

Supplementary Table 2: *In vivo* and *in vitro* analyses performed in South Africa to test water quality

Target mode of action	No.	<i>In vivo/in vitro</i>	Assay/Assay type	Endpoint	Biological agent	*Type of water	Reference
Non-specific toxicity (baseline toxicity)							
Toxicity	1	<i>In vitro</i>	Biotox assay	Bioluminescence inhibition	<i>Aliivibrio fischeri</i> (= <i>Vibrio fischeri</i>)	Wastewater effluent Carwash effluent	Surujjal-Naicker et al., 2015, Tekere et al., 2016
	2	<i>In vitro</i>	Mammalian cell colony formation inhibition test	Colony formation	Buffalo Green monkey (BGM) kidney cells and Chinese hamster V79 cells	Freshwater	Slabbert et al., 1998
	3	<i>In vitro</i>	Bacterial growth inhibition assay	Growth inhibition	<i>Pseudomonas putida</i>	Freshwater	Slabbert et al., 1998
	4	<i>In vivo</i>	Ostracod toxkit-F	Growth inhibition	<i>Heterocypris incongruens</i>	River	Singh et al., 2017
	5	<i>In vivo</i>	Microplate-based <i>Hydra attenuata</i> assay	Growth rate	<i>Hydra vulgaris</i> (= <i>Hydra attenuata</i>)	Wetland, lake, treated sewage and creek	Oberholster et al., 2008
	6	<i>In vivo</i>	Chronic acid tolerance bioassay	Growth rate (mass & length)	<i>Amietophrynus maculatus</i> , <i>Chiromantis xerampelina</i> , <i>Hildebrandtia ornata</i> , <i>Pyxicephalus edulis</i>	Rain water	Farquharson et al., 2016
	7	<i>In vivo</i>		Hatching rate	<i>A. maculatus</i> , <i>C. xerampelina</i> , <i>H. ornata</i> , <i>P. edulis</i>	Rain water	Farquharson et al., 2016
	8	<i>In vivo</i>		Hatching rate	<i>Pyxicephalus adspersus</i>	Wetland, lake, treated sewage and creek	Oberholster et al., 2008

	9	<i>In vivo</i>	Diptera assay	Mortality	<i>Chironomus caffrarius</i>	River	Singh et al., 2017
	10	<i>In vivo</i>	Fish lethality test	Mortality	<i>Danio rerio, Labeobarbus aeneus, Oreochromis mossambicus, Poecilia reticulata, Pseudocrenilabrus philander, Tilapia sparrmanii</i>	Freshwater, Carwash effluent,	Botha et al., 2015, Brand et al., 2020, Slabbert et al., 1998, Tekere et al., 2016
	11	<i>In vivo</i>	<i>Daphnia/Ceriodaphnia</i> lethality test	Mortality	<i>Daphnia magna Daphnia pulex Ceriodaphnia dubia</i>	Freshwater, Wetland, lake, treated sewage and creek, Carwash effluent	Botha et al., 2015, Oberholster et al., 2008, Slabbert et al., 1998, Tekere et al., 2016
	12	<i>In vivo</i>		Swimming behaviour	<i>D. rerio</i>	Freshwater	Brand et al., 2020
	13	<i>In vitro</i>	Resazurin cell proliferation assay	Mitochondrion activity	GH3 rat pituitary carcinoma cells	Drinking water, surface and treated wastewater, drinking water treatment plant	Simba, 2017
Staining assays	14	<i>In vitro</i>	MTT viability assay	Mitochondrion activity	HuTu-80 human duodenum adenocarcinoma cell H4IIE- <i>luc</i> rat hepatoma cell MDA-kb2 human breast carcinoma cells	Dam, River	Prinsloo et al., 2013 Pheiffer et al., 2019, Vogt et al., 2019 Powrie, 2016
	15	<i>In vitro</i>	WST-1 viability assay	Mitochondrion activity	RAW264.7 mouse macrophage cells	Treated sewage, wastewater	Makene and Pool, 2015, Makene et al., 2016
	16	<i>In vitro</i>	XTT viability assay	Mitochondrion activity	RAW264.7 mouse macrophage cells	Treated sewage	Makene and Pool, 2015
	Specific toxicity						
ENDOCRINE DISRUPTION							
<i>Androgen receptor (AR)</i>							

AR(ant)-agonism	17	<i>In vitro</i>	Reporter gene assay	Receptor mediated effects	MDA-kb2 human breast carcinoma cells	River	De Jager et al., 2011, Powrie, 2016
	18	<i>In vitro</i>	YAS	Receptor mediated effects	<i>Saccharomyces cerevisiae</i>	River	Truter et al., 2016
<i>Oestrogen receptor (ER)</i>							
ER(ant)-agonism	19	<i>In vitro</i>	E-screen (modified)	Proliferation	MCF-7 human breast carcinoma cells	River	Swart et al., 2011
	20	<i>In vitro</i>	YES	Receptor mediated effects	<i>S. cerevisiae</i>	Dam, Laboratory water, Treated drinking water, Wetland, Stream, wastewater, River, drinking water treatment plant	Aneck-Hahn et al., 2005, Aneck-Hahn et al., 2008, Aneck-Hahn et al., 2009, Archer et al., 2020, Du Preez and Slabbert, 2008, Patrick et al., 2020, Van Zijl et al., 2017,
	21	<i>In vitro</i>	Reporter gene assay	Receptor mediated effects	T47D-kBluc human breast carcinoma cells	Feedlot runoff, Treated drinking water	Patrick et al., 2020, Van Zijl et al., 2017
Alternative ER techniques	22	<i>In vitro</i>	MCF-7 ER α ELISA	ER α protein concentration	MCF-7 breast carcinoma cells	River	Swart and Pool, 2009, Swart et al., 2011
	23	<i>In vivo</i>	Gonadosomatic index Urogenital papilla length index	Gonad size; Urogenital papilla length	<i>Clarias gariepinus</i>	Dam	Kruger et al., 2013
	24	<i>In vivo</i>	Vtg ELISA	Vitellogenin production	<i>D. rerio</i> , <i>O. mosambicus</i>	Feedlot runoff, River	Du Preez and Slabbert, 2008, Swart et al., 2011
	25	<i>In vivo</i>	Primary rainbow trout hepatocyte assay	Vitellogenin (Vtg) production	<i>Oncorhynchus mykiss</i>	Feedlot runoff	Du Preez and Slabbert, 2008
	26	<i>In vivo</i>	Vtg ELISA	Vitellogenin production	<i>Xenopus laevis</i>	River, wastewater treatment plant	Pool, 2008
<i>Thyroid receptor (TR)</i>							

TR activity	27	<i>In vitro</i>	Reporter gene assay	Receptor mediated effects	GH3.TRE. <i>luc</i> rat pituitary tumour cells	Drinking water, surface and treated wastewater, drinking water treatment plant	Simba, 2017
DIOXIN-LIKE ACTIVITY							
<i>Aryl hydrocarbon receptor (AhR)</i>							
AhR activity	28	<i>In vitro</i>	Reporter gene assay	Receptor mediated effects	H4IIE- <i>luc</i> rat hepatoma cell	Dam, River	Pheiffer et al., 2019, Vogt et al., 2019
DEVELOPMENTAL TOXICITY							
	29	<i>In vivo</i>	Toad embryo teratogenicity test	Embryo development (size & length), pigmentation, head shape, form of spines and tails	<i>X. laevis</i>	Freshwater	Slabbert et al., 1998
	30	<i>In vivo</i>	Abalone embryo development test	Operculate veliger stage embryo	<i>Haliotis midae</i>	Industrial and domestic effluent, coastal waters	Shackleton et al., 2002
	31	<i>In vivo</i>	Invertebrate reproduction test	Reproduction	<i>D. magna</i> , <i>C. dubia</i>	Freshwater	Slabbert et al., 1998
HEPATOTOXICITY							
Cytochrome P450	32	<i>In vivo</i>	Demethylating fluorescent activity kit	Cytochrome P450 activity	<i>Caridina nilotica</i> , <i>C. gariepinus</i> , <i>Perna perna</i>	River	Coetzee, 2015, Pheiffer, 2017, Van Rensburg et al., 2020
METAL TOXICITY							
	33	<i>In vivo</i>	Metallothionein content	Metallothionein content	<i>Atractolytocestus huronensis</i> , <i>C. nilotica</i> , <i>C. gariepinus</i> , <i>Contraeaecum sp.</i> , <i>Cyprinus carpio</i> , <i>Dreissena polymorpha</i>	Dam, River, Freshwater	Brand et al., 2019, Erasmus et al., 2020, Van Rensburg et al., 2020
NEUROTOXICITY							

Acetylcholinesterase (AChE) activity	34	<i>In vivo</i>	AChE activity assay	AChE activity	<i>A. huronensis</i> , <i>C. nilotica</i> , <i>C. gariepinus</i> , <i>Contracaecum sp.</i> , <i>C. carpio</i> , <i>P. perna</i> , <i>T. sparmanii</i>	River	Coetzee, 2015, Erasmus et al., 2020, Malherbe, 2013, Pheiffer, 2017, Van Rensburg et al., 2020
IMMUNOTOXICITY							
Cytotoxicity	35	<i>In vitro</i>	ELISA	LDH concentration	Whole blood culture	River	Pool and Magcwebeba, 2009
Immunity (cell mediated)	36	<i>In vitro</i>	ELISA	IFN- γ concentration	Whole blood culture	River	Pool and Magcwebeba, 2009
Immunity (hormonal)	37	<i>In vitro</i>	ELISA	IL-10 concentration	Whole blood culture	River	Pool and Magcwebeba, 2009
Inflammatory activity	38	<i>In vitro</i>	ELISA	IL-6 concentration	RAW264.7 mouse macrophage cells Whole blood culture	River, Treated sewage, wastewater	Makene and Pool, 2015, Makene et al., 2016, Pool and Magcwebeba, 2009, Pool et al., 2000
	39	<i>In vitro</i>	Griess reaction	Nitric oxide concentration	RAW264.7 mouse macrophage cells	Treated sewage, wastewater	Makene and Pool, 2015, Makene et al., 2016
Reactive toxicity							
<i>Mutagenicity</i>							
	40	<i>In vitro</i>	Ames	Colony formation	<i>Salmonella typhimurium</i>	Freshwater	Slabbert et al., 1998
	41	<i>In vitro</i>	Cell transformation test	Evidence of malignancies	Hamster embryo cells		
<i>Oxidative stress</i>							
	42	<i>In vivo</i>	Cellular energy allocation	Energy consumption (energy needed to reduce oxygen)	<i>A. huronensis</i> , <i>C. nilotica</i> , <i>C. gariepinus</i> , <i>Contracaecum sp.</i> , <i>C. carpio</i> , <i>T. sparmanii</i>	River	Erasmus et al., 2020, Malherbe, 2013, Pheiffer, 2017, Van Rensburg et al., 2019

43	<i>In vivo</i>	Catalase	Enzyme activity	<i>A. huronensis, C. nilotica, C. gariepinus, Contracaecum sp., C. carpio, D. polymorpha, H. vittatus, P. perna, T. sparmanii</i>	Freshwater, River	Brand et al., 2019, Coetzee, 2015, Erasmus et al., 2020, Gerber et al., 2018, Malherbe, 2013, Pheiffer, 2017, Van Rensburg et al., 2019
44	<i>In vivo</i>	Superoxide dismutase	Enzyme activity	<i>A. huronensis, C. nilotica, C. gariepinus, Contracaecum sp., C. carpio, H. vittatus, P. perna</i>	River	Coetzee, 2015, Erasmus et al., 2020, Gerber et al., 2018, Pheiffer, 2017, Van Rensburg et al., 2019
45	<i>In vivo</i>	Glutathione-S-transferase	Enzyme activity	<i>D. polymorpha</i>	Freshwater	Brand et al., 2019
46	<i>In vivo</i>	Reduced glutathione	Glutathione content	<i>A. huronensis, C. nilotica, C. gariepinus, Contracaecum sp., C. carpio, H. vittatus</i>	River	Erasmus et al., 2020, Gerber et al., 2018, Van Rensburg et al., 2019
47	<i>In vivo</i>	Lipid peroxidation	Malondialdehyde production	<i>A. huronensis, C. nilotica, C. gariepinus, Contracaecum sp., C. carpio, D. polymorpha, H. vittatus, P. perna, T. sparmanii</i>	River	Brand et al., 2019, Coetzee, 2015, Erasmus et al., 2020, Gerber et al., 2018, Malherbe, 2013, Pheiffer, 2017, Van Rensburg et al., 2019
48	<i>In vivo</i>	Protein Carbonyl levels	Protein concentration	<i>A. huronensis, C. nilotica, C. gariepinus, Contracaecum sp., C. carpio, H. vittatus, P. perna, T. sparmanii</i>	River	Coetzee, 2015, Erasmus et al., 2020, Gerber et al., 2018, Malherbe, 2013, Pheiffer, 2017, Van Rensburg et al., 2019

Low-complexity *in vivo* plant assays

Algal growth and PSII inhibition	49	<i>In vivo</i>	Algal growth inhibition assay	Growth inhibition	<i>Selenastrum capricornutum</i>	Carwash effluent, Freshwater	Slabbert et al., 1998, Tekere et al., 2016
Cytotoxicity, growth	50	<i>In vivo</i>	Root growth	Root growth	<i>Allium cepa</i>	Wetland, lake, treated sewage and creek	Oberholster et al., 2008

inhibition							
Seed germination and root growth	51	<i>In vivo</i>	Seed germination	Seed germination	<i>Lactuca sativa</i>	Wetland, lake, treated sewage and creek	Oberholster et al., 2008
	52	<i>In vivo</i>	Phytotoxkit-F	Seed germination, root and shoot growth inhibition	<i>Lepidium sativum, Sinapis alba, Sorghum saccharatum</i>	River	Singh et al., 2017
Low complexity <i>in vivo</i> protozoan assay							
Oxygen uptake	53	<i>In vivo</i>	Protozoan oxygen uptake assay	Oxygen consumption rate	<i>Tetrahymena pyriformis</i>	Freshwater, River	Bulannga and Schmidt 2022, Slabbert et al., 1998
Biomonitoring							
	54	<i>In vivo</i>	Biological diatom index, Generic diatom index, Specific pollution index	Abundance and species richness	Diatoms	River	Holmes and Taylor, 2015, Kock et al., 2019, Schoeman, 1979, Taylor et al., 2007
	55	<i>In vivo</i>	Fish diversity type indices, eg. Fish Response Assessment Index (FRAI) & Fish Assemblage Integrity Index (FAII)	Abundance and species richness	Fish	River	Kleynhans 1999, Malherbe et al., 2015, Malherbe et al., 2016
	56	<i>In vivo</i>	South African Scoring System version 5 (SASS5)	Abundance and species richness	Macroinvertebrates	River	De Necker et al., 2016, Malherbe, 2013, Malherbe et al., 2015, Malherbe et al., 2018, Van Deventer et al., 2021
	57	<i>In vivo</i>	Fish Health Assessment Index	Fish health	Fish	Dam, River	Erasmus et al., 2019, Malherbe, 2013, Nibamureke

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