

FINAL REPORT

Evaluation of the Induction Potential of Metaxalone on the Activities of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 in Human Hepatocytes

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Study Dates and Data Retention

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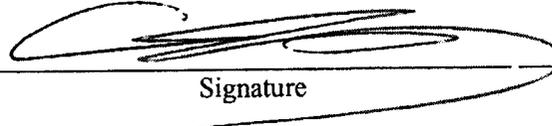
25 April 2006

In Vitro Technologies will retain all supporting documentation, including raw data and written records, for a period of up to five years following issuance of the final report. At the end of this period, Mutual Pharmaceuticals will be notified to determine whether the data (excluding proprietary information) will be transferred, retained, or destroyed.

Statement of Compliance

This study was conducted using good documentation practices. The study was conducted under my scientific guidance and management.

Genfu Chen, Ph.D.
Study Director



Signature

25 April 06
Date

Quality Assurance Statement

This study was inspected in accordance with In Vitro Technologies standard operating procedures. Based on audits conducted, the results reported accurately reflect the methods used and the data collected for this study.

All findings were reported to the Study Director and In Vitro Technologies Management.

Inspection/Audit Dates:	Study Phase Audited:	Date(s) reported to Study Director and Management:
26 October 2005	Incubations	26 October 2005
02 November 2005	Observation of cell morphology	09 November 2005
01 February 2006	Observation of cell morphology, dosing solution preparation, and dosing	01 February 2006
03 February 2006	Sample transfer	03 February 2006
01 March 2006	Harvest of paclitaxel incubation samples	02 March 2006
07, 10, 11 April 2006	Data and report	12 April 2006


Quality Assurance

25 April 06
Date

Glossary of Abbreviations

CYP..... cytochrome P450
DMEM.....Dulbecco's modified Eagle's medium
HEPESN-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonate)
HPLC high-performance liquid chromatography
HPLC-UV high-performance liquid chromatography with ultraviolet detection
KHB Krebs-Henseleit buffer
LC/MS..... liquid chromatography/mass spectrometry
MEM..... minimum essential medium
VC vehicle control

Summary

The objective of this study was to evaluate the potential of metaxalone to induce the activities of cytochrome P450 (CYP) isoforms CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 in human hepatocytes following *in vitro* exposure. Hepatocytes were incubated in the presence of metaxalone for 48 ± 3 hours, after which a selective substrate for each CYP isoform was added. The formation of a specific metabolite from its substrate was measured by high-performance liquid chromatography with ultraviolet detection or liquid chromatography/mass spectrometry.

Metaxalone at the tested concentration did not induce the activities of CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP2E1. Metaxalone at the concentrations of 0.4 and 4 μM did not induce CYP1A2 activity. However, metaxalone at the concentration of 40 μM induced CYP1A2 activity in all three donors. The induction of CYP1A2 activity ranged from about 2- to 3.3- fold. Metaxalone at the concentration of 40 μM slightly induced (about 21%) CYP3A4 activity in one of three donors tested. At the lower concentrations (0.4 and 4 μM), metaxalone did not induce CYP3A4 activity in any of the three donors.

Introduction

The liver represents the major organ for drug metabolism and contains the CYP enzymes, the major enzyme system for xenobiotic metabolism (1). Hepatocytes isolated from the liver constitute a physiologically relevant experimental model for the evaluation of potential drug-drug interactions related to the inhibition or induction of CYP enzyme activities.

Differences in drug-metabolizing enzymes among species, especially in CYP isoforms, often account for the inability to predict human clinical responses based on data obtained from laboratory animal studies. Human hepatocytes were used as an experimental model to reduce concerns about species differences (2). Cryopreserved hepatocytes provide a readily available and well-characterized biological model for use in CYP enzyme induction studies.

The objective of this study was to evaluate the potential of metaxalone to induce the activities of cytochrome P450 (CYP) isoforms CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 in human hepatocytes following *in vitro* exposure.

Experimental Methods

Description of Study

Hepatocytes were incubated in the presence of metaxalone for 48 ± 3 hours, after which a selective substrate for each CYP isoform was added. The formation of a specific metabolite from its substrate was measured by high-performance liquid chromatography with ultraviolet detection (HPLC-UV) or liquid chromatography/mass spectrometry (LC/MS).

Media

The following media, as prepared at In Vitro Technologies, was used in this study.

- DMEM Stock: Dulbecco's modified Eagle's medium (DMEM) supplemented with bovine serum albumin, fructose, N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonate) (HEPES), and sodium bicarbonate
- Hepatocyte Plating Medium: DMEM stock supplemented with antibiotics, bovine serum, hydrocortisone, insulin, and minimum essential medium (MEM) non-essential amino acids
- Sandwich Medium: Hepatocyte plating medium supplemented with Vitrogen™
- Incubation Medium: DMEM stock supplemented with antibiotics, hydrocortisone, insulin, and MEM non-essential amino acids
- Supplemented KHB: Krebs-Henseleit buffer (KHB) supplemented with antibiotics, calcium chloride, heptanoic acid, HEPES, and sodium bicarbonate

Test Article Preparation

The test article was identified in this study as follows:

- Metaxalone (molecular weight = 221 g/mol, purity: 99.9%, lot MTX-K4013)

Metaxalone stock solutions were prepared in methanol at 100 times (100X) the final concentration. The stock solutions were diluted with incubation medium to produce incubation solutions with final concentrations of 0.4, 4, and 40 μM , each containing 1% methanol. Stock solutions were prepared fresh prior to use.

Positive Control Article Preparation

Omeprazole and rifampin were prepared as 100X stock solutions in methanol. Positive control article stock solutions were diluted with incubation medium to the final concentrations listed below.

CYP isoform	Positive Control Article	Concentration
CYP1A2	Omeprazole	50 μM
CYP3A4	Rifampin	25 μM

Reference Control Article Preparation

Phenobarbital was prepared as a 100X stock solution in deionized water. All other reference control articles were prepared as 100X stock solutions in methanol. Reference control article stock solutions were diluted with incubation medium to the final concentrations listed below.

CYP isoform	Reference Control Article	Concentration
CYP2B6	Phenobarbital	1 mM*
CYP2C8	Rifampin	25 μM
CYP2C9	Rifampin	25 μM
CYP2C19	Rifampin	25 μM

* Methanol was added to the dosing solution to achieve a final methanol concentration of 1%.

CYP Isoform Substrate Preparation

The activity of each of the CYP isoforms was measured in the presence of the following isoform-selective substrates. Isoform-selective substrates were prepared as 100X stock solutions in acetonitrile and diluted with supplemented KHB to the final concentrations listed below.

CYP isoform	Isoform-selective substrate	Concentration
CYP1A2	Phenacetin	100 μM
CYP2A6	Coumarin	100 μM
CYP2B6	S-Mephenytoin	1 mM
CYP2C8	Paclitaxel	50 μM
CYP2C9	Tolbutamide	50 μM
CYP2C19	S-Mephenytoin	100 μM
CYP2D6	Dextromethorphan	16 μM
CYP2E1	Chlorzoxazone	300 μM
CYP3A4	Testosterone	125 μM

Hepatocyte Preparation

Hepatocytes were isolated and cryopreserved based on published methods (3–5). For this study, hepatocytes from four human donors were obtained from the cryopreserved hepatocyte bank maintained at In Vitro Technologies. Cryopreserved hepatocytes were thawed and counted to determine yield, viability was measured, and cell seeding density was adjusted accordingly. Hepatocytes were transferred to collagen-coated 48-well plates for attachment. After the hepatocytes attached to the collagen matrix, the plating medium was replaced with sandwich medium and the hepatocytes were incubated until use.

Donor Demographics and Medical Histories

The following information is provided as reported to In Vitro Technologies:

Donor 1, In Vitro Technologies lot AAS, was obtained from a 51-year-old Caucasian male who died from a stroke. Urinalyses and blood chemistries were within normal limits. Serologies were negative, except for cytomegalovirus. Medical history included diabetes, depression/anxiety, hypertension, and kidney disease. The donor had a history of alcohol and tobacco use, but no history of cannabinoid or other drug use. No chronic medications are listed.

Donor 2, In Vitro Technologies lot KCT, was obtained from a 83-year-old Caucasian female who died from a cerebrovascular accident. Urinalyses and blood chemistries were within normal limits. Serologies were negative, except for cytomegalovirus. Medical history included high cholesterol, kidney disease, and other heart disease. The donor had no history of alcohol, or other drug use. No chronic medications are listed.

Donor 3, In Vitro Technologies lot LOF, was obtained from a 54-year-old Caucasian female who died from cardiac arrest. Urinalyses and blood chemistries were within normal limits. Serologies were negative, except for cytomegalovirus. Medical history included hypertension and high cholesterol. The donor had a history of tobacco use, but no history of alcohol or other drug use. No chronic medications are listed.

Donor 4, In Vitro Technologies lot NPV, was obtained from a 40-year-old Caucasian female who died from a drug overdose. Urinalyses and blood chemistries were within normal limits. Serologies were negative, except for cytomegalovirus. Medical history included hypertension. The donor had a history of tobacco, marijuana, cocaine, and opiate use, but no history of alcohol use. No chronic medications are listed.

Test Article Incubations

All incubations were conducted at $37 \pm 1^\circ\text{C}$, 95% air/5% CO_2 , and saturating humidity. The sample size was $N = 3$ replicates for experimental groups.

After the cultures were established, the sandwich medium was removed and the hepatocytes were treated with an incubation solution containing metaxalone for 24 ± 1.5 hours. The incubation solution was aspirated and replaced with incubation solution containing the same

concentration of metaxalone as was used in the initial dosing and incubated for an additional 24 ± 1.5 hours. The total treatment period was 48 ± 3 hours.

After the treatment period of 48 ± 3 hours, the incubation solution was replaced with 150 μ L of supplemented KHB. The hepatocytes were incubated for 10 minutes to remove residual metaxalone. The supplemented KHB was replaced with 150 μ L of supplemented KHB containing an isoform-selective substrate. The hepatocytes were incubated for 4 hours.

CYP2C8 incubations were terminated by adding 150 μ L of acetonitrile. All other incubations were terminated by adding 150 μ L of ice-cold methanol. Samples were transferred to cryovials and stored at $-70 \pm 10^\circ\text{C}$ until analysis.

Control Incubations

All incubations were conducted at $37 \pm 1^\circ\text{C}$, 95% air/5% CO_2 , and saturating humidity. The sample size was $N = 4$ replicates for the vehicle control (VC), positive control, and reference control groups; and $N = 2$ replicates for the test article interference control groups.

Vehicle Control

VC samples were included to establish a baseline value for analysis. After the cultures were established, the sandwich medium was removed and the hepatocytes were treated with incubation medium containing 1% methanol for 24 ± 1.5 hours. The incubation medium containing 1% methanol was aspirated and replaced with incubation medium containing 1% methanol and incubated for an additional 24 ± 1.5 hours. The total treatment period was 48 ± 3 hours.

After the treatment period of 48 ± 3 hours, the incubation medium containing 1% methanol was replaced with 150 μ L of supplemented KHB and incubated for 10 minutes. The supplemented KHB was replaced with 150 μ L of supplemented KHB containing an isoform-selective substrate. The hepatocytes were incubated for 4 hours.

Positive Control

Positive controls samples were included to verify that the test system was responsive to known inducers. Omeprazole, a selective inducer of CYP1A2, was used to verify that the test system was responsive to CYP1A2 inducers. After the hepatocytes were established, the sandwich medium was replaced with incubation medium containing 50 μM omeprazole for 24 ± 1.5 hours. The incubation medium containing 50 μM omeprazole was aspirated and replaced with incubation medium containing 50 μM omeprazole and incubated for an additional 24 ± 1.5 hours. The total treatment period was 48 ± 3 hours.

After the treatment period of 48 ± 3 hours, the incubation solution was replaced with 150 μ L of supplemented KHB and incubated for 10 minutes to remove residual positive control article. The supplemented KHB was replaced with 150 μ L of supplemented KHB containing 100 μM phenacetin. The hepatocytes were incubated for 4 hours.

Rifampin, a selective inducer of CYP3A4, was used to verify that the test system was responsive to CYP3A4 inducers. After the hepatocytes were established, the sandwich medium was replaced with incubation medium containing 25 μM rifampin. The incubation medium containing 25 μM rifampin was aspirated and replaced with incubation medium containing 25 μM rifampin and incubated for an additional 24 ± 1.5 hours. The total treatment period was 48 ± 3 hours.

After the treatment period of 48 ± 3 hours, the incubation solution was replaced with 150 μL of supplemented KHB and incubated for 10 minutes to remove residual positive control article. The supplemented KHB was replaced with 150 μL of supplemented KHB containing 125 μM testosterone. The hepatocytes were incubated for 4 hours.

The test system was considered inducible since the mean specific activities of both CYP1A2 and CYP3A4 in the positive control samples treated with omeprazole and rifampin, respectively, were $\geq 200\%$ of the mean specific activities in the corresponding vehicle control samples.

Reference Control

Reference control samples were included to evaluate the inducibility of CYP2B6, CYP2C8, CYP2C9, and CYP2C19 in the test system.

After the hepatocytes were established, the sandwich medium was replaced with incubation medium containing reference control article for 24 ± 1.5 hours. The incubation medium containing reference control article was aspirated and replaced with incubation medium containing the same concentration of reference control article as was used in the initial dosing and incubated for an additional 24 ± 1.5 hours. The total treatment period was 48 ± 3 hours.

After the treatment period of 48 ± 3 hours, the incubation solution was replaced with 150 μL of supplemented KHB and incubated for 10 minutes to remove residual positive control article. The supplemented KHB was replaced with 150 μL of supplemented KHB containing an isoform-selective substrate. The hepatocytes were incubated for 4 hours.

Test Article Interference Control

Test article interference control samples were included to investigate the possibility of interference by metaxalone or their metabolites.

After the hepatocytes were established, the sandwich medium was removed and the hepatocytes were treated with an incubation solution containing 40 μM metaxalone for 24 ± 1.5 hours. The incubation solution was aspirated and replaced with incubation solution containing 40 μM metaxalone and incubated for an additional 24 ± 1.5 hours. The total treatment period was 48 ± 3 hours.

After the treatment period of 48 ± 3 hours, the incubation solution was replaced with 150 μL of supplemented KHB and incubated for 10 minutes to remove residual metaxalone. The supplemented KHB was replaced with 150 μL of supplemented KHB containing 1% acetonitrile. The hepatocytes were incubated for 4 hours.

Termination of Control Incubations

CYP2C8 incubations were terminated by adding 150 μ L of acetonitrile. All other incubations were terminated by adding 150 μ L of ice-cold methanol. Samples were transferred to cryovials and stored at $-70^{\circ}\text{C} \pm 10^{\circ}\text{C}$ until analysis.

Analyses

In Vitro Technologies measured the formation of metabolites from CYP isoform-selective substrates using the following bioanalytical procedures:

Phenacetin O-Deethylase (CYP1A2)

CYP1A2 activity was determined by measuring the formation of acetaminophen. Samples were analyzed using LC/MS.

Coumarin 7-Hydroxylase (CYP2A6)

CYP2A6 activity was determined by measuring the formation of 7-hydroxycoumarin and its conjugated derivatives, 7-hydroxycoumarin glucuronide and 7-hydroxycoumarin sulfate. Samples were analyzed using HPLC-UV.

S-Mephenytoin N-demethylase (CYP2B6)

CYP2B6 activity was determined by measuring the formation of nirvanol. Samples were analyzed using LC/MS.

Paclitaxel 6-Hydroxylase (CYP2C8)

CYP2C8 activity was determined by measuring the formation of 6-hydroxypaclitaxel. Samples were analyzed using HPLC-UV.

Tolbutamide 4'-Methyl Hydroxylase (CYP2C9)

CYP2C9 activity was determined by measuring the formation of 4'-methylhydroxytolbutamide. Samples were analyzed using LC/MS.

S-Mephenytoin 4'-Hydroxylase (CYP2C19)

CYP2C19 activity was determined by measuring the formation of 4'-hydroxymephenytoin. Samples were analyzed using LC/MS.

Dextromethorphan O-Demethylase (CYP2D6)

CYP2D6 activity was determined by measuring the formation of dextrorphan. Samples were analyzed using LC/MS.

Chlorzoxazone 6-Hydroxylase (CYP2E1)

CYP2E1 activity was determined by measuring the formation of 6-hydroxychlorzoxazone. Samples were analyzed using LC/MS.

Testosterone 6 β -Hydroxylase (CYP3A4)

CYP3A4 activity was determined by measuring the formation of 6 β -hydroxytestosterone. Samples were analyzed using HPLC-UV.

Description of Data Calculations

The concentration of metabolites is reported. Enzyme activity for each CYP isoform is reported as specific activity (pmol/minute/million cells) in the presence (SA_T) and absence (SA_C) of metaxalone. The data are expressed as mean \pm standard deviation and were calculated using Microsoft[®] Office Excel 2000. SA_T relative to SA_C for each CYP isoform is expressed as a percent using the following equation:

$$\% \text{ of vehicle control} = \frac{SA_T}{SA_C} \times 100$$

Except for test article interference samples, samples with back-calculated concentrations below the lower limit of quantitation were assigned the lower limit of quantitation value for calculation.

Criteria for Data Acceptance

The bioanalytical data for CYP isoforms were accepted in accordance with the In Vitro Technologies standard operating procedure(s) on bioanalytical data acceptance.

Results**Test System**

CYP1A2 activity in cryopreserved human hepatocytes was quantified by measuring the formation of acetaminophen from phenacetin. Following treatment with 50 μ M omeprazole, CYP1A2 activity was 2,027%, 854%, and 2,276% of the VC (1% methanol) in human hepatocytes prepared from Donors 1, 2, and 3, respectively (Table 1). CYP3A4 activity was quantified by measuring the formation of 6 β -hydroxytestosterone from testosterone. Following treatment with 25 μ M rifampin, CYP3A4 activity was >1,153%, >766%, and >1,511% of the VC in human hepatocytes prepared from Donors 1, 2, and 3, respectively (Table 2). The increase in activities of CYP1A2 and CYP3A4 following treatment with known inducers met the criteria set in the protocol; therefore, the hepatocytes from these donors were considered inducible. Additional hepatocyte preparations were used to evaluate the induction potential of

metaxalone on the CYP2C8 activity (see CYP2C8 sections for detail reasons). In preparation 2, following treatment with 50 μM omeprazole, CYP1A2 activity was 1,045%, 1,091%, and 1,847% of the VC in human hepatocytes prepared from Donors 1, 2, and 3, respectively (Table 3). Following treatment with 25 μM rifampin, CYP3A4 activity was 104%, 588%, and 2,069% of the VC in human hepatocytes prepared from Donors 1, 2, and 3, respectively (Table 4). Since CYP3A4 activity in hepatocytes treated with rifampin failed to meet the criteria in the protocol in preparations 2 and 3 of Donor 1, a different donor, Donor 4, was used to evaluate the induction potential of metaxalone on the CYP2C8 activity. Following treatment with its respective positive controls, CYP1A2 and CYP3A4 activity was 1,394% and 2,898% of the VC in human hepatocytes prepared from Donor 4 (Tables 5 and 6).

CYP1A2

Metaxalone at the tested concentration of 40 μM induced CYP1A2 activity in human hepatocytes prepared from Donors 1, 2, and 3. This conclusion is based on CYP1A2 activity (110, 131, and 332% of the VC in Donor 1; 108, 113, 197% of the VC in Donor 2; and 107, 108, and 297% of the VC in Donor 3) in hepatocytes treated with 0.4, 4, and 40 μM metaxalone (Table 7). The apparent increase of CYP1A2 activity by metaxalone at the concentrations of 0.4 and 4 μM was not statistically significant ($p > 0.05$; unpaired two-tailed t test). The assay method detected no chromatographic interference from metaxalone or its metabolite.

CYP2A6

CYP2A6 activity in cryopreserved human hepatocytes was quantified by adding coumarin to the hepatocytes and measuring the formation of 7-hydroxycoumarin and its conjugated derivatives, 7-hydroxycoumarin glucuronide and 7-hydroxycoumarin sulfate. CYP2A6 activity in the VCs from Donors 1, 2, and 3 was below the lower limit of quantitation (Tables 8a-d). Following treatment with metaxalone, CYP2A6 activity was still below the lower limit of quantitation. Therefore, metaxalone at the concentrations tested did not induce CYP2A6 activity in human hepatocytes isolated from these donors. The assay method detected no chromatographic interference from metaxalone or its metabolite.

CYP2B6

CYP2B6 activity in cryopreserved human hepatocytes was quantified by adding *S*-mephenytoin to the hepatocytes and measuring the formation of its metabolite, nirvanol. Metaxalone at the tested concentrations did not induce CYP2B6 activity in human hepatocytes prepared from Donors 1, 2, and 3. This conclusion is based on CYP2B6 activity (107, 95.7, and 97.1% of the VC in Donor 1; 106, 97.0, <86.5% of the VC in Donor 2; and 99.1, 99.1, and 99.1% of the VC in Donor 3) in hepatocytes treated with 0.4, 4, and 40 μM metaxalone (Table 9a). However, phenobarbital at the concentration of 1 mM induced CYP2B6 activity (335, 159, and >307% of the VC in hepatocytes from Donors 1, 2, and 3, respectively; Table 9b). The assay method detected no chromatographic interference from metaxalone or its metabolite.

CYP2C8

CYP2C8 activity in cryopreserved human hepatocytes was quantified by adding paclitaxel to the hepatocytes and measuring the formation of its metabolite, 6-hydroxypaclitaxel. Several incubations were conducted to evaluate the induction potential of metaxalone on the CYP2C8 activity. Data generated from the initial incubation varied dramatically due to lack of solubility of paclitaxel in supplemented KHB. It was determined that paclitaxel was soluble at the dosing concentration when prepared in DMEM-based incubation medium. Paclitaxel prepared in incubation medium was then used in subsequent incubations with preparation 2 from three same donors. A third preparation of hepatocytes from Donor 1 was included for the study since CYP3A4 activity following treatment with rifampin failed to meet the criteria set in the test system acceptance criteria. Incubations with hepatocytes from Donor 4 were conducted to replace Donor 1 for CYP2C8 since CYP3A4 activity following treatment with rifampin failed to meet the criteria set in the protocol in preparation 3 of Donor 1. Data reported here are from preparation 2 of Donors 2 and 3; and Donor 4. All other data generated for CYP2C8 are filed with the study documentation.

Metaxalone at the tested concentrations did not induce CYP2C8 activity in human hepatocytes isolated from all three donors tested. This conclusion is based on CYP2C8 activity (121, 122, and 108% of the VC in Donor 4; 103, 100, and 93.9% of the VC in Donor 2; and 101, 100, and 84.2% of the VC in Donor 3) in hepatocytes treated with 0.4, 4, and 40 μM metaxalone (Table 10a). The apparent increase of CYP2C8 activity following treatment with metaxalone was not statistically significant ($p > 0.05$; unpaired two-tailed t test). Rifampin, a reference control for CYP2C8, at the concentration of 25 μM induced CYP2C8 activity in hepatocytes prepared from two donors (260% and 156% of the VC in hepatocytes from Donors 4 and 3, respectively; Table 10b) but failed to induce CYP2C8 activity in a third donor (98.8% of the VC in Donor 2; Table 10b). The assay method detected no chromatographic interference from metaxalone or its metabolite.

CYP2C9

CYP2C9 activity in cryopreserved human hepatocytes was quantified by adding tolbutamide to the hepatocytes and measuring the formation of its metabolite, 4'-methylhydroxytolbutamide. Metaxalone at the concentrations tested did not induce CYP2C9 activity in human hepatocytes isolated from Donors 1, 2, and 3. This conclusion is based on CYP2C9 activity (108, 100, and <73.2% of the VC in Donor 1; 96.6, 92.9, and 60.4% of the VC in Donor 2; and 111, 103, and 78.4% of the VC in Donor 3) in hepatocytes treated with 0.4, 4, and 40 μM metaxalone (Table 11a). The apparent increase of CYP2C9 activity in Donors 1 and 3 following treatment with metaxalone was not statistically significant ($p > 0.05$; unpaired two-tailed t test). However, rifampin at the concentration of 25 μM induced CYP2C9 activity (213, 124, and 201% of the VC in hepatocytes from Donors 1, 2, and 3, respectively; Table 11b). The assay method detected no chromatographic interference from metaxalone or its metabolite.

CYP2C19

CYP2C19 activity in cryopreserved human hepatocytes was quantified by adding *S*-mephenytoin to the hepatocytes and measuring the formation of its metabolite, 4'-hydroxymephenytoin. CYP2C19 activity levels in hepatocytes isolated from all three donors were below the lower limit

of quantitation. Metaxalone at the concentrations tested did not induce CYP2C19 activity in human hepatocytes isolated from these donors since CYP2C19 activity in hepatocytes treated with 0.4, 4, and 40 μM metaxalone was undetectable or below the lower limit of quantitation (Table 12a). Rifampin increased 4'-hydroxymephenytoin concentrations from 0.00070 (mean of 0.00025, 0.00058, 0.0014, and 0.00058) to 0.01384 (mean of 0.00708, 0.01319, 0.01861, and 0.01649) μM in Donor 1 and from 0.00007 (mean of 0.00026, 0.00, 0.00, and 0.00) to 0.00118 (mean of 0.00142, 0.00191, 0.00119, and 0.00018) μM in Donor 3. Rifampin did not appear to induce CYP2C19 activity in Donor 2 (Table 12b). Therefore, lack of induction of CYP2C19 activity by metaxalone could be due to the unresponsiveness of hepatocytes to chemical treatment in Donor 2. The assay method detected no chromatographic interference from metaxalone or its metabolite.

CYP2D6

CYP2D6 activity in cryopreserved human hepatocytes was quantified by adding dextromethorphan to the hepatocytes and measuring the formation of its metabolite, dextrorphan. CYP2D6 activity was below the lower limit of quantitation in the VC from Donor 1. Metaxalone at the concentrations tested did not induce CYP2D6 activity since the activity following treatment with metaxalone was also below the lower limit of quantitation (Table 13). Metaxalone at the concentrations tested did not induce CYP2D6 activity in human hepatocytes isolated from Donors 2 and 3. This conclusion is based on CYP2D6 activity (95.9, 103, and <73.2% of the VC in Donor 2; and 98.8, 89.8, and 73.5% of the VC in Donor 3) in hepatocytes treated with 0.4, 4, and 40 μM metaxalone (Table 13). The assay method detected no chromatographic interference from metaxalone or its metabolite.

CYP2E1

CYP2E1 activity in cryopreserved human hepatocytes was quantified by adding chlorzoxazone to the hepatocytes and measuring the formation of its metabolite, 6-hydroxychlorzoxazone. Metaxalone at the concentrations tested did not induce CYP2E1 activity in human hepatocytes isolated from all three donors. This conclusion is based on CYP2E1 activity (97.8, 104, and 104% of the VC from Donor 1; 104, 89.6, 99.6% of the VC from Donor 2; and 90.7, 87.7, and 93.9% of the VC from Donor 3) in hepatocytes treated with 0.4, 4, and 40 μM metaxalone (Table 14). The apparent increase of CYP2E1 activity in Donors 1 and 2 following treatment with metaxalone was not statistically significant ($p > 0.05$; unpaired two-tailed t test). The assay method detected no chromatographic interference from metaxalone or its metabolite.

CYP3A4

CYP3A4 activity in the VC from Donor 1 was below the lower limit of quantitation. Metaxalone at the concentrations tested did not induce CYP3A4 activity, since the activity following treatment with metaxalone was still below the lower limit of quantitation (Table 15). Metaxalone at the tested concentration of 40 μM induced CYP3A4 activity in human hepatocytes prepared from Donor 2. The conclusion is based on CYP3A4 activity (>115, >98.7, and >121% of the VC) in hepatocytes treated with 0.4, 4, and 40 μM metaxalone (Table 15). The increase of CYP3A4 activity following treatment with metaxalone at the concentration of 0.4 μM was not statistically significant ($p > 0.05$; unpaired two-tailed t test). CYP3A4 activity in the VC from Donor 3 was below the lower limit of quantitation. Metaxalone at the tested

concentrations did not induce CYP3A4 activity, since the activity following treatment with metaxalone was still below the lower limit of quantitation (except in one of three replicates) for the concentration of 40 μ M. The assay method detected no chromatographic interference from metaxalone or its metabolite.

Conclusions

Metaxalone at the tested concentration did not induce the activities of CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP2E1. Metaxalone at the concentrations of 0.4 and 4 μ M did not induce CYP1A2 activity. However, metaxalone at the concentration of 40 μ M induced CYP1A2 activity in all three donors. The induction of CYP1A2 activity ranged from about 2- to 3.3- fold. Metaxalone at the concentration of 40 μ M slightly induced (about 21%) CYP3A4 activity in one of three donors tested. At the lower concentrations (0.4 and 4 μ M), metaxalone did not induce CYP3A4 activity in any of the donors.

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Table 1: CYP1A2 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with 50 μ M Omeprazole

Sample Identification	Acetaminophen Formation			Specific Activity (pmol/min/million cells)		Percent of VC
	Raw (μ M)	Individual	Adjusted (μ M) Mean \pm SD	Individual	Mean \pm SD	
Donor 1						
VC	0.05388	0.0539	0.0487 \pm 0.00543	0.481	0.435 \pm 0.0485	100
	0.05227	0.0523		0.467		
	0.04658	0.0466		0.416		
	0.04203	0.0420		0.375		
50 μ M Omeprazole	0.95167	0.952	0.987 \pm 0.0697	8.50	8.81 \pm 0.623	2,027
	1.07407	1.07		9.59		
	0.91389	0.914		8.16		
	1.00737	1.01		8.99		
Donor 2						
VC	0.03023	0.0302	0.0300 \pm 0.00305	0.270	0.267 \pm 0.0272	100
	0.03210	0.0321		0.287		
	0.03193	0.0319		0.285		
	0.02556	0.0256		0.228		
50 μ M Omeprazole	0.31279	0.313	0.256 \pm 0.0475	2.79	2.28 \pm 0.424	854
	0.21399	0.214		1.91		
	0.27687	0.277		2.47		
	0.21933	0.219		1.96		
Donor 3						
VC	0.04357	0.0436	0.0410 \pm 0.00447	0.389	0.366 \pm 0.0399	100
	0.04576	0.0458		0.409		
	0.03607	0.0361		0.322		
	0.03849	0.0385		0.344		
50 μ M Omeprazole	1.21524	1.22	0.932 \pm 0.192	10.9	8.33 \pm 1.72	2,276
	0.78444	0.784		7.00		
	0.87427	0.874		7.81		
	0.85595	0.856		7.64		

Abbreviations: SD, standard deviation; VC, vehicle control (1% methanol)

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Table 2: CYP3A4 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with 25 μ M Rifampin

Sample Identification	6 β -Hydroxytestosterone Formation			Specific Activity (pmol/min/million cells)		Percent of VC
	Raw (μ M)	Individual	Adjusted (μ M) Mean \pm SD	Individual	Mean \pm SD	
Donor 1						
VC	0.05693 ^a	<0.100	<0.100 \pm 0.000	<0.893	<0.893 \pm 0.000	100
	0.05726 ^a	<0.100		<0.893		
	0.05367 ^a	<0.100		<0.893		
	0.04590 ^a	<0.100		<0.893		
25 μ M Rifampin	1.22302	1.22	1.15 \pm 0.109	10.9	10.3 \pm 0.972	>1,153
	1.23746	1.24		11.0		
	1.14988	1.15		10.3		
	0.99967	1.00		8.93		
Donor 2						
VC	0.12401	0.124	<0.117 \pm 0.0141	1.11	<1.05 \pm 0.126	100
	0.13222	0.132		1.18		
	0.07973 ^a	<0.100		<0.893		
	0.11219	0.112		1.00		
25 μ M Rifampin	0.90574	0.906	0.898 \pm 0.120	8.09	8.01 \pm 1.08	>766
	1.05531	1.06		9.42		
	0.86362	0.864		7.71		
	0.76548	0.765		6.83		
Donor 3						
VC	0.06064 ^a	<0.100	<0.100 \pm 0.000	<0.893	<0.893 \pm 0.000	100
	0.05981 ^a	<0.100		<0.893		
	0.06402 ^a	<0.100		<0.893		
	0.08660 ^a	<0.100		<0.893		
25 μ M Rifampin	1.41954	1.42	1.51 \pm 0.220	12.7	13.5 \pm 1.97	>1,511
	1.83715	1.84		16.4		
	1.43319	1.43		12.8		
	1.35405	1.35		12.1		

Abbreviations: SD, standard deviation; VC, vehicle control (1% methanol)

^a The observed analyzed value (μ M) was below the lowest concentration on the standard curve (0.1 μ M).

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Table 3: CYP1A2 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with 50 μ M Omeprazole (Second Preparation)

Sample Identification	Acetaminophen Formation			Specific Activity (pmol/min/million cells)		Percent of VC
	Raw (μ M)	Individual	Adjusted (μ M) Mean \pm SD	Individual	Mean \pm SD	
Donor 1 (Second Preparation)						
VC	0.01946	0.0195	0.0187 \pm 0.00260	0.174	0.167 \pm 0.0232	100
	0.02177	0.0218		0.194		
	0.01773	0.0177		0.158		
	0.01564	0.0156		0.140		
50 μ M Omeprazole	0.19388	0.194	0.195 \pm 0.0113	1.73	1.74 \pm 0.101	1,045
	0.20277	0.203		1.81		
	0.20375	0.204		1.82		
	0.17938	0.179		1.60		
Donor 2 (Second Preparation)						
VC	0.02872	0.0287	0.0298 \pm 0.00108	0.256	0.266 \pm 0.00966	100
	0.03117	0.0312		0.278		
	0.03003	0.0300		0.268		
	0.02915	0.0292		0.260		
50 μ M Omeprazole	0.38828	0.388	0.325 \pm 0.0779	3.47	2.90 \pm 0.695	1,091
	0.37125	0.371		3.31		
	0.32416	0.324		2.89		
	0.21525	0.215		1.92		
Donor 3 (Second Preparation)						
VC	0.19864	0.199	0.183 \pm 0.0112	1.77	1.64 \pm 0.100	100
	0.17256	0.173		1.54		
	0.17818	0.178		1.59		
	0.18408	0.184		1.64		
50 μ M Omeprazole	3.13722	3.14	3.39 \pm 0.194	28.0	30.2 \pm 1.74	1,847
	3.60504	3.61		32.2		
	3.36530	3.37		30.0		
	3.44149	3.44		30.7		

Abbreviations: SD, standard deviation; VC, vehicle control (1% methanol)

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Table 4: CYP3A4 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with 25 μ M Rifampin (Second Preparation)

Sample Identification	6 β -Hydroxytestosterone Formation			Specific Activity (pmol/min/million cells)		Percent of VC
	Raw (μ M)	Individual	Adjusted (Mean \pm SD) (μ M)	Individual	Mean \pm SD	
Donor 1 (Second Preparation)						
VC	0.00000 ^a	<0.100	<0.100 \pm 0.000	<0.893	<0.893 \pm 0.000	100
	0.00000 ^a	<0.100		<0.893		
	0.00000 ^a	<0.100		<0.893		
	0.03661 ^a	<0.100		<0.893		
25 μ M Rifampin	0.10683	0.107	<0.104 \pm 0.00444	0.954	<0.927 \pm 0.0396	104
	0.10839	0.108		0.968		
	0.08994 ^a	<0.100		<0.893		
	0.08109 ^a	<0.100		<0.893		
Donor 2 (Second Preparation)						
VC	0.14737	0.147	0.144 \pm 0.0469	1.32	1.29 \pm 0.419	100
	0.11158	0.112		0.996		
	0.20923	0.209		1.87		
	0.10811	0.108		0.965		
25 μ M Rifampin	0.81654	0.817	0.847 \pm 0.162	7.29	7.56 \pm 1.45	588
	0.95081	0.951		8.49		
	0.99027	0.990		8.84		
	0.63133	0.631		5.64		
Donor 3 (Second Preparation)						
VC	0.34288	0.343	0.376 \pm 0.0590	3.06	3.36 \pm 0.527	100
	0.46316	0.463		4.14		
	0.36021	0.360		3.22		
	0.33717	0.337		3.01		
25 μ M Rifampin	7.83511	7.84	7.78 \pm 0.482	70.0	69.4 \pm 4.31	2,069
	8.14922	8.15		72.8		
	8.04003	8.04		71.8		
	7.07951	7.08		63.2		

Abbreviations: SD, standard deviation; VC, vehicle control (1% methanol)

^a The observed analyzed value (μ M) was below the lowest concentration on the standard curve (0.1 μ M).

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Table 5: CYP1A2 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with 50 μ M Omeprazole (Donor 4)

Sample Identification	Acetaminophen Formation			Specific Activity (pmol/min/million cells)		Percent of VC
	Raw (μ M)	Individual	Mean \pm SD	Individual	Mean \pm SD	
Donor 4						
VC	0.10396	0.104	0.0917 \pm 0.0131	0.928	0.818 \pm 0.117	100
	0.08076	0.0808		0.721		
	0.10194	0.102		0.910		
	0.07998	0.0800		0.714		
50 μ M Omeprazole	1.45568	1.46	1.28 \pm 0.154	13.0	11.4 \pm 1.37	1,394
	1.29021	1.29		11.5		
	1.08032	1.08		9.65		
	1.28295	1.28		11.5		

Abbreviations: SD, standard deviation; VC, vehicle control (1% methanol)

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Table 6: CYP3A4 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with 25 μ M Rifampin (Donor 4)

Sample Identification	6 β -Hydroxytestosterone Formation			Specific Activity (pmol/min/million cells)		Percent of VC
	Raw (μ M)	Individual	Mean \pm SD	Individual	Mean \pm SD	
Donor 4						
VC	0.25732	0.257	0.225 \pm 0.0376	2.30	2.00 \pm 0.336	100
	0.22842	0.228		2.04		
	0.24146	0.241		2.16		
	0.17103	0.171		1.53		
25 μ M Rifampin	7.10781	7.11	6.51 \pm 0.837	63.5	58.1 \pm 7.48	2,898
	7.18401	7.18		64.1		
	6.35636	6.36		56.8		
	5.38376	5.38		48.1		

Abbreviations: SD, standard deviation; VC, vehicle control (1% methanol)

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Table 7: CYP1A2 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with Metaxalone

Metaxalone (μM)	Acetaminophen Formation			Specific Activity (pmol/min/million cells)		Percent of VC
	Raw (μM)	Adjusted (μM)		Individual	Mean \pm SD	
Donor 1						
0 (VC)	0.05388	0.0539	0.0487 \pm 0.00543	0.481	0.435 \pm 0.0485	100
	0.05227	0.0523		0.467		
	0.04658	0.0466		0.416		
	0.04203	0.0420		0.375		
0.4	0.05121	0.0512	0.0537 \pm 0.00309	0.457	0.479 \pm 0.0276	110
	0.05264	0.0526		0.470		
	0.05714	0.0571		0.510		
4	0.07410	0.0741	0.0638 \pm 0.0193	0.662	0.570 \pm 0.172	131
	0.07581	0.0758		0.677		
	0.04160	0.0416		0.371		
40	0.15156	0.152	0.161 \pm 0.0133	1.35	1.44 \pm 0.119	332
	0.15617	0.156		1.39		
	0.17659	0.177		1.58		
Donor 2						
0 (VC)	0.03023	0.0302	0.0300 \pm 0.00305	0.270	0.267 \pm 0.0272	100
	0.03210	0.0321		0.287		
	0.03193	0.0319		0.285		
	0.02556	0.0256		0.228		
0.4	0.03165	0.0317	0.0323 \pm 0.000850	0.283	0.289 \pm 0.00759	108
	0.03208	0.0321		0.286		
	0.03329	0.0333		0.297		
4	0.03346	0.0335	0.0340 \pm 0.00198	0.299	0.304 \pm 0.0177	113
	0.03619	0.0362		0.323		
	0.03234	0.0323		0.289		
40	0.06015	0.0602	0.0589 \pm 0.00795	0.537	0.526 \pm 0.0710	197
	0.06616	0.0662		0.591		
	0.05040	0.0504		0.450		
Donor 3						
0 (VC)	0.04357	0.0436	0.0410 \pm 0.00447	0.389	0.366 \pm 0.0399	100
	0.04576	0.0458		0.409		
	0.03607	0.0361		0.322		
	0.03849	0.0385		0.344		
0.4	0.04030	0.0403	0.0438 \pm 0.00361	0.360	0.391 \pm 0.0322	107
	0.04347	0.0435		0.388		
	0.04750	0.0475		0.424		
4	0.04411	0.0441	0.0443 \pm 0.000214	0.394	0.396 \pm 0.00191	108
	0.04453	0.0445		0.398		
	0.04425	0.0443		0.395		
40	0.12276	0.123	0.122 \pm 0.00365	1.10	1.09 \pm 0.0326	297
	0.11776	0.118		1.05		
	0.12487	0.125		1.11		

Abbreviations: SD, standard deviation; VC, vehicle control (1% methanol)

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Table 8a: CYP2A6 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with Metaxalone

Metaxalone (μM)	Metabolite Formation			Specific Activity (pmol/min/million cells)		Percent of VC
	Raw (μM)	Adjusted (μM)		Individual	Mean \pm SD	
7-Hydroxycoumarin (7-HC) Formation: Donor 1						
0 (VC)	0.00000 ^a	<0.100	<0.100 \pm 0.000	<0.893	<0.893 \pm 0.000	100
	0.00000 ^a	<0.100		<0.893		
	0.00000 ^a	<0.100		<0.893		
	0.00000 ^a	<0.100		<0.893		
0.4	0.00000 ^a	<0.100	<0.100 \pm 0.000	<0.893	<0.893 \pm 0.000	100
	0.00000 ^a	<0.100		<0.893		
	0.00000 ^a	<0.100		<0.893		
4	0.00000 ^a	<0.100	<0.100 \pm 0.000	<0.893	<0.893 \pm 0.000	100
	0.00000 ^a	<0.100		<0.893		
	0.00000 ^a	<0.100		<0.893		
40	0.00000 ^a	<0.100	<0.100 \pm 0.000	<0.893	<0.893 \pm 0.000	100
	0.00000 ^a	<0.100		<0.893		
	0.00000 ^a	<0.100		<0.893		
7-Hydroxycoumarin (7-HC) Formation: Donor 2						
0 (VC)	0.00000 ^a	<0.100	<0.100 \pm 0.000	<0.893	<0.893 \pm 0.000	100
	0.00000 ^a	<0.100		<0.893		
	0.00000 ^a	<0.100		<0.893		
	0.00000 ^a	<0.100		<0.893		
0.4	0.00000 ^a	<0.100	<0.100 \pm 0.000	<0.893	<0.893 \pm 0.000	100
	0.00000 ^a	<0.100		<0.893		
	0.00000 ^a	<0.100		<0.893		
4	0.00000 ^a	<0.100	<0.100 \pm 0.000	<0.893	<0.893 \pm 0.000	100
	0.00000 ^a	<0.100		<0.893		
	0.00000 ^a	<0.100		<0.893		
40	0.00000 ^a	<0.100	<0.100 \pm 0.000	<0.893	<0.893 \pm 0.000	100
	0.00000 ^a	<0.100		<0.893		
	0.00000 ^a	<0.100		<0.893		
7-Hydroxycoumarin (7-HC) Formation: Donor 3						
0 (VC)	0.00000 ^a	<0.100	<0.100 \pm 0.000	<0.893	<0.893 \pm 0.000	100
	0.00000 ^a	<0.100		<0.893		
	0.00000 ^a	<0.100		<0.893		
	0.00000 ^a	<0.100		<0.893		
0.4	0.00000 ^a	<0.100	<0.100 \pm 0.000	<0.893	<0.893 \pm 0.000	100
	0.00000 ^a	<0.100		<0.893		
	0.00000 ^a	<0.100		<0.893		
4	0.00000 ^a	<0.100	<0.100 \pm 0.000	<0.893	<0.893 \pm 0.000	100
	0.00000 ^a	<0.100		<0.893		
	0.00000 ^a	<0.100		<0.893		
40	0.00000 ^a	<0.100	<0.100 \pm 0.000	<0.893	<0.893 \pm 0.000	100
	0.00000 ^a	<0.100		<0.893		
	0.00000 ^a	<0.100		<0.893		

Abbreviations: SD, standard deviation; VC, vehicle control (1% methanol)

^a The observed analyzed value (μM) was below the lowest concentration on the standard curve (0.1 μM).

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Table 8b: CYP2A6 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with Metaxalone

Metaxalone (μM)	Metabolite Formation			Specific Activity (pmol/min/million cells)		Percent of VC
	Raw (μM)	Adjusted (μM)		Individual	Mean \pm SD	
7-Hydroxycoumarin Glucuronide (7-HCG) Formation: Donor 1						
0 (VC)	0.01705 ^b	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.00000 ^b	<0.0500		<0.446		
	0.00000 ^b	<0.0500		<0.446		
	0.00000 ^b	<0.0500		<0.446		
0.4	0.00000 ^b	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.00000 ^b	<0.0500		<0.446		
	0.00000 ^b	<0.0500		<0.446		
4	0.00000 ^b	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.00000 ^b	<0.0500		<0.446		
	0.00000 ^b	<0.0500		<0.446		
40	0.00000 ^b	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.00000 ^b	<0.0500		<0.446		
	0.00000 ^b	<0.0500		<0.446		
7-Hydroxycoumarin Glucuronide (7-HCG) Formation: Donor 2						
0 (VC)	0.03808 ^b	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.04134 ^b	<0.0500		<0.446		
	0.03654 ^b	<0.0500		<0.446		
	0.03196 ^b	<0.0500		<0.446		
0.4	0.02245 ^b	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.03808 ^b	<0.0500		<0.446		
	0.03808 ^b	<0.0500		<0.446		
4	0.03437 ^b	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.03526 ^b	<0.0500		<0.446		
	0.02966 ^b	<0.0500		<0.446		
40	0.02926 ^b	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.02664 ^b	<0.0500		<0.446		
	0.03334 ^b	<0.0500		<0.446		
7-Hydroxycoumarin Glucuronide (7-HCG) Formation: Donor 3						
0 (VC)	0.00000 ^b	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.01964 ^b	<0.0500		<0.446		
	0.02373 ^b	<0.0500		<0.446		
	0.00000 ^b	<0.0500		<0.446		
0.4	0.02156 ^b	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.01820 ^b	<0.0500		<0.446		
	0.01816 ^b	<0.0500		<0.446		
4	0.00000 ^b	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.01965 ^b	<0.0500		<0.446		
	0.01624 ^b	<0.0500		<0.446		
40	0.00000 ^b	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.01883 ^b	<0.0500		<0.446		
	0.00000 ^b	<0.0500		<0.446		

Abbreviations: SD, standard deviation; VC, vehicle control (1% methanol)

^b The observed analyzed value (μM) was below the lowest concentration on the standard curve (0.05 μM).

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Table 8c: CYP2A6 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with Metaxalone

Metaxalone (μM)	Metabolite Formation			Specific Activity (pmol/min/million cells)		Percent of VC
	Raw (μM)	Adjusted (μM)		Individual	Mean \pm SD	
7-Hydroxycoumarin Sulfate (7-HCS) Formation: Donor 1						
0 (VC)	0.00000 ^c	<0.150	<0.150 \pm 0.000	<1.34	<1.34 \pm 0.000	100
	0.00000 ^c	<0.150		<1.34		
	0.00000 ^c	<0.150		<1.34		
	0.00000 ^c	<0.150		<1.34		
0.4	0.00000 ^c	<0.150	<0.150 \pm 0.000	<1.34	<1.34 \pm 0.000	100
	0.00000 ^c	<0.150		<1.34		
	0.00000 ^c	<0.150		<1.34		
4	0.00000 ^c	<0.150	<0.150 \pm 0.000	<1.34	<1.34 \pm 0.000	100
	0.00000 ^c	<0.150		<1.34		
	0.00000 ^c	<0.150		<1.34		
40	0.00000 ^c	<0.150	<0.150 \pm 0.000	<1.34	<1.34 \pm 0.000	100
	0.00000 ^c	<0.150		<1.34		
	0.00000 ^c	<0.150		<1.34		
7-Hydroxycoumarin Sulfate (7-HCS) Formation: Donor 2						
0 (VC)	0.00000 ^c	<0.150	<0.150 \pm 0.000	<1.34	<1.34 \pm 0.000	100
	0.00000 ^c	<0.150		<1.34		
	0.00000 ^c	<0.150		<1.34		
	0.00000 ^c	<0.150		<1.34		
0.4	0.00000 ^c	<0.150	<0.150 \pm 0.000	<1.34	<1.34 \pm 0.000	100
	0.00000 ^c	<0.150		<1.34		
	0.00000 ^c	<0.150		<1.34		
4	0.00000 ^c	<0.150	<0.150 \pm 0.000	<1.34	<1.34 \pm 0.000	100
	0.00000 ^c	<0.150		<1.34		
	0.00000 ^c	<0.150		<1.34		
40	0.00000 ^c	<0.150	<0.150 \pm 0.000	<1.34	<1.34 \pm 0.000	100
	0.00000 ^c	<0.150		<1.34		
	0.00000 ^c	<0.150		<1.34		
7-Hydroxycoumarin Sulfate (7-HCS) Formation: Donor 3						
0 (VC)	0.00000 ^c	<0.150	<0.150 \pm 0.000	<1.34	<1.34 \pm 0.000	100
	0.00000 ^c	<0.150		<1.34		
	0.00000 ^c	<0.150		<1.34		
	0.00000 ^c	<0.150		<1.34		
0.4	0.00000 ^c	<0.150	<0.150 \pm 0.000	<1.34	<1.34 \pm 0.000	100
	0.00000 ^c	<0.150		<1.34		
	0.00000 ^c	<0.150		<1.34		
4	0.00000 ^c	<0.150	<0.150 \pm 0.000	<1.34	<1.34 \pm 0.000	100
	0.00000 ^c	<0.150		<1.34		
	0.00000 ^c	<0.150		<1.34		
40	0.00000 ^c	<0.150	<0.150 \pm 0.000	<1.34	<1.34 \pm 0.000	100
	0.00000 ^c	<0.150		<1.34		
	0.00000 ^c	<0.150		<1.34		

Abbreviations: SD, standard deviation; VC, vehicle control (1% methanol)

^c The observed analyzed value (μM) was below the lowest concentration on the standard curve (0.15 μM).

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Table 8d: CYP2A6 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with Metaxalone

Metaxalone (μM)	Metabolite Formation			Specific Activity (pmol/min/million cells)		Percent of VC
	Raw (μM)	Adjusted (μM)		Individual	Mean \pm SD	
Total Metabolite Formation: Donor 1						
0 (VC)	0.0171 ^d	<0.300	<0.300 \pm 0.000	<2.68	<2.68 \pm 0.000	100
	0.000 ^d	<0.300		<2.68		
	0.000 ^d	<0.300		<2.68		
	0.000 ^d	<0.300		<2.68		
0.4	0.000 ^d	<0.300	<0.300 \pm 0.000	<2.68	<2.68 \pm 0.000	100
	0.000 ^d	<0.300		<2.68		
	0.000 ^d	<0.300		<2.68		
4	0.000 ^d	<0.300	<0.300 \pm 0.000	<2.68	<2.68 \pm 0.000	100
	0.000 ^d	<0.300		<2.68		
	0.000 ^d	<0.300		<2.68		
40	0.000 ^d	<0.300	<0.300 \pm 0.000	<2.68	<2.68 \pm 0.000	100
	0.000 ^d	<0.300		<2.68		
	0.000 ^d	<0.300		<2.68		
Total Metabolite Formation: Donor 2						
0 (VC)	0.0381 ^d	<0.300	<0.300 \pm 0.000	<2.68	<2.68 \pm 0.000	100
	0.0413 ^d	<0.300		<2.68		
	0.0365 ^d	<0.300		<2.68		
	0.0320 ^d	<0.300		<2.68		
0.4	0.0225 ^d	<0.300	<0.300 \pm 0.000	<2.68	<2.68 \pm 0.000	100
	0.0381 ^d	<0.300		<2.68		
	0.0381 ^d	<0.300		<2.68		
4	0.0344 ^d	<0.300	<0.300 \pm 0.000	<2.68	<2.68 \pm 0.000	100
	0.0353 ^d	<0.300		<2.68		
	0.0297 ^d	<0.300		<2.68		
40	0.0293 ^d	<0.300	<0.300 \pm 0.000	<2.68	<2.68 \pm 0.000	100
	0.0266 ^d	<0.300		<2.68		
	0.0333 ^d	<0.300		<2.68		
Total Metabolite Formation: Donor 3						
0 (VC)	0.000 ^d	<0.300	<0.300 \pm 0.000	<2.68	<2.68 \pm 0.000	100
	0.0196 ^d	<0.300		<2.68		
	0.0237 ^d	<0.300		<2.68		
	0.000 ^d	<0.300		<2.68		
0.4	0.0216 ^d	<0.300	<0.300 \pm 0.000	<2.68	<2.68 \pm 0.000	100
	0.0182 ^d	<0.300		<2.68		
	0.0182 ^d	<0.300		<2.68		
4	0.000 ^d	<0.300	<0.300 \pm 0.000	<2.68	<2.68 \pm 0.000	100
	0.0197 ^d	<0.300		<2.68		
	0.0162 ^d	<0.300		<2.68		
40	0.000 ^d	<0.300	<0.300 \pm 0.000	<2.68	<2.68 \pm 0.000	100
	0.0188 ^d	<0.300		<2.68		
	0.000 ^d	<0.300		<2.68		

Abbreviations: SD, standard deviation; VC, vehicle control (1% methanol)

^d The observed analyzed value (μM) for all metabolites was below the lowest concentration on the corresponding standard curve.

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Table 9a: CYP2B6 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with Metaxalone

Metaxalone (μM)	Nirvanol Formation			Specific Activity (pmol/min/million cells)		Percent of VC
	Raw (μM)	Adjusted (μM)		Individual	Mean \pm SD	
Donor 1						
0 (VC)	0.03230	0.0323	0.0319 \pm 0.00156	0.288	0.285 \pm 0.0139	100
	0.03384	0.0338		0.302		
	0.03014	0.0301		0.269		
	0.03141	0.0314		0.280		
0.4	0.03380	0.0338	0.0340 \pm 0.000883	0.302	0.304 \pm 0.00789	107
	0.03329	0.0333		0.297		
	0.03501	0.0350		0.313		
4	0.02742	0.0274	0.0305 \pm 0.00272	0.245	0.273 \pm 0.0243	95.7
	0.03241	0.0324		0.289		
	0.03178	0.0318		0.284		
40	0.03233	0.0323	0.0310 \pm 0.00204	0.289	0.277 \pm 0.0182	97.1
	0.03203	0.0320		0.286		
	0.02866	0.0287		0.256		
Donor 2						
0 (VC)	0.02927	0.0293	0.0289 \pm 0.00230	0.261	0.258 \pm 0.0205	100
	0.02920	0.0292		0.261		
	0.03137	0.0314		0.280		
	0.02582	0.0258		0.231		
0.4	0.02544	0.0254	0.0306 \pm 0.00559	0.227	0.273 \pm 0.0499	106
	0.02986	0.0299		0.267		
	0.03654	0.0365		0.326		
4	0.02852	0.0285	0.0281 \pm 0.000884	0.255	0.250 \pm 0.00790	97.0
	0.02703	0.0270		0.241		
	0.02860	0.0286		0.255		
40	0.00341 ^a	<0.0250	<0.0250 \pm 0.000	<0.223	<0.223 \pm 0.000	<86.5
	0.00320 ^a	<0.0250		<0.223		
	0.00330 ^a	<0.0250		<0.223		
Donor 3						
0 (VC)	0.02349 ^a	<0.0250	<0.0252 \pm 0.000435	<0.223	<0.225 \pm 0.00388	100
	0.02587	0.0259		0.231		
	0.02376 ^a	<0.0250		<0.223		
	0.02236 ^a	<0.0250		<0.223		
0.4	0.02177 ^a	<0.0250	<0.0250 \pm 0.000	<0.223	<0.223 \pm 0.000	99.1
	0.02343 ^a	<0.0250		<0.223		
	0.02326 ^a	<0.0250		<0.223		
4	0.02392 ^a	<0.0250	<0.0250 \pm 0.000	<0.223	<0.223 \pm 0.000	99.1
	0.02490 ^a	<0.0250		<0.223		
	0.02229 ^a	<0.0250		<0.223		
40	0.02005 ^a	<0.0250	<0.0250 \pm 0.000	<0.223	<0.223 \pm 0.000	99.1
	0.01976 ^a	<0.0250		<0.223		
	0.02169 ^a	<0.0250		<0.223		

Abbreviations: SD, standard deviation; VC, vehicle control (1% methanol)

^a The observed analyzed value (μM) was below the lowest concentration on the standard curve (0.025 μM).

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Table 9b: CYP2B6 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with 1 mM Phenobarbital

Sample Identification	Nirvanol Formation			Specific Activity (pmol/min/million cells)		Percent of VC
	Raw (μM)	Individual	Adjusted (μM) Mean \pm SD	Individual	Mean \pm SD	
Donor 1						
VC	0.03230	0.0323	0.0319 \pm 0.00156	0.288	0.285 \pm 0.0139	100
	0.03384	0.0338		0.302		
	0.03014	0.0301		0.269		
	0.03141	0.0314		0.280		
1 mM Phenobarbital	0.10875	0.109	0.107 \pm 0.00957	0.971	0.955 \pm 0.0854	335
	0.11143	0.111		0.995		
	0.11462	0.115		1.02		
	0.09308	0.0931		0.831		
Donor 2						
VC	0.02927	0.0293	0.0289 \pm 0.00230	0.261	0.258 \pm 0.0205	100
	0.02920	0.0292		0.261		
	0.03137	0.0314		0.280		
	0.02582	0.0258		0.231		
1 mM Phenobarbital	0.04920	0.0492	0.0461 \pm 0.00398	0.439	0.411 \pm 0.0355	159
	0.04148	0.0415		0.370		
	0.04957	0.0496		0.443		
	0.04398	0.0440		0.393		
Donor 3						
VC	0.02349 ^a	<0.0250	<0.0252 \pm 0.000435	<0.223	<0.225 \pm 0.00388	100
	0.02587	0.0259		0.231		
	0.02376 ^a	<0.0250		<0.223		
	0.02236 ^a	<0.0250		<0.223		
1 mM Phenobarbital	0.08499	0.0850	0.0775 \pm 0.00718	0.759	0.692 \pm 0.0641	>307
	0.07505	0.0751		0.670		
	0.08123	0.0812		0.725		
	0.06864	0.0686		0.613		

Abbreviations: SD, standard deviation; VC, vehicle control (1% methanol)

^a The observed analyzed value (μM) was below the lowest concentration on the standard curve (0.025 μM).

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Table 10a: CYP2C8 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with Metaxalone (Additional Preparations)

Metaxalone (μM)	6-Hydroxypaclitaxel Formation			Specific Activity (pmol/min/million cells)		Percent of VC
	Raw (μM)	Adjusted (μM)		Individual	Mean \pm SD	
Donor 4						
0 (VC)	0.08285	0.0829	0.0921 \pm 0.0161	0.740	0.822 \pm 0.144	100
	0.08009	0.0801		0.715		
	0.11542	0.115		1.03		
	0.08994	0.0899		0.803		
0.4	0.10107	0.101	0.111 \pm 0.00917	0.902	0.993 \pm 0.0819	121
	0.11888	0.119		1.06		
	0.11380	0.114		1.02		
4	0.14559	0.146	0.112 \pm 0.0295	1.30	1.00 \pm 0.263	122
	0.10099	0.101		0.902		
	0.08983	0.0898		0.802		
40	0.09865	0.0987	0.0997 \pm 0.0199	0.881	0.890 \pm 0.177	108
	0.08039	0.0804		0.718		
	0.12010	0.120		1.07		
Donor 2 (Second Preparation)						
0 (VC)	0.06388	0.0639	0.0794 \pm 0.0130	0.570	0.709 \pm 0.116	100
	0.09357	0.0936		0.835		
	0.07434	0.0743		0.664		
	0.08562	0.0856		0.764		
0.4	0.07293	0.0729	0.0815 \pm 0.00865	0.651	0.728 \pm 0.0772	103
	0.09023	0.0902		0.806		
	0.08141	0.0814		0.727		
4	0.07943	0.0794	0.0794 \pm 0.00668	0.709	0.709 \pm 0.0596	100
	0.08606	0.0861		0.768		
	0.07270	0.0727		0.649		
40	0.07115	0.0712	0.0745 \pm 0.00292	0.635	0.665 \pm 0.0261	93.9
	0.07602	0.0760		0.679		
	0.07637	0.0764		0.682		
Donor 3 (Second Preparation)						
0 (VC)	0.08170	0.0817	0.104 \pm 0.0171	0.729	0.928 \pm 0.152	100
	0.12268	0.123		1.10		
	0.10896	0.109		0.973		
	0.10226	0.102		0.913		
0.4	0.09108	0.0911	0.105 \pm 0.0131	0.813	0.934 \pm 0.117	101
	0.11734	0.117		1.05		
	0.10528	0.105		0.940		
4	0.10611	0.106	0.104 \pm 0.0109	0.947	0.929 \pm 0.0969	100
	0.09227	0.0923		0.824		
	0.11368	0.114		1.02		
40	0.08248	0.0825	0.0875 \pm 0.0188	0.736	0.78 \pm 0.167	84.2
	0.10821	0.108		0.966		
	0.07170	0.0717		0.640		

Abbreviations: SD, standard deviation; VC, vehicle control (1% methanol)

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Table 10b: CYP2C8 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with 25 μ M Rifampin (Additional Preparations)

Sample Identification	6-Hydroxypaclitaxel Formation			Specific Activity (pmol/min/million cells)		Percent of VC
	Raw (μ M)	Individual	Adjusted (μ M) Mean \pm SD	Individual	Mean \pm SD	
Donor 4						
VC	0.08285	0.0829	0.0921 \pm 0.0161	0.740	0.822 \pm 0.144	100
	0.08009	0.0801		0.715		
	0.11542	0.115		1.03		
	0.08994	0.0899		0.803		
25 μ M Rifampin	0.18993	0.190	0.239 \pm 0.0566	1.70	2.14 \pm 0.505	260
	0.23044	0.230		2.06		
	0.21642	0.216		1.93		
	0.32028	0.320		2.86		
Donor 2 (Second Preparation)						
VC	0.06388	0.0639	0.0794 \pm 0.0130	0.570	0.709 \pm 0.116	100
	0.09357	0.0936		0.835		
	0.07434	0.0743		0.664		
	0.08562	0.0856		0.764		
25 μ M Rifampin	0.07811	0.0781	0.0784 \pm 0.00304	0.697	0.700 \pm 0.0271	98.8
	0.08002	0.0800		0.714		
	0.08120	0.0812		0.725		
	0.07426	0.0743		0.663		
Donor 3 (Second Preparation)						
VC	0.08170	0.0817	0.104 \pm 0.0171	0.729	0.928 \pm 0.152	100
	0.12268	0.123		1.10		
	0.10896	0.109		0.973		
	0.10226	0.102		0.913		
25 μ M Rifampin	0.13762	0.138	0.162 \pm 0.0178	1.23	1.45 \pm 0.159	156
	0.16532	0.165		1.48		
	0.16664	0.167		1.49		
	0.17996	0.180		1.61		

Abbreviations: SD, standard deviation; VC, vehicle control (1% methanol)

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Table 11a: CYP2C9 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with Metaxalone

Metaxalone (μM)	4'-Methylhydroxytolbutamide Formation			Specific Activity (pmol/min/million cells)		Percent of VC
	Raw (μM)	Individual	Adjusted (μM) Mean \pm SD	Individual	Mean \pm SD	
Donor 1						
0 (VC)	0.01215	0.0122	0.0137 \pm 0.00161	0.108	0.122 \pm 0.0144	100
	0.01502	0.0150		0.134		
	0.01513	0.0151		0.135		
	0.01245	0.0125		0.111		
0.4	0.01557	0.0156	0.0147 \pm 0.000753	0.139	0.132 \pm 0.00672	108
	0.01410	0.0141		0.126		
	0.01455	0.0146		0.130		
4	0.01331	0.0133	0.0137 \pm 0.00136	0.119	0.122 \pm 0.0121	100
	0.01523	0.0152		0.136		
	0.01261	0.0126		0.113		
40	0.00931 ^a	<0.0100	<0.0100 \pm 0.0000346	<0.0893	<0.0895 \pm 0.000309	<73.2
	0.00952 ^a	<0.0100		<0.0893		
	0.01006	0.0101		0.0898		
Donor 2						
0 (VC)	0.05192	0.0519	0.0491 \pm 0.00479	0.464	0.438 \pm 0.0428	100
	0.04864	0.0486		0.434		
	0.05325	0.0533		0.475		
	0.04250	0.0425		0.379		
0.4	0.04819	0.0482	0.0474 \pm 0.00223	0.430	0.423 \pm 0.0200	96.6
	0.04489	0.0449		0.401		
	0.04915	0.0492		0.439		
4	0.04634	0.0463	0.0456 \pm 0.000864	0.414	0.407 \pm 0.00772	92.9
	0.04581	0.0458		0.409		
	0.04465	0.0447		0.399		
40	0.02917	0.0292	0.0296 \pm 0.000651	0.260	0.265 \pm 0.00581	60.4
	0.02936	0.0294		0.262		
	0.03038	0.0304		0.271		
Donor 3						
0 (VC)	0.02021	0.0202	0.0181 \pm 0.00206	0.180	0.162 \pm 0.0184	100
	0.01700	0.0170		0.152		
	0.01952	0.0195		0.174		
	0.01586	0.0159		0.142		
0.4	0.02067	0.0207	0.0201 \pm 0.00125	0.185	0.179 \pm 0.0111	111
	0.02096	0.0210		0.187		
	0.01867	0.0187		0.167		
4	0.01807	0.0181	0.0187 \pm 0.00235	0.161	0.167 \pm 0.0210	103
	0.02129	0.0213		0.190		
	0.01671	0.0167		0.149		
40	0.01364	0.0136	0.0142 \pm 0.000560	0.122	0.127 \pm 0.00500	78.4
	0.01432	0.0143		0.128		
	0.01475	0.0148		0.132		

Abbreviations: SD, standard deviation; VC, vehicle control (1% methanol)

^a The observed analyzed value (μM) was below the lowest concentration on the standard curve (0.01 μM).

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Table 11b: CYP2C9 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with 25 μ M Rifampin

Sample Identification	4'-Methylhydroxytolbutamide Formation			Specific Activity (pmol/min/million cells)		Percent of VC
	Raw (μ M)	Individual	Adjusted (μ M) Mean \pm SD	Individual	Mean \pm SD	
Donor 1						
VC	0.01215	0.0122	0.0137 \pm 0.00161	0.108	0.122 \pm 0.0144	100
	0.01502	0.0150		0.134		
	0.01513	0.0151		0.135		
	0.01245	0.0125		0.111		
25 μ M Rifampin	0.02792	0.0279	0.0291 \pm 0.00481	0.249	0.260 \pm 0.0429	213
	0.03387	0.0339		0.302		
	0.03168	0.0317		0.283		
	0.02289	0.0229		0.204		
Donor 2						
VC	0.05192	0.0519	0.0491 \pm 0.00479	0.464	0.438 \pm 0.0428	100
	0.04864	0.0486		0.434		
	0.05325	0.0533		0.475		
	0.04250	0.0425		0.379		
25 μ M Rifampin	0.05908	0.0591	0.0608 \pm 0.00254	0.528	0.543 \pm 0.0227	124
	0.06386	0.0639		0.570		
	0.06191	0.0619		0.553		
	0.05839	0.0584		0.521		
Donor 3						
VC	0.02021	0.0202	0.0181 \pm 0.00206	0.180	0.162 \pm 0.0184	100
	0.01700	0.0170		0.152		
	0.01952	0.0195		0.174		
	0.01586	0.0159		0.142		
25 μ M Rifampin	0.03543	0.0354	0.0364 \pm 0.00192	0.316	0.325 \pm 0.0172	201
	0.03771	0.0377		0.337		
	0.03829	0.0383		0.342		
	0.03419	0.0342		0.305		

Abbreviations: SD, standard deviation; VC, vehicle control (1% methanol)

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Table 12a: CYP2C19 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with Metaxalone

Metaxalone (μM)	4'-Hydroxymephenytoin Formation			Specific Activity (pmol/min/million cells)		Percent of VC
	Raw (μM)	Individual	Mean \pm SD	Individual	Mean \pm SD	
Donor 1						
0 (VC)	0.00025 ^a	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.00058 ^a	<0.0500		<0.446		
	0.00114 ^a	<0.0500		<0.446		
	0.00058 ^a	<0.0500		<0.446		
0.4	0.00708 ^a	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.01319 ^a	<0.0500		<0.446		
	0.01861 ^a	<0.0500		<0.446		
4	0.01649 ^a	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.00029 ^a	<0.0500		<0.446		
	0.00064 ^a	<0.0500		<0.446		
40	0.00057 ^a	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.00031 ^a	<0.0500		<0.446		
	0.00037 ^a	<0.0500		<0.446		
Donor 2						
0 (VC)	N/A*	N/A	<0.0500 \pm N/A	N/A	<0.446 \pm N/A	100
	0.01146 ^a	<0.0500		<0.446		
	0.01456 ^a	<0.0500		<0.446		
	N/A*	N/A		N/A		
0.4	0.00765 ^a	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.00779 ^a	<0.0500		<0.446		
	0.00808 ^a	<0.0500		<0.446		
4	0.00775 ^a	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.00744 ^a	<0.0500		<0.446		
	0.00773 ^a	<0.0500		<0.446		
40	0.00697 ^a	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.00840 ^a	<0.0500		<0.446		
	0.00790 ^a	<0.0500		<0.446		
Donor 3						
0 (VC)	0.00026 ^a	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.00000 ^a	<0.0500		<0.446		
	0.00000 ^a	<0.0500		<0.446		
	0.00000 ^a	<0.0500		<0.446		
0.4	0.00000 ^a	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.00000 ^a	<0.0500		<0.446		
	0.00023 ^a	<0.0500		<0.446		
4	0.00000 ^a	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.00000 ^a	<0.0500		<0.446		
	0.00000 ^a	<0.0500		<0.446		
40	0.00191 ^a	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.00000 ^a	<0.0500		<0.446		
	0.00000 ^a	<0.0500		<0.446		

Abbreviations: SD, standard deviation; VC, vehicle control (1% methanol), N/A, not applicable

^a The observed analyzed value (μM) was below the lowest concentration on the standard curve (0.05 μM).

* Samples were lost during analysis and therefore could not be analyzed.

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Table 12b: CYP2C19 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with 25 μ M Rifampin

Sample Identification	4'-Hydroxymephenytoin Formation			Specific Activity (pmol/min/million cells)		Percent of VC
	Raw (μ M)	Individual	Adjusted (μ M) Mean \pm SD	Individual	Mean \pm SD	
Donor 1						
VC	0.00025 ^a	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.00058 ^a	<0.0500		<0.446		
	0.00114 ^a	<0.0500		<0.446		
	0.00058 ^a	<0.0500		<0.446		
25 μ M Rifampin	0.00708 ^a	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.01319 ^a	<0.0500		<0.446		
	0.01861 ^a	<0.0500		<0.446		
	0.01649 ^a	<0.0500		<0.446		
Donor 2						
VC	N/A*	N/A	<0.0500 \pm N/A	N/A	<0.446 \pm N/A	100
	0.01146 ^a	<0.0500		<0.446		
	0.01456 ^a	<0.0500		<0.446		
	N/A*	N/A		N/A		
25 μ M Rifampin	0.00986 ^a	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.01018 ^a	<0.0500		<0.446		
	0.01272 ^a	<0.0500		<0.446		
	0.00915 ^a	<0.0500		<0.446		
Donor 3						
VC	0.00026 ^a	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.00000 ^a	<0.0500		<0.446		
	0.00000 ^a	<0.0500		<0.446		
	0.00000 ^a	<0.0500		<0.446		
25 μ M Rifampin	0.00142 ^a	<0.0500	<0.0500 \pm 0.000	<0.446	<0.446 \pm 0.000	100
	0.00191 ^a	<0.0500		<0.446		
	0.00119 ^a	<0.0500		<0.446		
	0.00018 ^a	<0.0500		<0.446		

Abbreviations: SD, standard deviation; VC, vehicle control (1% methanol)

^a The observed analyzed value (μ M) was below the lowest concentration on the standard curve (0.05 μ M).

* Samples were lost during analysis and therefore could not be analyzed.

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Table 13: CYP2D6 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with Metaxalone

Metaxalone (μM)	Dextrorphan Formation			Specific Activity (pmol/min/million cells)		Percent of VC
	Raw (μM)	Adjusted (μM)		Individual	Mean \pm SD	
Donor 1						
0 (VC)	0.00772 ^a	<0.0100	<0.0100 \pm 0.000	<0.0893	<0.0893 \pm 0.000	100
	0.00796 ^a	<0.0100		<0.0893		
	0.00736 ^a	<0.0100		<0.0893		
	0.00724 ^a	<0.0100		<0.0893		
0.4	0.00809 ^a	<0.0100	<0.0100 \pm 0.000	<0.0893	<0.0893 \pm 0.000	100
	0.00749 ^a	<0.0100		<0.0893		
	0.00853 ^a	<0.0100		<0.0893		
4	0.00832 ^a	<0.0100	<0.0100 \pm 0.000	<0.0893	<0.0893 \pm 0.000	100
	0.00721 ^a	<0.0100		<0.0893		
	0.00744 ^a	<0.0100		<0.0893		
40	0.00398 ^a	<0.0100	<0.0100 \pm 0.000	<0.0893	<0.0893 \pm 0.000	100
	0.00205 ^a	<0.0100		<0.0893		
	0.00520 ^a	<0.0100		<0.0893		
Donor 2						
0 (VC)	0.01286	0.0129	0.0139 \pm 0.00152	0.115	0.124 \pm 0.0136	100
	0.01432	0.0143		0.128		
	0.01581	0.0158		0.141		
	0.01247	0.0125		0.111		
0.4	0.01302	0.0130	0.0133 \pm 0.000485	0.116	0.119 \pm 0.00433	95.9
	0.01302	0.0130		0.116		
	0.01386	0.0139		0.124		
4	0.01361	0.0136	0.0143 \pm 0.000589	0.122	0.128 \pm 0.00526	103
	0.01468	0.0147		0.131		
	0.01457	0.0146		0.130		
40	0.00998 ^a	<0.0100	<0.0102 \pm 0.000260	<0.0893	<0.0906 \pm 0.00232	<73.2
	0.00956 ^a	<0.0100		<0.0893		
	0.01045	0.0105		0.0933		
Donor 3						
0 (VC)	0.07011	0.0701	0.0665 \pm 0.00607	0.626	0.594 \pm 0.0542	100
	0.05856	0.0586		0.523		
	0.07219	0.0722		0.645		
	0.06505	0.0651		0.581		
0.4	0.06218	0.0622	0.0657 \pm 0.00305	0.555	0.586 \pm 0.0272	98.8
	0.06688	0.0669		0.597		
	0.06789	0.0679		0.606		
4	0.06071	0.0607	0.0597 \pm 0.00164	0.542	0.533 \pm 0.0146	89.8
	0.06060	0.0606		0.541		
	0.05782	0.0578		0.516		
40	0.05087	0.0509	0.0489 \pm 0.00347	0.454	0.436 \pm 0.0310	73.5
	0.05088	0.0509		0.454		
	0.04486	0.0449		0.401		

Abbreviations: SD, standard deviation; VC, vehicle control (1% methanol)

^a The observed analyzed value (μM) was below the lowest concentration on the standard curve (0.01 μM).

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Table 14: CYP2E1 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with Metaxalone

Metaxalone (μM)	6-Hydroxychlorzoxazone Formation			Specific Activity (pmol/min/million cells)		Percent of VC
	Raw (μM)	Adjusted (μM)		Individual	Mean \pm SD	
Donor 1						
0 (VC)	0.28067	0.281	0.283 \pm 0.00460	2.51	2.53 \pm 0.0411	100
	0.28793	0.288		2.57		
	0.28627	0.286		2.56		
	0.27817	0.278		2.48		
0.4	0.28854	0.289	0.277 \pm 0.0279	2.58	2.47 \pm 0.249	97.8
	0.29749	0.297		2.66		
	0.24529	0.245		2.19		
4	0.28784	0.288	0.295 \pm 0.0236	2.57	2.64 \pm 0.210	104
	0.27623	0.276		2.47		
	0.32160	0.322		2.87		
40	0.28453	0.285	0.294 \pm 0.00876	2.54	2.63 \pm 0.0782	104
	0.29753	0.298		2.66		
	0.30121	0.301		2.69		
Donor 2						
0 (VC)	0.07385	0.0739	0.0748 \pm 0.00211	0.659	0.668 \pm 0.0188	100
	0.07610	0.0761		0.679		
	0.07690	0.0769		0.687		
	0.07229	0.0723		0.645		
0.4	0.07071	0.0707	0.0776 \pm 0.00753	0.631	0.693 \pm 0.0673	104
	0.07649	0.0765		0.683		
	0.08565	0.0857		0.765		
4	0.06315	0.0632	0.0670 \pm 0.00355	0.564	0.598 \pm 0.0317	89.6
	0.06775	0.0678		0.605		
	0.07013	0.0701		0.626		
40	0.06247	0.0625	0.0745 \pm 0.0141	0.558	0.665 \pm 0.126	99.6
	0.07091	0.0709		0.633		
	0.09003	0.0900		0.804		
Donor 3						
0 (VC)	0.05899	0.0590	0.0570 \pm 0.00420	0.527	0.509 \pm 0.0375	100
	0.06077	0.0608		0.543		
	0.05718	0.0572		0.511		
	0.05110	0.0511		0.456		
0.4	0.05031	0.0503	0.0517 \pm 0.00140	0.449	0.462 \pm 0.0125	90.7
	0.05310	0.0531		0.474		
	0.05169	0.0517		0.462		
4	0.05245	0.0525	0.0500 \pm 0.00389	0.468	0.446 \pm 0.0348	87.7
	0.05202	0.0520		0.464		
	0.04550	0.0455		0.406		
40	0.05260	0.0526	0.0535 \pm 0.00164	0.470	0.478 \pm 0.0146	93.9
	0.05541	0.0554		0.495		
	0.05254	0.0525		0.469		

Abbreviations: SD, standard deviation; VC, vehicle control (1% methanol)

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Table 15: CYP3A4 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with Metaxalone

Metaxalone (μM)	6 β -Hydroxytestosterone Formation			Specific Activity (pmol/min/million cells)		Percent of VC
	Raw (μM)	Adjusted (μM)		Individual	Mean \pm SD	
Donor 1						
0 (VC)	0.05693 ^a	<0.100	<0.100 \pm 0.000	<0.893	<0.893 \pm 0.000	100
	0.05726 ^a	<0.100		<0.893		
	0.05367 ^a	<0.100		<0.893		
	0.04590 ^a	<0.100		<0.893		
0.4	0.05415 ^a	<0.100	<0.100 \pm 0.000	<0.893	<0.893 \pm 0.000	100
	0.06053 ^a	<0.100		<0.893		
	0.05911 ^a	<0.100		<0.893		
4	0.05783 ^a	<0.100	<0.100 \pm 0.000	<0.893	<0.893 \pm 0.000	100
	0.05948 ^a	<0.100		<0.893		
	0.05705 ^a	<0.100		<0.893		
40	0.06888 ^a	<0.100	<0.100 \pm 0.000	<0.893	<0.893 \pm 0.000	100
	0.06424 ^a	<0.100		<0.893		
	0.06511 ^a	<0.100		<0.893		
Donor 2						
0 (VC)	0.12401	0.124	<0.117 \pm 0.0141	1.11	<1.05 \pm 0.126	100
	0.13222	0.132		1.18		
	0.07973 ^a	<0.100		<0.893		
	0.11219	0.112		1.00		
0.4	0.12083	0.121	0.134 \pm 0.0122	1.08	1.20 \pm 0.109	>115
	0.14424	0.144		1.29		
	0.13828	0.138		1.23		
4	0.10953	0.110	0.116 \pm 0.00524	0.978	1.03 \pm 0.0468	>98.7
	0.11883	0.119		1.06		
	0.11837	0.118		1.06		
40	0.14198	0.142	0.141 \pm 0.00273	1.27	1.26 \pm 0.0244	>121
	0.14356	0.144		1.28		
	0.13824	0.138		1.23		
Donor 3						
0 (VC)	0.06064 ^a	<0.100	<0.100 \pm 0.000	<0.893	<0.893 \pm 0.000	100
	0.05981 ^a	<0.100		<0.893		
	0.06402 ^a	<0.100		<0.893		
	0.08660 ^a	<0.100		<0.893		
0.4	0.05106 ^a	<0.100	<0.100 \pm 0.000	<0.893	<0.893 \pm 0.000	100
	0.08255 ^a	<0.100		<0.893		
	0.05998 ^a	<0.100		<0.893		
4	0.06298 ^a	<0.100	<0.100 \pm 0.000	<0.893	<0.893 \pm 0.000	100
	0.05381 ^a	<0.100		<0.893		
	0.07264 ^a	<0.100		<0.893		
40	0.05587 ^a	<0.100	<0.101 \pm 0.00238	<0.893	<0.905 \pm 0.0213	101
	0.10413	0.104		0.930		
	0.08088 ^a	<0.100		<0.893		

Abbreviations: SD, standard deviation; VC, vehicle control (1% methanol)

^a The observed analyzed value (μM) was below the lowest concentration on the standard curve (0.1 μM).

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Appendix 1: In Vitro Technologies Protocol No. 1188

In Vitro Technologies, Inc.
Protocol No. 1188
Version: Final (20 October 2005)

**Evaluation of the Induction Potential of Metaxalone on the
Activities of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9,
CYP2C19, CYP2D6, CYP2E1, and CYP3A4 in Human Hepatocytes**

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Objective

The objective of this study is to evaluate the potential of metaxalone to induce the activities of cytochrome P450 (CYP) isoforms CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 in human hepatocytes following *in vitro* exposure.

Test Article Information

The test article will be identified in this study as follows:

- Metaxalone (molecular weight = 221 g/mol)

Mutual Pharmaceutical Company will provide metaxalone and will be responsible for the derivation, characterization, retention, and stability testing of metaxalone. Additionally, Mutual Pharmaceutical will be responsible for providing In Vitro Technologies with detailed information regarding handling and storage requirements, diluents or cosolubilizers, and safety hazards and precautions (Material Safety Data Sheet or other documentation) for metaxalone, before or upon initiation of this study.

Test System Identification

The test system that will be used in this study is cryopreserved human hepatocytes.

Test System Justification

The liver represents the major organ for drug metabolism and contains the CYP enzymes, the major enzyme system for xenobiotic metabolism (1). Hepatocytes isolated from the liver constitute a physiologically relevant experimental model for the evaluation of potential drug-drug interactions related to the inhibition or induction of CYP enzyme activities.

Differences in drug-metabolizing enzymes among species, especially in CYP isoforms, often account for the inability to predict human clinical responses based on data obtained from laboratory animal studies. Human hepatocytes can be used as an experimental model to reduce concerns about species differences (2). Cryopreserved hepatocytes provide a readily available and well-characterized biological model for use in CYP enzyme induction studies.

Description of Study

Hepatocytes will be incubated in the presence of metaxalone for 48 ± 3 hours, after which a selective substrate for each CYP isoform will be added. The formation of a specific metabolite

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from its substrate will be measured by high-performance liquid chromatography with UV detection (HPLC-UV) or liquid chromatography/mass spectrometry (LC/MS).

Experimental Methods

Media

The following media, as prepared at In Vitro Technologies, will be used in this study.

- DMEM Stock: Dulbecco's modified Eagle's medium (DMEM) supplemented with bovine serum albumin, fructose, N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonate) (HEPES), and sodium bicarbonate
- Hepatocyte Plating Medium: DMEM stock supplemented with antibiotics, bovine serum, hydrocortisone, insulin, and minimum essential medium (MEM) non-essential amino acids
- Sandwich Medium: Hepatocyte plating medium supplemented with Vitrogen™
- Incubation Medium: DMEM stock supplemented with antibiotics, hydrocortisone, insulin, and MEM non-essential amino acids
- Supplemented KHB: Krebs-Henseleit buffer (KHB) supplemented with antibiotics, calcium chloride, heptanoic acid, HEPES, and sodium bicarbonate

Test Article Preparation

Metaxalone stock solutions will be prepared in methanol at 100 times (100X) the final concentration. The stock solutions will be diluted with incubation medium to produce incubation solutions with final concentrations of 0.4, 4, and 40 μM , each containing 1% methanol. Stock solutions will be prepared fresh prior to use. Modifications in test article preparation, which pertain to changes in solvent used or changes in incubation concentrations, may be made with the approval of the Study Director. These modifications and their rationale will be communicated to Mutual Pharmaceutical and will be described in the study report.

Positive Control Article Preparation

Omeprazole and rifampin will be prepared as 100X stock solutions in methanol. Positive control article stock solutions will be diluted with incubation medium to the final concentrations listed below.

CYP isoform	Positive Control Article	Concentration
CYP1A2	Omeprazole	50 μM
CYP3A4	Rifampin	25 μM

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Reference Control Article Preparation

Phenobarbital will be prepared as a 100X stock solution in deionized water. All other reference control articles will be prepared as 100X stock solutions in methanol. Reference control article stock solutions will be diluted with incubation medium to the final concentrations listed below.

CYP isoform	Reference Control Article	Concentration
CYP2B6	Phenobarbital	1 mM*
CYP2C8	Rifampin	25 μ M
CYP2C9	Rifampin	25 μ M
CYP2C19	Rifampin	25 μ M

* Methanol will be added to the dosing solution to achieve a final methanol concentration of 1%.

CYP Isoform Substrate Preparation

The activity of each of the CYP isoforms will be measured in the presence of the following isoform-selective substrates. Isoform-selective substrates will be prepared as 100X stock solutions in acetonitrile and diluted with supplemented KHB to the final concentrations listed below.

CYP isoform	Isoform-selective substrate	Concentration
CYP1A2	Phenacetin	100 μ M
CYP2A6	Coumarin	100 μ M
CYP2B6	S-Mephenytoin	1 mM
CYP2C8	Paclitaxel	50 μ M
CYP2C9	Tolbutamide	50 μ M
CYP2C19	S-Mephenytoin	100 μ M
CYP2D6	Dextromethorphan	16 μ M
CYP2E1	Chlorzoxazone	300 μ M
CYP3A4	Testosterone	125 μ M

Hepatocyte Preparation

Hepatocytes were isolated and cryopreserved based on published methods (3–5). For this study, hepatocytes from three human donors will be obtained from the cryopreserved hepatocyte bank maintained at In Vitro Technologies. Donor demographics and medical histories will be provided in the study report. Cryopreserved hepatocytes will be thawed and counted to determine yield, viability will be measured, and cell seeding density will be adjusted accordingly. Hepatocytes will be transferred to collagen-coated 48-well plates for attachment. After hepatocytes attach to the collagen matrix, plating medium will be replaced with sandwich medium and the hepatocytes will be incubated until use.

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Test Article Incubations

All incubations will be conducted at 37 ± 1 °C, 95% air/5% CO₂, and saturating humidity. The sample size will be N = 3 replicates for experimental groups.

After the cultures are established, sandwich medium will be removed and the hepatocytes will be treated with an incubation solution containing metaxalone for 24 ± 1.5 hours. The incubation solution will be aspirated and replaced with incubation solution containing the same concentration of metaxalone as was used in the initial dosing and incubated for an additional 24 ± 1.5 hours. The total treatment period will be 48 ± 3 hours.

After the treatment period of 48 ± 3 hours, the incubation solution will be replaced with 150 µL of supplemented KHB. The hepatocytes will be incubated for 10 minutes to remove residual metaxalone. The supplemented KHB will be replaced with 150 µL of supplemented KHB containing an isoform-selective substrate. The hepatocytes will be incubated for 4 hours.

CYP2C8 incubations will be terminated by adding 150 µL of acetonitrile. All other incubations will be terminated by adding 150 µL of ice-cold methanol. Samples will be transferred to cryovials. If analysis does not occur immediately after incubation, samples will be stored at -70 °C \pm 10 °C.

Control Incubations

All incubations will be conducted at 37 ± 1 °C, 95% air/5% CO₂, and saturating humidity. The sample size will be N = 4 replicates for the vehicle control, positive control, and reference control groups; and N = 2 replicates for the test article interference control groups.

Vehicle Control

Vehicle control samples will be included to establish a baseline value for analysis.

After the cultures are established, sandwich medium will be removed and the hepatocytes will be treated with incubation medium containing 1% methanol for 24 ± 1.5 hours. The incubation medium containing 1% methanol will be aspirated and replaced with incubation medium containing 1% methanol and incubated for an additional 24 ± 1.5 hours. The total treatment period will be 48 ± 3 hours.

After the treatment period of 48 ± 3 hours, the incubation medium containing 1% methanol will be replaced with 150 µL of supplemented KHB and will be incubated for 10 minutes. The supplemented KHB will be replaced with 150 µL of supplemented KHB containing an isoform-selective substrate. The hepatocytes will be incubated for 4 hours.

Positive Control

Positive controls samples will be included to verify that the test system is responsive to known inducers.

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Omeprazole is a selective inducer of CYP1A2 and will be used to verify that the test system is responsive to CYP1A2 inducers. After the hepatocytes are established, sandwich medium will be replaced with incubation medium containing 50 μ M omeprazole for 24 ± 1.5 hours. The incubation medium containing 50 μ M omeprazole will be aspirated and replaced with incubation medium containing 50 μ M omeprazole and incubated for an additional 24 ± 1.5 hours. The total treatment period will be 48 ± 3 hours.

After the treatment period of 48 ± 3 hours, the incubation solution will be replaced with 150 μ L of supplemented KHB and will be incubated for 10 minutes to remove residual positive control article. The supplemented KHB will be replaced with 150 μ L of supplemented KHB containing 100 μ M phenacetin. The hepatocytes will be incubated for 4 hours.

Rifampin is a selective inducer of CYP3A4 and will be used to verify that the test system is responsive to CYP3A4 inducers. After the hepatocytes are established, sandwich medium will be replaced with incubation medium containing 25 μ M rifampin. The incubation medium containing 25 μ M rifampin will be aspirated and replaced with incubation medium containing 25 μ M rifampin and incubated for an additional 24 ± 1.5 hours. The total treatment period will be 48 ± 3 hours.

After the treatment period of 48 ± 3 hours, the incubation solution will be replaced with 150 μ L of supplemented KHB and will be incubated for 10 minutes to remove residual positive control article. The supplemented KHB will be replaced with 150 μ L of supplemented KHB containing 125 μ M testosterone. The hepatocytes will be incubated for 4 hours.

The test system will be considered inducible if the mean specific activities of both CYP1A2 and CYP3A4 in the positive control samples treated with omeprazole and rifampin, respectively, are $\geq 200\%$ of the mean specific activities in the corresponding vehicle control samples. If these criteria are not met, the study will be repeated.

Reference Control

Reference control samples will be included to evaluate the inducibility of CYP2B6, CYP2C8, CYP2C9, and CYP2C19 in the test system.

After the hepatocytes are established, sandwich medium will be replaced with incubation medium containing reference control article for 24 ± 1.5 hours. The incubation medium containing reference control article will be aspirated and replaced with incubation medium containing the same concentration of reference control article as was used in the initial dosing and incubated for an additional 24 ± 1.5 hours. The total treatment period will be 48 ± 3 hours.

After the treatment period of 48 ± 3 hours, the incubation solution will be replaced with 150 μ L of supplemented KHB and will be incubated for 10 minutes to remove residual positive control article. The supplemented KHB will be replaced with 150 μ L of supplemented KHB containing an isoform-selective substrate. The hepatocytes will be incubated for 4 hours.

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Test Article Interference Control

Test article interference control samples will be included to investigate the possibility of interference by metaxalone or their metabolites.

After the hepatocytes are established, sandwich medium will be removed and the hepatocytes will be treated with an incubation solution containing 40 μM metaxalone for 24 ± 1.5 hours. The incubation solution will be aspirated and replaced with incubation solution containing 40 μM metaxalone and incubated for an additional 24 ± 1.5 hours. The total treatment period will be 48 ± 3 hours.

After the treatment period of 48 ± 3 hours, the incubation solution will be replaced with 150 μL of supplemented KHB and will be incubated for 10 minutes to remove residual metaxalone. The supplemented KHB will be replaced with 150 μL of supplemented KHB containing 1% acetonitrile. The hepatocytes will be incubated for 4 hours. If interference is observed in these samples, then Mutual Pharmaceuticals will be notified to determine a course of action.

Termination of Control Incubations

CYP2C8 incubations will be terminated by adding 150 μL of acetonitrile. All other incubations will be terminated by adding 150 μL of ice-cold methanol. Samples will be transferred to cryovials. If analysis does not occur immediately after incubation, samples will be stored at $-70^\circ\text{C} \pm 10^\circ\text{C}$.

Analyses

In Vitro Technologies will measure the formation of metabolites from CYP isoform-selective substrates using the following bioanalytical procedures:

Phenacetin O-Deethylase (CYP1A2)

The activity of CYP1A2 in hepatocytes will be determined by measuring the formation of acetaminophen. Samples will be analyzed using an LC/MS method.

Coumarin 7-Hydroxylase (CYP2A6)

The activity of CYP2A6 in hepatocytes will be determined by measuring the formation of 7-hydroxycoumarin and its conjugated derivatives, 7-hydroxycoumarin glucuronide and 7-hydroxycoumarin sulfate. Samples will be analyzed using an HPLC-UV method.

S-Mephenytoin N-demethylase (CYP2B6)

The activity of CYP2B6 in hepatocytes will be determined by measuring the formation of nirvanol. Samples will be analyzed using an LC/MS method.

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Paclitaxel 6-Hydroxylase (CYP2C8)

The activity of CYP2C8 in hepatocytes will be determined by measuring the formation of 6-hydroxypaclitaxel. Samples will be analyzed using an HPLC-UV method.

Tolbutamide 4'-Methyl Hydroxylase (CYP2C9)

The activity of CYP2C9 in hepatocytes will be determined by measuring the formation of 4'-methylhydroxytolbutamide. Samples will be analyzed using an LC/MS method.

S-Mephenytoin 4'-Hydroxylase (CYP2C19)

The activity of CYP2C19 in hepatocytes will be determined by measuring the formation of 4'-hydroxymephenytoin. Samples will be analyzed using an LC/MS method.

Dextromethorphan O-Demethylase (CYP2D6)

The activity of CYP2D6 in hepatocytes will be determined by measuring the formation of dextrorphan. Samples will be analyzed using an LC/MS method.

Chlorzoxazone 6-Hydroxylase (CYP2E1)

The activity of CYP2E1 in hepatocytes will be determined by measuring the formation of 6-hydroxychlorzoxazone. Samples will be analyzed using an LC/MS method.

Testosterone 6 β -Hydroxylase (CYP3A4)

The activity of CYP3A4 in hepatocytes will be determined by measuring the formation of 6 β -hydroxytestosterone. Samples will be analyzed using an HPLC-UV method.

Description of Data Calculations

The concentration of metabolites will be reported. Enzyme activity for each CYP isoform will be reported as specific activity (pmol/minute/million cells) in the presence (SA_T) and absence (SA_C) of metaxalone. The data will be expressed as mean \pm standard deviation. SA_T relative to SA_C for each CYP isoform will be expressed as a percent using the following equation:

$$\% \text{ of vehicle control} = \frac{SA_T}{SA_C} \times 100$$

Except for test article interference samples, samples with back-calculated concentrations below the lower limit of quantitation (LLOQ) will be assigned the LLOQ value for calculation.

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Criteria for Data Acceptance

The bioanalytical data for CYP isoforms will be accepted in accordance with the In Vitro Technologies standard operating procedure(s) on bioanalytical data acceptance.

The test article may be cytotoxic at one or more of the concentrations tested. This is an acceptable outcome of induction studies.

Study Report

A copy of the final study report will be issued to Mutual Pharmaceuticals and will include, but not be limited to, the following information:

- Participating Personnel
- Study Dates and Data Retention
- Statement of Compliance
- Quality Assurance Statement
- Summary
- Introduction
- Experimental Methods
- Results
- Conclusions
- Description of Data Calculations
- Copy of study protocol

Data Retention

In Vitro Technologies will retain all supporting documentation, including raw data and written records, for a period of up to five years following issuance of the final report. At the end of this period, Mutual Pharmaceuticals will be notified to determine whether the data (excluding proprietary information) will be transferred, retained, or destroyed.

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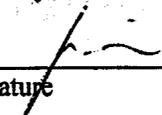
Protocol Approval

Sponsor Approval

This protocol has been reviewed and approved by the following:

Jie Du, Ph.D.

Sponsor Representative
Mutual Pharmaceuticals Company

Signature 

Date

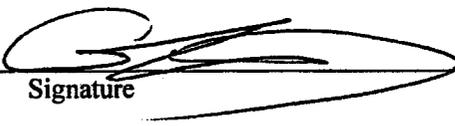
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Study Director Review

This study will be conducted using good documentation practices and using equipment that is properly maintained and calibrated in accordance with In Vitro Technologies standard operating procedures. The study will be conducted under my scientific guidance and management. I have reviewed the procedures outlined in this protocol.

Genfu Chen, Ph.D.

Study Director
In Vitro Technologies

Signature 

Date

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~~20 OCT 05~~

References

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2. Li, A. P.; Lu, C.; Brent, J. A.; Pham, C.; Fackett, A.; Ruegg, C. E.; Silber, P. M. Cryopreserved human hepatocytes: characterization of drug-metabolizing enzyme activities and applications in higher throughput screening assays for hepatotoxicity, metabolic stability, and drug-drug interaction potential. *Chem. Biol. Interact.* 1999, 121, 17-35.
3. Li, A. P.; Roque, M. A.; Beck, D. J.; Kaminski, D. L. Isolation and culturing of hepatocytes from human liver. *J. Tiss. Culture Methods* 1992, 14, 139-146.
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5. Ruegg, C. E.; Silber, P. M.; Mughal, R. A.; Ismail, J.; Lu, C.; Bode, D. C.; Li, A. P. Cytochrome-P450 induction and conjugated metabolism in primary human hepatocytes after cryopreservation. *In Vitro Toxicol.* 1997, 10, 217-222.

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Appendix 2: Copy of Protocol Amendment PAM06-006

PROTOCOL AMENDMENT FORM

IVT Study Number: 305-1188-03

Document Number: PAM 06-006

Date of Sponsor's Verbal Approval: 25Jan2006

Briefly describe the amendment:

The protocol is amended as follows: 100X paclitaxel stock will be diluted with incubation medium to prepare the 1X substrate solution. 1X test article interference control solution for CYP2C8 is amended as follows: 1X test article interference control solution for CYP2C8 will be incubation medium containing 1% acetonitrile.

Briefly describe the reason for the amendment:

Paclitaxel is not soluble in supplemented KHB at 50 micromolar containing 1% acetonitrile.

Approved by: _____

Sponsor Representative

Date: 1/31/06

Approved by: _____

Study Director

Date: 02Feb06

Effective Date: 04 June 2002