Water quality monitoring with *in vitro* bioassays to compare untreated oil sands process-affected water with unimpacted rivers

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Table S1: Location Details of Sampling Sites

Site	Latitude (°)	Longitude (°)	Sampling method
June 2021 Sampling			
M1	56.709425	-111.441239	West, by foot
M2	56.720824	-111.405641	West, by foot
FMO	56.769322	-111.408084	Collected directly at the outfall
M3	56.839889	-111.411878	East, by boat
M4	57.127226	-111.602073	Directly below sandbar, by boat
M5	57.158124	-111.628559	Centre, by boat
T1	57.193911	-111.625830	Side stream (west), by boat
M6	57.215791	-111.61250	Centre, by boat
M7	57.313621	-111.670648	Centre, by boat
M8	57.557581	-111.504948	East, by boat
August 2021 Sampling			
T2	56.940291	-111.439454	Thalweg, by boat
S2W	57.034967	-111.504514	West, by boat
S2E	57.035504	-111.501486	East, by boat
MSO	57.035927	-111.506772	Collected directly at the outfall
S4E	57.039686	-111.504488	East, by boat
S1E	57.049622	-111.504956	Thalweg, by boat
M4’	57.127898	-111.600994	Thalweg, by boat

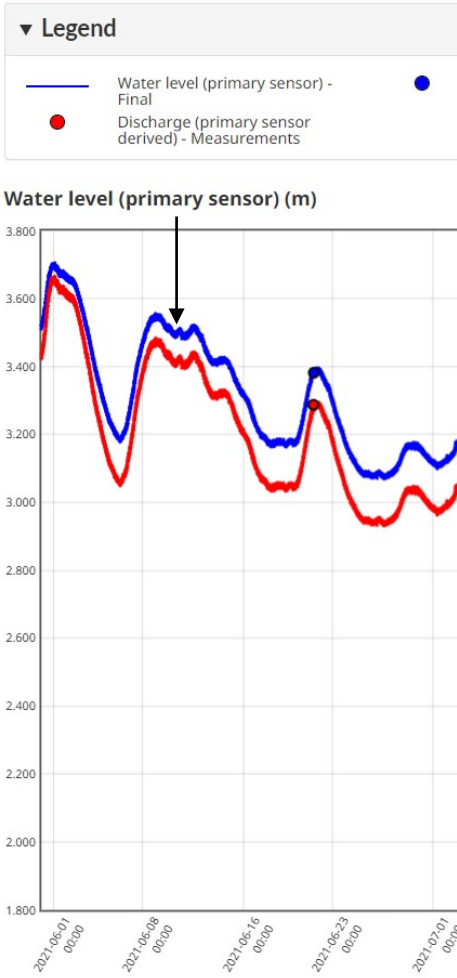

Figure S1. Hydrometric data for Athabasca River (station 07DA001) retrieved from https://wateroffice.ec.gc.ca. Arrows denote our sampling days.

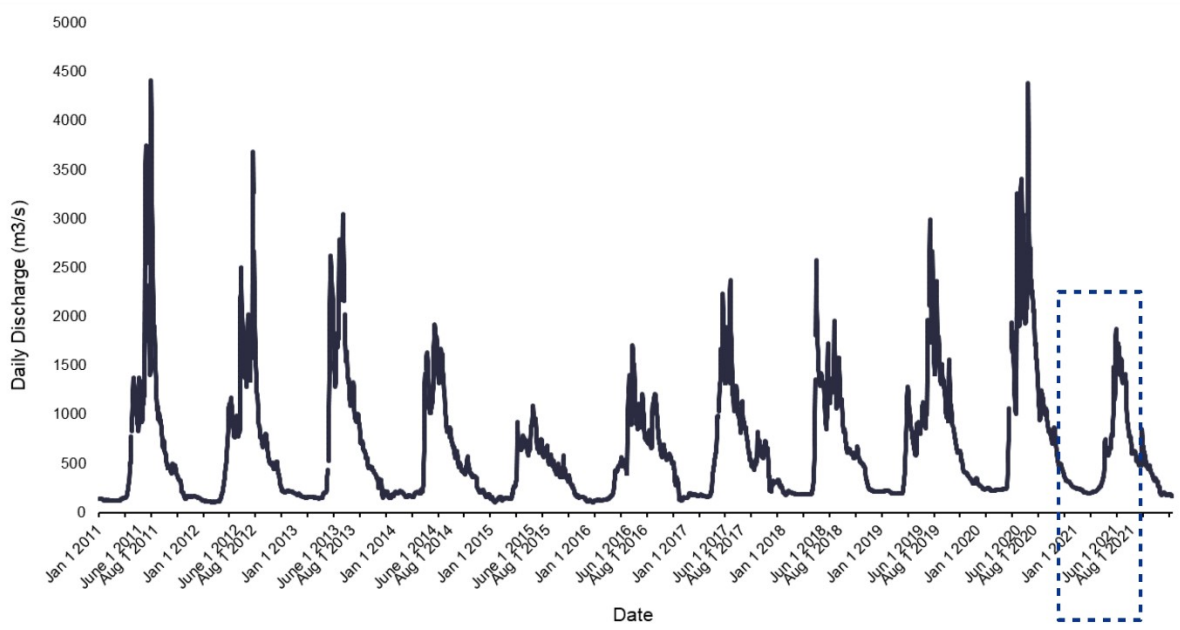

Figure S2. Daily discharge for 2011-2021 in Lower Athabasca River (station 07DA001) retrieved from https://wateroffice.ec.gc.ca. Dashed box denotes our sampling period.

Table S2: Field parameters for samples collected in June 2021. See https://aws.kisters.net[1] database for Aug 2021 sampling. TSS = total suspended solids, TDS = total dissolved solids, TOC = total organic carbon, DOC = dissolved organic carbon, TIC = total inorganic carbon, TN = total nitrogen, DN = dissolved nitrogen, Cl = chloride, PO4-P=phosphate, SO4 = sulfate, NO2 = nitrite-nitrogen, NH4-N = ammonia-nitrogen

Site	pH	Conductivity µs/cm	TDS ppm	Salinity psu	Water temperature °C	Air temperature °C	Humidity %	Wind speed km/h	Atmospheric pressure mm Hg	Altitude ft
M1	7.91	218.3	107.5	0.154	21.2	N/A	N/A	N/A	N/A	N/A
M2	7.82	396.5	194.5	0.242	25	N/A	N/A	N/A	N/A	N/A
FMO	7.02	1584	776.6	0.847	25	N/A	N/A	N/A	N/A	N/A
M3	8.08	183.3	90.8	0.137	17	10.6	69.9	N/A	20.99	877
M4	7.95	443.3	217.7	0.261	17.5	11.3	76	5.7	29.04	835
M5	7.95	248.4	122.2	0.168	17.1	10.9	75.8	8.2	29.05	824
T1	8.05	217.1	106.9	0.153	17.1	11.2	68.2	5.5	29.05	817
M6	8.04	223.9	110.9	0.157	17.3	11.8	72.2	8.5	29.06	811
M7	8.03	211.5	104.1	0.151	17.1	11.3	69.2	10.4	29.08	800
M8	N/A	269.8	132.7	0.178	16.9	11.4	74.8	4.7	29.1	772

Table S3. Water chemistry and physicochemical parameters of samples collected in June 2021

Site	TSS mg/L	TDS mg/L	TOC mg/L	DOC mg/L	TIC mg/L	TN mg/L	DN mg/L	Cl- mg/L	PO4-P µg/L	SO4-S mg/L	TON-N mg/L	NO2-N µg/L	NH4-N µg/L
FMO	59.6	580.4	11.13	11.06	36.05	10.170	9.805	125.4	8	25.1	8.63	14.6	13.7
M1	245.2	152.0	14.19	14.79	18.52	0.474	0.6041	2.3	<LOD	8.2	0.115	<LOD	6.1
M2	277.6	154.0	14.94	15.43	18.48	0.518	0.6422	2.2	14	8.2	0.112	<LOD	5.4
M3	121.6	132.8	13.56	14.26	12.84	0.434	0.521	11.3	13	3.5	0.040	<LOD	5.6
M4	106.0	156.4	13.18	14.47	17.98	0.435	0.605	3.1	9	7.9	0.097	<LOD	5.6
M5	226.8	154.8	13.36	15.00	17.64	0.466	0.6509	3.4	8	7.6	0.112	1.4	5.9
M6	250.4	156.0	13.88	15.06	17.90	0.499	0.6735	3.1	13	7.8	0.114	<LOD	5.5
M7	134.0	148.8	14.01	15.20	16.56	0.472	0.6603	6.1	11	6.1	0.092	<LOD	7.3
M8	172.0	156.8	13.73	15.68	16.11	0.463	0.643	6.4	10	6.6	0.089	<LOD	6.9
T1	160.4	165.6	17.66	19.15	17.34	0.575	0.8055	2.9	10	7.9	0.102	<LOD	5.4

Table S3. Continued. Al = Aluminum, B = Boron, Ca = Calcium, Cu = Copper, Fe = Iron, K = potassium, Mg = Magnesium, Mn = Manganese, Na = Sodium, Ni = Nickel, P = Phosphorus, Pb = lead, S = sulfur, Se = Selenium, Si = Silicon, V = Vanadium, Zn = Zinc

Sites	CaCO3 equiv. mg/L	Al mg/L	B mg/L	Ca mg/L	Cu mg/L	Fe mg/L	K mg/L	Mg mg/L	Mn mg/L	Na mg/L	Ni mg/L	P mg/L	Pb mg/L
FMO	222.10	0.031	0.189	58.047	<LOD	0.054	12.444	18.736	0.098	82.791	0.003	0.047	<LOD
M1	91.75	0.866	0.026	25.182	<LOD	0.701	1.717	7.011	0.007	7.346	0.003	0.025	<LOD
M2	92.34	0.707	0.032	25.324	<LOD	0.609	1.601	7.069	0.006	7.575	0.003	0.024	<LOD
M3	61.31	0.405	0.028	16.423	<LOD	0.637	1.184	4.930	0.007	11.070	<LOD	0.029	0.004
M4	88.81	0.416	0.023	24.429	<LOD	0.417	1.644	6.753	0.006	7.450	0.002	0.022	<LOD
M5	88.91	0.456	0.023	24.473	<LOD	0.454	1.567	6.752	0.006	7.673	0.003	0.023	<LOD
M6	90.00	0.464	0.023	24.794	<LOD	0.449	1.535	6.820	0.006	7.659	0.002	0.023	<LOD
M7	80.26	0.670	0.026	22.117	<LOD	0.656	1.449	6.080	0.008	8.808	0.002	0.028	<LOD
M8	82.77	0.620	0.025	22.910	<LOD	0.622	1.569	6.209	0.008	9.185	0.003	0.029	<LOD
T1	86.89	0.536	0.035	23.496	<LOD	0.560	1.555	6.852	0.007	9.173	0.002	0.027	<LOD

Table S3. continued

Sites	S mg/L	Se mg/L	Si mg/L	V mg/L	Zn mg/L
FMO	25.192	<LOD	2.437	<LOD	0.032
M1	7.179	<LOD	4.412	0.003	<LOD
M2	7.107	<LOD	4.072	0.002	<LOD
M3	3.288	<LOD	3.596	0.002	<LOD
M4	6.795	<LOD	3.282	<LOD	<LOD
M5	6.740	<LOD	3.467	0.002	<LOD
M6	6.866	<LOD	3.434	0.002	<LOD
M7	5.446	<LOD	4.153	0.002	<LOD
M8	5.919	<LOD	3.990	0.002	<LOD
T1	6.946	<LOD	3.395	0.002	<LOD

A. Sample Extraction

Water samples were filtered onsite using glass fiber filters (VWR Glass Fiber Filters, diameter = 4.7cm, particle retention = 1.5 µm, CA28333-129) and acidified to a pH of 2 using formic acid to extract the organic acids during solid phase extraction (SPE). The untreated OSPW was filtered using syringe filters (Basix™ Syringe Filters, Nylon, diameter = 25 mm, pore size = 0.45µm) and acidified similar to the river samples. The SPE vacuum manifold (24-position Supelco Visiprep) was set up with the Oasis HLB cartridges (6 cc, 500 mg), which were first conditioned with 5 mL methanol, followed by 5 mL ultrapure water (Milli-Q system, 18.2 resistance). Samples were then introduced to the cartridges using a vacuum pump (GAST Model DOA-P704-AA). After sample introduction, the cartridges were washed with 10 mL ultrapure water and dried under vacuum for 1 h. For the river and WWTP samples, the SPE cartridges were eluted with 10 mL methanol followed by 10 mL ethyl acetate whereas the OSPW SPE cartridges were eluted with 5 mL methanol then 5 mL methanol: ethyl acetate (1:1 v/v). Eluents were then evaporated, dried (via nitrogen blow down evaporation, Organomation), and reconstituted for chemical and bioanalysis.

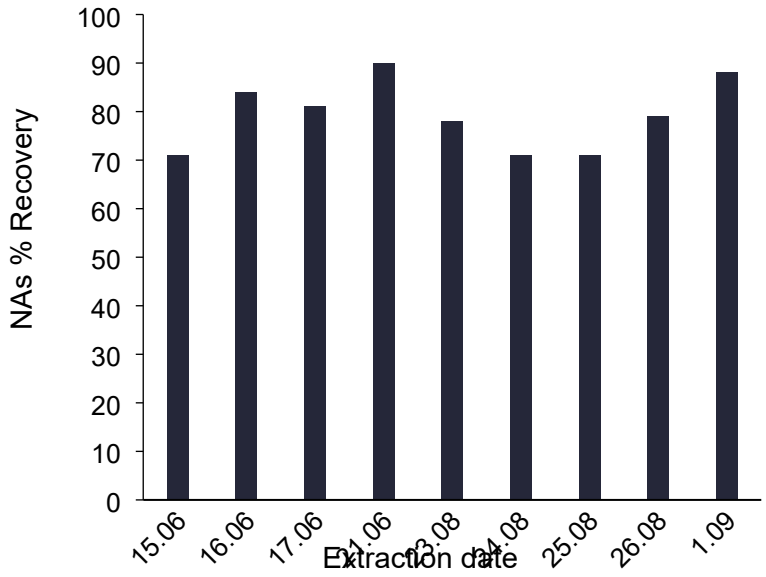

Figure S3. % Recovery of naphthenic acids from spiked samples.

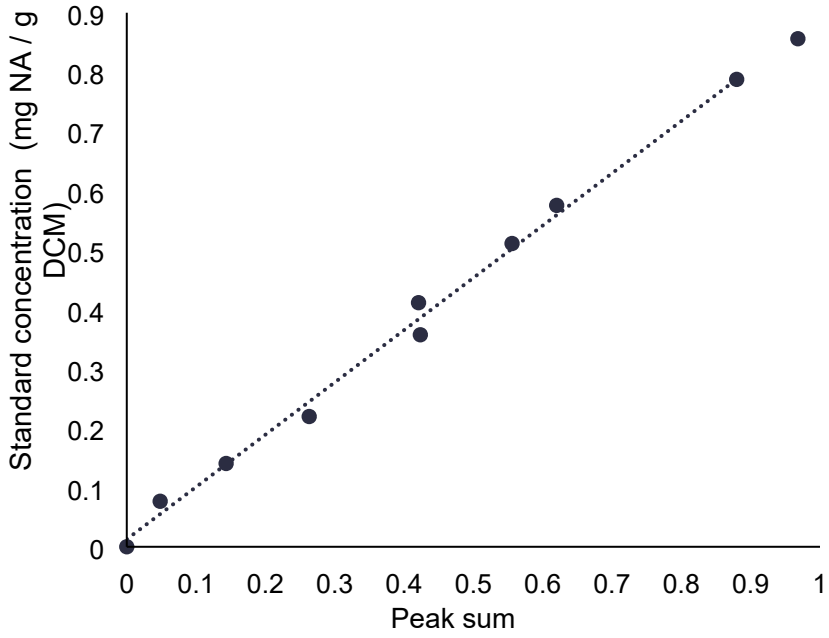

Figure S4. Example of calibration curve for FTIR using commercial Sigma-Aldrich NAs. LOD was calculated using an approach described in detail elsewhere Vlachos *et al.* (2006) [2]. LOD ranges from 0.02 – 0.061 mg/L

B. Selection of the battery of *in vitro* bioassays

(This text was directly taken from MSc Thesis by Kia Barrow -- Barrow (2022) [3])

A battery of *in vitro* bioassays was chosen for this study based off the following criteria. The first criterion was to include toxicity pathways that cover the three classes of modes of action (MOA): non-specific, specific, and reactive toxicity, as recommended by [4]. A MOA refers to a common set of signs that characterize a particular adverse biological response caused by a range of biochemical processes and/or interactions between xenobiotics and an organism [4]. This criterion is important as groups of chemicals with a common MOA act together in mixtures, and a single chemical can act through different MOAs based on exposure duration and target organism. Therefore, by covering the three classes of MOAs, it is more likely to capture a wider picture of the potential adverse effects, allowing for a more comprehensive toxicity assessment of a mixture.

Secondly, a comprehensive literature review was conducted which looked at the toxicity pathways that have been previously identified as being relevant to untreated OSPW through similar and other forms of bioanalyses (Table 1.1). Next, the test battery was chosen based on the protection goal of the samples. Most of the samples used in this study (i.e., surface water and municipal effluents) have the potential to threaten aquatic ecosystem health. Therefore, an emphasis was placed on including relevant pathways that may potentially be impacted upon exposure to these samples. Moreover, it was important to also include bioassays using human cell lines to obtain information on potential impacts on human health as there are estimated 155,000 Indigenous residents living within or adjacent to the Lower Athabasca region [5]. Ultimately, the final battery of bioassays consisted of seven *in vitro* tests (Figure S5).

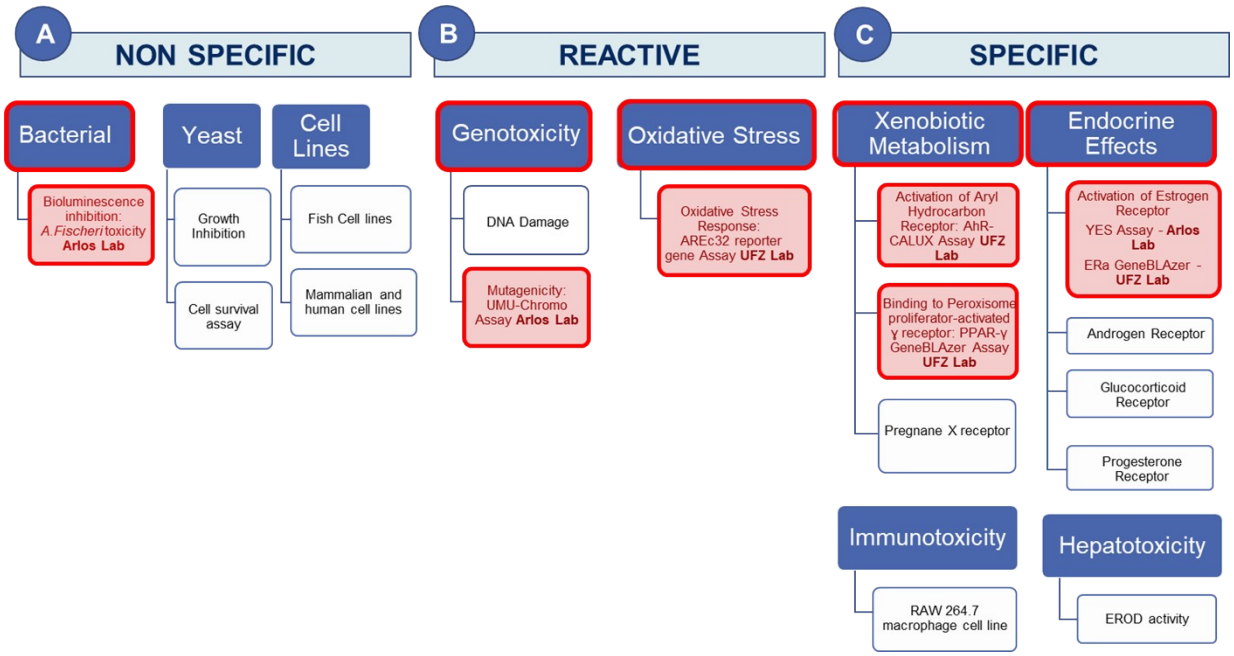

Figure S5. Selection of toxicity pathways. *In vitro* bioanalyses highlighted in red were run in this study based on selection criteria (please see text above). Each assay is discussed in detail subsequently below.

Non-specific toxicity includes all cytotoxic responses that lead to the dysregulation of normal cellular activity [4]. For this MOA, cytotoxicity was measured using *Aliivibrio fischeri* bacteria. This well-established method is used to determine the overall toxic effect of the mixture where cytotoxicity is calculated based on the inhibition of luminescence of the bacteria[6]. This assay is the most frequently employed bioassay in the assessment of OSPW toxicity due to its simplicity, quick results, and high sensitivity to organic compounds (see Table S4)[7].

Reactive toxicity refers to the chemical reactions that occur between the chemical and biological molecules [4]. These MOAs include mutagenicity and oxidative stress. Mutagenicity is the effect caused by a physical or chemical agent that changes the genetic material (e.g., DNA), resulting in a higher-than-normal frequency of mutations in an organism [8]. In this study,

mutagenicity is assessed using the UMU-ChromoTest assay – a method which uses genetically engineered *Salmonella typhimurium* TA1535 to measure the response to genetic damage through colorimetric evaluation. This method is based on the principle that the umuC gene is directly involved and responsible for the induction of mutagenesis [9]. This assay was included due to the known mutagenicity of PACs which are found in OSPW, and municipal WWTP effluents [9, 10]. Based on this, mutagenicity was a critical endpoint to contrast the responses between the types of water samples used in this study.

Oxidative stress response (OSR) is a type of adaptive stress response – a pathway that plays a critical role in returning a cell to homeostasis after damage by stressors [11]. Typically, the presence of electrophilic chemicals and chemicals that produce reactive oxygen species (e.g., disinfection by-products, pesticides, pharmaceuticals) releases the Nrf2 transcription factor which activates the antioxidant response element (ARE) in mammals, therefore inducing the OSR [12-14]. Escher et al. (2013) [14] found that the OSR can be induced by a wide range of chemicals that can directly or indirectly produce reactive oxygen species, and as such the induction of the OSR may be better suited as an early warning of potential adverse effects due to its increased sensitivity. The AREc32 reporter cell line was generated by Wang et al (2006) and adopted by Escher et al. (2013) for water quality assessment [14, 15]. This cell line uses breast cancer cells and allows for the luminescence measurement in response to various chemicals [14]. The AREc32 reporter gene assay was used to measure the OSR in this study.

Specific toxicity refers to all mechanisms by which specific groups of contaminants (e.g., endocrine disrupting compounds) selectively bind to a receptor or interfere with an enzyme function [11]. Typical bioassays employed for this type of toxicity target endocrine effects (e.g., activation of the estrogen receptor) and xenobiotic metabolism as in the detection of the

induction of the aryl-hydrocarbon nuclear receptor (AhR) and binding to the peroxisome proliferator-activated receptor (PPAR).

Endocrine disrupting compounds (e.g., natural and synthetic hormones, alkylphenols, phytoestrogens, pharmaceuticals) are commonly found in wastewater and environmental samples and are known for their toxic effects on the hormonal systems of aquatic organisms which may lead to issues with sexual, development and behavioral patterns [16-18]. Estrogenicity is a relevant pathway for the health of both aquatic ecosystems and humans. It has been well studied that WWTP effluents are a significant source of estrogenic compounds in surface waters, therefore this endpoint was critical in distinguishing between the responses of municipal effluents and untreated OSPW [18, 19]. Moreover, recent studies have shown that OSPW exhibits estrogenic behavior and thus, estrogenicity is an important endpoint when evaluating the toxicity of OSPW-derived extracts [20]. Commonly employed assays targeting the estrogen receptor (ER) include the yeast estrogen screen (YES) and ERα-GeneBLAzer tests [16].

The YES assay uses recombinant *Saccharomyces cerevisiae* yeast which has been transfected with the human estrogen receptor and an expression plasmid carrying the reporter gene *lac-Z* encoding for the enzyme β-galactosidase. Once the yeast is exposed to estrogenic compounds, the β-galactosidase metabolizes the ONPG substrate producing a quantifiable luminescence response [19]. Although the YES assay is simple and inexpensive, there are some limitations such as its relatively high limit of detection, and potential matrix interferences from compounds such as anti-estrogens [21]. These factors motivated the decision to also include the ERα-GeneBLAzer to compare the results of estrogenic activity.

The ERα-GeneBLAzer assay uses a mammalian cell line and is based on the quantification of β-lactamase with fluorescence measurement. This assay is more sensitive to

estrogenic activity than the YES assay, and therefore has a lower limit of detection [22, 23]. This assay has been used for the analysis of estrogenic activity of treated wastewater effluents, surface waters and drinking water [22]. Based on this, it was interesting to compare the estrogenicities of the samples especially OSPW using these assays.

Table S4: Published studies using *in vitro* bioassays for OSPW research. The type of OSPW (fresh, aged, treated, fractionated) are also indicated. WIP: west-in-pit, OF: organic fraction, AEOs: acid extractable organics, IF: inorganic fraction, RCW- recycle water, BMDM: bone marrow-derived macrophages, MLSB- Mildred Lake Settling Basin, PC- petroleum coke, AOP: advanced oxidation process. Table reprinted from Barrow (2022) [3].

OSPW type	Test organism	Toxicity endpoint	Reference
Fresh & aged - fractionated	3T3-L1 preadipocytes cells	Activation of PPARɣ signalling	Peng *et al.* (2016) [24]
WIP- OF- untreated and treated using ozonation	C57BL mouse BMDM	Immunotoxicity	Garcia-Garcia *et al.* (2011) [25]
WIP- untreated and treated using ozonation	C57BL mouse BMDM	Immunotoxicity	Wang *et al.* (2013) [26]
Fractionated & AEOs	*Escherichia coli* strain PQ37	Cytotoxicity and genotoxicity	Zetouni *et al.* (2017) [10]
Untreated and treated using AOP	Goldfish primary kidney macrophage	Acute toxicity and antimicrobial response	Shu *et al.* (2014) [27]
WIP- untreated and treated using ozonation	H295R cells	Cytotoxicity and disruption of sex hormone production	He *et al.* (2010) [28]
Fractionated aged tailings water	H295R cells	Disruption of sex hormone production	Leclair *et al.* (2015) [29]
Fractionated aged tailings water	H4IIE-*luc* cells	Cytotoxicity and binding to the aryl hydrocarbon receptor	Leclair *et al.* (2015) [29]
WIP- untreated & treated using ozonation	MDA-kb2 cells	Androgenic response	He *et al.* (2011) [30]
Whole, OF, IF and reconstituted-OF-IF	RAW 246.7 mouse macrophage	Acute toxicity	Qin *et al.* (2019) [31]
Whole, OF, IF	RAW 246.7 mouse macrophage	Immunotoxicity	Phillips *et al.* (2020) [32]
Fractionated aged tailings water	Recombinant yeast *Saccharomyces cerevisiae* cells	Induction of estrogen and androgen receptor	Leclair *et al.* (2015) [29]
WIP- fractionated- untreated and biologically treated	Recombinant yeast *Saccharomyces cerevisiae* cells	Activation of estrogen receptor	Yue *et al.* (2015) [33]
WIP- Fractionated	RTgill-W1 cell line	Cytotoxicity and uptake of ionizable organic chemicals	Brinkmann *et al.* (2020) [34]
MLSB- fractionated	*Salmonella* strains TA98 and TA100	Mutagenicity	Madill *et al.* (1999) [35]
Fractionated & AEOs	*Salmonella* strains TA98 and TA100	Cytotoxicity and mutagenicity	Zetouni *et al.* (2017) [10]
WIP- untreated & treated using ozonation	T47D-kbluc cells	Estrogenic response	He *et al.* (2011) [30]
Untreated and treated using photocatalytic degradation	THP-1 cell	Detection of immune cell activating compounds	Suara *et al.* (2022) [36]
MLSB- fractionated	*Vibrio fischeri* strain M169	Mutagenicity	Madill *et al.* (1999) [35]
RCW pond- untreated and treated using ozonation	*Vibrio fischeri* bacteria	Acute toxicity	Scott *et al.* (2008) [37]
WIP- fractionated	*Vibrio fischeri* bacteria	Acute toxicity	Frank *et al.* (2009) [38]
WIP- untreated and treated using ozonation	*Vibrio fischeri* bacteria	Acute toxicity	Martin *et al.* (2010) [39]
WIP & coke-treated	*Vibrio fischeri* bacteria	Acute toxicity	Gamal El-Din *et al.* (2011) [40]
WIP, RCW pond, MLSB- untreated and treated with PC adsorption	*Vibrio fischeri* bacteria	Acute toxicity	Zubot *et al.* (2021) [41]

Untreated and treated using simulated wetland	*Vibrio fischeri* bacteria	Acute toxicity	Toor *et al.* (2013) [42]
Fresh- AEOs- untreated	*Vibrio fischeri* bacteria	Acute toxicity	Sohrabi *et al.* (2013) [43]
WIP- untreated and treated using ozonation	*Vibrio fischeri* bacteria	Acute toxicity	Wang *et al.* (2013) [26]
Untreated and treated using AOP	*Vibrio fischeri* bacteria	Acute toxicity	Shu *et al.* (2014) [27]
WIP- untreated and treated using ozonation	*Vibrio fischeri* bacteria	Acute toxicity	Sun *et al.* (2014) [44]
WIP- fractionated	*Vibrio fischeri* bacteria	Acute toxicity	Morandi *et al.* (2015) [45]
Untreated and treated using coagulation/flocculation process	*Vibrio fischeri* bacteria	Acute toxicity	Wang *et al.* (2015) [46]
Untreated and treated using UV/oxidation	*Vibrio fischeri* bacteria	Acute toxicity	Fang *et al.* (2019) [6]
Whole & AEOs	*Vibrio fischeri* bacteria	Acute toxicity	Miles *et al.* (2019) [47]
Untreated and treated using photocatalytic degradation	*Vibrio fischeri* bacteria	Acute toxicity	Suara *et al.* (2022) [36]

C. Methodology

1. Cytotoxicity - *Aliivibrio fischeri* bioluminescence inhibition assay

On the morning of the assay, the *Aliivibrio fischeri* lyophilized bacteria was reconstituted with the reagent diluent (provided by Environmental Bio-detection Products Inc [EBPI]) and equilibrated at 4 °C for at least 30 minutes. 70 µL and 45 µL from the reconstituted SPE extract of the river/WWTP samples and OSPW respectively was transferred into a test tube and evaporated to dryness using a nitrogen evaporator. The dried sample was reconstituted in 900 µL ultrapure water and 100 µL OAS Solution (provided by EBPI). The pH of the sample solution was adjusted to 7 ± 0.2, using 1N sodium hydroxide. A 96-well plate was prepared with a 1:2 serial dilution of 100 µL sample and 100 µL Sample Diluent (provided by EBPI). The final concentration in the wells ranged from 93 to 1 REF and 4.5 to 0.07 REF for the river/WWTP samples and OSPW respectively. Positive control wells were used to validate each run using 3,5-dichlorophenol (DCP) with well concentrations ranging from 67.5 to 1 mg/L. All samples and the positive control were run in duplicate. After the 96-well plate was prepared, the plate and the *Aliivibrio fischeri* bacteria were equilibrated on a chill block at 15 °C for 30 minutes. Next, 100 µL bacteria was pipetted into each well. The luminescence of the 96-well plate was measured at time intervals 0, 5, 10, 15 and 30 minutes. The plate was placed on the chill block in between measurements.

2. Estrogenicity – Yeast estrogen screen (YES)

All chemicals were purchased from Sigma-Aldrich. The GOLD solution, GOLD media and Minimal media were prepared as stock solutions and stored at 4°C prior to running the assay.

Table S5: Preparation of GOLD solution

Compound	Concentration (g/L)	Storage	Volume to make GOLD solution (mL)
Adenine hydrochloride hydrate	1.2	Room Temperature	75
L-Histidine-HCl	2.4	4 °C	50
L-Arginine-HCl	2.4	4 °C	25
L-Methionine	2.4	4 °C	25
L-Tyrosine	0.9	Room Temperature	25
L-Isoleucine	3.6	4 °C	25
L-Lysine-HCl	3.6	4 °C	100
L-Phenylalanine	3	Room Temperature	25
L-Glutamic Acid	6	Room Temperature	25
L-Aspartic Acid	4	Room Temperature	25
L-Valine	18	4 °C	25
L-Threonine	24	4 °C	25
L-Serine	45	4 °C	50
L-Leucine	3.6	Room Temperature	25
L-Tryptophan	4.8	4 °C	50
Uracil	2.4	Room Temperature	25

Table S6: Preparation of GOLD medium

Solution	Volume (mL)
20% Dextrose stock	60
10X YNB without amino acids	60
GOLD solution	110
Ultrapure water	370

Table S7: Preparation of Minimal medium

Solution	Volume (mL)
20% Dextrose stock	100
10X YNB without amino acids	100
L-Lysine-HCl	10
Ultrapure water	790

Agar solution was made by combining 78 mL ultrapure water, 10 mL-10X YNB without amino acids and 2 g of bactoagar into a glass media round bottle. The solution was autoclaved on a liquid cycle. Once the bottle was cool to touch, the following was added and mixed into the

agar solution: 10 mL 20% dextrose, 1 mL L-Histidine-HCl and 1 mL L-Lysine-HCl. From one batch of agar solution, about 6 petri plates were prepared;10-15 mL of the agar solution was poured onto each petri plate and left to solidify at room temperature. When the agar has solidified, the plate was streaked with cells from a previous stock. The streaked plate was then inverted and incubated at 30 °C for 3-4 days. The plate with the grown cells was stored at 4°C for 2 weeks until it was discarded.

Running the assay:

A colony of cells from the previously streaked plate was isolated and transferred to a 15 mL conical tube with 1 mL of GOLD medium. The tube was incubated at 30 °C, 300 rpm for 18-24 hours. The next day, a spectrophotometer was used to check the optical density at 660 nm (OD_{660}) of the cells was checked to ensure that it was approximately 1. Then, the cells were transferred to a flat bottom flask, with 9 mL of minimal media and incubated at 30 °C, 300 rpm for 18-24 hours. After incubation, 100 µL of cells was added to a microcentrifuge tube with 100 µL of 30% glycerol. This cell stock was stored at -80°C for future assays. Next, 10 mL of minimal media was added to the flask and incubated at 30 °C, 300 rpm for 4-6 hours. At the end of incubation, the seeding media was prepared using 100 µL 10mM copper (II) sulphate pentahydrate, 20 mL minimal media and cells. The cells were added to the seeding media until the OD_{660} reached 0.03 ± 0.002. Immediately after, the samples and controls were prepared in duplicates. 5 µL of the previously prepared samples were added to 2 mL amber vials and left for the methanol to evaporate. Similarly, the positive control, 17β-estradiol (E2) had been previously prepared in stock solutions in methanol. 10 µL of each concentration of E2 was added to a 2 mL amber glass vial and left to dry before the cell solution was added. Once the methanol had

evaporated, 200 µL of cells was added to each vial. The amber vials were incubated at 30 °C, 300 rpm for 18-24 hours. The exposure concentrations ranged from 3.13×10^{-9} M to 2.44×10^{-11} M for E2; 66 to 0.5 REF for river/WWTP samples and 5 to 0.04 REF for OSPW. The next day, 25 µL of the exposed cells were transferred to a 96-well plate, along with 75 µL of minimal media in each well. The cell density at 660 nm was read using a plate reader (Synergy LX), with Gen 5.3.11 software programmed to a 10-minute kinetic mode read with 50 second intervals. Next, each well received 100 µL of 1:1 YPER-β-Galactosidase solution (Thermo Scientific), and the plate was read at an optical density of 420 nm using a 1-hour kinetic mode read with 50 second intervals. Blank and solvent control wells were also included in each plate to ensure that there were no sources of contamination during the bioassay. For all plates, these wells showed signals below the detection limit.

3. ERα-GeneBLAzer:

GeneBLAzer ERα-UAS-bla GripTite Cells are stably expressing the β-lactamase reporter gene. The test system is based on the GeneBLAzer® FRET Assay from Thermo Fischer Scientific, which quantifies the amount of formed β-lactamase with fluorescence measurement. The method is based on a specific substrate for the β-lactamase, whose implementation depends on the Forster resonance energy transfer (FRET). The substrate readily enters the cell, where endogenous esterases rapidly convert it into a fluorescent precursor (blue fluorescence) that is then transformed into an enzyme product by β-lactamase (green fluorescence).

5×10^3 cells per well were seeded in 30 µL of medium per well (Opti-MEM, 2 % csFBS, 100 U/mL penicillin, 100 µg/mL streptomycin) in black, clear bottom poly-D-lysine coated 384-well plates (Corning). Cells were treated with 10 µL/well of the dosing medium containing the

samples, blanks, and the reference estradiol (E2) and incubated for 24 h. For the detection of the expression of β-lactamase the ToxBLAzer detection reagent was prepared according to the instructions of the manufacturer and 8µl of the reagent were added per well. Fluorescence was read with excitation at 409 nm and emission at 460 nm (blue) and 530 nm (green) immediately after addition of the reagent (t = 0h) and after 2h of incubation at room temperature in the dark. To determine cell viability of treated cells and unexposed cells as control, confluency was measured based on phase contrast images acquired using an Incucyte Zoom S3 (Essen BioScience, Ann Arbor, Michigan, USA). Cell viability assessment using IncuCyte and the commonly used PrestoBlue cell viability reagent was compared previously for the AhR CALUX assay in Nivala *et al.* (2018) [48] with IncuCyte found to be a more reliable cell viability measurement. Cell viability was expressed as percentage of the control value.

4. AhR CALUX:

The rat hepatoma cell line H4L7.5c2 stably expressing the luciferase reporter gene plasmid pGudLuc7.5 containing a total of 20 XREs was used in the CALUX assays [49, 50]. 3.5 x 10^3 cells per well were seeded in 30 µL of medium per well (DMEM with Glutamax, 10 % FBS, 100 U/mL penicillin, 100 µg/mL streptomycin, 0.4 mg/mL geneticin) in black, clear bottom poly-D-lysine coated 384-well plates (Corning). Plates were incubated for 24 h. Cells were treated with 10 µL/well of the dosing medium containing the samples, blanks or controls and incubated for 24 h. Luminescence was measured and the AHR activity potential of the samples evaluated against the reference TCDD. To measure luciferase activity, cells were washed twice with PBS and subsequently 20 µL of lysis buffer was added (25 mM Tris, 1 % Triton-X 100, 2 nM EDTA, 2 mM DTT, 10 % glycerol). After a 10 min incubation period at RT 20 µL of luciferase substrate

buffer (20 mM Tricine, 2.67 mM MgSO4, 33.3 mM DTT, 0.1 mM EDTA, 0.261 mM coenzyme A, 0.53 mM ATP, 0.47 mM D-luciferin was added to each well and luminescence was read. Cell viability was determined following the same principle as for the ERα-GeneBLAzer assay.

5. PPARɣ-GeneBLAzer:

GeneBLAzer PPARγ-UAS-bla 293H cells are based on the same reporter gene system as ERα-GeneBLAzer assay and the assay was performed in a similar way except that the cells were seeded with 6.5 x 10^3 cells per well and rosiglitazone was used as reference compound.

6. UMU-ChromoTest:

The night before the assay was run, the freeze-dried bacteria (*Salmonella typhimurium* TA1535) was rehydrated using growth media, reagent V and 1x glucose solution (provided by EBPI). The reconstituted bacteria was incubated at 37 °C, 100 rpm for 16-18 hours. The next morning, the optical density at 600 nm (OD_{600}) of the overnight growth media was measured against a fresh medium blank to ensure that the OD_{600} was more than 0.1. Next, the overnight bacteria was diluted using fresh growth media. The inoculated bacteria were incubated at 37 °C, 100 rpm for 1.5 hours. During this time, the samples were prepared. 325 µL and 80 µL from the reconstituted SPE extract of the river/WWTP samples and OSPW respectively was transferred into a test tube. The contents of the test tube were evaporated to dryness using a nitrogen evaporator and the dried sample was reconstituted in 1.2 mL of 10% dimethyl sulfoxide (DMSO) in sterile 0.85% saline. The pH of the sample was adjusted to 7 ± 0.2 using 1N sodium hydroxide. Samples were run in duplicate with well concentrations ranging from 481 to 15 REF and 8 to 0.28 REF for the river/WWTP samples and OSPW respectively. A positive control, 4-nitroquinoline 1-oxide (4-NQO), was included in each run with well concentrations ranging from 5.26 to 0.16 µM. 4-NQO

is used to validate the results of each run, where the induction ratio at the well concentration of 5.26 µM must be at least 2. The first 96 well-plate (Plate A) was prepared with samples and controls following the procedure described by ISO 13829. After the incubation of the inoculated bacteria, the OD_{600} was measured to ensure that it was at least 80% of the overnight OD_{600}. 70 µL of the bacteria was added into all wells except the blank wells. The well-plate was incubated at 37 °C, 100 rpm for 2 hours.

Near the end of incubation of Plate A, 270 µL of growth medium was pipetted into each well of Plate B, which was then placed in the incubator at 37 °C, 100 rpm with the lid on. At the end of incubation of Plate A, 30 µL from each well was transferred to the corresponding well in Plate B. The absorbance at 600 nm of Plate B was measured using the microplate reader (Synergy LX) and then this plate was incubated at 37 °C, 100 rpm for 2 hours.

Near the end of incubation of Plate B, the ONPG powder was dissolved in a phosphate buffer (provided by EBPI) and stored in the dark at room temperature until use. The B-Buffer was brought down to room temperature. Once at room temperature, 35 µL of 2-mercaptoethanol was added to the B-Buffer. 120 µL of the B-buffer solution was pipetted into each well of Plate C, which was incubated at 37°C, 100 rpm. At the end of incubation of plate B, the absorbance at 600 nm was measured. Next, 30 µL from each well was transferred to the corresponding well in plate C, immediately followed by 30 µL ONPG solution. Plate C was incubated at 37 °C, 100 rpm for 30 minutes. Once the yellow color was developed, 120 µL of stop solution (provided by EBPI) was added to each well. Lastly, the absorbance at 420 nm of Plate C was measured.

UMU-Express P450 1A2 bacterial strain

The P450 1A2 bacterial strain was used as an alternative to S9 activation. The human cytochrome P450s are involved in the metabolism of drugs, carcinogens, mutagens, steroids, and

prostaglandins. The role of the P450s is to either direct substrates for detoxification or activate substrates to produce carcinogenic or mutagenic intermediates [51]. Therefore, the P450 strain can be used to determine the mutagenic activity of a compound with significance to human exposure. In this study, five samples (M4, M4', M6, FMO and MSO) were analyzed using this bacterial strain as a confirmatory test to investigate the mutagenic potential of PAHs.

The night before the assay is run, reagents V, W, X, Y and Z (provided by EBPI) are added to the growth media prior to rehydrating the lyophilized bacteria. Once the growth media has been added to the bacteria, the bacterial solution is incubated for 14-16 hours at 37°C, 100 rpm. At the end of incubation, the optical density (OD_{600}) of the overnight growth media was measured against a fresh medium blank. The OD_{600} should reach a value between 0.15-0.20. The remaining steps follow the same procedure as described for the method using the standard bacterial strain.

M6 was the only river site that showed activity using this bacterial strain with a 2-aminoanthracene (2AA)-EQ value of 1.76 µg/L. On the other hand, both WWTPs showed activities of 13.71 µg/L and 11.95 µg/L 2AA-EQ for FMO and MSO respectively. OSPW did not show any activity with this strain at well concentrations ranging from 7.41 to 0.93 REF. Although OSPW was not analyzed for the PAH concentrations, OSPW typically has high PAH concentrations ranging from 2048 to 5252 µg/L [50]. It is possible then that the high exposure concentrations of PAHs led to a cytotoxic environment, as observed by the low growth factors ($G < 0.26$). From these results, further analysis should be conducted using a wider range of smaller well concentrations of OSPW to eliminate any interfering cytotoxicity effects.

7. AREc32 reporter gene assay:

The AREc32 cell line is a cell line, which expresses luciferase stably under the antioxidant response element-driven NRF-2 line based on the MCF7 breast cancer cells [50]. 2.65 x 10^3 cells per well were seeded in 30 µL of medium per well (DMEM with Glutamax, 10 % FBS, 100 U/mL penicillin, 100 µg/mL streptomycin) in black, clear bottom poly-D-lysine coated 384-well plates (Corning). The assay was performed similar to AhR-CALUX except for a higher concentration of 1.9 mM D-luciferin in the luciferase substrate buffer. Luminescence was measured and the AHR activity potential of the samples evaluated against the reference tert-butylhydroquinone (tBHQ).

Table S8. Data analysis steps completed for each assay. OD = optical density, RLU = relative light units, IC = inhibition concentration; EC = effect concentration; YES = yeast estrogen screen assay. B = blue, G = green, E=effect. IR = induction ratio.

Assay Type	Validation	Data Analysis
Aliivibrio Fischeri toxicity assay	Positive control is 3,5-dichlorophenol. In this study, $IC_{10,15\ min}$ = 2.7 ± 1.2 mg/L.	1. Calculate % Inhibition from the raw RLU using the equation below: $\% Inhibition = 1 - \frac{RLU_{sample,\ t\ min}}{RLU_{sample,\ 0} * \frac{RLU_{blank,\ t\ min}}{RLU_{blank,\ 0}}}$ 2. Normalize % Inhibition from 0 - 100% 3. Complete a Ligand Binding-Sigmoidal Dose response regression using log concentration and average normalized % Inhibition (on Sigmaplot) 4. Calculate IC_{10} using parameters obtained from regression fitting
YES assay	Positive control and reference compound is 17β-estradiol (E2). In this study, EC_{10} = 1.01× 10^{-10} ± 3.65 × 10^{-11} M.	1. Calculate the β-Galactosidase (β-Gal) response using the raw cell density (OD_{660}) and raw β-Gal data (OD_{420}). Note that at OD_{420} only absorbance values between 0.2 to 1.0 were included in the analysis. $\beta - Gal\ response = \frac{1000 * slope(raw\ \beta - gal\ data)}{volume\ of\ cells\ plated\ (mL) * average\ OD_{660}}$ 2. Normalize β-Gal response from 0 – 100% 3. Remove concentrations affected by cytotoxicity from the data set 4. Model the data using a 4-Parametric Logistic Equation using concentration and average normalized β-Gal response (on Sigmaplot) 5. Calculate EC_{10} using parameters obtained from regression fitting 6. Calculate the BEQ of each sample using the EC_{10} of E2
All	No positive control	1. Calculate the % cytotoxicity from the confluency data

mammalian cell lines		$\%\ Cytotoxicity = 1 - \frac{Confluency(sample)}{Confluency(unexposed\ cells)}$ 2. Data in the linear range up to the 30% cytotoxicity were fitted to a linear trendline with a slope and an y-intercept of 0 3. Determine the cytotoxicity IC_{10} of each sample $IC_{10} = \frac{10}{slope}$
ERα-GeneBLAzer; PPARɣ-GeneBLAzer	Positive control and reference compound is 17β-estradiol (E2). ERα: EC_{10} for E2 $1.3 \times 10^{-11} \pm 1.3 \times 10^{-12}$ M PPARɣ: EC_{10} for rosiglitazone $3.5 \times 10^{-10} \pm 1.3 \times 10^{-10}$	1. Calculate the blue: green ratio using the following equation: $\frac{B}{G} = \frac{\left(E_{460\ nm}(2\ h) - \left(E_{460\ nm}(0\ h, unexposed\ cells)\right)\right) - E_{460\ nm}(2\ h, cellfree)}{\left(E_{530\ nm}(2\ h) - \left(E_{530\ nm}(0\ h, unexposed\ cells)\right)\right) - E_{530\ nm}(2\ h, cellfree)}$ 2. Calculate the % effect using the following equation: $\%\ effect = \frac{\frac{B}{G}ratio\ (sample) - \frac{B}{G}ratio\ (unexposed\ cells)}{\frac{B}{G}ratio\ (maximum) - \frac{B}{G}ratio\ (unexposed\ cells)}$ 3. Data with concentrations lower than the cytotoxicity IC_{10} and in the linear range up to the 30% effect were fitted to a linear trendline with a slope and a "0" y-intercept. 4. Find the EC_{10} for each sample using the following equation: $EC_{10} = \frac{10}{slope}$ 5. Calculate the BEQ using the EC_{10} of E2 or rosiglitazone, respectively. $\mathrm{BEQ_{bio}} = \frac{\mathrm{EC_{reference}}}{\mathrm{EC_{sample}}} = \frac{\mathrm{slope_{sample}}}{\mathrm{slope_{reference}}}$

AhR-CALUX assay	Reference compound is benzo[a]pyrene (B[a]P); EC_{10} = 8.38×10^{-6} M.	1. Calculate the % effect using the following equation: $\%\ effect = \frac{RLU - RLU(min)}{RLU(max) - RLU(min)}$ 2. Data with concentrations lower than the cytotoxicity IC_{10} and in the linear range up to the 30% effect was fitted to a linear trendline with an y-intercept of 0 3. Find the EC_{10} for each sample using the following equation: $EC_{10} = \frac{10}{slope}$ 4. Calculate the BEQ using the appropriate reference compound
AREc32 reporter gene assay	Reference compound is dichlorvos; $EC_{IR1.5}$ of 7.70×10^{-6} M	1. Calculate the induction ratio IR $IR = \frac{RLU\ (sample)}{RLU(unexposed\ cells)}$ 2. Data with concentrations lower than the cytotoxicity IC_{10} and in the linear range up to 3-4 was fitted to a linear trendline with a slope an y-intercept of 1 3. Find the effect concentration triggering an IR of 1.5 (50% over control) $EC_{IR1.5}$ for each sample using the following equation: $EC_{IR1.5} = \frac{0.5}{slope}$ 4. Calculate the BEQ using the appropriate reference compound
UMU-ChromoTest	Reference compound & positive control is 4-nitroquinoline 1-oxide	1. Determine the β-Galactosidase (β-Gal) activity using the following equation:

assay	(4-NQO). For test validation, the IR of 4-NQO at well concentration of 5.26 µM must be at least 2. In this study, IR = 8.8 ± 2.9.	$\beta - Galactosidase\ activity = \frac{A_{420}sample - A_{420}blank}{A_{420}negative\ control - A_{420}blank}$ 2. Determine the growth factor (G) using the following equation: $Growth\ factor = \frac{A_{600}sample - A_{600}blank}{A_{600}negative\ control - A_{600}blank}$ Note: G must be greater than 0.5 for results to be considered valid 3. Find the IR by dividing the β-Gal by G. Note: For a sample to be considered mutagenic, IR must be > 1.5 4. Find the slope by fitting the data to a linear trendline with a y-intercept of 1 5. Find the $EC_{IR1.5}$ for each sample using the following equation: $EC_{IR_{1.5}} = \frac{0.5}{slope}$ 6. Calculate the BEQ using the $EC_{IR1.5}$ of NQO-EQ as $EC_{reference}$.

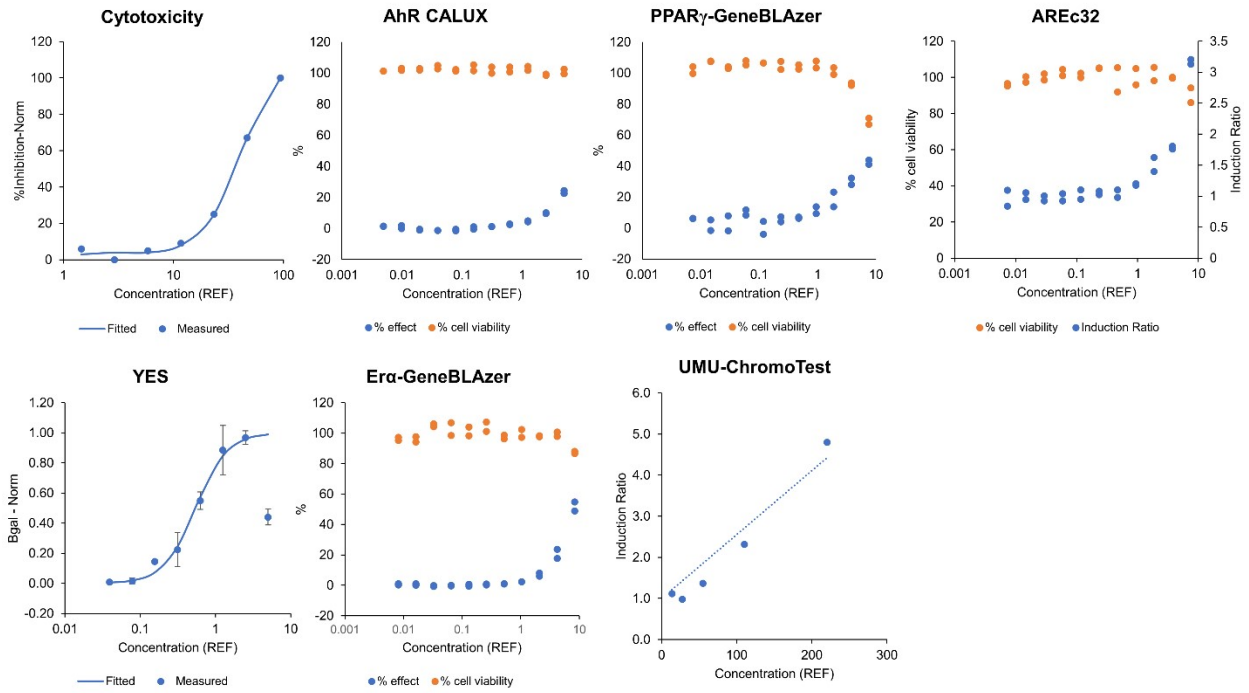

Figure S6. Examples of the concentration-response curves for each bioassay.

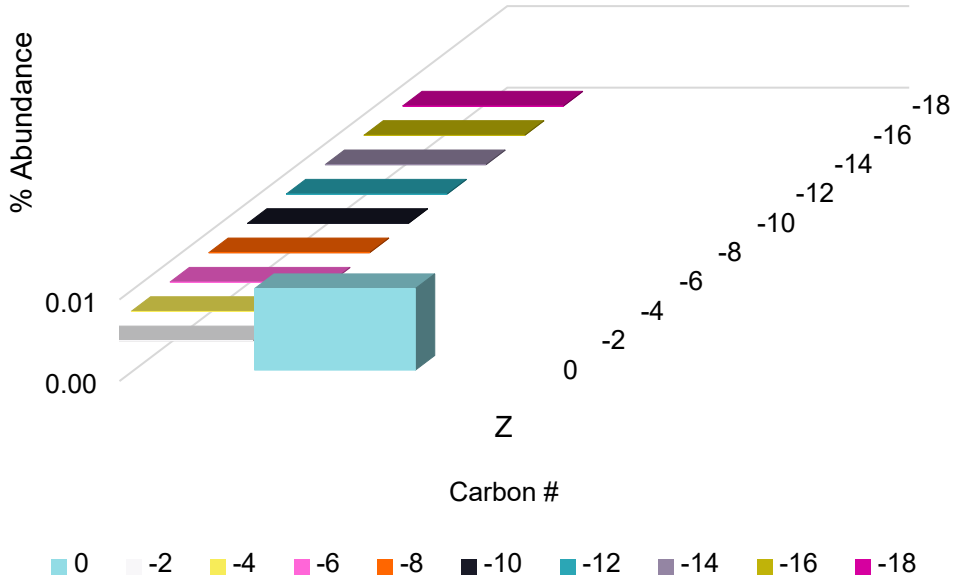

Figure S7. NA speciation of untreated OSPW. 'Z' represents the unsaturation or number of rings in each compound.

Table S9. Summarized results for bioassays – AEO concentrations & BEQ values.

	AEOs (mg/L)	PPARɣ [a]	AhR [b]	AREc32 [c]	ERα [b]	YES [b]	UMU [b]	Cytotoxicity [b]
June								
M8	0.6	*	53.2	*	*	1.3	*	13.3
M7	1.6	*	*	*	0.92	2.7	0.5	23.9
M6	2.4	*	46.4	*	*	7.5	0.5	24.8
M5	0.4	83.7	*	*	*	7.1	*	21.9
T1	1.1	130.9	*	*	*	2.7	*	6.59
M4	0.4	65.7	*	248.1	*	2.6	0.3	13.5
M3	0.5	*	*	*	*	1.5	*	22.7
FMO	1.3	89.0	94.9	333.8	1.53	2.2	2.6	19.2
M2	1.2	233.9	*	*	*	3.8	*	13.1
M1	0.7	74.2	*	*	*	2.1	0.2	10.6
August								
M4’	0.9	*	*	*	*	0.7	*	33.5
S1E	0.2	*	*	*	*	2.7	*	32.6
S4E	0.1	*	*	*	*	2.6	*	***
MSO	3.3	94.8	106.5	559.7	*	19.9	0.7	12.0
S2E	<LOD	*	*	*	*	3.7	0.2	***
S2W	<LOD	*	*	*	*	0.8	*	***
T2	0.3	*	*	*	0.57	n.p.	*	4.2
OSPW	52.2 ± 8.0	2824.2 ± 589.8	172.4 ± 142.8	774.7 ± 292.8	6.4 ± 0.3	133.8 ± 34.8	6.4	1.0 ± 0.7

[a] Units = ng/L rosiglitazone-EQ. [b] Units are the same as Table S8. [c] Units = µg/L dichlorvos-EQ. * = no activity; *** = cannot be determined; n.p. = not processed.

Table S10. Summarized results for bioassays - EC10, $EC_{IR1.5}$, IC10 (REF) values.
* = no activity, ** = no cytotoxicity, *** = cannot be determined; n.p. = not processed.

	PPARɣ		AhR		AREc32	
	EC10	IC10	EC10	IC10	EC(IR1.5)	IC10
June						
M8	*	1.8	4.0	**	*	**
M7	*	1.9	*	**	*	**
M6	*	2.0	4.6	**	*	**
M5	1.1	2.1	*	**	*	**
T1	0.7	0.8	*	3.9	*	**
M4	1.4	2.3	*	**	6.9	**
M3	*	2.0	*	**	*	**
FMO	1.0	**	2.2	**	5.1	**
M2	0.4	1.3	*	**	*	**
M1	1.2	1.5	*	**	*	**
August						
M4’	*	**	*	**	*	**
S1E	*	5.4	*	**	*	**
S4E	*	**	*	**	*	**
MSO	0.9	3.0	2.0	**	3.0	**
S2E	*	**	*	**	*	**
S2W	*	**	*	**	*	**
T2	*	**	*	**	*	**
OSPW	0.04 ± 0.01	2.7 ± 0.3	2.3 ± 2.2	3.7 ± 1.0	2.5 ± 1.2	5.1

Table S10 continued.

	ERα		YES	UMU	Cytotoxicity
	EC10	IC10	EC10	EC(IR1.5)	IC10
June					
M8	*	1.9	16.1	*	13.3
M7	3.7	4.8	11.1	208	23.9
M6	*	1.3	3.0	100	24.8
M5	*	2.0	3.0	*	21.9
T1	*	0.8	8.3	*	6.59
M4	*	5.2	7.2	161	13.5
M3	*	2.2	13.4	*	22.7
FMO	2.2	7.8	10.6	21	19.2
M2	*	1.4	9.2	*	13.1
M1	*	1.8	16.6	385	10.6
August					
M4’	*	**	27.9	*	33.5
S1E	*	8.6	17.0	*	32.6
S4E	*	**	10.3	*	***
MSO	*	4.8	1.5	74	12.0
S2E	*	**	9.4	455	***
S2W	*	**	25.2	*	***
T2	6.0	**	n.p.	*	4.2
OSPW	0.7 ± 0.3	1.6 ± 0.04	0.2 ± 0.07	5.5 ± 3.5	1.0 ± 0.7

D. Calculation of Dilution Factor (DF)

$$Dilution\ factor = \frac{Flowrate\ of\ river}{Flowrate\ of\ WWTP\ effluent}$$

Flow rate for LAR June sampling = 1565 m^3/s (retrieved from https://wateroffice.ec.gc.ca)

Flow rate for LAR August sampling = 522 m^3/s (retrieved from https://wateroffice.ec.gc.ca)

Average daily flow of FMO = 0.233 m^3/s) [52].

E. Polycyclic Aromatic Compounds (PACs)

PACs is a broad group of organic chemicals that include polycyclic aromatic hydrocarbons (PAHs). PACs data for select river samples was obtained from AEP (retrieved from https://aws.kisters.net) and are presented in Table S11. The total PACs concentrations in June 2021 (mean = 445.7 ± 204.6 ng/L) are statistically significantly higher than that of the samples collected in August 2021 (mean = 73.9 ± 15.7 ng/L) (ANOVA, $p = 0.001$, $\alpha = 0.05$). This supports the trend observed in the bioassay and AEOs data, where there are higher concentrations during higher flow conditions of the LAR. This trend correlates with the findings of Droppo *et al.* (2018) [53] who investigated the temporal influence on contaminant transport in two tributaries of the Lower Athabasca River and found that higher daily loadings of PACs occurred in the high flow seasons (May-July) compared to low flow seasons (July to October). The authors further reported that the daily PACs loads decreased 10 to 100 times from May to October and that the highest PAC loads occurred during the spring melt [53].

Although the WWTP effluents collected in June and August were not sent for PACs analysis, we collected samples at a later date (October 2021) to assess the PACs concentration in these point sources. The total PACs concentrations are 62.8 ng/L for FMO and 151.3 ng/L for MSO. The PAC concentrations reported here are lower than numerous studies which reported PACs levels ranging from 864 to 4700 ng/L, which may be related to variations in the wastewater treatment process and differences in influent concentrations, service populations and volume of water to be treated [54]. The untreated OSPW used in this study was not analyzed for PACs.

Table S11. PACs Data for Selected Samples

Sample	Alkylated PAHs (ng/L)		Parent PAHs (ng/L)		Dibenzothiophenes (ng/L)	
	June	August	June	August	June	August
M4’	387.6	66.6	34.2	10.1	117.6	17.0
S1E	570.7	62.0	37.0	9.7	211.7	18.7
S4E	214.0	45.9	30.5	8.2	59.1	10.9
S2E	219.5	49.5	29.3	11.4	64.5	15.9
S2W	210.4	38.8	29.5	8.9	60.9	10.2
M3’	282.7	38.8	29.2	8.9	85.8	11.7
FMO*	37.2		9.2		16.5	
MSO*	85.7		20.9		44.8	

Data retrieved from Alberta Environment & Parks – Enhanced Monitoring Program. *- Samples were collected in October 2021

Table S12. Effects-based trigger (EBT) for surface water used for comparison to sample bioassay responses

Assay	EBT value	Units
Cytotoxicity	<20 [a]	REF
Mammalian cell line cytotoxicity	<10 [b]	REF
YES Assay	1.07 [c]	ng/L 17β-Estradiol EQ
ERα-GeneBLAzer	0.34 [c]	ng/L 17β-Estradiol EQ
AhR activation	250 [b]	ng/L benzo[a]pyrene EQ
PPAR-ɣ activation	1.2 [b]	µg/L rosiglitazone EQ
Mutagenicity	0.64 [d]	µg/L 4-nitroquinoline 1-oxide EQ
Oxidative stress response	1.4 [b]	mg/L dichlorvos EQ

[a] Threshold for chronic toxicity effects obtained from van der Oost *et al.* (2017) [55]. [b] EBT values obtained from Escher and Neale (2021) [56]. [c] EBT values obtained from Escher *et al.* (2018) [57]. [d] Predicted no-effect concentration obtained from Xu *et al.* (2014) [58]. The BEQ values from Table S9 was compared with these values (i.e., by getting the ratio of BEQ:EBT values). For cytotoxicity, the EBT-IC_{10} (REF) of <10 REF was used as the threshold in this study.

F. Additional evidence related to impacts of hydrologic conditions

It is difficult for us to directly assess the specific impacts of hydrologic conditions on the results of the bioassay as there is only one sampling site (M4) which overlapped both sampling campaigns (labelled as M4 and M4'in June and August respectively). Table S13 shows the summarized results for the June and August sampling of this river site.

With the exception of AEO concentrations, there is a greater response from the M4 sampled in high flow conditions (June) than the low flow conditions for the parameters (chemical and bioassays) included in this study. This comparison indicates the potential value in a year-round monitoring using a test battery of bioassays to properly establish a temporal trend of the bioactivity of the river.

Table S13. Data comparison for M4 in June and August 2021

Parameter	Units	June 2021	August 2021
AEOs concentration	mg/L	0.40	0.86
Total PACs concentration [a]	ng/L	217.6	36.7
Cytotoxicity: *Aliivibrio fischeri*	EC10 (REF)	13.5	33.5
Cytotoxicity: ERαGeneBLAzer	IC10 (REF)	5.20	no cytotoxicity
Cytotoxicity: PPARɣ- GeneBLAzer	IC10 (REF)	2.30	no cytotoxicity
Cytotoxicity: AhR CALUX	IC10 (REF)	no cytotoxicity	no cytotoxicity
Cytotoxicity: AREc32 reporter gene	IC10 (REF)	no cytotoxicity	no cytotoxicity
YES	EEQ (ng/L)	2.57	0.66
ERα-GeneBLAzer assay	EEQ (ng/L)	no activation	no activation
AhR CALUX	B[a]p-EQ (µg/L)	no activation	no activation
PPARɣ-GeneBLAzer assay	Rosiglitazone-EQ (µg/L)	0.07	no activation
UMU-ChromoTest	4NQO-EQ (µg/L)	0.33	no activation
AREc32 reporter gene assay	Dichlorvos-EQ (mg/L)	0.25	no activation

[a] Data obtained from Alberta Environment & Parks- Enhanced Monitoring Program.

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