Supporting Information

Mixture Toxicity of Reactive Chemicals by Using Two Bacterial Growth Assays as Indicators of Protein and DNA Damage

8 pages, three figures and two tables

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Working Hypotheses

The working hypotheses for the present study are presented in a flow chart for better orientation (Figure S-1).

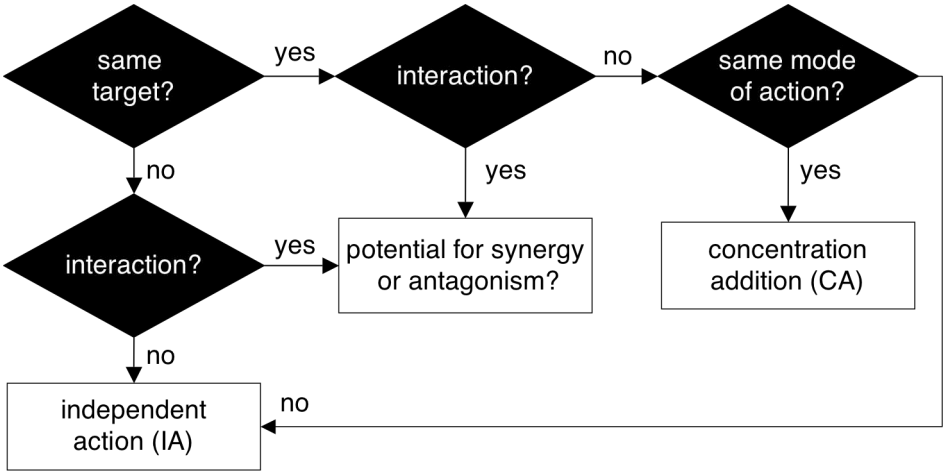

Figure S-1. Working hypotheses.

Additional Information on the reference chemical CTP

4-Chlorothiophenol (CTP) was used as model compound for a nucleophilic molecule. CTP has an acidity constant of 6.13 (1). Its thiol group is therefore deprotonated at ambient pH and the negatively charged sulfide is highly nucleophilic (just like the cysteine groups in proteins and peptides). Substituted thiophenols have been shown to be highly cytotoxic (1). They inhibited the proliferation of rapidly growing mouse leukemia cells *in vitro* and this effect could be related to electronic parameters in a QSAR (1). The high activity of CTP in the cell proliferation test was attributed to the formation of sulfur-centered radicals, which induce oxidative stress (1). The EC_{50} in the GSH+ strain was 0.033 mM (Table 1), i.e. it is among the more toxic chemicals in the test set, but it is also more hydrophobic with an octanol-water partition coefficient $\log K_{ow}$ of 3.39 (1). When comparing the EC_{50} value with that of baseline toxicants in this test set (2), a preliminary QSAR analysis indicated an excess of baseline toxicity (Figure S-2).

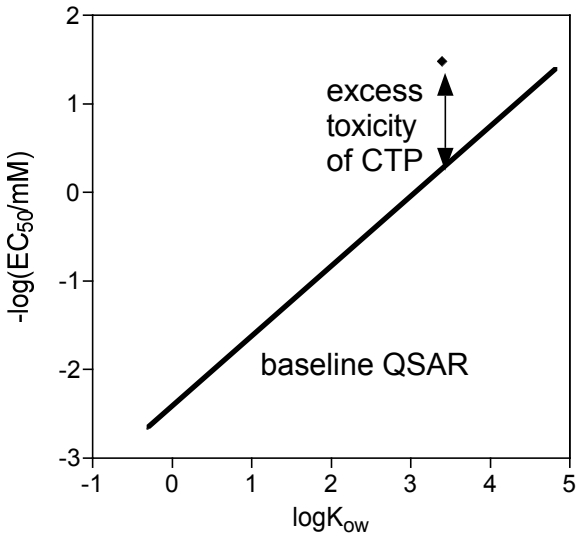

Figure S-2. QSAR analysis indicative of excess toxicity of CTP.

Two-Stage Predictions

In two-stage predictions (TSP) groups of similarly acting compounds were described with CA and the predictions of these groups were fed in models for IA using the different CA-groups as one component each in modeling the IA prediction. This method was successfully applied to predict the toxicity of a 40-component mixture of herbicides with four different modes of action in algae (3) and helped to explain mixture effects of a complex mixture of pollutants in contaminated sediments (4).

The TSP approach can also be exploited as a diagnostic tool for mode of action analysis. In a group of 14 nitrobenzenes, Altenburger et al. (5) identified three compounds that apparently did not act through the same mode of toxic action as the other nitrobenzenes (baseline toxicity) by comparing different TSP with experimental mixture effects.

A similar approach was used for the identification of the specific mode of toxic action of the β-blocker propranolol in algae (2). QSAR (quantitative structure-activity relationship) analysis revealed a specific effect of propranolol in green algae. The following exploration of TSP of mixtures of baseline toxicants, specific inhibitors of photosystem II and propranolol clearly showed that propranolol exhibits another yet unidentified mode of toxic action because it was modeled best with a TSP using all baseline toxicants as one CA group and combining these with propranolol and the inhibitor of photosystem II through the model of IA.

Particularly in mixtures with a low number of components and in biotests with high inherent variability it is often not possible to resolve the different predictions of CA and IA sufficiently to distinguish quantitatively between them (6). Thus such approaches are valuable diagnostic tools only if predictions can be quantitatively discriminated.

For the mixtures of similarly and dissimilarly acting compounds in the test for glutathione-depletion related toxicity we mentioned in the MS, that a TSP might be the appropriate model. The TSP lay between the predictions for CA and IA (Figure S-3) and did not describe better the experimental data. Considering the variability of the experimental data and the small differences in the predictions, the hypothesis of predictability by TSP cannot be refuted, though. In case of mixtures of nonspecifically acting chemicals, a TSP is not reasonable because all mixture components act via the same two toxic mechanisms.

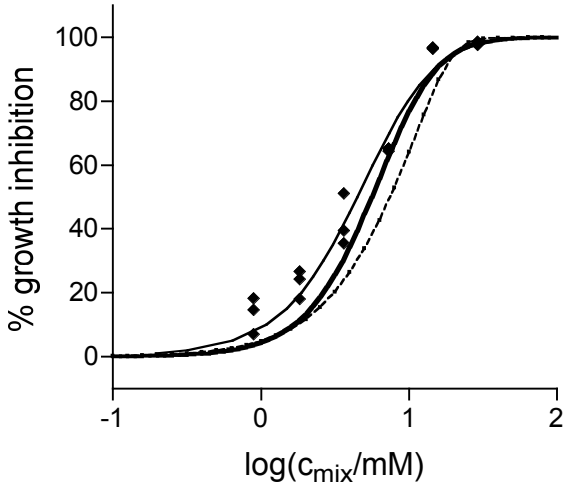

Figure S-3. Two-stage prediction of mixture toxicity for the test with GSH+ (*E.coli* strain MJF 276) and the mixture of 3 acrylates and 3 epoxides (0.02% ACR, 5.2% ACN, 2.2%EA, 78.8% EOX, 5.3% EPOX, 8.5% SOX). The solid line is the prediction for CA, the broken line the prediction for IA and the bold line the TSP.

Additional Information

Statistical information complementing Tables 1 and 2 is given in Tables S-1 and S-2.

Compound	*E. coli* strain	EC_{50} (mM)	95% confidence interval of EC_{50} (mM)	slope	95% confidence interval of slope	Number of data points	r^2
ACR	GSH+	0.01	0.008 to 0.011	2.19	1.19 to 3.19	31	0.880
ACA	GSH+	30.67	28.04 to 33.55	1.41	1.22 to 1.61	38	0.969
ACN	GSH+	1.88	1.79 to 1.98	1.42	1.30 to 1.53	40	0.999
EA	GSH+	0.72	0.66 to 0.78	1.44	1.23 to 1.65	44	0.965
HEA	GSH+	0.66	0.62 to 0.70	1.47	1.33 to 1.61	43	0.981
IBA	GSH-	0.82	0.66 to 1.0	0.97	0.76 to 1.18	26	0.925
EPOX	GSH+	1.01	0.95 to 1.07	4.30	2.79 to 5.80	37	0.941
SOX	GSH+	1.57	1.44 to 1.72	1.23	1.07 to 1.39	37	0.967
EOX	GSH+	19.11	16.61 to 21.99	1.43	1.10 to 1.77	37	0.908
EPI	GSH+	4.37	4.22 to 4.54	1.96	1.78 to 2.14	34	0.995
BCl	GSH+	0.41	0.37 to 0.46	1.30	1.04 to 1.56	37	0.966
DClP	GSH+	0.06	0.05 to 0.06	1.96	1.40 to 2.52	31	0.949
DClB	GSH-	0.09	0.08 to 0.09	2.81	2.07 to 3.55	31	0.966
NBCl	GSH+	0.08	0.07 to 0.09	1.69	1.30 to 2.08	35	0.961
MBCl	GSH+	0.69	0.61 to 0.77	2.22	1.49 to 2.94	56	0.891
CTP	GSH+	0.03	0.030 to 0.037	1.37	1.19 to 1.55	48	0.933

ACR	GSH-	0.003	0.0025 to 0.0030	2.35	1.79 to 2.91	67	0.904
ACN	GSH-	0.88	0.83 to 0.93	1.38	1.23 to 1.54	54	0.976
EA	GSH-	0.37	0.35 to 0.40	1.32	1.13 to 1.50	50	0.962
EPOX	GSH-	1.16	0.94 to 1.4	1.33	0.823 to 1.83	52	0.733
SOX	GSH-	1.38	1.26 to 1.52	1.25	1.05 to 1.45	34	0.957
EOX	GSH-	15.24	14.10 to 16.47	1.58	1.32 to 1.84	29	0.963
ACN	DNA+	5.36	3.61 to 7.96	1.34	0.63 to 2.06	14	0.764
HEA	DNA+	1.26	0.46 to 3.40	0.82	0.21 to 1.43	10	0.628
SOX	DNA+	0.03	0.015 to 0.064	0.93	0.267 to 1.59	12	0.662
EOX	DNA+	2.41	1.46 to 3.96	2.11	0.129 to 4.08	12	0.796
ACN	DNA-	14.84	7.20 to 30.59	0.31	0.12 to 0.49	18	0.443
HEA	DNA-	7.75	5.671 to 10.60	0.71	0.49 to 0.93	17	0.827
SOX	DNA-	6.48	5.877 to 7.141	2.24	1.55 to 2.92	47	0.846
EOX	DNA-	137.3	89.52 to 210.5	1.66	0.57 to 2.70	12	0.803

Table S-1. Descriptors and statistics of the concentration-effect curves of the single compounds (extension of Table 1 in the manuscript)

Mixture composition p_i (mol%)	*E. coli* strain	EC_{50} (mM)	95% confidence interval of EC_{50} (mM)	slope	95% confidence interval of slope	Degrees of freedom	r^2
0.4% ACR, 68% ACN, 31.6%EA	GSH+	1.03	0.93 to 1.14	1.98	1.63 to 2.34	16	0.978
0.4% ACR, 68% ACN, 31.6%EA	GSH-	0.26	0.23 to 0.29	1.68	1.31 to 2.04	22	0.932
0.02% ACR, 5.2% ACN, 2.2%EA, 78.8% EOX, 5.3% EPOX, 8.5% SOX	GSH+	4.16	3.65 to 4.75	1.58	1.28 to 1.89	16	0.965
0.02% ACR, 5.2% ACN, 2.2%EA, 78.8% EOX, 5.3% EPOX, 8.5% SOX	GSH-	2.45	2.21 to 2.72	1.27	1.10 to 1.44	28	0.958
91% EOX, 3% EPOX, 6% SOX	GSH+	9.28	8.76 to 9.83	1.81	1.63 to 1.98	28	0.986
91% EOX, 3% EPOX, 6% SOX	GSH-	8.81	7.76 to 10.0	1.90	1.48 to 2.32	16	0.963
0.01% ACR, 1.3%ACA, 23.1% ACN, 3.1% EA, 1.1%HEA, 0.7% IBA, 64.8% EOX, 4.6% EPOX, 1.3% SOX	GSH+	2.99	2.85-3.14	1.89	1.72 to 2.06	34	0.984
80.3% EPI, 6.3% BCl, 1.0% DClP, 1.5% DClB, 1.4% NBCl, 9.6% MBCl	GSH+	1.41	1.32 to 1.51	1.90	1.67 to 2.12	28	0.982

Table S-2. Descriptors and statistics of the concentration-effect curves of the mixtures (extension of Table 2 in the manuscript)

Additional References

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(2) Escher, B. I.; Bramaz, N.; Eggen, R. I. L.; Richter, M. In-vitro Assessment of Modes of Toxic Action of Pharmaceuticals in Aquatic Life. *Environ. Sci. Technol.* **2005**, 39, 3090-3100.

(3) Junghans, M. *Studies on combination effects of environmentally relevant toxicants: Validation of prognostic concepts for assessing the algal toxicity of realistic aquatic pesticide mixtures,* Ph D. Thesis, University of Bremen, Germany, http://elib.suub.uni-bremen.de, 2004.

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(5) Altenburger, R.; Schmitt, H.; Schüürmann, G. Algal toxicity of nitrobenzenes: Combined effect analysis as a pharmacological probe for similar modes of interaction. *Environ. Toxicol. Chem*. **2005**, 24, 324-333.

(6) Faust, M. *Kombinationseffekte von Schadstoffen auf aquatische Organismen: Prüfung der Vorhersagbarkeit am Beispiel einzelliger Grünalgen (Combination effects of pollutants on aquatic organisms: Assessment of predictability on the example of unicellular green algae),* Ph D. Thesis, University of Bremen, Germany, 1999.