

Electronic Supplementary Information: Exploring the oxidative stress response mechanism triggered by environmental water samples

Peta A. Neale, Maud E. S. Achard, Beate I. Escher, Frederic D. L. Leusch

Table S1: Properties of chemicals detected in the water samples and known to be active in the ARE GeneBLAzer assay.

Chemical	CAS No.	Molecular Weight (g/mol)	EC_{IR1.5,ox stress} (M) ± standard deviation*
2,4-Dinitrophenol	51-28-5	184.11	$4.34 \times 10^{-5} \pm 1.14 \times 10^{-5}$
Caffeine	58-08-2	194.19	$9.33 \times 10^{-6} \pm 9.57 \times 10^{-7}$
Diclofenac	15307-86-5	296.15	$3.98 \times 10^{-5} \pm 1.05 \times 10^{-5}$
Genistein	446-72-0	270.24	$3.32 \times 10^{-6} \pm 7.68 \times 10^{-7}$
Metolachlor	51218-45-2	283.80	$6.90 \times 10^{-6} \pm 1.07 \times 10^{-6}$

*EC_{IR1.5,ox stress} calculated from raw fluorescence data in the US EPA ToxCast database ¹.

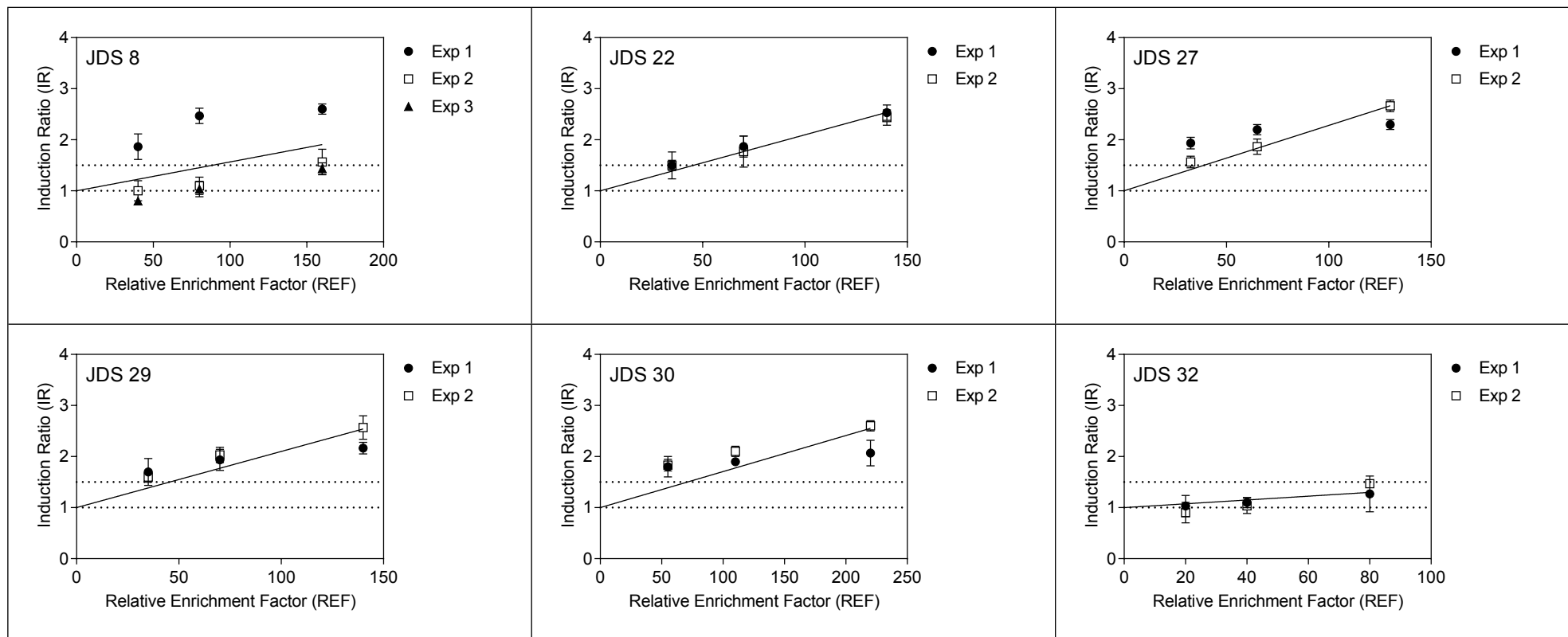
Section S1: Preparation of Krebs-Henseleit buffer

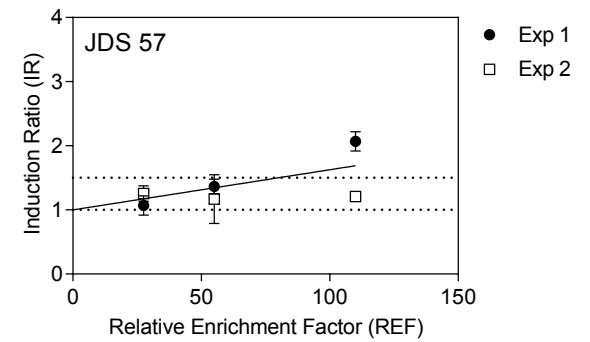
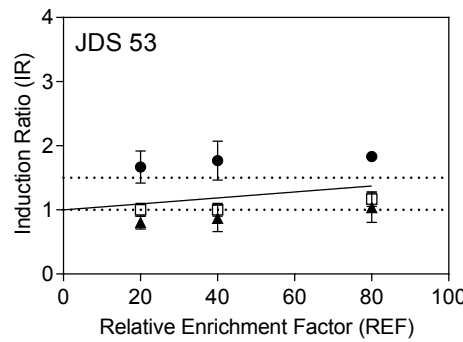
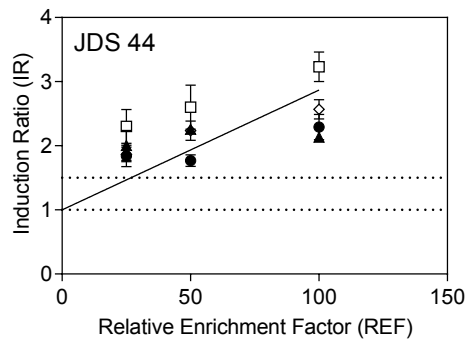
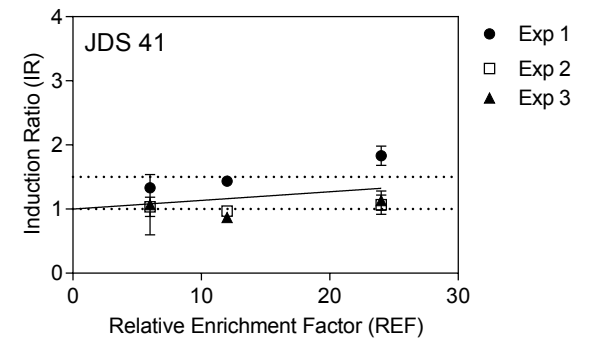
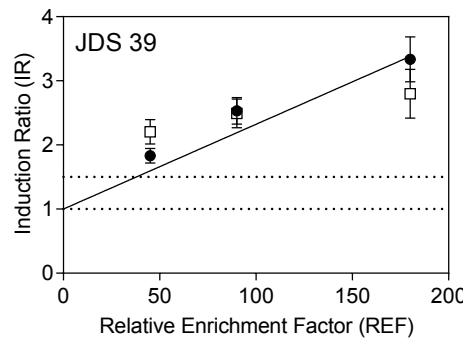
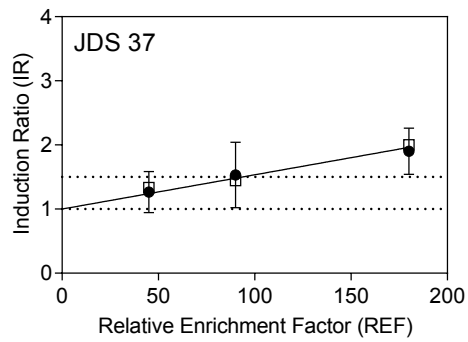
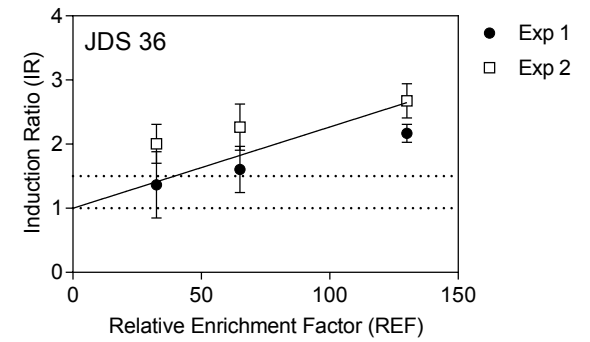
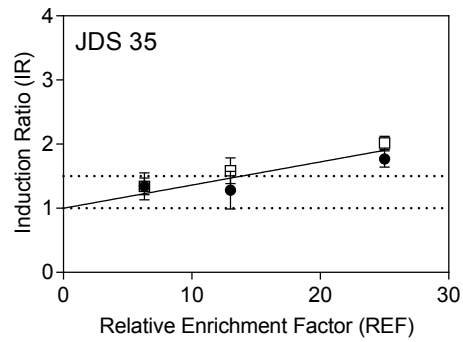
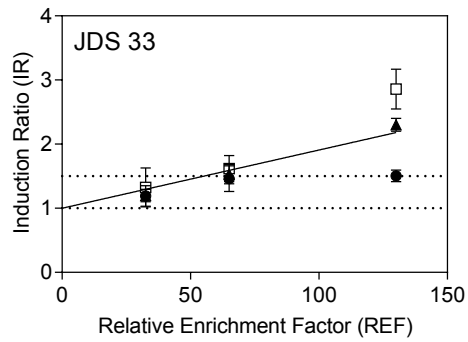
Krebs-Henseleit buffer was prepared prior to the experiment using concentrated stock solutions of the different components (Table S2). One millilitre of each stock solution was added to 27 mL of sterile ultrapure water and the pH was adjusted to 7.16 with 240 μ L 1 M hydrochloric acid. The solution was filter sterilised using 0.22 μ m Millex-GS syringe filter (Merck Millipore, Bayswater, Australia) and final volume was made up to 40 mL using sterile ultrapure water. The final pH was 7.4.

Table S2: Krebs-Henseleit buffer composition.

Chemical	CAS No.	Molecular Weight (g/mol)	Stock concentration (mM)	Final concentration (mM)
D-glucose	50-99-7	180.16	444	11.1
Magnesium sulphate (anhydrous)	7487-88-9	120.37	46.9	1.17
Potassium phosphate monobasic	7778-77-0	136.09	47.0	1.18
Potassium chloride	7447-40-7	74.55	188	4.69
Sodium chloride	7647-14-5	58.44	4723	118
Calcium chloride dihydrate	10035-04-8	147.01	101	2.54
Sodium bicarbonate	144-55-8	84.01	1000	25.0

Figure S1: Concentration-effect curves for the water samples in the DCFH-DA assay. The samples were collected during a previous study on the Joint Danube Survey (JDS), with description of sampling sites provided in Neale *et al.* ²





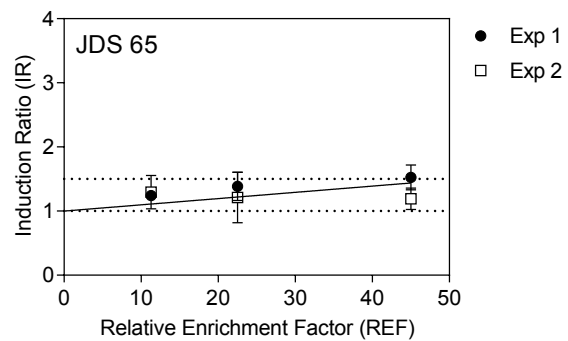
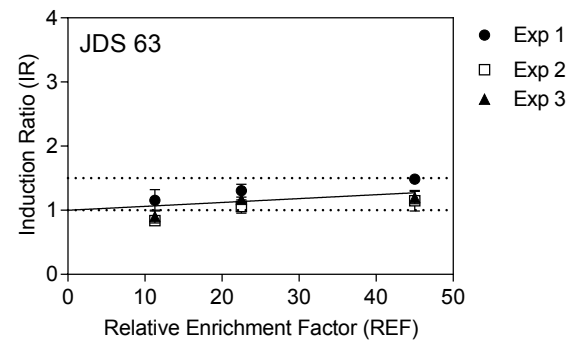
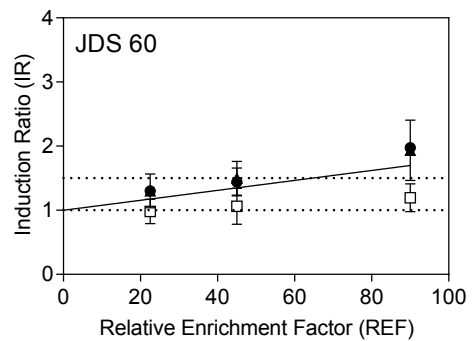
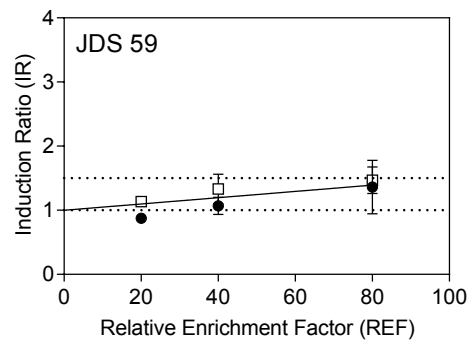
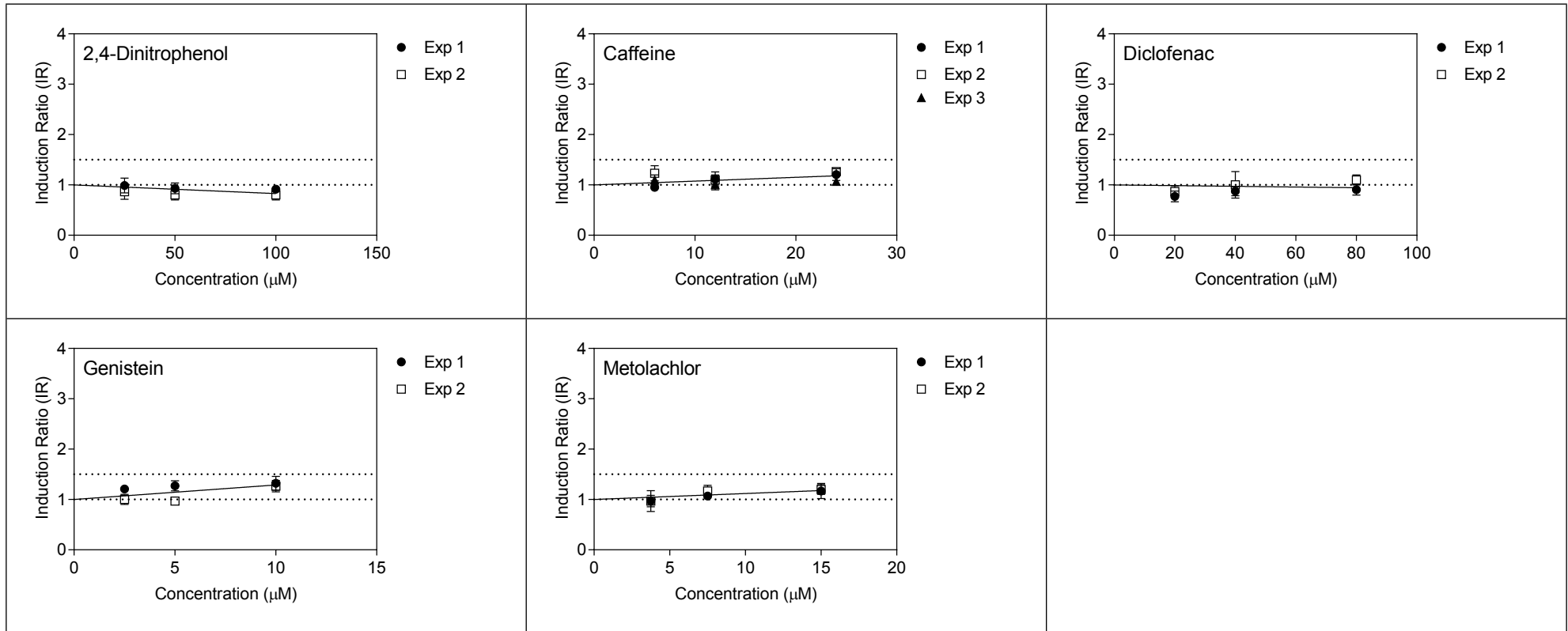


Figure S2: Concentration-effect curves for the studied chemicals in the DCFH-DA assay.



References

1. US EPA, Interactive Chemical Safety for Sustainability (iCSS) Dashboard v2, <http://actor.epa.gov/dashboard/>, Accessed 5th Jan 2016, <http://actor.epa.gov/dashboard/>, Accessed 5th Jan 2016.
2. P. A. Neale, S. Ait-Aissa, W. Brack, N. Creusot, M. S. Denison, B. Deutschmann, K. Hilscherova, H. Hollert, M. Krauss, J. Novak, T. Schulze, T. B. Seiler, H. Serra, Y. Shao and B. I. Escher, *Environ. Sci. Technol.*, 2015, **49**, 14614-14624.