

**Evaluation of a Novel Renewable Hepatic Cell Model for Prediction of Clinical CYP3A4
Induction Using a Correlation-based RIS Approach**

Rongjun Zuo, Feng Li, Sweta Parikh, Li Cao, Kirsten L. Cooper, Yulong Hong, Jin Liu, Ronald
A. Faris, Daochuan Li, Hongbing Wang

Corning Life Sciences, Bedford, MA USA (R.Z., F.L., S.P., L.C., K.L.C.); Corning Incorporated,
Science and Technology, Corning, NY USA (Y.H., J.L., R.A.F.); University of Maryland,
School of Pharmacy, Baltimore, MD USA (D.L., H.W.)

Journal Title: Drug Metabolism & Disposition

Table S1 Table S1 Induction response in HepatoCells measured as CYP3A4 mRNA fold increase

Rifampicin				Phenytoin				Phenobarbital				Terbinafine			
Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B	Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B	Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B	Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B
0.023	<u>4.1</u>	0.04	1.4	0.06	0.13	0.04	-0.38	0.91	4.9	2.3	0.09	0.046	-0.12	-0.53	-0.15
0.069	2.2	0.33	1.7	0.17	-0.41	<u>-0.63</u>	-0.60	2.7	1.3	-0.54	0.17	0.14	-0.04	-0.53	0.10
0.21	6.5	8.4	6.0	0.51	0.50	-0.11	-0.20	8.2	1.2	<u>-0.07</u>	0.00	0.41	0.47	0.72	0.03
0.62	17	25	15	1.5	0.52	0.54	0.65	25	1.5	0.26	-0.34	1.2	3.1	1.9	6.9
1.9	24	32	23	4.6	2.7	0.62	3.4	74	6.2	4.8	1.00	3.7	9.8	22	13
5.6	20	23	22	14	11	15	5.4	222	19	14	8.7	11	18	24	21
17	21	<u>31</u>	28	42	20	37	32	667	64	62	31	33	19	14	14
				125	23	<u>39</u>	35	2000	110	135	73	100	14	11	4

Sulfinpyrazone				Probenecid				Pioglitazone				Dexamethasone			
Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B	Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B	Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B	Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B
0.091	<u>2.6</u>	<u>5.3</u>	6.4	0.14	0.08	-0.19	0.40	0.0057	0.50	0.55	-0.07	0.11	-0.15	-0.30	<u>-0.31</u>
0.27	-0.16	<u>0.26</u>	1.1	0.41	-0.15	0.42	0.74	0.017	-0.06	0.20	-0.17	0.34	-0.25	-0.59	-0.55
0.82	0.14	2.7	1.2	1.2	-0.16	-0.40	-0.04	0.051	0.66	<u>0.91</u>	0.60	1.0	0.01	0.63	-0.02
2.47	0.99	2.7	1.5	3.7	0.02	-0.13	-0.35	0.15	3.9	3.4	3.2	3.1	0.31	0.22	0.40
7.41	5.1	4.2	8.0	11	0.34	-0.24	0.52	0.46	3.5	<u>5.4</u>	2.9	9.3	1.1	<u>0.86</u>	1.9
22	15	27	<u>19</u>	33	1.9	1.9	1.5	1.4	6.9	6.4	5.1	28	2.2	2.9	2.6
67	31	38	28	100	4.6	6.2	3.8	4.2	20	27	12	83	<u>16</u>	17	8.4
200	28	53	38	300	25	26	22	13	33	31	15	250	32	53	29

Underscored data not used for curve fitting due to replicate data points not able to reach <40% CV.

Bold data are not used for curve fitting since at such concentrations toxicity or enzyme inhibition appeared to be obvious.

Table S1 Table S1 Induction response in HepatoCells measured as CYP3A4 mRNA fold increase (Cont'd)

Omeprazole				Clotrimazole				Nifedipine				Flumazenil			
Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B	Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B	Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B	Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B
0.05	1.8	-0.05	<u>-0.07</u>	0.005	-0.06	0.13	0.44	0.046	0.06	0.47	0.35	0.02	-0.26	-0.70	0.02
0.14	0.04	-0.28	-0.14	0.014	0.01	-0.32	0.41	0.14	0.44	1.41	0.72	0.07	-0.20	-0.52	-0.16
0.41	0.06	-0.52	-0.17	0.041	0.38	0.24	0.94	0.41	1.4	4.6	1.6	0.21	-0.18	-0.33	-0.43
1.2	0.46	1.3	0.49	0.12	2.0	4.0	3.0	1.2	4.8	9.9	6.2	0.62	-0.23	-0.71	0.13
3.7	2.5	1.7	2.9	0.37	5.7	8.4	9.5	3.7	9.1	17.5	15	1.9	-0.05	-0.65	-0.15
11	10	12	12	1.1	12	16	17	11	22	22	19	5.6	-0.02	-0.57	-0.34
33	24	29	24	3.3	16	18	17	33	28	40	27	17	-0.13	-0.40	-0.14
100	19	45	27	10	0.78	0.09	0.32	100	16	27	26	50	0.08	0.04	0.12

Quinidine				Methotrexate				Digoxin				Carbamazepine			
Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B	Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B	Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B	Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B
0.11	0.55	0.14	1.12	0.009	-0.31	0.15	-0.55	9.1E-05	-0.35	0.01	-0.25	0.23	0.62	-0.16	2.26
0.34	-0.36	0.78	1.12	0.03	-0.49	-0.63	-0.43	0.0003	-0.61	-0.64	-0.53	0.69	-0.15	-0.61	0.16
1.0	-0.42	0.90	0.34	0.082	-0.47	-0.29	-0.41	0.0008	0.00	0.39	-0.45	2.1	-0.08	-0.24	0.19
3.1	0.24	0.71	0.96	0.25	-0.43	-0.30	-0.63	0.0025	-0.33	-0.52	0.06	6.2	0.12	0.00	1.33
9.3	0.44	0.18	0.58	0.74	-0.50	-0.74	-0.27	0.0074	-0.03	-0.47	-0.05	19	2.07	0.62	2.60
28	1.04	2.04	0.08	2.2	-0.56	-0.35	-0.39	0.0222	-0.22	-0.53	-0.40	56	4.7	4.5	7.5
83	0.71	0.57	0.90	6.7	-0.62	-0.79	-0.68	0.0667	-0.57	-0.24	-0.55	166.7	10.6	8.0	14.2
250	-0.55	0.00	-0.44	20	-0.61	-0.40	-0.59	0.20	-0.91	-0.87	-0.89	500	16.2	9.9	13.0

Underscored data not used for curve fitting due to replicate data points not able to reach <40% CV.

Bold data are not used for curve fitting since at such concentrations toxicity or enzyme inhibition appeared to be obvious.

Table S1 Table S1 Induction response in HepatoCells measured as CYP3A4 mRNA fold increase (Cont'd)

Rosiglitazone				Primaquine			
Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B	Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B
0.046	0.82	0.29	-0.1	0.055	1.32	-0.23	0.72
0.137	0.97	1.24	1.1	0.49	0.11	-0.05	0.24
0.412	1.47	2.81	3.9	4.4	0.02	-0.35	-0.15
1.2	9.47	9.81	15	40	-0.51	-0.15	0.13
3.7	22	36	38				
11	47	75	82				
33	46	83	91				
100	5.8	7.8	7.3				

Bold data are not used for curve fitting since at such concentrations toxicity or enzyme inhibition appeared to be obvious.

Table S2 Induction response in HepatoCells measured as CYP3A4 enzyme activity fold increase

Rifampicin			Phenytoin			Phenobarbital			Terbinafine						
Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B	Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B	Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B	Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B
0.02	1.3	0.03	1.3	0.06	0.19	0.19	0.71	0.91	0.4	0.4	0.44	0.05	0.21	0.17	0.47
0.07	3.5	1.7	2.2	0.17	0.11	0.37	0.87	2.7	0.4	0.0	0.60	0.14	0.39	0.58	0.51
0.21	7.8	5.1	4.5	0.51	0.33	0.43	0.73	8.2	0.7	0.61	0.77	0.41	0.81	1.14	0.89
0.62	16	14	11	1.5	0.48	0.67	0.80	25	0.9	1.0	0.8	1.2	2.9	3.3	4.4
1.9	21	21	15	4.6	0.6	0.91	1.3	74	2.4	2.7	3	3.7	11.3	17	12
5.6	30	29	28	14	2	2	2.8	222	11	14	14.0	11	15	19	15
16.7	27	31	24	42	6	7	8	667	31	47	42	33	10	12	10
				125	16	19	19	2000	33	52	43	100	5	7	3

Sulfinpyrazone			Probenecid			Pioglitazone			Dexamethasone						
Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B	Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B	Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B	Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B
0.091	0.27	0.03	0.0	0.14	0.19	-0.1	-0.1	0.006	0.32	0.69	0.96	0.11	-0.27	-0.31	-0.27
0.27	0.34	-0.2	0.3	0.41	-0.03	0.1	0.0	0.017	0.52	0.75	1.24	0.34	-0.24	-0.24	-0.21
0.82	0.53	0.44	0.23	1.2	0.19	0.0	0.0	0.051	0.52	0.89	1.35	1.03	-0.07	-0.16	-0.11
2.5	1.8	1.3	1.2	3.7	0.39	0.0	0.0	0.154	0.95	1.52	1.71	3.09	0.01	-0.16	-0.33
7.4	5.4	3.9	5.6	11	0.41	0.0	0.2	0.46	1.41	2.55	3.91	9.26	0.44	0.16	0.34
22	17	16	12	33	1.3	0.7	0.72	1.4	3.1	5.3	6.5	27.8	1.3	0.7	0.8
67	25	22	20	100	3.3	2.3	1.81	4.2	8.8	15.7	20.6	83.3	4.0	3.2	3.4
200	18	21	18	300	12	8.0	5.88	12.5	18.8	37.9	34.8	250.0	6.9	6.2	7.0

Bold data are not used for curve fitting since at such concentrations toxicity or enzyme inhibition appeared to be obvious.

Table S2 Induction response in HepatoCells measured as CYP3A4 enzyme activity fold increase (Cont'd)

Omeprazole			Clotrimazole			Nifedipine			Flumazenil						
Conc. (μM)	Lot 2B	Lot 3A	Lot 3B	Conc. (μM)	Lot 2B	Lot 3A	Lot 3B	Conc. (μM)	Lot 2B	Lot 3A	Lot 3B	Conc. (μM)	Lot 2B	Lot 3A	Lot 3B
0.05	-0.05	-0.09	-0.11	0.005	-0.43	-0.29	-0.28	0.046	-0.56	-0.55	-0.60	0.023	-0.01	-0.35	-0.16
0.14	0.02	-0.13	-0.15	0.014	-0.35	-0.30	-0.28	0.14	0.03	-0.07	-0.30	0.069	0.02	-0.12	-0.21
0.41	0.07	-0.09	0.00	0.041	-0.34	-0.26	-0.31	0.41	0.88	0.91	0.13	0.21	-0.09	-0.15	-0.17
1.23	0.16	0.19	0.10	0.12	-0.38	-0.34	-0.43	1.2	2.43	2.24	1.21	0.62	0.07	-0.17	0.04
3.70	0.75	0.27	0.21	0.37	-0.64	-0.45	-0.54	3.7	3.84	3.26	1.98	1.9	-0.05	-0.25	-0.12
11.1	1.25	0.94	1.41	1.11	-0.74	-0.54	-0.60	11	2.99	0.96	0.43	5.6	0.16	-0.18	-0.12
33.3	1.25	0.92	0.65	3.33	-0.77	-0.57	-0.65	33	0.69	0.59	0.08	17	0.21	-0.01	-0.08
100.0	0.61	1.09	0.49	10.0	-0.76	-0.57	-0.64	100	-0.35	-0.31	0.80	50	0.29	0.18	0.30

Quinidine			Methotrexate			Digoxin			Carbamazepine						
Conc. (μM)	Lot 2B	Lot 3A	Lot 3B	Conc. (μM)	Lot 2B	Lot 3A	Lot 3B	Conc. (μM)	Lot 2B	Lot 3A	Lot 3B	Conc. (μM)	Lot 2B	Lot 3A	Lot 3B
0.11	-0.25	0.00	-0.03	0.01	-0.19	0.00	-0.28	9.1E-05	-0.10	-0.02	-0.10	0.23	-0.08	0.16	0.22
0.34	-0.30	-0.15	-0.04	0.03	-0.15	-0.08	-0.30	0.0003	-0.11	-0.01	-0.30	0.69	-0.02	0.01	0.20
1.0	-0.13	-0.21	-0.03	0.08	-0.19	-0.21	-0.26	0.001	0.21	0.06	-0.17	2.1	0.11	0.18	0.18
3.1	0.04	0.22	0.08	0.25	-0.30	-0.27	-0.43	0.002	0.02	-0.16	-0.24	6.2	0.14	0.37	0.59
9.3	0.05	-0.10	0.03	0.74	-0.35	-0.32	-0.35	0.007	0.09	-0.19	-0.29	18.5	1.10	1.33	1.57
27.8	0.00	0.21	-0.31	2.22	-0.37	-0.21	-0.37	0.022	0.00	-0.17	-0.36	55.6	3.82	4.66	5.34
83.3	-0.47	-0.30	-0.39	6.67	-0.41	-0.35	-0.42	0.067	-0.21	-0.20	-0.30	167	6.70	6.20	7.85
250	-0.83	-0.65	-0.70	20.00	-0.37	-0.29	-0.40	0.2	-0.73	-0.57	-0.62	500	2.44	2.81	3.25

Bold data are not used for curve fitting since at such concentrations toxicity or enzyme inhibition appeared to be obvious.

Table S2 Induction response in HepatoCells measured as CYP3A4 enzyme activity fold increase (Cont'd)

Rosiglitazone				Primaquine			
Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B	Conc. (μ M)	Lot 2B	Lot 3A	Lot 3B
0.05	0.42	0.62	0.44	0.05	0.20	0.11	0.05
0.14	0.53	0.96	0.90	0.49	-0.18	-0.05	-0.06
0.41	1.06	1.49	1.61	4.44	-0.02	0.13	0.06
1.23	3.9	4.27	4.64	40.00	-0.17	0.00	0.36
3.70	11	16	14				
11	25	36	33				
33	15	21	23				
100	0.02	0.73	0.45				

Bold data are not used for curve fitting since at such concentrations toxicity or enzyme inhibition appeared to be obvious.

Table S3 Slope factors using Sigmoidal Hill 4 parameter function for curving fitting of concentration dependent induction response

Compound	Enzyme activity fold increase			mRNA expression fold increase		
	Lot 2B	Lot 3A	Lot 3B	Lot 2B	Lot 3A	Lot 3B
Rifampicin	1.0	1.0	1.2	2.4	3.1	1.3
Phenytoin	1.4	1.4	1.3	1.7	3.6	3.6
Carbamazepine	1.6	2.1	1.9	1.1	1.6	2.5
Phenobarbital	3.1	2.8	3.7	1.7	1.6	1.4
Terbinafine	2.4	3.6	1.8	1.7	4.2	1.0
Sulfinpyrazone	1.6	2.5	1.6	1.2	1.3	1.2
Probenecid	1.2	1.1	1.0	1.5	1.3	1.6
Pioglitazone	1.2	1.1	1.5	1.3	3.1	0.8
Dexamethasone	1.2	1.4	1.3	1.2	1.8	1.1
Rosiglitazone	1.2	1.5	1.2	2.0	2.0	1.7
Omeprazole	2.2	1.4	1.4	1.8	1.3	1.9
Nifedipine	0.9	1.0	1.3	1.3	0.4	1.1
Clotrimazole	na	na	na	1.2	1.4	2.0
Quinidine	na	na	na	na	na	na
Flumazenil	na	na	na	na	na	na
Primaquine	na	na	na	na	na	na
Methotrexate	na	na	na	na	na	na
Digoxin	na	na	na	na	na	na

na: no slope factors reported since no concentration-dependent curve was obtained.