Supporting Information

Kinetics of Glutathione Depletion and Antioxidant Gene Expression as Indicators of Chemical Modes of Action Assessed *in vitro* in Mouse Hepatocytes with Enhanced Glutathione Synthesis.

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Concentration-response fit details. Cytotoxicity Log AC_{50} estimates were determined based on absolute concentration-response curve (CRC).[1] The curve plateaus were set to average reading from empty media plates and control cell wells. CRC were extrapolated beyond tested concentration ranges when E_{MAX} was not reached in the concentration range tested. For example, tBHP is the least toxic of the 7 analyzed chemicals and does not reach 100% lethality in millimolar concentrations. On the contrary, Dinoseb was extremely potent but MTT fluorescence reading indicated that it did not decrease survival below 50% across wide concentration range above 30 uM. Upon manual examination it was clear that the cells were damaged and not functional – an observation that was not adequately captured by fluorescent reading. Thus, for DNSB cytotoxicity assessment, MTT fluorescence microplate reading from cell exposures to concentration above 30 uM were not use in the CRC fitting and the CRC was allowed to extrapolate the curve into concentrations above 30 uM. The consistent CRC boundaries allow for more exact AC_{50} comparison, less influenced by solubility or selected concentration range.

Table S1: Multivariate changes in antioxidant gene expression between HV and CR cell lines for each chemical.

Chemical	Pillai Statistic	F-score	Pr(>F)
tBHQ	1.00	1104.59	0.023
HQ	1.00	2365881.61	0.001
CHP	1.00	103.08	0.076
tBHP	0.94	2.02	0.498
PFOA	0.27	0.45	0.767
BPA	0.85	6.94	0.028
DNSB	0.24	0.40	0.802

Difference in overall gene expression profiles were established with Multivariate ANalysis of VAriance (MANOVA) using Pillai's trace for significance testing. P-values are corrected for multiple comparisons using family-wise error rate.

Table S2: Univariate changes in antioxidant gene expression between HV and CR cell lines

Chem	Endpoint	Time	Effect in CR ΔΔE	Effect in HV ΔΔE	Difference in ΔΔE at (HV-CR)	p.adj
BPA	*gclc*	24	-0.22	-0.05	0.17	0.156
BPA	*gclm*	24	-0.07	-0.04	0.03	0.834
BPA	*hmox*	24	-0.14	0.11	0.25	0.101
BPA	*nqo1*	24	-0.01	0.21	0.22	0.130
CHP	*gclc*	6	0.12	0.12	0.00	0.998
CHP	*gclc*	24	0.19	0.44	0.25	0.121
CHP	*gclm*	6	0.31	0.28	-0.03	0.885
CHP	*gclm*	24	0.26	0.53	0.26	0.109
CHP	*hmox*	6	0.88	1.15	0.27	0.330
CHP	*hmox*	24	0.52	0.93	0.41	0.101
CHP	*nqo1*	6	0.23	0.26	0.03	0.834
CHP	*nqo1*	24	0.30	0.64	0.34	0.162
DNSB	*gclc*	24	0.19	0.31	0.12	0.376
DNSB	*gclm*	24	0.21	0.25	0.04	0.772
DNSB	*hmox*	24	0.56	0.62	0.06	0.834
DNSB	*nqo1*	24	0.18	0.27	0.08	0.543
HQ	*gclc*	6	0.31	0.79	0.48	0.005
HQ	*gclc*	24	0.08	0.46	0.38	0.101
HQ	*gclm*	6	0.57	0.77	0.20	0.083
HQ	*gclm*	24	0.38	0.64	0.26	0.101
HQ	*hmox*	6	1.82	2.01	0.19	0.049
HQ	*hmox*	24	1.17	1.82	0.65	0.005
HQ	*nqo1*	6	0.38	0.35	-0.02	0.834
HQ	*nqo1*	24	0.88	1.18	0.30	0.112
PFOA	*gclc*	24	-0.14	-0.14	0.00	0.998
PFOA	*gclm*	24	-0.07	-0.10	-0.02	0.812
PFOA	*hmox*	24	-0.17	-0.14	0.04	0.568
PFOA	*nqo1*	24	-0.03	-0.06	-0.03	0.830
tBHP	*gclc*	6	0.00	0.05	0.06	0.475
tBHP	*gclc*	24	-0.29	-0.16	0.12	0.376
tBHP	*gclm*	6	0.20	0.28	0.08	0.346
tBHP	*gclm*	24	-0.15	-0.01	0.14	0.374
tBHP	*hmox*	6	0.59	0.86	0.28	0.169
tBHP	*hmox*	24	-0.25	0.06	0.31	0.186
tBHP	*nqo1*	6	0.13	0.19	0.06	0.341
tBHP	*nqo1*	24	-0.13	0.06	0.19	0.374
tBHQ	*gclc*	6	0.12	0.69	0.57	0.001

tBHQ	*gclc*	24	-0.02	0.22	0.24	0.070
tBHQ	*gclm*	6	0.43	0.66	0.23	0.036
tBHQ	*gclm*	24	0.36	0.55	0.19	0.011
tBHQ	*hmox*	6	1.42	1.88	0.46	0.005
tBHQ	*hmox*	24	0.75	1.30	0.55	0.101
tBHQ	*nqo1*	6	0.22	0.47	0.25	0.101
tBHQ	*nqo1*	24	0.73	1.13	0.40	0.002

Table S3: Multivariate changes in antioxidant gene expression across all chemicals from 6 to 24 hr in response to sublethal chemical exposure.

Gene	**Cell line**	**Pillai**	**F-Score**	**Pr(>F)**
Gclc	HV	0.913446	13.19192	0.036
Gclc	CR	0.778847	4.402198	0.18
Gclm	HV	0.709742	3.056517	0.18
Gclm	CR	0.895029	10.65803	0.046
Hmox	HV	0.790072	4.70442	0.18
Hmox	CR	0.96225	31.86248	0.006
Nqo1	HV	0.978375	56.55384	0.002
Nqo1	CR	0.978272	56.27903	0.002

Difference in overall gene expression profiles were established with Multivariate ANalysis of VAriance (MANOVA) using Pillai's trace for significance testing. P-values are corrected for multiple comparisons using family-wise error rate.

Table S4: Univariate changes in antioxidant gene expression from 6 to 24 hr in response to sublethal chemical concentrations and separated by cell line.

Chem	Endpoint	Cell line	Effect at 6 hr ΔΔE	Effect at 24 hr ΔΔE	Difference in ΔΔE (24 hr - 6 hr)	p.adj
HQ	nqo1	HV	0.35	1.18	0.83	0.000
HQ	nqo1	CR	0.38	0.88	0.50	0.019
HQ	hmox	HV	2.01	1.82	-0.19	0.109
HQ	hmox	CR	1.82	1.17	-0.65	0.001
HQ	gclc	HV	0.79	0.46	-0.33	0.008
HQ	gclc	CR	0.31	0.08	-0.22	0.211
HQ	gclm	HV	0.77	0.64	-0.13	0.100
HQ	gclm	CR	0.57	0.38	-0.19	0.157
tBHQ	nqo1	HV	0.47	1.13	0.66	0.002
tBHQ	nqo1	CR	0.22	0.73	0.51	0.002
tBHQ	hmox	HV	1.88	1.30	-0.58	0.060
tBHQ	hmox	CR	1.42	0.75	-0.67	0.001
tBHQ	gclc	HV	0.69	0.22	-0.47	0.003
tBHQ	gclc	CR	0.12	-0.02	-0.14	0.086
tBHQ	gclm	HV	0.66	0.55	-0.11	0.109
tBHQ	gclm	CR	0.43	0.36	-0.07	0.336
CHP	nqo1	HV	0.26	0.64	0.38	0.100
CHP	nqo1	CR	0.23	0.30	0.07	0.633
CHP	hmox	HV	1.15	0.93	-0.22	0.432
CHP	hmox	CR	0.88	0.52	-0.35	0.070
CHP	gclc	HV	0.12	0.44	0.31	0.100
CHP	gclc	CR	0.12	0.19	0.06	0.643
CHP	gclm	HV	0.28	0.53	0.25	0.162
CHP	gclm	CR	0.31	0.26	-0.04	0.713
tBHP	nqo1	HV	0.19	0.06	-0.13	0.451
tBHP	nqo1	CR	0.13	-0.13	-0.26	0.070
tBHP	hmox	HV	0.86	0.06	-0.81	0.008
tBHP	hmox	CR	0.59	-0.25	-0.84	0.001
tBHP	gclc	HV	0.05	-0.16	-0.22	0.060
tBHP	gclc	CR	0.00	-0.29	-0.28	0.049
tBHP	gclm	HV	0.28	-0.01	-0.30	0.041
tBHP	gclm	CR	0.20	-0.15	-0.36	0.022

Figure S1. Schematic of Experimental Design

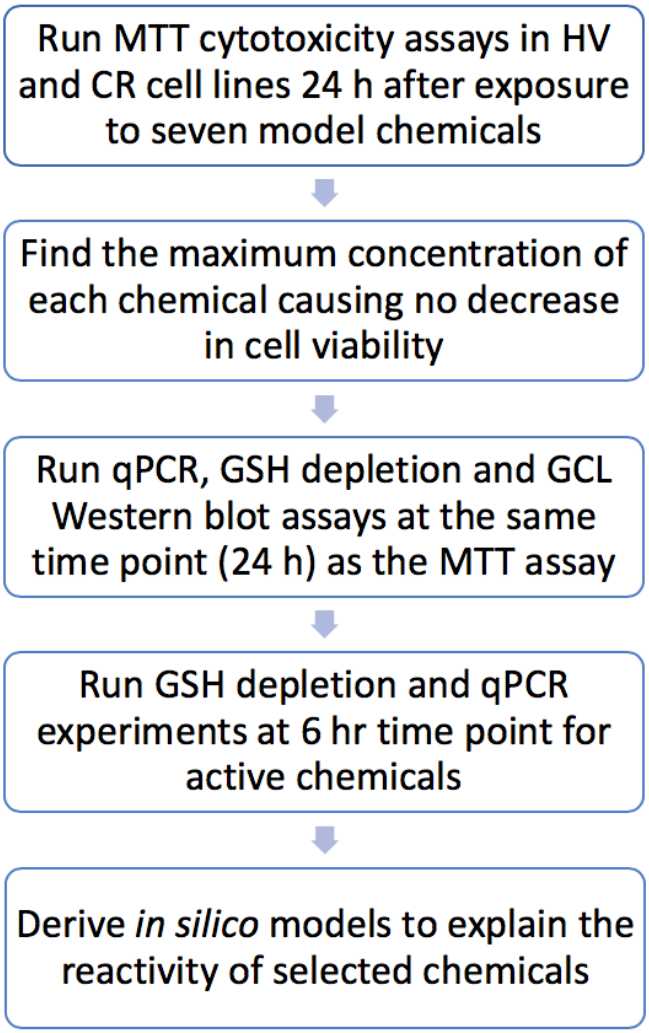
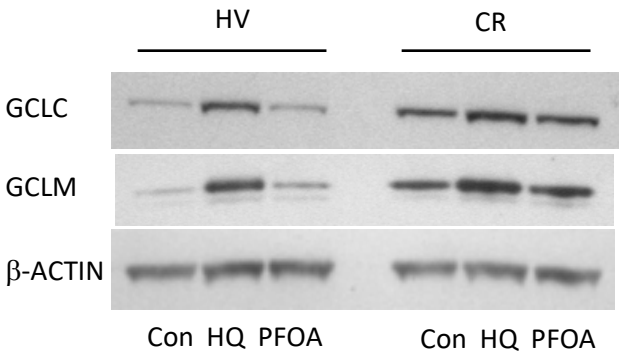


Figure S2. GCLC and GCLM protein expression in HV and CR cell lines treated with vehicle control, 100 uM hydroquinone or 100 uM perfluorooctanoic acid. Panel A: Representative Western immunoblot showing bands for GCLC, GCLM and β–Actin. Lanes for vehicle control (Con), hydroquinone (HQ) and perfluorooctanoic acid (PFOA) are indicated. Quantitation of GCLC (Panel B) and GCLM (Panel C) band optical density represented as a percentage of β–Actin optical density. N=3 independent experiments. *Statistically significant difference ($p<0.05$) between HV and CR cell lines within treatments. #Statistically significant difference ($p<0.05$) between control and treated samples within HV or CR cell lines.

A.



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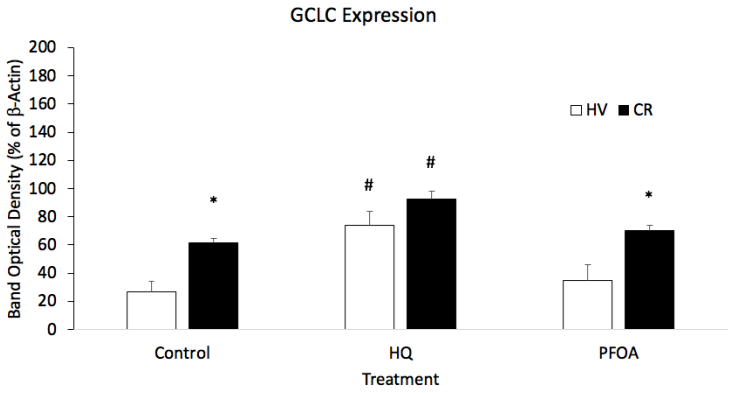

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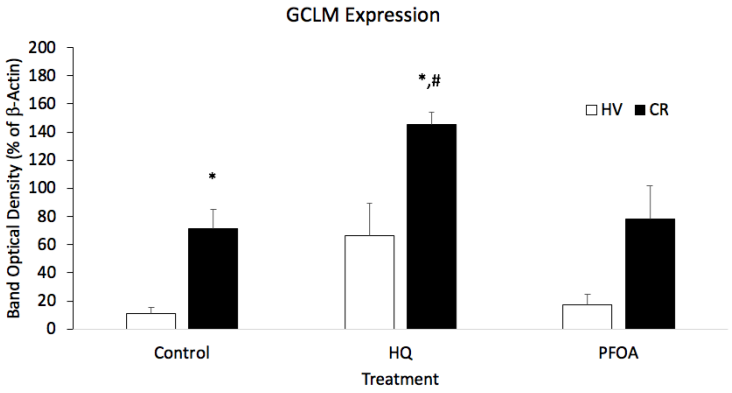
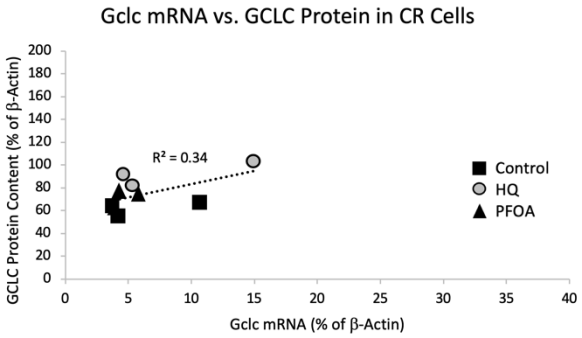


Figure S3. Correlations between GCLC and GCLM mRNA expression, and GCLC and GCLM protein expression in HV and CR cell lines exposed to vehicle control, 100 uM hydroquinone or 100 uM perfluorooctanoic acid. GCLC and GCLM mRNA expression are plotted as a percentage of β-Actin mRNA. GCLC and GCLM protein expression are plotted as a percentage of β-Actin protein. Symbols for vehicle control (Control), hydroquinone (HQ) and perfluorooctanoic acid (PFOA) are indicated. N=3 independent experiments.

A.



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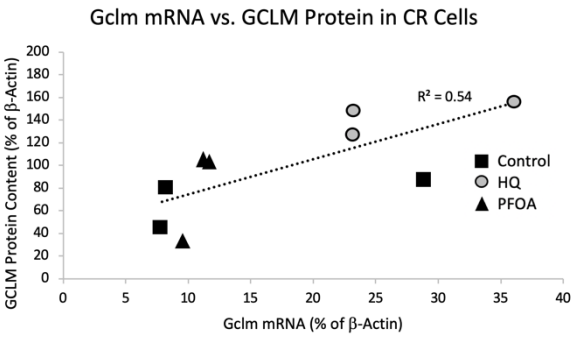

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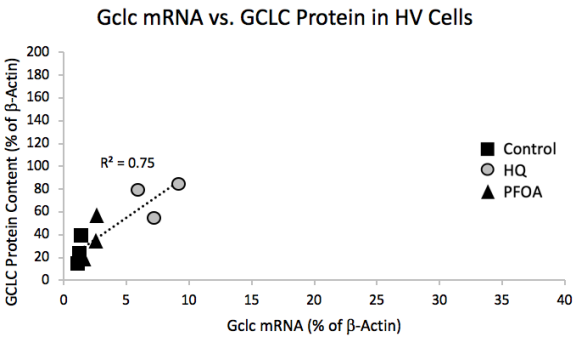

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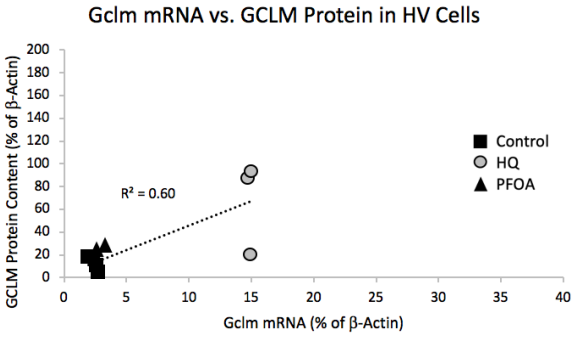
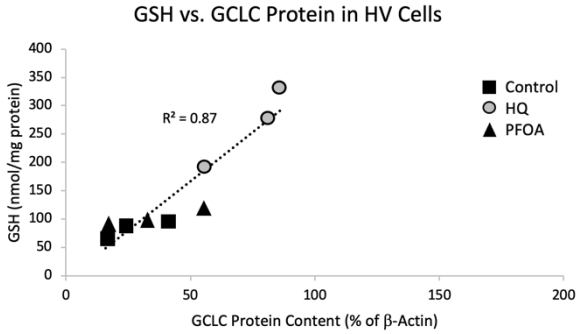


Figure S4. Correlations between GCLC and GCLM protein expression and GSH content in HV and CR cell lines exposed to vehicle control, 100 uM hydroquinone or 100 uM perfluorooctanoic acid. GCLC and GCLM protein expression are plotted as a percentage of β-Actin protein. GSH levels were measured by the NDA assay. Symbols for vehicle control (Control), hydroquinone (HQ) and perfluorooctanoic acid (PFOA) are indicated. N=3 independent experiments.

A.



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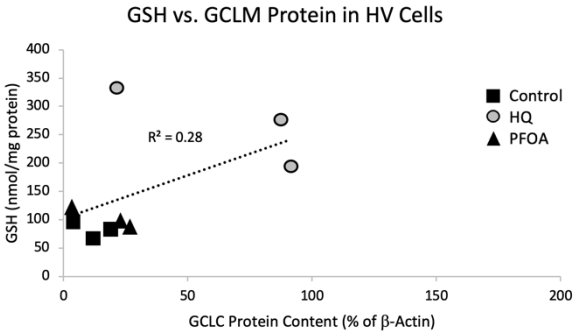

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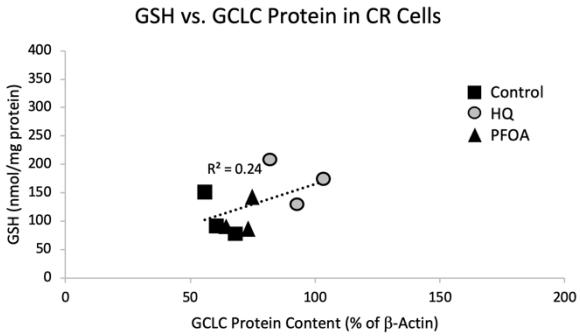

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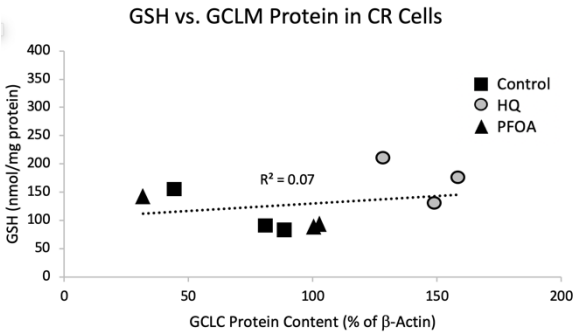

References

(1) Sebaugh, J. L. (**2011**) Guidelines for accurate EC50/IC50 estimation. Pharm. Stat. 10, 128–134.