged at low density, they are able to recover and differentiate into both hepatocytes and biliary epithelial cells and are thus considered to be progenitor cells². HepaRG™ cells do not grow in serum-free medium and show moderate anchorage-independent growth in soft agar. When injected into mice, p53-competent HepaRG™ cells did not form tumours and repopulated damaged livers in mice, and demonstrated differentiated hepatocyte functions 6 weeks after implantation^{3,4}.

Hepatocyte-like differentiation of HepaRG™ cells

In vitro, maximum cell differentiation is reached after 2 weeks of exposure to DMSO and 40 to 50% of the confluent cell population are hepatocyte-like in nature, with morphology close to that of PHHs. Differentiated HepaRG™ cells stop proliferating and retain their hepatocyte-like features. Genes up-regulated during differentiation are those related to cell cycle inhibition, increased susceptibility to apoptosis, innate immunity and liver-enriched transcripts involved in lipid homeostasis and drug metabolism⁵. The expression of different hepatic nuclear factors (HNFs) involved in hepatic-specific gene expression changes as the cells grow and differentiate³. The cells surrounding the hepatocyte-like cells are biliary epithelial cells.

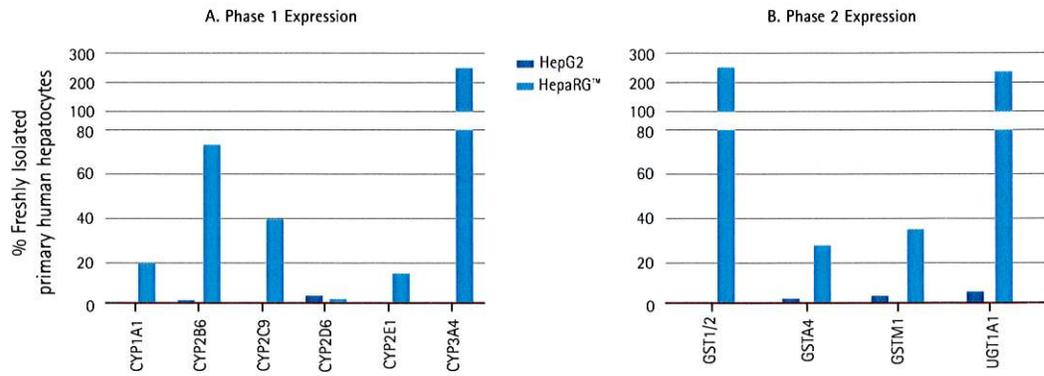
Although HepaRG™ cells do not produce urea (due to poor or disturbed nitrogen elimination via the urea cycle), they are able to regulate carbohydrate metabolism (glycogenolysis and/or gluconeogenesis), produce lactate (a product of anaerobic metabolism) and albumin, and eliminate galactose and sorbitol at comparable rates to PHHs. These features, along with the limited chromosomal rearrangements in these cells, make HepaRG™ cells much more differentiated than any other human hepatocyte cell line reported thus far⁶.

Gene expression profiling has revealed that HepaRG™ cells are remarkably close to certain hepatocyte populations which have a low expression of several genes involved in xenobiotic (CYP3A4 and CYP2C9), lipid, and carbohydrate metabolism⁷. It is therefore suggested that HepaRG™ cells behave as a PHH population, and can be classified as an "average human hepatocyte population." However, some drug metabolizing enzymes (DMEs) may be expressed at different levels than in PHHs and are sensitive to DMSO (see Figure 2), making direct comparisons with some drugs not possible.

Expression of drug metabolizing enzymes (DMEs)

There have been a number of comprehensive analyses of Phase 1 and 2 DME expression in HepaRG™ cells. A summary of the DMEs, regulation pathways and transporter characteristics is shown in Table 2. A genome-wide gene expression profiles analysis showed that for most genes encoding phase 1 and 2 DMEs and drug transporters, the differences between HepaRG™ cells and PHHs were much smaller than between HepG2 cells and PHHs⁸. The expression of DMEs is greater when they are cultured for longer times and high densities⁹. Moreover, inclusion of 2% DMSO in the culture medium caused the expression of some DMEs (CYP2B6, CYP2C9, CYP2E1, CYP3A4, UGT1A1, GSTA4, GSTM1, and GSTA1/A2) to be increased further, such that the levels were comparable with those measured in 3 to 5 day cultures of PHHs (which themselves are only 10 to 60% of the expression levels in freshly isolated hepatocytes, depending on the CYP). The expression of the CYP enzymes is maintained in the differentiated HepaRG™ cells for at least one month when cultured in the presence of DMSO^{9,10,11}. Removal of 2% DMSO reverses the effect; whereby the expression and activities of major CYPs, especially CYP3A4, decreases significantly¹².

Figure 2. HepaRG[™] cells express phase 1 (A.) and phase 2 (B.) drug metabolism enzymes at higher levels than do HepG2 cells. Gene expression studies were conducted to measure the transcript levels of DMEs in HepaRG[™] and HepG2 cells, shown here as a percentage of the transcript levels in freshly isolated PHHs. (Data from Aninat C. et al, 2006.⁹)



CYP2D6 expression in highly differentiated HepaRG[™] cells is less than 10% of PHHs, suggesting this donor is polymorphic for this CYP¹³. These cells are also polymorphic for CYP2C9 which is reflected in the relatively low CYP2C9-mediated metabolism of diclofenac and tolbutamide^{9,12}. CYP2E1 and CYP1A2 activities are also relatively low in the differentiated HepaRG[™] cells cultured with or without DMSO when compared with freshly isolated PHHs^{9,12}. The levels of these CYPs can be increased in HepaRG[™] cells without altering the levels of other highly expressed CYPs (such as CYP3A4) using a transient transfection method

involving electroporation, as demonstrated for CYP2E1¹⁴. CYP4F3B and CYP4A11 are expressed and functional in HepaRG[™] cells^{15,16}. Although these are not drug metabolizing CYPs, they are important to note because they catalyse ω -hydroxylation, a pathway accounting for 5–10% of total fatty acid oxidation¹⁷. The presence of these enzymes is especially relevant to the use of these cells as a model for steatosis (See section entitled "Drug-induced liver injury").

Table 2. DMEs, transporters and transcription factors in HepaRG[™] cells and other cell lines

Characteristic	HepaRG [™] cells		HepG2 cells	
	Basal expression	Inducibility	Basal expression	Inducibility
Phase 1 DMEs				
CYP1A1	+	✓	+	✓
CYP1A2	-/+	✓	+	✓
CYP2B6	+++	✓	+	Poor
CYP2C8	+	✓	ND	ND
CYP2C9	+ (PM)	✓	-	ND
CYP2C19	+++	✓	ND	ND
CYP2E1	+	✓	-	x
CYP3A4	+++	✓	-	x
CYP2D6	+ (PM)	NI	+	NI
CYP4F3B/CYP4A11	++	✓	ND	ND
FM01/2/3	+++	x	++	x
Aldehyde dehydrogenases	+++	ND	ND	ND
Alcohol dehydrogenases	+++	ND	ND	ND
Phase 2 DMEs				
UGT1A1	+++	✓	+	ND
GSTM1	++	ND	+	ND
GSTA4	+++	ND	+	ND
GSTA1/A2	+++	✓	+	ND
SULT1B1/SULT1E1	+++	x	++	x
NAT1/2	+++	ND	ND	ND

Characteristic	HepaRG™ cells		HepG2 cells	
	Basal expression	Inducibility	Basal expression	Inducibility
DME regulation transcription factors				
AhR	+++	ND	++	ND
CAR	++	ND	+/-	ND
PXR	+++	ND	++	ND
PPARs	+++	ND	+++	ND
Drug transporters				
MRP1/MPR3	+++	ND	ND	ND
MRP2	++	✓	++	✓
MDR1	+++	✓	++	x
MDR3	++	ND	ND	ND
BSEP	+	ND	ND	ND
BCRP	+++	✓	+++	ND
OCT1	+++	x	-	x
OATP1B1/OATP1B3/OATP2B1	+	ND	-	ND
OAT2	+++	ND	++	ND
OCT1	+++	ND	-	x
NTCP	++	x	+	x
PepT1	++	ND	ND	ND

Taken from Kanebratt and Andersson (12,19); Hart et al. (8); Wilkening et al. (69) and Gerets et al. (70).

- = absent, + = present at low levels, ++ = present at measurable levels, +++ = present at levels comparable to PHHs, ✓ = inducible; PM = polymorphic; NI = not inducible in PHHs; ND = Not determined, x = little or no induction measured.

DME activity

Because relevant DMEs are generally expressed and active in HepaRG™ cells, this model has been successfully used for studies of enzyme induction and inhibition. A panel of DME activities in HepaRG™ cells have also been determined (Table 2)^{9,11,18,19,20}. The levels of activities correlate with the expression level of each CYP. A major difference between HepaRG™ cells and PHHs is that CYP1A1 is continuously expressed in the former; but is attributed to the transformed state of HepaRG™ cells rather than to the presence of biliary cells¹⁰. CYP2C19 activity is much higher in differentiated HepaRG™ cells than PHHs and CYP2C8 and CYP2C9 activities were also present at levels equivalent to those in PHHs used for comparison in the study¹⁸. Likewise, bupropion 6-hydroxylation (CYP2B6) and testosterone 6β-hydroxylation (CYP3A4) are present in HepaRG™ cells at levels reported to be present in PHH cultures^{9,18}. In accordance with the low expression of CYP2A6 and CYP2E1, metabolism of coumarin and chlorzoxazone was equally low or lacking. The characterization of Phase 2 DMEs in HepaRG™ cells is relatively limited compared to that of CYPs. GSTs are expressed at levels comparable to PHHs (Table 2) and were stable for up to 1 month^{9,10}. GSTA1/2 and UGT1A1 expression was induced by omeprazole, phenobarbital and rifampicin in low- and high-density cultures.

Transcription factors

Any cell model proposed as an alternative to PHHs should express transcription factors involved in the regulation of DMEs and transporters. The nuclear receptor, CAR, is present in HepaRG™ cells and the addition of 2% DMSO causes CAR to be increased further; which is important because this nuclear receptor is responsible for "phenobarbital-like" induction of CYP2B6, an enzyme not expressed in Fa2N4 and HepG2 cells^{9,21}. FDA compliance requires that CAR-mediated CYP2B6 induction should be demonstrated by the hepatic test model²². Other important transcription factors include AhR, PXR and PPAR, and all are expressed in high levels in differentiated HepaRG™ cells. In contrast to HepaRG™ cells and PHHs, HepG2 cells lack or have only low expression of DMEs and express less than 50% of PHH levels of AhR, CAR and PXR.

Drug transporters

The presence of drug transporters in HepaRG™ cells was investigated at the mRNA level and compared with the levels in PHHs and HepG2 cells (Table 2)¹². The expression of many efflux transporters was equivalent to or higher than PHHs; whereas mRNA levels of bile salt export pump were at 5- to 100-fold lower than in PHHs. The expression levels of the uptake transporters were also 5-fold lower than in PHHs. In order to achieve significant expression of some liver-specific transporters (OCT1, OATP-C, NTCP and BSEP) the HepaRG™ cells had to be grown to confluence. Functional bile canalicular structures in differentiated HepaRG™ cells have been demonstrated using fluorescent substrates^{3,10}.

Applications

Initially, HepaRG™ cells required a number of weeks of culture to bring them to a differentiated state, which was considered somewhat of a disadvantage in terms of end user planning and resources; however, now HepaRG™ cells are available as cryopreserved differentiated cells (i.e. they are differentiated in 2% DMSO and then frozen as a suspension). The freeze/thaw process does not alter their functional activities and the inter-batch reproducibility is excellent; therefore, these cells are ready to use in diverse applications. Some of these are outlined below.

Metabolism studies

PHHs are the standard by which to measure metabolism, since they have a full complement of DMEs, cofactors and transporter proteins²³. Hepatoblastoma cell lines have low or lack important DMEs required and therefore are poor substitutes for PHHs. The high levels of relevant DMEs and transporters present in HepaRG™ cells makes them a much better model than any other cell line currently available. The relative mRNA content of CYPs in HepaRG™ cells is similar to that in PHHs, suggesting that HepaRG™ cells represent a model which is not overabundant in a single CYP isoform and therefore representative of the distribution of CYPs present in PHHs¹². The intrinsic clearance of a number of compounds in differentiated HepaRG™ cells has been compared with that in PHHs^{12,24,25}. In line with basal CYP3A4 activities, midazolam clearance (CYP3A4) was much higher in differentiated HepaRG™ cells than PHHs, but was equivalent in HepaRG™ cells which had been cultured without DMSO. The clearance of compounds metabolized by UGTs, CYP1A2, CYP2C19, CYP3A4, and UGT1A4 in differentiated HepaRG™ cells was comparable to their clearance in PHHs. While the prediction of *in vivo* intrinsic clearance was mostly within 2-fold, there was a greater under-prediction of more highly cleared compounds by HepaRG™ cells, although this observation is also true for PHHs and is thought to be an inherent feature of cell-based clearance models²⁵. The clearance of compounds metabolized mainly by CYP2D6 and CYP2A6 was lower in HepaRG™ cells than in PHHs, but correlated with the lower mRNA expression of the DMEs responsible for the metabolism of these compounds. The long-term stability of CYPs in differentiated HepaRG™ cells makes them a suitable model for metabolism of low clearance compounds which require at least one day of incubation.

Enzyme inhibition

HepaRG™ cells are unique among hepatocyte cell lines in that they can be used for CYP inhibition studies, because they have sufficient levels of DMEs. This is not possible for cell lines which already have low or no activities.

Early studies showed a very good correlation between the IC_{50} values of eight inhibitors of different CYPs in HepaRG™ cells and PHHs, suggesting that HepaRG™ cells represent a promising model for this important endpoint¹⁸.

Enzyme induction

HepaRG™ cells represent a relevant alternative model for induction since they express functional transcription factors involved in the induction of the main CYP enzymes, as well as good DME activities and transporter functions^{9,26}. Another advantage of a cell model that has reproducible and marked CYP induction responses is that the effects of different compounds tested at different times can be directly compared, and inducers with low induction responses can be detected, thus avoiding false negative results.

HepaRG™ cells respond to prototypical inducers of CYP1A1, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP3A4 (Table 2)^{10,18,19}. As with PHHs, CYP2D6 activities were not inducible in HepaRG™ cells¹⁸. The CYP induction responses were also shown to be stable over one month¹⁰. Importantly, responsiveness to prototypical inducers of CYPs was similar in different passages of HepaRG™ cells; therefore, large amounts of these cells can be produced at later passages which retain the same fold-induction values as earlier passages. The inter-batch reproducibility in these induction studies was shown to be very good¹⁹. The known lower level of induction of CYP2C9 compared with that of CYP3A4 *in vivo* was also reflected in HepaRG™ cells (CYP2C9 was induced by ~2-fold and CYP3A4 by 24-fold by rifampicin). CYP3A4 was also inducible by rifampicin in HepaRG™ cells cultured in conditions resulting in sub-maximal basal CYP3A4 activities. There was no CYP3A4 induction response in fully differentiated HepaRG™ cells, most likely because the basal activities had reached the maximal level obtainable (a normal phenomenon when maximum transcription activity is reached)²⁷. Kanebratt and Andersson¹² recommended the removal of DMSO for one day to decrease CYP3A4 activities before treatment with test compounds and prototypical inducers. With this in mind, HepaRG™ cells which are cultured under conditions that result in lower basal activities will likely result in induction responses that are much higher than in PHHs⁹.

There are reports of an excellent correlation between the *in vitro* and *in vivo* induction of CYP3A4 selected compounds. These predictions of *in vivo* induction were determined using different calculation models, namely the AUC/ F_2 prediction model, the Relative Induction Score model and the "Relative Factor" model^{12,28,29}. EC_{50} values for CYP3A4 induction in HepaRG™ cells treated with rifampicin also correlated well with those reported in

PHHs and PXR reporter gene assays³⁰. The time-dependent inhibition of CYP3A4 could also be monitored in HepaRG™ cells by measuring the ratio between the mRNA and activity of this CYP. The prediction model incorporating opposing effects of induction and inhibition was developed further using PHHs and HepaRG™ cells²⁸. Furthermore, these studies underlined the fact that HepaRG™ cells and PHHs could be used interchangeably. A method measuring CYP1A2, CYP2B6 and CYP3A4 induction, using a substrate cocktail incubation for activity and real-time PCR for mRNA detection, has been described in detail by Andersson³¹. The method for CYP induction using HepaRG™ cells is currently part of a validation phase at the European Centre for the Validation of Alternative Methods³².

As with basal activities, the measurement of Phase 2 enzyme induction in HepaRG™ cells and PHHs is still relatively limited³³. Phenobarbital, rifampicin and omeprazole increased both UGT1A1 and GSTA1/A2 transcripts in differentiated HepaRG™ cells, which was comparable to the level of induction of these DMEs measured in PHHs¹⁰. Likewise, the levels of induction of the transporters, MDR1, MRP2 and BCRP, were also much lower than CYP induction¹⁰ and comparable with the induction levels measured in PHHs³⁴.

Drug-induced liver injury

The stable metabolic activities in HepaRG™ cells make them ideal for long-term and repeated dose toxicity studies in which the toxic effects are metabolism-dependent and/or only evident after days or weeks^{12,35}. In addition, the presence of both biliary cells and hepatocytes provides information as to whether toxicity is specific to one cell type or whether both are affected. There have been a number of studies which have shown that HepaRG™ cells were able to predict drug-induced liver toxicity in humans due to their unique stable differentiated functions and ability to mirror *in vivo* effects. The toxicity of many compounds has been tested using rodent models but important species differences in metabolism and DME regulation result in conclusions which are not human-specific. For example, phenobarbital hepatotoxicity is much lower in humans than in rodents; and reasons for this are related to the gene expression profile over time, as shown in PHHs and HepaRG™ cells³⁶. Moreover, the expression profiles measured in HepaRG™ cells were comparable to PHHs, supporting the use of these cells for dose- and time-dependent studies. The use of HepaRG™ cells as a model for cytotoxicity assays has recently been investigated using a number of compounds. For example, two hepatotoxicants which require bioactivation are aflatoxin B₁ and acetaminophen and the IC₅₀s for these in HepaRG™ cells were similar to those measured in PHHs⁹.

Moreover, HepaRG™ cells were sensitive to the cytotoxic effect of these compounds at concentrations which were completely non-toxic to HepG2 cells^{13,20}. Aflatoxin B₁ cytotoxicity was time-dependent and specific to hepatocyte-like cells in the culture, such that depletion of ATP did not exceed 60% of the control levels (equivalent to the percentage of hepatocyte-like cells in the culture)²⁰. Thus HepaRG™ cells were able to differentiate between metabolism-dependent effects of compounds within the same culture. Studies on HepaRG™ cells treated with acetaminophen showed these cells demonstrated the key features of toxicity of this compound, namely marked glutathione depletion, adduct formation and mitochondrial dysfunction. Moreover, the release of LDH over time reflected the *in vivo* release of aminotransferase activity seen in humans as a result of acetaminophen overdose³⁷. Other hepatotoxicants, such as iron citrate, have been shown to be accumulated in HepaRG™ cells and PHHs, which only occurs in cells which have hepatocyte-like phenotypes (such as the presence of CYP2E1 and 3A4, lipid metabolism (e.g. fatty acid binding protein 1) and stress responses (e.g. superoxide dismutase)). Unlike the HepaRG™ hepatocyte-like cells, the HepaRG™ biliary cells do not accumulate iron³⁸. In another study, the relative metabolism-dependent hepatotoxic effects of three different thiopurines were demonstrated to be similar in HepaRG™ cells and PHHs³⁹. The sensitivity of both PHHs and HepaRG™ cells was lower than in rat hepatocytes, demonstrating a significant species difference in toxicity and the importance of using a human cell type. However, the human cell line, HepG2, was insensitive to the thiopurine, azathioprine, probably due to the lack of GST activity (mainly GSTM1) which catalyses the first metabolic step in the bioactivation of this compound⁴⁰. HepaRG™ cells have been used to determine the anti-tumor cytotoxicity of doxorubicin, delivered as free compound or loaded into nanoparticles⁴¹. The nanoparticles bypass the chemoresistant effects of MDR1-mediated efflux of the compound and thus making the cells more sensitive to toxicity. HepaRG™ cultures have also been shown to be adaptable to higher throughout cytotoxicity screening⁴².

Until now, there have been no *in vitro* hepatic models to predict steatosis (the accumulation of triglycerides) caused by drugs. However, this process was demonstrated in HepaRG™ cells treated with a number of polyunsaturated fatty acids and derivatives, suggesting that these cells may represent the first model for steatosis^{43,44}. HepaRG™ cells were shown to accumulate neutral lipids after treatment with the steatogenic drugs, tetracycline and amiodarone⁴³. Lipid accumulation increased after repeated dosing with relatively low concentrations of these drugs for two weeks.

Moreover, there was a direct correlation between lipid accumulation and levels of mRNA encoding for lipid synthesis markers in response to steatogenic drugs⁴³. The *in vitro* effects shown in HepaRG™ cells were predictive of the *in vivo* microvesicular steatosis reported in patients chronically treated with amiodarone and tetracycline.

Another mechanism of liver damage is phospholipidosis (accumulation of phospholipids and formation of lamellar bodies) and this liver injury has also been shown to occur in HepaRG™ cells after treatment with amiodarone^{11,43}. The HepaRG™ cells were able to discriminate between amiodarone which causes steatosis (after 24 h) and phospholipidosis (evident after 2 weeks) and tetracycline, which causes steatosis only. Similar to steatosis, the accumulation of phospholipids was concomitant with an up-regulation of lipid synthesis genes (and genes encoding proteins involved in the formation of vesicles) and was cumulative when the cells were dosed chronically over 2 weeks with non-cytotoxic concentrations of this drug. Similar studies using HepG2 cells showed that none of the genes involved in the formation of droplets were altered after amiodarone treatment, suggesting that HepG2 cells are poor models for this liver injury⁴⁵. It has been suggested that the gene markers involved in the formation of lamellar bodies and in the synthesis of phospholipids could represent potential biomarkers of drug-induced phospholipidosis in humans¹¹.

HepaRG™ cells have been used to investigate the effect of "glitazones" and "glitazars" on gene expression profiles using a whole genome transcriptomic approach⁴⁶. The first PPAR γ agonists with the intended therapeutic target of being antidiabetics were ciglitazone and troglitazone which were dropped during clinical trials and from the market, respectively, due to severe liver failure and death. Subsequent follow-up compounds, rosiglitazone and pioglitazone, were much less hepatotoxic. The dual PPAR α and PPAR γ agonists, muraglitazar and tesaglitazar, were not hepatotoxic but they were cardiotoxic and nephrotoxic⁴⁷. Incubations of troglitazone, rosiglitazone, muraglitazar and tesaglitazar with PHHs and HepaRG™ cells showed that troglitazone was the most toxic, causing loss of ATP, a decrease in NTCP uptake activity and inducing apoptosis. All four drugs altered genes regulating lipid, carbohydrate, xenobiotic and cholesterol metabolism, as well as inflammation and immunity in PHHs and HepaRG™ cells. These studies provided a comprehensive assessment of the effects of these drugs on both PHHs and HepaRG™ cells and demonstrated the relative contribution of different gene pathways involved in hepatotoxicity.

Genotoxicity and carcinogenicity

There are a number of studies which have focused on the possibility of using HepaRG™ cells for genotoxicity assays, since they are able to proliferate and they express significantly higher levels of DMEs than rodent cell lines or HepG2 cells. These cells have been developed and successfully used in the micronucleus and Comet assays^{20,48,49,50}. HepaRG™ cells therefore represent a promising cell model for the micronucleus test and have been recommended to be tested for use in this assay by ECVAM and IWGT workshops^{51,52}.

Phenotypic changes to HepaRG™ cells leading to malignant cell clones may also provide information on potential carcinogens⁵³. Whole genome expression analysis of HepaRG™ cells has been used to identify a set of genes linked to genotoxic and non-genotoxic compounds⁴⁹. Using this technology, HepaRG™ cells may be used as an alternative to PHHs to discriminate between genotoxic and non-genotoxic compounds and to provide information on compound and time dependent effects on cell cycle and apoptosis signaling pathways.

Transporter studies

The presence and function of important efflux and uptake transporters, together with the formation of tight junctions and correct location of canaliculi in cultured HepaRG™ cells, makes these cells suitable for biliary secretion studies². These studies were designed to reflect *in vivo*-like uptake, metabolism and secretion of both the parent and metabolites. Although the expression and activity of sinusoidal drug transporters such as OCT1, OATPs and NTCP are lower in HepaRG™ cells than in PHHs, the levels expressed are far higher than other cell lines, such as HepG2 cells, in which these transporters are low or lacking⁵⁴.

Transporters and their inhibition are thought to play a key role in cholestasis, in which the liver retains compounds that would normally be excreted into the bile. There is a lack of a model that can accurately predict the cholestatic effects of drugs in humans. Chlorpromazine, a known cholestatic drug, inhibited bile acid efflux in HepaRG™ cells, resulting in the accumulation of tritiated-taurocholic acid and oxidative stress (Antherieu et al., unpublished). Cholestasis can also be caused by inflammatory mediators, via down-regulation of transporters, as described below. Therefore, drugs causing cholestasis indirectly via inflammation can potentially be detected using HepaRG™ cells.

Inflammation

The liver plays an important role in sepsis and subsequent multiple organ failure. Sepsis is accompanied by a release of lipopolysaccharide (LPS), which compromises hepatocellular functions by upregulating

Kupffer cell tumor necrosis factor (TNF α), interleukin-1 β , (IL-1 β), and IL-6 production via the Toll-like receptor-4 and its co-receptor CD14 (which are both expressed in HepaRG™ cells⁵⁵). HepaRG™ cells have been shown to be responsive to LPS and exhibit classical responses of PHHs to inflammatory stimulation⁵⁵. Moreover, HepaRG™ cells are able to differentiate between the β -receptor-specific modulations of inflammatory responses and provide important information on the most suitable catecholamine to use for treatment of sepsis.

Human-specific effects of IL-1 β on transporter expression, function and liver cholestasis have been studied in HepaRG™ cells and compared with those in PHHs⁵⁶. The changes in uptake and efflux transporter profiles in PHHs were largely mirrored by those in HepaRG™ cells. Notably, Ntcp and BSEP mRNA and protein expression and uptake of taurocholate were markedly decreased in both cell types by IL-1 β , suggesting that this inflammatory mediator participates in both cholestasis and perturbation of hepatic detoxification pathways caused in humans.

Viral infections

There is a huge demand for better viral infection models. Specifically, more information on the processes involved in virus receptor binding, uptake, and membrane fusion is needed so that treatments to prevent virus entry into cells can be developed. Hepatoma cells, such as HepG2, have a limited use in viral infection research since they have impaired antiviral responses. Animal models are less relevant and, for ethical reasons, should be avoided. Until now, no hepatoma cell line has been shown to be susceptible to viral infection and support viral replication. Although Huh-7 cells are used for hepatitis C virus (HCV) infection and replication they are poorly differentiated and so do not reflect effects seen *in vivo*. By contrast, HepaRG™ cells retain functional and efficient signalling pathways involved in pro-inflammatory effects and are susceptible to viral infection^{5,57}. Markers of viral infections, such as IFN- β and a number of chemokines involved in inflammation and chemotaxis were strongly induced in HepaRG™ cells exposed to extracellular stimuli and the appearance of newly synthesized hepatitis B virus (HBV) transcripts was demonstrated^{5,58,59}. Modulation of the small surface protein of the HBV envelope has been shown to interfere with HBV infections of PHHs and HepaRG™ cells, thus giving vital information on the mechanisms of infection and the cell surface receptor^{60,61}. Studies investigating the infection of HepaRG™ cells with HCV have provided information on the mechanisms involved in the internalization and replication of this virus⁶². Unlike HBV, the permissiveness of HepaRG™ cells to HCV is much lower and not as long-lasting, because HepaRG™ cells

have a strong type I interferon-mediated anti-viral response⁴. These findings regarding the permissiveness of HepaRG™ cells to HBV and HCV infection support the use of HepaRG™ cells as an important model for viral hepatitis research.

Drug target identification

HepaRG™ cells have been used in investigations into lipid metabolism and its regulation by PPAR α in order to identify novel therapeutic targets for dyslipidemia⁶³. In these studies, expression of the apoA-IV gene was significantly increased by the compound KRP-101 via PPAR α upregulation in HepaRG™ cells. The therapeutic relevance was confirmed *in vivo*, in which dogs treated with KRP-101 had decreased serum triglyceride levels and increased serum apoA-IV levels.

3D cultures and bioreactors

HepaRG™ cells have been cultured in three-dimensional (3D) bioreactors in volumes ranging from 800 mL to 2 mL^{64,65}. These cultures were subsequently used for drug metabolism studies. The morphology of the cells inside the bioreactor resembled the *in vivo* arrangement of the cells and the expression of transporter proteins reflected appropriate cell polarization. CYP2C9, CYP1A1/2, and CYP3A4-mediated activities were similar 2 and 17 days after the cells were differentiated with DMSO, while CYP2B6 activities increased over this time. The bioreactor model was also able to demonstrate the CYP3A4 selective inhibition by ketoconazole (a decrease of 69%) and a 6-fold induction of CYP3A4 and CYP2B6 activities. Both these inhibitory and induction responses were similar to those measured *in vivo*; moreover, all the results from this study were carried out in one bioreactor, indicating that sequential experiments can be successfully conducted using the same dynamic cell model.

HepaRG™ cells have been tested for their application to bioartificial liver (BAL) models⁶⁶. A main function of a BAL model is to remove ammonia via the urea cycle and conjugation with amino acids, especially glutamine by glutamine synthetase. HepG2 cells have a limited use in BAL models because they have such low DME activities and lack a functional urea cycle⁶⁷. The low urea production measured in 2D monolayer cultures of HepaRG™ cells was improved by preconditioning the BAL with carbamoylglutamate, an analogue of N-acetylglutamate. This compound activates carbamoylphosphate synthetase, which is the rate-determining enzyme of the urea cycle under normal physiological conditions⁶⁸. HepaRG™ cells are a promising cell type for use in BAL models; however, in order to generate a fully effective BAL, the urea cycle in these cells would have to be improved further⁶⁸.

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Related Products

Product	Size	Catalogue No.
Cryopreserved HepaRG™ Cells	1 mL in vial with $\geq 8 \times 10^6$ viable cells	MMHPR116
HepaRG™ Thawing/Plating Medium Supplement	12.5 mL	MMADD671
HepaRG™ Tox Medium Supplement	12.5 mL	MMADD631
HepaRG™ Induction Medium Supplement	2.6 mL	MMADD641
HepaRG™ Serum Free Induction Medium Supplement	0.6 mL	MMADD651
HepaRG™ Culture Medium Supplement	14.0 mL	MMADD621

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Cryopreserved **HepaRG™** Cells

- [Overview](#)
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Applications of HepaRG™ Cells

Cytochrome P450 Genotyping Data Obtained in Collaboration with N. Picard at Limoges Hospital (France) using a validated TaqMan allelic discrimination assay (ABI PRISM 7000 Sequence Detection System, Applied Biosystems)

SNP Description

SNPs: Single

Nucleotide

Polymorphisms

wt: wild type

CYP2D6

Mut: mutation

** : wt = G

T = minor allele in

Caucasians

-	*2 (2850C>T)	*2/wt
-	*3 (2549delA)	wt/wt
-	*4 (1846G>A)	wt/wt
-	*7 (2935A>C)	wt/wt
-	*10 (100C>T)	wt/wt
-	CYP3A4	
-	*1B (392G>A)	wt/wt
-	OATP1B1 (=OATP2)	
-	*5 (521T>C)	wt/*5
-	OATP1B3 (=OATP8)	
-	334T>C	wt/wt**
-	MRP2	
-	-24C>T	wt/wt
-	1249G>A	wt/mut

3972C>T		wt/mut	
Vmax value of Phase I dependent activities (nmole/h/million cells)			
Activity	Enzyme	Method	Specification*
After thawing cells were incubated in suspension for 1 hour at 37°C with test compounds.			
Phenacetin O-deethylase activity	CYP1A2	- phenacetin (200 µM) - midazolam (50 µM) - bupropion (100 µM) - dextrometorphan (100 µM)	≥0.2
Metabolites formed were measured by LC-MS/MS			
Midazolam 1' hydroxylase activity	CYP3A4/5	-	≥1
Bupropion hydroxylase activity	CYP2B6	-	≥0.05
Dextrometorphan o demethylase activity	CYP2D6	-	≥0.05

*Activities are expressed as nanomole of metabolite formed/h/million cells. To convert to pmole of metabolite/min/million cells multiply the result by 16.66.

Induction of metabolic activity

Inducer	Enzyme	Assay	Method	Fold Induction*
				Fold induction: activity of treated cells/activity of control cells
				Specification
After thawing cells were incubated for 48 hours with test inducers. Cells were then incubated with the test substrates:				
Omeprazole, 50 µM	CYP1A2	Phenacetin O-deethylation	- for 2 hours with midazolam (50 µM) and bupropion (100 µM) - for 5 hours with phenacetin (200 µM)	≥1
The formation of metabolites was measured by LC-MS/MS				
Phenobarbital, 1 µM	CYP2B6	Bupropion hydroxylation	-	≥1
Rifampicin, 10 µM	CYP3A4/5	Midazolam 1' hydroxylation	-	≥1

*The fold induction in CYP1A2, CYP2B6 and CYP3A4/5 activity was determined as a measure of specific metabolite formation relative to vehicle control cells (un-induced).

Vmax value of uptake transporter activities (pmole/min/million cells)

Substrate	Transporter	Method	Specification
After thawing cells were incubated in suspension for 3 minutes at 37°C in uptake medium with substrate. The uptake transporter activity (Vmax) is measured as cell associated radioactivity with a liquid scintillation counter and expressed in pmol/min/million cells.			
[3H] MPP+, 250 µM	OCT	-	≥0.50
[3H] Taurocholate, 25 µM	NTCP	-	≥6

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Cell Biology

ATCC® Number:

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[Biosafety Level:](#)

2 [Cells contain human papilloma virus]

Shipped:

frozen

Medium & Serum:

[See Propagation](#)

Growth Properties:

adherent

Organism:

Homo sapiens

epithelial

Morphology:

**Organ:** cervix

Source:

Disease: adenocarcinoma**Cell Type:** epithelial

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.

Permits/Forms:

Applications:

transfection host
 screening for *Escherichia coli* strains with invasive potential

Human adenovirus 3

Encephalomyocarditis virus

Virus Susceptibility:

Human poliovirus 1

Human poliovirus 2

Human poliovirus 3

DNA Profile (STR):

Amelogenin: X

CSF1PO: 9,10

D13S317: 12,13.3

D16S539: 9,10

D5S818: 11 12

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1. PRODUCT AND COMPANY IDENTIFICATION

Product name : MDCK-II Cell Line canine

Product Number : 00062107

Brand : Sigma

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

Target Organs

Eyes, Skin

WHMIS Classification

Not WHMIS controlled.

Not WHMIS controlled.

Not a dangerous substance or mixture according to the Globaly Harmonised System (GHS).

HMIS Classification

Health hazard: 0

Chronic Health Hazard: *

Flammability: 0

Physical hazards: 0

Potential Health Effects

Inhalation May be harmful if inhaled. May cause respiratory tract irritation.

Skin May be harmful if absorbed through skin. May cause skin irritation.

Eyes May cause eye irritation.

Ingestion May be harmful if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

CAS-No.	EC-No.	Index-No.	Concentration
Cells, non human, non primate			
no data available	-	-	<= 1 %
Fetal bovine serum			
no data available	-	-	>= 89 - <= 90 %

Dimethyl sulfoxide			
67-68-5	200-664-3	-	10 %

4. FIRST AID MEASURES

General advice

Move out of dangerous area.

If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration.

In case of skin contact

Wash off with soap and plenty of water.

In case of eye contact

Flush eyes with water as a precaution.

If swallowed

Never give anything by mouth to an unconscious person. Rinse mouth with water.

5. FIRE-FIGHTING 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 fire-fighters

Wear self contained breathing apparatus for fire fighting if necessary.

Hazardous combustion products

Hazardous decomposition products formed under fire conditions. - Carbon oxides, Sulphur oxides

Explosion data - sensitivity to mechanical impact

no data available

Explosion data - sensitivity to static discharge

no data available

6. ACCIDENTAL RELEASE MEASURES

Personal precautions

Avoid breathing vapors, mist or gas.

Environmental precautions

Do not let product enter drains.

Methods and materials for containment and cleaning up

Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Conditions for safe storage

Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.

Recommended storage temperature: -196 °C

Store under inert gas. Hygroscopic.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection

Respiratory protection not required. For nuisance exposures use type OV/AG (US) or type ABEK (EU EN 14387) respirator cartridges. 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

Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin and body protection

impervious clothing, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Hygiene measures

General industrial hygiene practice.

Specific engineering controls

Use mechanical exhaust or laboratory fumehood to avoid exposure.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	liquid
Colour	no data available

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	no data available
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

no data available

Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Carbon oxides, Sulphur oxides

Other decomposition products - no data available

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Oral LD50

no data available

Inhalation LC50

no data available

Dermal LD50

no data available

Other information on acute toxicity

no data available

Skin corrosion/irritation

no data available

Serious eye damage/eye irritation

Eyes: no data available

Respiratory or skin sensitization

no data available

Germ cell mutagenicity

no data available

Carcinogenicity

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

no data available

Specific target organ toxicity - single exposure (Globally Harmonized System)

no data available

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. May cause respiratory tract irritation.
Ingestion	May be harmful if swallowed.
Skin	May be harmful if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.

Synergistic effects

no data available

Additional Information

RTECS: Not available

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.

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

Not WHMIS controlled.

Not WHMIS controlled.

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

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