Bale et al.:

Correlating *In Vitro* Data to *In Vivo* Findings for Risk Assessment

Supplementary Data

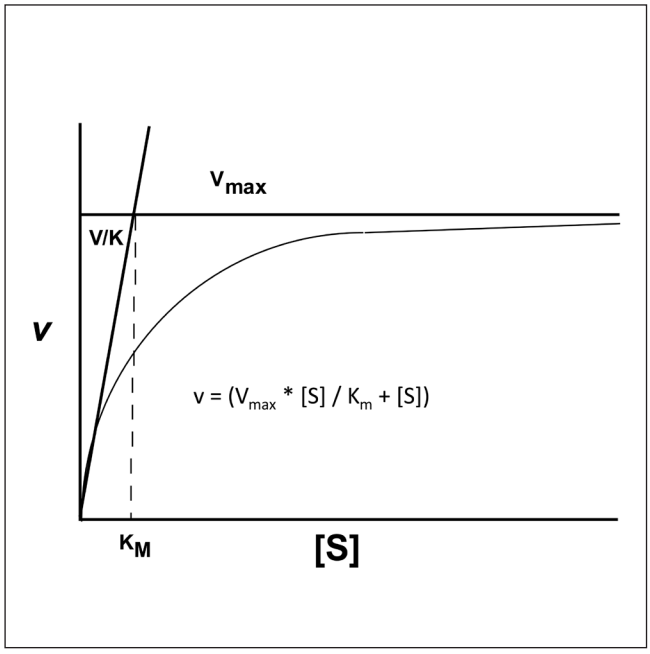

Fig. S1: Determination of metabolic rate constants by kinetic analysis (Lineweaver-Burke plot)
V is the rate of metabolism determined at a specific substrate concentration [S]. Clint is a measure of metabolic potential; $Cl_{int} = V_{max}/ K_m$.

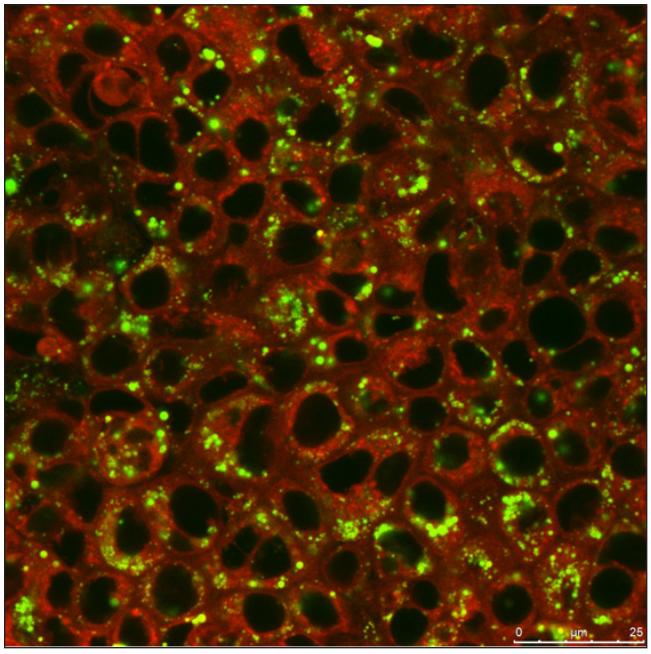

Fig. S2: Confluent HepG2/C3A cells stained with Nile red
Cells were photographed under a laser-scanning confocal microscope. For neutral lipids (yellow-green), emission and excitation wavelengths were 488 and 495-560 nm. For polar lipids (red), emission and excitation wavelengths were 561 and 615-700 nm. Photo courtesy of Dr Michael Santillo.

Tab. S1: Published values for microsomal protein per g liver (MMPGL)

	Rat	Human
Houston et al., 1994	45	
Carlile et al., 1997	60.1	
Lipscomb et al., 1998*		20.8 (n=4)
Lipscomb et al., 2003		52.2 (n=20)
Barter et al., 2007*		29-34 (95% CI)
Barter et al., 2008		27-32 (@ 22 years); 29 (@ 65 years)

*Also addressed hepatocyte yield (HPGL)

Tab. S2: Total and form-specific CYP content of human MSP

CYP enzyme	Specific content (pmol/mg MSP)	% spectrally-determined CYP
Total – spectrally determined	446 ±171	100
Total – sum of forms quantified	303 ±173	69 ±21
CYP1A	42 ±27	8.0 ±5.5
CYP2B6*	2.7 ±1.9	0.6 ±0.4
CYP2C**	64 ±16	14 ±6
CYP2E1	52 ±25	13 ±6
CYP3A	142 ±105	31 ±16

Adapted from Snawder and Lipscomb (2000), n=40, except *n=23 and ** sum of immunologically quantified CYP2C8, CYP2C9, CYP2C18, and CYP2C19

Tab. S3: Correlations of endpoint assays

Pearson Correlation Coefficients; Probability > |r| under H0: Rho=0

Endpoint	DNA	DCF	EROD	BROD	NR	R123
DNA	1.00000	**0.55402*** <0.0001	0.03609 0.3386	0.2345 <0.0001	**0.65634*** <0.0001	0.37094 <0.0001
DCF		1.00000	0.09716 0.0024	0.22811 <0.0001	0.28143 <0.0001	0.14830 <0.0001
EROD			1.00000	0.39875 <0.0001	-0.19144 <0.0001	-0.13787 <0.0001
BROD				1.00000	0.07733 0.0153	-0.19220 <0.0001
NR					1.00000	0.38519 <0.0001
R123						1.00000

*Strongly correlated

Data from Flynn and Ferguson (2008).

Tab. S4: Predictions of liver-active concentrations for unknown compounds using the discriminant analysis model
(percentage of Y=1 listed beneath each qualifier)

Compound	Concentration (μg/ml)													
	0	0.1	0.25	0.5	1	2.5	5	10	25	50	100	250	500	1000
AND	N (0%)			N (33%)	N (0%)	N (40%)	A (50%)	N (40%)	N (33%)	A (50%)	A (50%)			
ANF	N 0%)	A (100%)	A (100%)	A (100%)	A (100%)	A (100%)	A (100%)	A (100%)	A (100%)					
BNF	N (0%)	A (100%)	A (100%)	A (100%)	A (100%)	A (100%)	A (100%)							
DAI	N (0%)		N (33%)	A (100%)	N (33%)	N (17%)	A (60%)	A (60%)	N (40%)	A (80%)				
DEX	N (0%)			A (80%)	A (60%)	N (40%)	N (40%)	N (25%)	N (40%)	A (60%)	N (25%)			
EST	N (0%)			N (33%)	A (100%)	N (33%)	A (83%)	A (67%)	A (67%)	A (67%)	A (67%)			
GEN	N (0%)			N (0%)	N (0%)	N (0%)	N (0%)	N (17%)	N (0%)	N (0%)	A (100%)			
PRO	N (0%)						N (33%)	A (50%)	A (100%)	A (100%)	A (100%)	A (100%)	*	*
QUE	N (0%)			N (0%)	N (0%)	N (17%)	N (0%)	N (0%)	N (17%)	A (83%)	A (100%)			
TES	N (0%)					A (50%)	A (67%)	A (83%)	A (100%)	A (100%)	A (100%)			

Using 100% or all 420 observations as the training dataset, the average 'toxicity' (i.e. proportion of observations with Y=1) was predicted for the unknown compounds androstenedione (AND), α-naphthoflavone (ANF), β-naphthoflavone (BNF), daidzein (DAI), dexamethasone (DEX), estriol (EST), genistein (GEN), propranolol (PRO), quercetin (QUE), and testosterone (TES) using the discriminant analysis method.
N=not liver active, A=liver active. *100% cytolethality. Data from Flynn and Ferguson (2008).