Environ. Chem. **2015**, *12*, 267–285
doi:10.1071/EN14054_AC
©CSIRO 2015

Supplementary material

Chemical and bioanalytical assessment of coal seam gas associated water

Janet Y. M. Tang[A,G], Mauricio Taulis[B], Jacinta Edebeli[A], Frederic D.L. Leusch[C], Paul Jagals[D], Gregory P. Jackson[E] and Beate I. Escher[A,F]

[A]The University of Queensland, National Research Centre for Environmental Toxicology (Entox), Coopers Plains, Qld 4108, Australia.

[B]Queensland University of Technology, School of Earth, Environmental, and Biological Sciences, Brisbane, Qld 4001, Australia.

[C]Griffith University, Smart Water Research Centre, Southport, Qld 4222, Australia.

[D]The University of Queensland, School of Population Health, Herston, Qld 4006, Australia.

[E]Department of Health, Health Protection Unit, Herston, Qld 4029, Australia.

[F]Helmholtz Centre for Environmental Research – UFZ, D-04318 Leipzig, Germany.

[G]Corresponding author. Email: y.tang@uq.edu.au

 ©CSIRO 2015

Table S1. Supplementary information on preservatives and sample containers used for chemical analysis

Analytes	Container	Washing	Preservative
pH, conductivity, colour, Na, K, Ca, Mg, fluoride, hardness, SiO_2, turbidity	125-mL HDPE	Reverse osmosis water	1 mL of ethylene diamine (0.5 % w/v)
Heavy metals	250-mL HDPE	Acid	2.5 mL of nitric acid
Dissolved metals	1-L HDPE	Detergent	None
Sulfide	500-mL HDPE	Detergent	2 mL of zinc acetate (10 % solution)
Phenols, total petroleum hydrocarbons	1-L amber glass	Solvent	80 mg of sodium thiosulfate
VOC, BTEX	2 × 40-mL glass vials with septum cap	None	Sodium bisulfate
PAHs	1-L amber glass	Solvent	80 mg of sodium thiosulfate

Supplementary information on the chemical analysis of the organic compounds at QHFSS

The total petroleum hydrocarbons (TPHs) were analysed by Gas Chromatography–Flame Ionisation Detector (GC/FID) after solid phase extraction according to QHFSS method QIS 16308. The phenols and PAHs were analysed by Gas Chromatography–Mass Spectrometry (GC-MS) after solvent extraction according to QHFSS method QIS 16647. The volatile organics were analysed by purge and trap GC-MS, which were subcontracted to Advanced Analytical P/L.

Supplementary information on bioanalytical tools used in this study

Induction of xenobiotic metabolism

AhR-CAFLUX

The hepatoma H4IIE cells, which are stably transfected with the dioxin control elements and green fluorescent protein (GFP),[1–3] were seeded for 24 h at 3×10^5 cells mL^{-1} in 96-well black, clear-bottom microtitre plates (Corning). This cell line was a gift from Prof Michael Dension from UC Davis. Prior to dosing the sample extracts were evaporated to near dryness and reconstituted with MEM α medium consisted of 10 % foetal bovine serum (FBS) (GIBCO BRL Life Technologies) and were serially diluted before transferring to the plates containing the seeded cells. Sixteen concentrations were used in each experiment. Concentrations of reference compound 2,3,7,8-tetrachloro-dibenzo-dioxin (TCDD, Novachem) ranging from 0.1 pM to 10 nM were run on each plate. The GFP signal was measured after 24 h of incubation at 485-nm excitation and 520-nm emission on a FluoStar Omega plate reader (BMG Labtech). The induction of AhR is proportional to the quantity of GFP expressed.

Specific modes of action

ERα-, AR, GR, PR and TRβ-CALUX

The U2OS human osteosarcoma cells had been stably transfected with either estrogen receptor α (ERα),[4] androgen receptor (AR),[5] glucocorticoid receptor (GR),[6] progesterone receptor (PR)[6] or thyroid receptor β (TRβ)[7] with a luciferase reporter gene. Upon exposure to endocrine active compounds, the enzyme luciferase is induced and the endocrine activity can be quantified as luminescence production after adding the substrate luciferin. The CALUX cells were seeded at 8×10^4 to 1.2×10^5 cells mL^{-1} (depending on the specific assay) in 96-well clear microtitre plates (Greiner Bio-One) with DMEM/F12 medium without phenol red supplemented with 5 % stripped FBS and 100-μM non-essential amino acids (GIBCO BRL Life Technologies) for 24 h (except for the ERα-CALUX cells that were incubated for a further 24 h with a change of medium after the first 24 h). The sample extracts were serially diluted and transferred to the seeded cells, the final methanol concentration was below 0.1 %. Three concentrations in two replicate wells were run in each independent experiment. After incubation for 24 h the medium was completely removed and the cells were lysed with 30 μL of lysis reagent (25 mM Tris, 2 mM dithiothreitol, 2 mM EDTA, 10 %

glycerol and 1 % Triton X-100 in MilliQ water). The luciferase activity was measured using a FluoStar Omega plate reader (BMG Labtech) at the luminescence mode after injection of the glowmix as described in Van der Linden et al.[6] Reference compounds were run on each plate, 17*β*-estradiol (E_2) and tamoxifen for ER*α*-CALUX in agonist and antagonist modes respectively. Dihydrotestosterone and flutamide were used for AR-CALUX in agonist and antagonist modes respectively. Dexamethasone and mifepristone were used for GR-CALUX in agonist and antagonist modes respectively. Levonorgestrel and mifepristone were used for PR-CALUX in agonist and antagonist modes respectively. Triiodothyronine (T_3) was used for TRβ-CALUX in agonist mode. All reference compounds were purchased from Sigma–Aldrich.

GH3.TRE-luc

The GH3.TRE-luc cells were grown in DMEM/F12 supplemented with 10 % FBS (GIBCO BRL Life Technologies), the medium was replaced with serum free medium PCM 24 h before plating.[8] The PCM medium contained DMEM/F12 without phenol red (GIBCO BRL Life Technologies), 10 µg mL^{-1} bovine insulin, 10 µM ethanolamine, 10 ng mL^{-1} sodium selenite, 10 µg human apotrasferrin, and 500 µg mL^{-1} bovine serum albumin (all components were purchased from Sigma–Aldrich). The cells were seeded for 24 h at 3×10^5 cells mL^{-1} in 96-well white, clear-bottom microtitre plates (Corning). Prior to dosing the sample extracts were evaporated to near dryness and reconstituted with PCM medium and were serially diluted before transferring to the seeded cells. Fourteen concentrations in duplicate wells were performed in each experiment. Reference compound T_3 for ranging from 1 fM to 8.4 nM was run on each plate. At the end of a 24 h incubation 50 µL of lysis reagent (125 mM Tris, 10 mM dithiothreitol, 10 mM EDTA, 50 % glycerol and 5 % Triton X-100 in MilliQ water) was added to each well. The luciferase activity was measured using a FluoStar Omega plate reader (BMG Labtech) at the luminescence mode after addition of 100 µL of luciferase reagent (60 mM Tricine, 8 mM magnesium sulfate heptahydrate, 0.3 M EDTA, 100 mM DTT, 0.83 mM coenzyme A, 1.6 mM ATT, 1.4 mM luciferin).

Reactive modes of action

umuC *assay*

The bacteria *Salmonella typhimurium* TA1535 were stably transfected with the plasmid pSK 1002 carrying a *lacZ* gene under the control of the *umu* regulatory region.[9] The *lacZ* reporter gene encodes for proteins with *β*-galactosidase activity that can metabolise a colourless substrate ortho-nitrophenyl-*β*-galactoside (ONPG) into a yellow-coloured product ortho-nitrophenol (ONP). The induced enzyme activity was measured at 420 nm in the absorbance mode on a FluoStar Optima plate reader (BMG Labtech). The assay was also run in the presence of a rat liver metabolic enzyme mix (S9 fraction) to differentiate between chemicals that require metabolic activation and those that are detoxified by metabolism. Six concentrations were run on each experiment. Reference compound for the non-

metabolic mode was 4-nitroquinoline-1-oxide (4NQO) and for the metabolic active mode was 2-aminoanthracene (2AA). Both 4NQO and 2AA were purchased from Sigma–Aldrich.

E. coli *GSH^+/GSH^- assay*

The *E.coli* GSH^+/GSH^- assay for protein damage is based on the growth inhibition differences between a strain that is glutathione-deficient (GSH^-) and the corresponding parent strain (GSH^+).[10,11] In this assay the parent strain MJF276 (GSH^+) is capable of synthesising GSH whereas the mutant strain MJF335 (GSH^-) lacks both *γ*-glutamylcysteine synthase and GSH synthase. The GSH^- strain has the same sensitivity as the GSH^+ strain to non-reactive and DNA damaging chemicals but the GSH^- strain is more sensitive to chemicals that attack cysteine in proteins. The difference in effect concentrations for 50 % inhibition of growth assessed as biomass increase between GSH^+ and GSH^- indicates the relevance of GSH conjugation as a detoxification step as well as direct reactivity with cysteine-containing proteins. The growth measurements were measured at 600-nm absorbance mode on a FluoStar Omega plate reader (BMG Labtech). Sixteen concentrations were run on each experiment. The reference compound for this assay was Sea-Nine (4,5-dichloro-2-*n*-octyl-4-isothiazolin-3-one, CAS 64359–81–5, >97 %, Rohm and Haas Co.).

Adaptive stress response

Induction of oxidative stress responses (AREc32)

The AREc32 cells[12] were seeded for 24 h at 1.2×10^5 cells mL^{-1} in 96-well white, clear-bottom microtitre plates (Corning). Prior to dosing the sample extracts were evaporated to near dryness and reconstituted with DMEM supplemented with 10 % FBS (GIBCO BRL Life Technologies) and were serially diluted before transferring to the seeded cells. Sixteen concentrations were run on each experiment. Reference compound tert-butylhydroquinone (tBHQ, Sigma–Aldrich) ranging from 1 to 8.6 μM was run on each plate. At the end of a 24-h incubation the medium was completely removed and the cells were lysed with 30 μL of lysis reagent (25 mM Tris, 2 mM dithiothreitol, 2 mM EDTA, 10 % glycerol and 1 % Triton X-100 in MilliQ water). The luciferase activity was measured using a FluoStar Omega plate reader (BMG Labtech) at the luminescence mode after addition of 100 μL of luciferase reagent (20 mM Tricine, 2.67 mM magnesium sulfate heptahydrate, 0.1 M EDTA, 33.3 mM DTT, 261 μM coenzyme A, 530 μM ATT, 470 μM luciferin).

Cell viability/cytotoxicity

Microtox assay or bioluminescence inhibition test with bacteria Vibrio fischeri

The Microtox assay[13] measures the relative decrease in light output in luminescence mode following exposure to water extracts after 30-min incubation. Damage occurring at any cellular level including the disruption of membranes, electron transport chain, enzymes or cytoplasmic composition can result in decreased light output. Phenol was used as the reference chemical but a ‘virtual baseline

toxicant' with a $\log K_{ow}$ of 3 and a molecular weight of 300 $g\,mol^{-1}$ was used to describe the bioanalytical equivalents.[14]

Caco2-NRU (neutral red uptake) assay

The Caco2-NRU assay was adapted from Konsoula and Barile.[15] In brief, Caco2 cells were grown in DMEM/F12 with phenol red supplemented with 8 % FBS and 100 μM non-essential amino acids (GIBCO BRL Life Technologies), the cells were seeded at 2×10^5 cells mL^{-1} in 96 well plates (Greiner Bio-One) before dosing with the sample extracts with a final methanol concentration below 1 %. Methanol at this concentration failed to produce toxicity. Three concentrations in two replicate wells were run in each experiment. After 21 h of incubation the medium was removed and replaced with neutral red medium (50 μg mL^{-1} neutral red). After a further 3 h incubation the neutral red medium was removed and replaced with neutral red desorbing fixative (1 % acetic acid, 50 % ethanol) before shaking on an orbital shaker for 10 min at 600 rpm at room temperature. The absorbance was read in a FluoStar Omega plate reader (BMG Labtech) at 540 nm.

AREc32 cell viability assay

In the AREc32 cell viability assay the medium used and the cell density were the same as described above for the AREc32 induction assay, except the cells were seeded on 96-well clear microtitre plates (Corning). Sixteen concentrations were run on each experiment. After 21 h of incubation the medium was removed and replaced with MTS solution (CellTiter Aqueous One Solution Cell Proliferation Assay, Promega) for further 2-h incubation. The absorbance was read in a FluoStar Omega plate reader (BMG Labtech) at 492 nm.

THP1 cytokine production (TP1-CPA) assay

The THP1-CPA methodology was adapted from Baqui *et al*.[16] In brief, THP1 cells were seeded at 1×10^6 cells mL^{-1} in 96 well plates (Greiner Bio-One) in DMEM/F12 with phenol red supplemented with 8 % FBS, 100 μM non-essential amino acids (GIBCO BRL Life Technologies) and 1 μg mL^{-1} *E. coli* lipopolysaccharide (LPS, Sigma–Aldrich). The sample extracts were serially diluted and transferred to the cells for 24-h incubation and the final methanol concentration was below 0.1 %. Methanol at this concentration failed to produce toxicity. Three concentrations in two replicate wells were run in each experiment. After 24 h the cells were transferred to a V-bottom 96 well plate (Corning), centrifuged at 300*g* for 5 min and the supernatant was transferred to a new flat-bottom 96 well plate. The IL1β concentration in the supernatant was quantified by a Human IL1β Quantikine ELISA kit (R&D Systems).

 ©CSIRO 2015

Table S2. On-site measurements of physicochemical properties and sample electroneutrality assessed in the laboratory

Sample ID	Sampling date	Conductivity at t = 25°C (µS cm^{-1})	pH	Sample temperature (°C)	Sample electroneutrality (%)
RN 15811	20-Mar-12	2746	9.42	19.7	0.48
RN 15811	14-Nov-13	2560	7.15	25.2	1.01
RN 107761	20-Mar-12	3250	8.18	26.0	3.56
RN 107761	14-Nov-13	3820	9.8	27.5	0.60
RN 100739	20-Mar-12	2006	8.53	23.6	0.73
RN 24467	14-Nov-13	3950	8.99	26.8	0.30

Table S3. Phenols by GCMS (both 2012 and 2013 sampling campaigns)

WB, non-CSG water bore

	Concentration units	LOR	Public Health Regulation Standard	RN 15811		RN 107761		RN 107739	RN 24467	WB	Field Blank	Trip Blank
Sampling year				2012	2013	2012	2013	2012	2013	2013	2013	2013
Phenol	µg L^{-1}	0.25	150	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	0.5	<0.25	<0.25
2-Chlorophenol	µg L^{-1}	1	300	<1	<1	<1	<1	<1	<1	<1	<1	<1
4-Chlorophenol	µg L^{-1}	0.3	10	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
2-Methylphenol	µg L^{-1}	1		<1	<1	<1	<1	<1	<1	<1	<1	<1
4-Methylphenol	µg L^{-1}	1	600	<1	<1	<1	<1	<1	<1	<1	<1	<1
2-Nitrophenol	µg L^{-1}	1		<1	<1	<1	<1	<1	<1	<1	<1	<1
2,4-Dimethylphenol	µg L^{-1}	1		<1	<1	<1	<1	<1	<1	<1	<1	<1
2,4-Dichlorophenol	µg L^{-1}	1	200	<1	<1	<1	<1	<1	<1	<1	<1	<1
4-Chloro-3-methylphenol	µg L^{-1}	1	10	<1	<1	<1	<1	<1	<1	<1	<1	<1
2,4,6-Trichlorophenol	µg L^{-1}	1		<1	<1	<1	<1	<1	<1	<1	<1	<1
2,4,5-Trichlorophenol	µg L^{-1}	1	20	<1	<1	<1	<1	<1	<1	<1	<1	<1
2,4-Dinitrophenol	µg L^{-1}	1	350	<1	<1	<1	<1	<1	<1	<1	<1	<1
4-Nitrophenol	µg L^{-1}	1		<1	<1	<1	<1	<1	<1	<1	<1	<1
2,3,4,6-Tetrachlorophenol	µg L^{-1}	1	30	<1	<1	<1	<1	<1	<1	<1	<1	<1
2-Methyl-4,6-dinitrophenol	µg L^{-1}	1		<1	<1	<1	<1	<1	<1	<1	<1	<1
Pentachlorophenol	µg L^{-1}	1	10	<1	<1	<1	<1	<1	<1	<1	<1	<1
Coumarin	µg L^{-1}	0.3	0.5	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3

©CSIRO 2014
doi:10.1071/EN14054_AC

Table S4. Volatile organic carbons (VOCs) by purge and trap GCMS (2012 sampling campaign only)

	Units	LOR	RN 15811	RN 107761	RN 100739
BTEX (benzene, toluene, ethylbenzene, xylene)					
Benzene	µg L^{-1}	1	<1	<1	<1
Toluene	µg L^{-1}	2	<2	<2	<2
Ethylbenzene	µg L^{-1}	2	<2	<2	<2
m- & *p*-Xylenes	µg L^{-1}	4	<4	<4	<4
o-Xylene	µg L^{-1}	2	<2	<2	<2
Total Xylenes	µg L^{-1}	6	<6	<6	<6
Total BTEX	µg L^{-1}	11	<11	<11	<11
THMs (trihalomethanes)					
Chloroform	µg L^{-1}	2	<2	<2	<2
Bromodichloromethane	µg L^{-1}	2	<2	<2	<2
Dibromochloromethane	µg L^{-1}	2	<2	<2	<2
Bromoform	µg L^{-1}	2	<2	<2	<2
Total Trihalomethanes	µg L^{-1}	8	<8	<8	<8
Halogenated Aliphatic Chemicals					
1,1,1,2-Tetrachloroethane	µg L^{-1}	2	<2	<2	<2
1,1,1-Trichloroethane	µg L^{-1}	2	<2	<2	<2
1,1,2,2-Tetrachloroethane	µg L^{-1}	2	<2	<2	<2
1,1,2-Trichloroethane	µg L^{-1}	2	<2	<2	<2
1,1-Dichloroethane	µg L^{-1}	2	<2	<2	<2
1,1-Dichloroethene	µg L^{-1}	2	<2	<2	<2
1,1-Dichloropropene	µg L^{-1}	2	<2	<2	<2
1,2,3-Trichloropropane	µg L^{-1}	2	<2	<2	<2
1,2-Dibromo-3-chloropropane	µg L^{-1}	2	<2	<2	<2
1,2-Dibromoethane (EDB)	µg L^{-1}	1	<1	<1	<1
1,2-Dichloroethane	µg L^{-1}	2	<2	<2	<2
cis-1,2-Dichloroethene	µg L^{-1}	2	<2	<2	<2
trans-1,2-Dichloroethene	µg L^{-1}	2	<2	<2	<2
1,2-Dichloropropane	µg L^{-1}	2	<2	<2	<2
1,3-Dichloropropane	µg L^{-1}	2	<2	<2	<2
cis-1,3-Dichloropropene	µg L^{-1}	2	<2	<2	<2
trans-1,3-Dichloropropene	µg L^{-1}	2	<2	<2	<2
trans-1,4-Dichloro-2-butene	µg L^{-1}	2	<2	<2	<2
1-Chlorobutane	µg L^{-1}	2	<2	<2	<2
2,2-Dichloropropane	µg L^{-1}	2	<2	<2	<2
Allyl chloride	µg L^{-1}	2	<2	<2	<2
Bromochloromethane	µg L^{-1}	2	<2	<2	<2
Carbon tetrachloride	µg L^{-1}	2	<2	<2	<2
Dibromomethane	µg L^{-1}	2	<2	<2	<2
Dichlorodifluoromethane	µg L^{-1}	10	<10	<10	<10
Dichloromethane	µg L^{-1}	4	<4	<4	<4
Hexachlorobutadiene	µg L^{-1}	1	<2	<2	<2
Hexachloroethane	µg L^{-1}	2	<2	<2	<2
Iodomethane	µg L^{-1}	2	<2	<2	<2
Pentachloroethane	µg L^{-1}	2	<2	<2	<2
Tetrachloroethene	µg L^{-1}	2	<2	<2	<2
Trichloroethene	µg L^{-1}	2	<2	<2	<2
Trichlorofluoromethane	µg L^{-1}	10	<10	<10	<10
Vinyl chloride	µg L^{-1}	10	<10	<10	<10
Halogenated aromatics					
1,2,3-Trichlorobenzene	µg L^{-1}	2	<2	<2	<2
1,2,4-Trichlorobenzene	µg L^{-1}	2	<2	<2	<2
1,2-Dichlorobenzene	µg L^{-1}	2	<2	<2	<2
1,3-Dichlorobenzene	µg L^{-1}	2	<2	<2	<2
1,4-Dichlorobenzene	µg L^{-1}	2	<2	<2	<2
2-Chlorotoluene	µg L^{-1}	2	<2	<2	<2
4-Chlorotoluene	µg L^{-1}	2	<2	<2	<2

	Units	LOR	RN 15811	RN 107761	RN 100739
Bromobenzene	μg L^{-1}	2	<2	<2	<2
Chlorobenzene	μg L^{-1}	2	<2	<2	<2
Other aromatics					
1,2,4-Trimethylbenzene	μg L^{-1}	2	<2	<2	<2
1,3,5-Trimethylbenzene	μg L^{-1}	2	<2	<2	<2
4-Isopropyltoluene	μg L^{-1}	2	<2	<2	<2
n-Butylbenzene	μg L^{-1}	2	<2	<2	<2
sec-Butylbenzene	μg L^{-1}	2	<2	<2	<2
tert-Butylbenzene	μg L^{-1}	2	<2	<2	<2
Isopropylbenzene	μg L^{-1}	2	<2	<2	<2
n-Propylbenzene	μg L^{-1}	2	<2	<2	<2
Naphthalene	μg L^{-1}	4	<4	<4	<4
Nitrobenzene	μg L^{-1}	1	<1	<1	<1
O-, N-, S- compounds					
2-Butanone (MEK)	μg L^{-1}	4	<4	<4	<4
2-Hexanone (MBK)	μg L^{-1}	4	<4	<4	<4
2-Nitropropane	μg L^{-1}	1	<1	<1	<1
4-Methyl-2-pentanone (MIBK)	μg L^{-1}	4	<4	<4	<4
Acetone	μg L^{-1}	4	<4	<4	<4
Acrylonitrile	μg L^{-1}	0.1	<0.1	<0.1	<0.1
Carbon disulfide	μg L^{-1}	2	<2	<2	<2
Chloroacetonitrile	μg L^{-1}	1	<1	<1	<1
Ethyl methacrylate	μg L^{-1}	4	<4	<4	<4
Methacrylonitrile	μg L^{-1}	0.1	<0.1	<0.1	<0.1
Methyl acrylate	μg L^{-1}	0.1	<0.1	<0.1	<0.1
Methyl methacrylate	μg L^{-1}	0.1	<0.1	<0.1	<0.1
Methyl t-butyl ether (MTBE)	μg L^{-1}	4	<4	<4	<4

References

[1] S. R. Nagy, J. R. Sanborn, B. D. Hammock, M. S. Denison, Development of a green fluorescent protein-based cell bioassay for the rapid and inexpensive detection and characterization of Ah receptor agonists. *Toxicol. Sci.* **2002**, *65*, 200–10. doi:10.1093/toxsci/65.2.200

[2] B. Zhao, D. S. Baston, E. Khan, C. Sorrentino, M. S. Denison, Enhancing the response of CALUX and CAFLUX cell bioassays for quantitative detection of dioxin-like compounds. *Sci. China – Chem.* **2010**, *53*, 1010–6. doi:10.1007/s11426-010-0142-8

[3] L. Jin, L. van Mourik, C. Gaus, B. Escher, Applicability of passive sampling to bioanalytical screening of bioaccumulative chemicals in marine wildlife. *Environ. Sci. Technol.* **2013**, *47*, 7982–8. doi:10.1021/es401014b

[4] B. van der Burg, R. Winter, M. Weimer, P. Berckmans, G. Suzuki, L. Gijsbers, et al., Optimization and prevalidation of the *in vitro* ER alpha CALUX method to test estrogenic and antiestrogenic activity of compounds. *Reprod. Toxicol.* **2010**, *30*, 73–80. doi:10.1016/j.reprotox.2010.04.007

[5] B. van der Burg, R. Winter, H. Y. Man, C. Vangenechten, P. Berckmans, M. Weimer, et al., Optimization and prevalidation of the *in vitro* AR CALUX method to test androgenic and antiandrogenic activity of compounds. *Reprod. Toxicol.* **2010**, *30*, 18–24. doi:10.1016/j.reprotox.2010.04.012

[6] S. C. van der Linden, M. B. Heringa, H. Y. Man, E. Sonneveld, L. M. Puijker, A. Brouwer, et al., Detection of multiple hormonal activities in wastewater effluents and surface water, using a panel of steroid receptor CALUX bioassays. *Environ. Sci. Technol.* **2008**, *42*, 5814–20. doi:10.1021/es702897y

[7] K. Bekki, H. Takigami, G. Suzuki, N. Tang, K. Hayakawa, Evaluation of Toxic Activities of polycyclic aromatic hydrocarbon derivatives using in vitro bioassays. *J. Health Sci.* **2009**, *55*, 601–10. doi:10.1248/jhs.55.601

[8] A. C. Gutleb, I. A. T. M. Meerts, J. H. Bergsma, M. Schriks, A. J. Murk, T-Screen as a tool to identify thyroid hormone receptor active compounds. *Environ. Toxicol. Pharmacol.* **2005**, *19*, 231–8. doi:10.1016/j.etap.2004.06.003

[9] Y. Oda, S.-i. Nakamura, I. Oki, T. Kato, H. Shinagawa, Evaluation of the new system (umu-test) for the detection of environmental mutagens and carcinogens. *Mutat. Res.* **1985**, *147*, 219–29. doi:10.1016/0165-1161(85)90062-7

[10] A. Harder, B. I. Escher, P. Landini, N. B. Tobler, R. P. Schwarzenbach, Evaluation of bioanalytical assays for toxicity assessment and mode of toxic action classification of reactive chemicals. *Environ. Sci. Technol.* **2003**, *37*, 4962–70. doi:10.1021/es034197h

[11] J. Y. M. Tang, E. Glenn, H. Thoen, B. I. Escher, *In vitro* bioassay for reactive toxicity towards proteins implemented for water quality monitoring. *J. Environ. Monit.* **2012**, *14*, 1073–81. doi:10.1039/c2em10927a

[12] X. J. Wang, J. D. Hayes, C. R. Wolf, Generation of a stable antioxidant response element-driven reporter gene cell line and its use to show redox-dependent activation of Nrf2 by cancer chemotherapeutic agents. *Cancer Res.* **2006**, *66*, 10983–94. doi:10.1158/0008-5472.CAN-06-2298

[13] J. Y. M. Tang, S. McCarty, E. Glenn, P. A. Neale, M. S. Warne, B. I. Escher, Mixture effects of organic micropollutants present in water: towards the development of effect-based water quality trigger values for baseline toxicity. *Water Res.* **2013**, *47*, 3300–14. doi:10.1016/j.watres.2013.03.011

[14] B. I. Escher, N. Bramaz, J. F. Mueller, P. Quayle, S. Rutishauser, E. L. M. Vermeirssen, Toxic equivalent concentrations (TEQs) for baseline toxicity and specific modes of action as a tool to improve interpretation of ecotoxicity testing of environmental samples. *J. Environ. Monit.* **2008**, *10*, 612–21. doi:10.1039/b800949j

[15] R. Konsoula, F. A. Barile, Correlation of *in vitro* cytotoxicity with paracellular permeability in Caco-2 cells. *Toxicol. In Vitro* **2005**, *19*, 675–84. doi:10.1016/j.tiv.2005.03.006

[16] A. Baqui, T. F. Meiller, J. J. Chon, B. F. Turng, W. A. Falkler, Granulocyte-macrophage colony-stimulating factor amplification of interleukin-1 beta and tumor necrosis factor alpha production in THP-1 human monocytic cells stimulated with lipopolysaccharide of oral microorganisms. *Clin. Diagn. Lab. Immunol.* **1998**, *5*, 341–7.