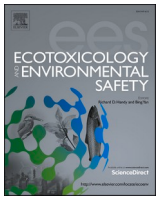
Ecotoxicology and Environmental Safety 302 (2025) 118726

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv



# Comprehensive cytotoxicity assessment of treated produced water from thermal distillation using human cell lines

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ARTICLE INFO

*Keywords:*
Produced water
Thermal distillation
Cell viability
Estrogenic activity
Activation of aryl hydrocarbon receptor

ABSTRACT

Produced water (PW) could be an alternative water resource after treatment for fit-for-purpose applications. However, comprehensive studies assessing the impact of treated PW exposure on human health are still lacking. Herein, we evaluated the effect of untreated and treated PW from the Permian Basin after two thermal distillation systems followed by granular activated carbon (GAC) and zeolite on human cell lines, including intestinal epithelial (Caco-2) cells, breast cancer cells (MCF-7), and embryonic kidney (HEK293) cells. Cells were exposed to untreated PW, thermal distillate, and post-treated PW after GAC and zeolite treatment. Various *in vitro* toxicity assays were conducted to assess cell viability, necrosis, apoptosis, oxidative stress, estrogenicity, and aryl hydrocarbon receptor (AhR) activation after PW exposure. Overall, untreated PW caused significant cytotoxicity, reduced cell viability, induced oxidative stress, and apoptosis, whereas distillate and GAC+zeolite-treated PW did not induce significant toxicity. As shown by E-screen assays, significant estrogenic activity was observed in feed PW and the distillate but not in the post-treated PW. CYP1A1 gene upregulation was observed in the distillate, suggesting activation of AhR by residual organic compounds. Post-treated PW did not induce AhR activation, highlighting the need for post-treatment following thermal distillation to mitigate residual xenobiotic organic compounds. Given that thermal distillation followed by GAC and zeolite filtration eliminated all adverse impacts on human cell lines, this integrated treatment process demonstrates strong potential for safe discharge and beneficial reuse of treated PW.

## 1. Introduction

In 2022, unconventional hydrocarbon resources made a major contribution to the U.S. energy landscape, with approximately 66 % of U.S. crude oil production, totaling 2.84 billion barrels, derived from tight oil reserves (EIA, 2024). The Permian Basin, located in Texas and New Mexico, accounted for most of its production. While this surge of unconventional production has enhanced both the global energy landscape and regional economies, it also poses significant environmental challenges. Produced water (PW) is the water generated from subterranean formations brought to the surface during oil and gas extraction. Globally, the water-to-oil production ratio is typically 3:1, leading to the extraction of approximately 300 million barrels of water daily alongside oil and gas (Liang et al., 2018). The substantial amount of PW necessitates effective management and disposal strategies. PW has certain chemical traits from the formations and may include any chemicals introduced during hydraulic fracturing procedures (Clark and Veil, 2009; Jiang et al., 2021). The physical and chemical properties of PW depend on factors such as the geographic location of the field, its geological formation, the lifetime of its reservoirs, and the type of hydrocarbon product being produced (Fakhru'l-Razi et al., 2009; Jiang et al., 2022a). PW comprises high levels of total dissolved solids (TDS), oil and grease, suspended solids, heavy metals, naturally occurring radioactive material (NORM), organic compounds, microorganisms, and chemical additives of hydraulic fracturing (HF) fluids, such as biocides and surfactants (Delanka-Pedige et al., 2024; Jiang et al., 2022b; Scanlon et al., 2020). The current PW management practices are mainly PW reinjection for enhanced oil recovery (48.3 %) and deep-well injection (47.4 %) for disposal (GWPC, 2022). However, significant seismic risks are associated with deep-well injection of PW (Gregory and Murali Mohan, 2015). On the other hand, water resources management in arid and semiarid southwestern United States has been a significant

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https://doi.org/10.1016/j.ecoenv.2025.118726
Received 12 May 2025; Received in revised form 8 July 2025; Accepted 19 July 2025
Available online 26 July 2025

challenge due to limited freshwater supplies and chronic droughts. Therefore, reusing PW beyond the oil and gas sector offers a viable solution to water shortages and disposal challenges (Delanka-Pedige et al., 2023).

Thermal distillation has emerged as one of the promising treatment processes for hypersaline PW treatment and reuse. Compared to most membrane desalination technologies, thermal distillation is capable of treating hypersaline PW (TDS 100,830–201,474 mg/L) generated in the Permian Basin. Moreover, it is resistant to fouling and scaling and has shown robust performance with minimal pretreatment (Tarazona et al., 2024a). Several lab studies have demonstrated the potential of membrane distillation (MD) in PW desalination when combined with membrane bioreactors (MBR), GAC adsorption, photocatalysis, or oxidation process to enhance contaminants removal and minimize membrane fouling (Chen et al., 2023, 2022, 2024; Hsieh and Malmali, 2023; Lin et al., 2020; Van Houghton et al., 2024; Zhang et al., 2021). However, due to the complexity of PW composition, PW may still contain certain levels of organic pollutants, ammonia, NORM, and metals that pose significant health impacts even after treatment with advanced technologies (Delanka-Pedige et al., 2023). For instance, some volatile organic compounds (VOCs) in PW may still enter the thermal distillate during evaporation due to their low boiling points and high vapor pressures. Exposure to those VOCs, such as BTEX (benzene, toluene, ethylbenzene, xylenes) and formaldehyde, could cause effects such as reduced fertility and fecundity, lower semen quality, disrupted menstrual cycles, and elevated risk of miscarriage, stillbirth, and preterm birth (Kassotis et al., 2016b). The trace amount of petrogenic alkylphenols (AP), naphthenic acids, and total petroleum hydrocarbons (TPH) in treated PW could disrupt the endocrine system while interfering with hormone action (Kassotis et al., 2016a). As a result of these concerns, the reuse of treated PW beyond the oil and gas sector has raised significant public concern due to potential risks to human health and the environment. Characterization studies have identified 1198 constituents in PW, with toxicity data available for 527 of them in public databases (Danforth et al., 2020). Identifying and quantifying all of these constituents is not feasible, particularly given the presence of unknown or non-target analytes. The reuse of treated PW beyond the oil and gas sectors for industrial, commercial, agricultural, and environmental applications remains a developing area, with evolving regulatory frameworks and scientific understanding. Currently, the U.S. Environmental Protection Agency (EPA) is revising the Oil and Gas Extraction Effluent Guidelines and Standards (40 CFR Part 435) to support environmentally sustainable reuse of produced water (USEPA, 2025a), including applications such as agricultural irrigation and wildlife water uses. Under the Clean Water Act, the EPA is required to revise point source effluent limitations guidelines to reflect advancements in pollution control technologies. Certain reuse scenarios are considered lower-risk and more feasible in the near term, especially where there are fewer sensitive environmental or human receptors, such as industrial uses (e.g., cooling water, manufacturing process water) and construction use (e.g., concrete mixing, roadbed compaction). These pathways are attractive because they typically occur in controlled environments with limited direct exposure risks. Other reuse pathways, such as agricultural irrigation and discharge to surface or groundwater, are subject to greater scrutiny due to the potential for direct human and ecological exposure. For example, agricultural irrigation could introduce the possibility of chemical uptake into crops and soil accumulation. These applications require more extensive characterization of treated PW and development of risk-based toxicity assessment framework and monitoring protocols (Cooper et al., 2021; Danforth et al., 2019; Delanka-Pedige et al., 2023; GWPC, 2019; GWPC, 2022; GWPC, 2023; Jiang et al., 2022a). Therefore, in addition to conventional water quality parameters, toxicity assays are essential for evaluating the potential risks of constituents in treated PW.

Although the toxicity of many individual chemicals in PW has been studied and documented, there is limited information regarding the toxicity of actual PW. Several studies using marine luminescent bacteria, fish, and aquatic invertebrates such as *Daphnia* have been conducted to assess the toxicity of untreated PW, and have reported substantial sublethal toxicity as well as significant developmental and metabolic alterations (Blewett et al., 2017; He et al., 2017b; Hull et al., 2018; Wiltse et al., 2025). Comprehensive toxicological studies on treated PW are more limited. One study employed the marine luminescent bacterium *Vibrio fischeri* to assess changes in acute toxicity in PW samples collected before and after filtration (Alzahrani et al., 2013). The study showed that nanofiltration-treated water retained a median effective concentration ($EC_{50}$) of 13.65 %, while no toxicity was detected in the RO-treated water. *Ceriodaphnia dubia, Raphidocelis subcapitata,* zebrafish embryos, and *Vibrio fischeri* were used to assess the performance of a pilot-scale thermal distillation system for PW treatment (Tarazona et al., 2024b). It was demonstrated that thermal distillation alone cannot remove all toxic constituents affecting aquatic organisms (Tarazona et al., 2024a). Thermal distillation followed by post-treatment resulted in no observable adverse effects on *R. subcapitata, C. dubia,* and zebrafish embryos (Tarazona et al., 2024b).

In addition to conventional aquatic toxicity testing, assays using human cell lines can help identify the potential human health impacts of PW reuse, including DNA damage, endocrine disruption, oxidative stress, necrosis, and apoptosis. Different cell lines may respond differently to specific toxicants; therefore, employing a diverse set of cell lines enhances the reliability and scope of toxicity evaluations, ensuring a thorough assessment of potential health risks (Allen et al., 2005). Very few existing studies primarily focus primarily on a limited set of biological endpoints, such as cytotoxicity, and typically use only a single human or animal cell line (Hu et al., 2022; Van Houghton et al., 2024; Wiltse et al., 2025). Hu et al. employed the RTgill-W1 fish gill cell line to evaluate cell viability and membrane damage induced by raw PW from the Permian Basin, identifying high salinity as the primary toxicological driver, with organic compounds, heavy metals, and ammonia also contributing to toxicity (Hu et al., 2022). PW from conventionally drilled wells in the Denver-Julesburg Basin exhibited low salinity; however, activation of the aryl hydrocarbon receptor (AhR) in proprietary mammalian cells revealed toxicity not detected by the *Daphnia magna* $EC_{50}$ assay, suggesting the need to address organic contaminants in PW (Wiltse et al., 2025). Similarly, another study reported that PW salinity from the Permian Basin reduced MCF-7 cell viability and showed that membrane distillation effectively eliminated cytotoxicity and prevented AhR activation (Van Houghton et al., 2024). However, previous studies have been limited to single cell lines, and lack systematic evaluation across multiple endpoints and diverse cell models to fully assess the risk of treated PW on public health and ecological environment.

This study addresses this knowledge gap by evaluating multiple toxicological responses of thermally treated PW from the pilot-scale treatment facilities in the Permian Basin using several human cell lines. Due to the complexity of PW composition, multiple toxicity endpoints were used in this study to comprehensively assess the risk of untreated and treated PW exposure, including cell viability, proliferation, membrane integrity, oxidative stress, apoptosis, and endocrine-disrupting activity across various human cell lines. Moreover, the expression of the AhR gene was evaluated after exposure to untreated and treated PW. AhR plays a crucial role in sensing environmental xenobiotics. Thus, the expression of AhR was used to verify the existence of unknown aromatic compounds in the final effluent.

## 2. Materials and methods

### 2.1. Produced water collection and treatment technologies

PW samples used in this study were collected in the Permian Basin. Two pilot-scale thermal desalination treatment systems were used to treat the PW, including low-temperature thermal desalination (LTD) and mechanical vapor recompression (MVR). The distillate from the two

thermal desalination systems was polished using granular activated carbon (GAC) and zeolite to remove residual ammonia and volatile organic compounds. Details of the thermal distillation and post-treatment processes are provided below.

The thermal desalination treatment system in Orla, Texas was a pilot-scale LTD unit that treats 500 barrels of PW per day (79.5 $m^3$/d). The raw PW with TDS 100,000–170,000 mg/L was pretreated with 30 % hydrogen peroxide ($H_2O_2$) and filtered using a basket strainer (1/16" mesh screen) to remove hydrogen sulfide ($H_2S$) and suspended solids in the raw PW before treating it in the thermal desalination system. The distillation unit was a modular thermal system powered by low-grade exhaust waste heat from a gas compressor. The details of LTD system can be found in our previous study (Tarazona et al., 2024a). The second set of samples was collected from an MVR system in Big Springs, Texas. The raw PW had TDS levels between 130,000–170,000 mg/L, and it was used as MVR feed without pretreatment. Feed and distillate PW samples were collected and stored at 4°C prior to analysis.

High concentrations of ammonium and organic compounds have been reported in PW from the Permian Basin (Chen et al., 2023; Hu et al., 2020). Due to their boiling points and the operational conditions of the thermal systems, fractions of ammonia and volatile organic compounds could be partitioned to the distillate during thermal distillation processes. Therefore, even after thermal distillation, further treatment is often required to remove ammonia and residual organics before safe disposal or reuse of the treated PW (Tarazona et al., 2024b). Zeolite is a porous aluminosilicate mineral with unique adsorption and cation exchange properties that enable it to be used as an adsorbent for ammonia removal. In this study, the clinoptilolite (Double Eagle, Casper, WY), the most abundant natural zeolite type, was packed in a cylindrical column to remove ammonia. GAC has been widely used to remove organic compounds from water due to its high surface area and strong adsorption capacity. GAC (Aqua-Tech, Spectrum Brand, Inc.) was packed in a column in this study to remove the residual organics in the distillate. GAC, followed by zeolite (GAC+ zeolite) as a unified treatment train, was set up to remove organics, metals, and ammonia. The height and diameter of the columns were 30 cm and 2 cm, respectively, with empty bed contact times of 2.6 and 2.725 h for GAC and zeolite columns, respectively. The details regarding the GAC and zeolite post-treatment can be found in our previous study (Tarazona et al., 2024b).

The untreated samples (the feed of the thermal system), thermal distillate, and post-treated PW were collected for chemical characterization in a commercial lab. Details of the specific chemical characterization methods and protocols are presented in Table S1 in the supplementary information. Toxicity characterization of PW samples was conducted using human cell lines listed below.

### 2.2. Cell culture and sample preparation for cytotoxicity assays

The toxicity effect of different PW samples was tested on human intestinal epithelial (Caco-2) cells, human breast cancer cells (MCF-7), and a specific immortalized cell line derived from an aborted fetus or human embryonic kidney cells (HEK293). These cells were originally obtained from the American Type Culture Collection (ATCC). The cells were cultured in 75 $cm^2$ sterile tissue culture flasks containing Dulbecco's Modified Eagle Medium (DMEM) with phenol red supplemented with 10 % Fetal Bovine Serum (FBS) and 1 % penicillin-streptomycin solution (10,000 U/mL) (Limam et al., 2014). They were incubated in an atmosphere of 5 % $CO_2$ at 37 °C. When the confluency of cells surpassed 80 %, the cell layer was washed twice with phosphate buffer saline (PBS) and then dispersed using 0.25 % Trypin-EDTA solution for subculture and toxicity assays. To assess the cytotoxic effects of PW samples, each of the three cell types was exposed to the following PW dilutions: 6.25 %, 12.5 %, 25 %, and 50 % for dose-response assessment. The feed, distillate, and GAC+zeolite post-treated PW were analyzed in this study to compare their cytotoxicity. All treatments, controls, and blanks were performed in replicates.

### 2.3. Cell viability assays

#### 2.3.1. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay

The CyQUANT™ MTT cell viability Assay Kit (Thermo Fisher Scientific, USA) is a colorimetric assay that measures cellular metabolic activity during exposure to PW samples. It is based on the cleavage of the yellow tetrazolium salt to purple formazan crystals. A cell density of $1 \times 10^5$ cells/well was seeded in a 96-well plate (100 µL per well). The cells were left to recover and adhere at 37 °C for 24 h. Next, the culture medium was removed and replaced with 100 µL of PW dilutions (involves 50 µL of 2 ×concentrated cell culture medium, deionized water, and various volumes of PW to achieve a final 6.25–50 % PW dilution). The cells were incubated at 37 °C for 24 h. Afterward, 10 µL of MTT labeling reagent was added to each well and incubated for 4 h at 37 °C. After adding solubilization solution and being incubated for another 4 h at 37 °C, the optical density (OD) was measured at 570 nm and 690 nm utilizing the Synergy HTX Multimode Reader (BioTek, USA) to calculate the percentage of cell viability.

#### 2.3.2. CellTiter-Glo® 2.0 cell viability assay

The CellTiter-Glo® 2.0 assay (Promega, Madison, USA) assesses viable cell count in a cell culture. The adenosine triphosphate (ATP) reacts with luciferase, producing luminescence proportional to the ATP concentration, which correlates to the number of live cells (Bertoletti et al., 2022). Cells were seeded in 96-well white opaque-walled plate at a density of $1 \times 10^5$ cells/well and left to adhere at 37 °C for 24 h. Next, the medium was removed and replaced with 100 µL of PW dilutions ranging from 6.25–50 %. The cells were incubated at 37 °C for 24 h. Afterward, 100 µL of CellTiter-Glo® 2.0 reagent was added to each well. The contents were shaken on an orbital shaker to induce cell lysis, and the plate was incubated at room temperature to stabilize the luminescent signal. Finally, the luminescence was recorded using the same microplate reader (Nowak et al., 2018). The cell viability was presented as a percentage of luminescence from control cells (Malinowski et al., 2022).

### 2.4. LDH (Lactate Dehydrogenase) cytotoxicity assay

LDH is a cytosolic enzyme released into the cell culture medium upon damage to the cell membrane, which is a typical characteristic of cells undergoing apoptosis and necrosis (Kamiloglu et al., 2020). This study used the CyQuantTM LDH cytotoxicity assay kit (Thermo Fisher Scientific, USA) to measure cell lysis. Cells were seeded at a cell density of 10,000 cells/100 µL ($1 \times 10^5$ cells/mL) in a 96-well plate and allowed to attach in an incubator at 37 °C for 24 h. Afterward, the manufacturer's instructions were followed, and the optical density (OD) was measured at 490 nm and 680 nm utilizing the same Multimode Reader (BioTek, USA) to calculate the percentage of cytotoxicity (Huang et al., 2021).

### 2.5. Caspase-Glo® 3/7 apoptosis assay

Caspase-3 and caspase-7 are both activated universally during apoptosis. The Caspase-Glo® 3/7 Assay (Promega, Madison, USA) kit is a homogeneous, luminescent assay. The MCF-7 cell lines were seeded in a white opaque 96-well plate at a cell density of $10^4$ cells/well and left to adhere for 24 h at 37 °C (Gökhan, 2022). Next, the medium was removed and replaced with 100 µL of 50 % PW (distillate and GAC+zeolite samples) and incubated at 37 °C for 24 h. The Caspase-Glo® 3/7 reagent was prepared, and 100 µL of the reagent was added to each well containing 100 µL of blank, negative control, and cells treated with PW. The plate was gently mixed and incubated according to the manufacturer's guidelines. The luminescence was recorded every 30 min for several hours using the same microplate reader mentioned above to obtain the

maximum luminescence. The apoptosis was represented as a percentage of the negative control (Mfotie Njoya et al., 2018).

### 2.6. Cell oxidative stress based on nitrite assays

Nitric oxide (NO) serves as a molecular mediator in various physiological processes, including vasodilation, inflammation, thrombosis, immunity, and neurotransmission. The immune cells release various inflammatory mediators, such as NO, detected by the Griess reagent kit assay (G-7921, Invitrogen, Thermo Fisher Scientific). A calibration curve was created to convert absorbance readings to nitrite concentrations. Sodium nitrite solutions (1–100 μM) were prepared by diluting the standard solution with DI water (Privat et al., 1997). The Griess reagent, consisting of 10 μL N-(1-naphthyl) ethylenediamine and 10 μL sulfanilic acid, was prepared (20 μL per well). The components were added to a 96-well plate, totaling 300 μL (20 μL Griess reagent, 150 μL nitrite-containing sample, 130 μL DI water). The 96-well plate was incubated at room temperature for 30 min after adding all components. The absorbance in each well was measured using the optimum measurement wavelength of 548 nm. Next, the absorbance readings were converted to nitrite concentration using the calibration curve (Berisha et al., 2020; Guevara et al., 1998).

### 2.7. Expression of AhR genes

AhR, a xenobiotic sensor, could metabolize polycyclic aromatic hydrocarbons (PAHs) and other aromatic compounds into carcinogenic intermediates (Barouki et al., 2007). The AhR regulates the expression of the CYP1A1 and CYP1B1 genes, which encode important enzymes in xenobiotic metabolism (Lin et al., 2003). In this study, the activation of AhR was evaluated by measuring the expression level of target genes (CYP1A1 and CYP1B1) after exposure of MCF-7 cells to PW samples.

MCF-7 cells were seeded in a 24-well plate at a cell density of $10^5$ cells/mL and incubated in 5 % $CO_2$ at 37°C for 24 h to allow them to adhere. After 24 h, the cells were exposed to 80 % PW and incubated at 37°C for 24 h. RNA extraction was conducted using the PureLink® RNA Mini Kit (Thermo Fisher Scientific, United States). After 24 h, PW samples were removed from the 24-well plate before adding the prepared lysis buffer. Homogenization was carried out by transferring the lysate to a 1.5 mL RNase-free tube and passing it 5–10 times through an 18-gauge needle attached to an RNase-free syringe. The RNA concentration was measured using the BioTek Epoch Microplate Spectrophotometer with a Take3 microvolume plate. The RNA's purity was analyzed using the ratio of the absorbance value measured at 260 and 280 nm. The remaining RNA was stored at −80°C for future use (Zhao et al., 2023).

All RNA samples were treated with DNase I (Amplification Grade, Thermo Fisher Scientific) to eliminate DNA via hydrolysis before cDNA synthesis. Afterward, reverse transcription was done to form cDNA using the iScriptTM cDNA Synthesis Kit made by Bio-Rad. qPCR reactions were performed in 96-well plates with a system volume of 20 μL, containing 10 μL of 2 × SsoAdanved Universal SYBR Green Supermix (Bio-Rad), 1 μL of each forward and reverse primer (10 μM), 2 μL of diluted DNA sample and 6 μL of DNA/RNA free water as described in a previous study (Van Houghton et al., 2024). Amplification and detection were carried out in a CFX Connect Real-Time system (Bio-Rad, Hercules, CA). The reaction began with an initial denaturation of 10 min at 95°C, followed by 40 cycles of 95°C for 15 s, primer annealing, and extension at 60 °C for 1 min. The gene expression was determined using the ddCT (Delta-Delta-CT) method with GAPDH (glyceraldehyde-3-phosphate dehydrogenase) as the housekeeping gene. The fold change was calculated and normalized relative to the control values for cells grown without PW.

### 2.8. E-Screen assay for the determination of estrogenicity

The E-Screen assay measures enhanced cell proliferation in the presence of estrogen-active substances, which assesses the estrogenicity of environmental chemicals (Soto et al., 1995). E-screen assays were conducted based on the protocol in the previous study with modifications (Körner et al., 1999). The cells were cultured in a 96-well plate at a MCF-7 cell density of 10,000 cells/well and left to adhere at 37°C for 24 h. The cells were exposed to 80 % PW dilutions in phenol-red free DMEM containing 10 % estrogen-free charcoal-stripped bovine serum, 1 % penicillin-streptomycin solution, and 2 mM L-glutamine. The negative control was prepared without hormones, and the internal positive control had 17β-Estradiol (E2) in five concentrations between $10^{-12}$ - $10^{-9}$ M. The assay was terminated on day 6, and the cell proliferation was assessed by using the colorimetric Sulforhodamine B (SRB) Cell Cytotoxicity Assay Kit following the manufacturer's instructions. SRB solution binds to basic amino acids in fixed cells to reflect the cell mass after PW exposure. The absorbance was measured using the Synergy HTX Multimode Reader (BioTek, USA) at 565 nm.

### 2.9. Statistical analysis

Results from the cytotoxicity assays and endocrine-disrupting activity evaluations were expressed as percentages relative to the untreated control. One-way ANOVA was used to identify statistically significant differences in cell proliferation relative to the positive control. An alpha value of 0.05 was used for all statistical tests. Differences with $p \leq 0.05$ were considered statistically significant and are marked with an asterisk (*). Results were visualized using Prism 10 (GraphPad Software, San Diego, CA, USA).

## 3. Results and discussion

### 3.1. The chemical characterization of PW samples from two distillation systems

Thermal distillation can convert water to vapor and then condense the vapor back into liquid water. During evaporation, constituents that cannot evaporate with the water remain in the concentrate. Therefore, thermal distillation can effectively remove most dissolved salts, heavy metals, microorganisms, and non-volatile organic contaminants, thereby reducing PW toxicity. The two PW treatment systems effectively removed the majority of the constituents in the raw PW with a salt removal efficiency of 99.72 % for LTD and 99.95 % for MVR, as shown in Table 1. The salinity levels in both distillates meet the secondary drinking water standards at 500 mg/L for TDS. Removal efficiencies for organics were comparable between the two thermal distillation processes, ranging from 43.15 % to 49.87 %. Although the ammonia removal by both two thermal systems was higher than 85 %, ammonia concentrations in the distillate were relatively high for discharge purposes, with 46 mg $NH_3$-N/L in the LTD system and 21 mg $NH_3$-N/L in the MVR system, which may pose a toxicity risk to aquatic organisms and promote algal blooms in the receiving waters (USEPA, 2025b). Moreover, the distillates from both LTD and MVR contain VOCs such as benzene, toluene, ethylbenzene, and xylene. As mentioned in Section 2.1, the high temperatures within the LTD and MVR units could volatilize some VOCs, resulting in their existence in the distillate. Our previous study also showed that the distillate after this LTD process inhibited *V. fischeri* bioluminescence and *R. subcapitata* growth, and caused 100% mortality in *C. dubia* neonates and *D. rerio* embryos (Tarazona et al., 2024a). Therefore, further treatment using GAC, followed by zeolite post-treatment, is essential to remove the VOCs, ammonia, and other harmful compounds to ensure no potential health risks. GAC treatment has been widely used for the removal of organic compounds from water due to its high specific surface area and strong adsorption capacity. Consequently, passing the distillate through GAC

**Table 1**
**Characteristics of feed, distillate, and post-treated PW for LTD and MVR.**

| | Units | LTD | | | MVR | | |
|---|---|---|---|---|---|---|---|
| | | Feed | Distillate | GAC +zeolite | Feed | Distillate | GAC +zeolite |
| pH | S.U. | 6.58 | 8.52 | 7.4 | 6.1 | 10.2 | 8.2 |
| $NH_3$-N | mg/L | 611 | 46 | BDL | 139 | 21 | BDL |
| TOC | mg/L | 74.4 | 42.3 | 5.4 | 79.8 | 40.0 | 10.0 |
| TDS | mg/L | 103552 | 287 | NA | 172000 | 80 | 106 |
| TPH | mg/L | 28.0 | 3.0 | NA | 59.0 | 4.6 | 1.2 |
| $Na^+$ | mg/L | 24795 | 72.5 | 59.7 | 47600 | 0.9 | 26.4 |
| $Cl^-$ | mg/L | 63756 | 84.0 | 2.0 | 72100 | 1.5 | 27.2 |
| $Cd^{2+}$ | mg/L | NA | 0.0011 | BDL | 0.0416 | BDL | BDL |
| $Ca^{2+}$ | mg/L | 4491 | 0.61 | 4.7 | 8200 | 1.0 | 2.6 |
| $Mg^{2+}$ | mg/L | 701 | 1.4 | 509 | 1100 | BDL | 0.4 |
| $Ba^{2+}$ | mg/L | 5.9 | 0.007 | 0.006 | 31 | 0.005 | 0.005 |
| $Li^+$ | mg/L | 30.8 | 0.004 | 0.04 | 25.9 | BDL | BDL |
| Benzene | mg/L | NA | 0.501 | NA | 0.445 | 0.0213 | BDL |
| Toluene | mg/L | NA | 0.548 | NA | 0.307 | 0.0236 | BDL |
| Ethylbenzene | mg/L | NA | 0.0214 | NA | 0.0208 | 0.00234 | BDL |
| Xylene | mg/L | NA | 0.377 | NA | 0.128 | 0.0181 | BDL |
| Phenol | mg/L | NA | 0.026 | NA | 0.021 | 0.440 | BDL |
| Naphthalene | mg/L | NA | 0.0131 | NA | 0.0306 | 0.0200 | BDL |
| Fluorene | mg/L | NA | 0.00147 | NA | 0.0103 | 0.00508 | BDL |
| Phenanthrene | mg/L | NA | 0.00141 | NA | 0.0145 | 0.00406 | BDL |
| Acenaphthene | mg/L | NA | 0.00043 | NA | BDL | BDL | BDL |
| 2-Nitrophenol | mg/L | NA | 0.007 | NA | BDL | BDL | BDL |
| Acetone | mg/L | NA | NA | NA | 0.618 | 0.829 | BDL |
| 2-Propanol | mg/L | NA | NA | NA | 2.04 | 4.26 | BDL |

NA: not available; BDL: below detection limit

columns effectively removed a significant amount of organic compounds from the distillate (Tarazona et al., 2024b). As shown in Table 1, GAC+zeolite post-treatment after LTD and MVR reduced the ammonia and VOC concentrations to trace amounts.

### 3.2. Cell viability change after PW exposure

MTT assay evaluates the cellular metabolic activity by measuring the conversion of the MTT tetrazolium dye to the amount of purple formazan formed with the help of the enzyme oxidoreductase. The amount of purple formazan generated is directly proportional to the cell viability in each well (Berehu et al., 2021). Fig. 1 shows a similar trend in the cell viability in cells exposed to PW samples from two systems. Cell metabolic activity was suppressed substantially when they were exposed to a PW feed percentage of 12.5 and above. The mean cell viability at 6.25 % feed PW in MCF-7, HEK293, and Caco-2 cell lines was 79.5 %, 83.7 %,

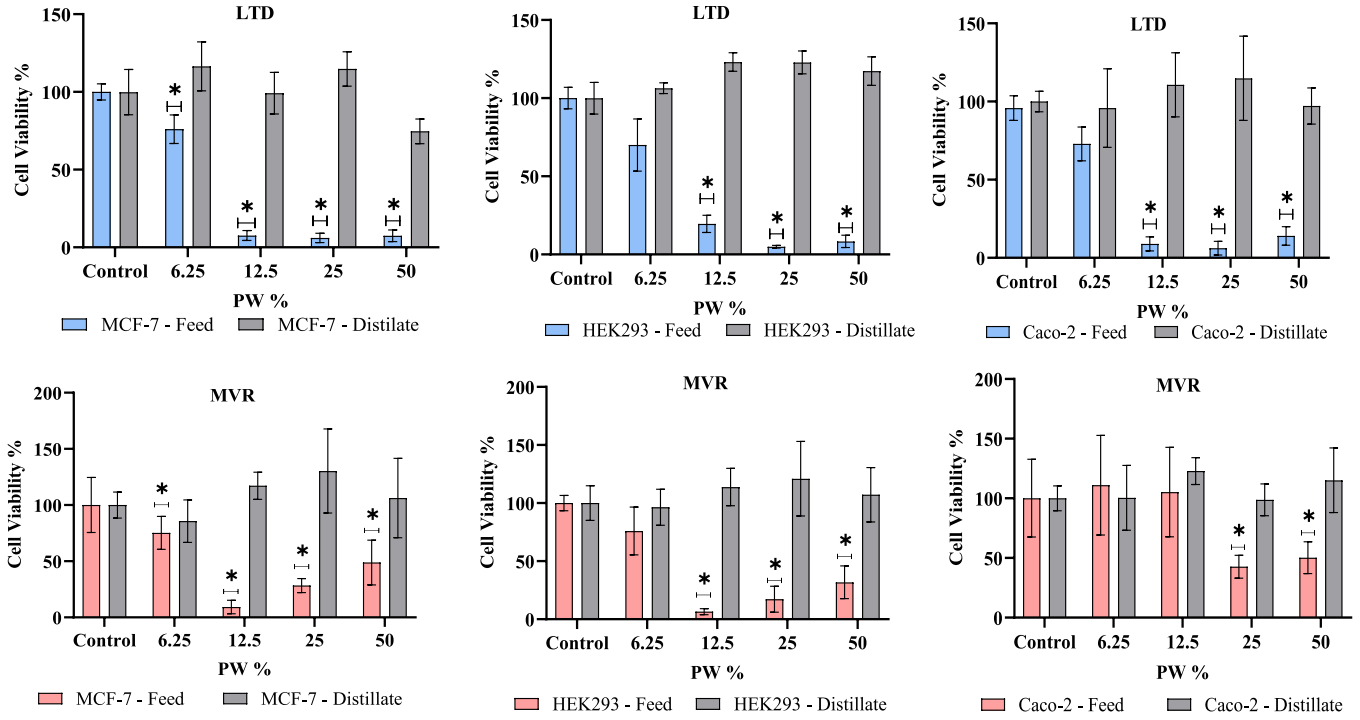


**Fig. 1.** MCF-7, HEK293 & Caco-2 cell viability after exposure to feed and distillate from LTD and MVR units based on MTT assay. The * represents the statistical difference (P-value $< 0.05$) in contrast to the control.

and 88.5 % in LTD samples, and 73.1 %, 85.9 %, and 86.5 % in MVR samples, respectively. The 50 % distillate has no significant difference in cell viability compared to the control (P-value > 0.05). After increasing the percentage of distillate to 80 %, the cell viability was still not changed compared to the control (Figure S1).

In this study, the CellTiter-Glo® 2.0 Assay was also employed as an alternative method to measure cell viability based on ATP quantification. This assay was used to address the limitations of the MTT assay, which might show inconsistent results caused by chemical interference (Ghasemi et al., 2021). The CellTiter-Glo® assay produced comparable results for cell viability, as shown in Fig. 2. The distillate from both LTD and MVR systems did not reduce the viability of any of the three cell types. A key difference between the MTT and CellTiter-Glo® 2.0 assays was evident at 6.25 % feed concentration, where the latter detected a significant reduction in cell viability across all three cell lines in both LTD and MVR samples, highlighting its greater sensitivity. The *in vivo* studies also demonstrate the toxicity of untreated PW. For instance, the acute, chronic, and pulse toxicity of flowback and PW releases to aquatic systems was studied using the model benthic invertebrate *Lumbriculus variegatus* in Alberta (Mehler et al., 2020). Although *L. variegatus* is thought to have a high tolerance to many stressors, the acute toxicity was still significant at low concentrations (i.e., high dilutions) of flowback and PW ($LC_{50}$ at 48-h exposure: 4–5 %). In another study, the acute toxicity of a real flowback and PW sample from the Duvernay Formation in Canada was assessed using the zebrafish embryo exposure model (He et al., 2017a). The results reveal that the acute toxicity of zebrafish embryos was attributable to high salinity and organic contaminants in flowback and PW with $LC_{50}$ values ranging from 0.6 % to 3.9 %, depending on the hydraulic fracturing flowback and PWs fractions and embryo developmental stages.

The salinity in feed water was the major contributor to its cytotoxicity. Our previous study revealed that high salinity was the foremost toxicological driver in PW from the Permian Basin, and > 30 g/L of TDS could cause significant inhibition in tested marine bacteria and fish cell lines (Hu et al., 2022). The 50 % feed in our study contained approximately a TDS of 51,776 and 86,000 mg/L in the LTD and MVR samples, respectively. The LTD and MVR processes removed 99.54 % and 99.95 % of TDS from feed PW, respectively. Thus, no cytotoxicity was caused by the salinity in the distillate.

Previous studies have shown that ammonia concentrations at or above 5 mM significantly reduce the viability of bovine mammary epithelial cells ($p < 0.05$) (Wang et al., 2018). In this study, the LTD and MVR distillates contained approximately 46 and 21 mg/L $NH_3$-N (3.3 and 1.5 mM), both below the reported toxicity threshold. To confirm the effects of ammonia on cell viability, a solution of 3.3 mM ammonia was prepared in deionized water and used for cell exposure. Cell viability remained at 100 %, indicating that ammonia at this concentration did not cause observable toxicity.

Table 1 presents the organic compounds found in the feed, distillate, and post-treated PW collected from LTD and MVR. The distillate from LTD contains BTEX, acenaphthene, TPH ($C_6$ to $C_{35}$), fluorene, naphthalene, and phenol. Similarly, the PW for MVR treatment contains BTEX, anthracene, naphthalene, fluorene, acetone, phenanthrene, and other compounds in both feed and distillate. Organic compounds can cause cytotoxicity in human cell lines, depending on the concentration to which the cells are exposed. A study conducted to assess the cytotoxicity of benzene on a human myeloid cell line (HL-60) demonstrated that benzene induced cytotoxicity, apoptosis, and changes in gene expression levels even at concentrations of 0.05 mM after 8-hour exposure to benzene (Nishikawa et al., 2011). Additionally, another study reported that exposure to benzene, toluene, and o-xylene induced HL-60 cell death, and the $IC_{50}$ values for benzene, toluene, and o-xylene were 8.75 mM, 2.74 mM, and 0.84 mM, respectively (Sarma et al., 2011). PAHs are also known to exert adverse toxic effects on human cell lines. A study revealed that exposure to PAHs, such as benzo[*a*]pyrene (BaP), phenanthrene (PHE), and fluoranthene (FLA), reduced cell viability in human epithelial lung cells (A549). The half-maximal inhibitory concentrations ($IC_{50}$) for PHE, FLA, and BaP were approximately 407 μM, 424 μM, and 846 μM, respectively (Takam et al., 2024). Despite the lower concentrations of BTEX and PAHs in the feed PW

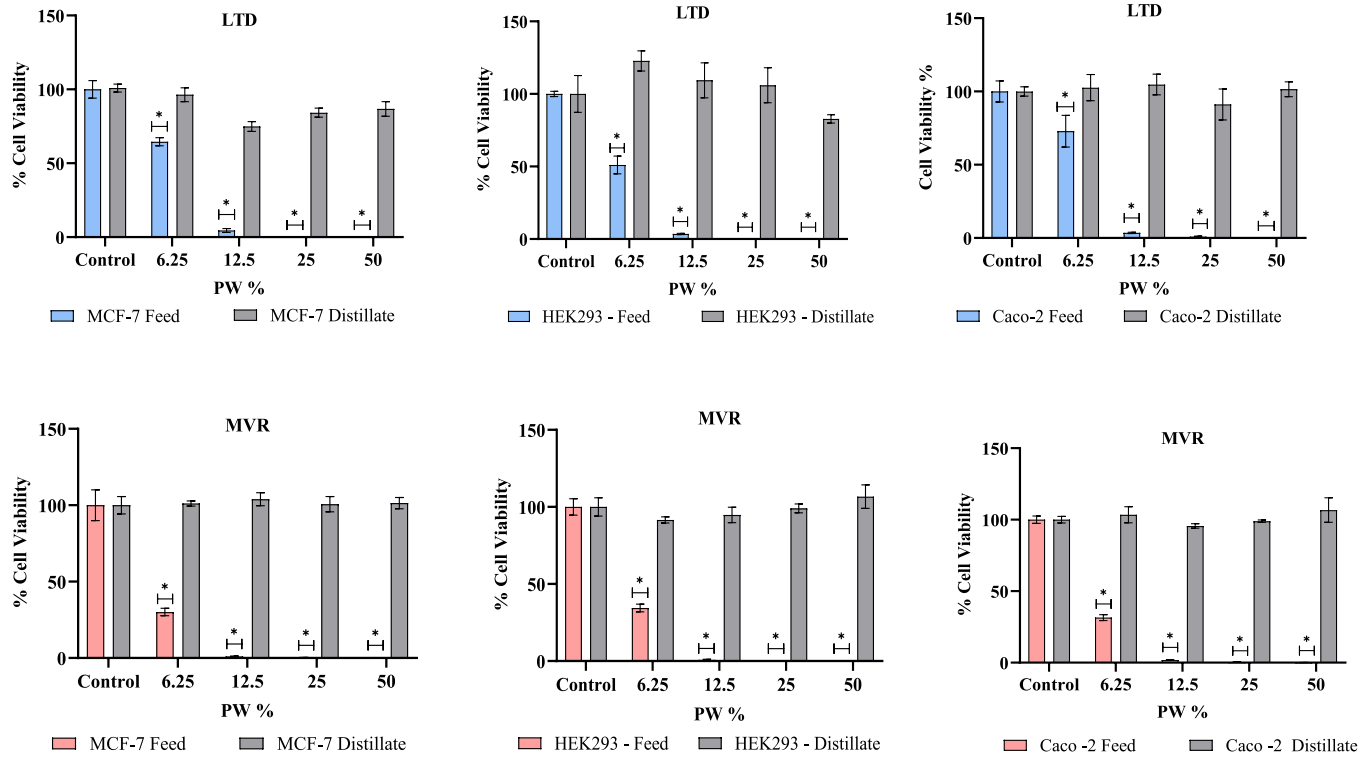


**Fig. 2.** MCF-7, HEK293 & Caco-2 cell viability after exposure to feed and distillate from LTD and MVR units based on CellTiter-Glo® 2.0 assay. The * represents the statistical difference (P-value < 0.05) in contrast to the control.

compared to reported values, the combined effects of multiple PAHs may still exert cytotoxic effects. The contribution of organic compounds to the toxicity of raw PW was also demonstrated in the previous study (Hu et al., 2022). Both thermal distillation processes reduced the concentrations of organic compounds, thereby eliminating the cytotoxicity indicated by MTT and CellTiter-Glo® 2.0 assays. A recent study conducted by Van Houghton et al. also revealed that the distillate from MD did not cause cytotoxicity in MCF-7 cells, whereas raw PW exhibited significant toxicity at concentrations higher than 3.13 % and 6.25 % (Van Houghton et al., 2024).

### 3.3. Cell membrane damage after PW exposure

Fig. 3 shows the cytotoxicity based on cell membrane damage after exposure to feed and distillate PW. Similar to cell viability results, exposure to an increasing percentage of feed PW caused significant cell membrane disruption. LDH test indicates irreversible cell death due to cell membrane disruption (Fotakis and Timbrell, 2006). High levels of TDS in the feed PW may have contributed to membrane damage in exposed cells. Exposure to 6.25 % feed PW from the LTD system caused significant cytotoxicity in MCF-7 and Caco-2 cell lines, while 6.25 % feed PW from the MVR system caused significant membrane damage in MCF-7 and HEK293 cells ($p < 0.05$). However, no significant cytotoxicity was observed with the distillates from either thermal system. The observed cytotoxicity in feed PW can largely be attributed to high salinity. High concentrations of salts could induce cell swelling due to osmotic stress and result in cell growth inhibition, cell senescence, and reduced cell adhesion (Sharma et al., 2024; Yamakami et al., 2016). A study conducted an LDH assay to test the impact of PW on gill cell line RTgill-W1 of rainbow trout, and it concluded that high salinity was the major cause of cell membrane damage in their study (Hu et al., 2022). No cell membrane damage was observed in the distillate, likely due to the low TDS, TOC, and salinity levels after the distillation processes.

### 3.4. Cell apoptosis after exposure to treated PW

Cell apoptosis, also known as cell-programmed death, occurs when the cells are active participants in their own death under normal physiological conditions. When cell apoptosis occurs, both caspase-3 and caspase-7 are universally activated during apoptosis (Manzano et al., 2024). Apoptosis can be assessed by measuring the activity of caspase-3 and caspase-7. Exposure to 50 % distillate and GAC+zeolite-treated PW from LTD showed no significant apoptosis compared to the control (Figure S2), suggesting that residual constituents did not induce apoptotic responses in MCF-7. A previous study demonstrated that with an ammonia concentration of 5 mM, the rate of apoptosis increased significantly in MAC-T cells in contrast to the control (Wang et al., 2018). Exposure to 50 % distillate (containing 1.65 mM ammonia) did not induce significant apoptosis, suggesting that ammonia-induced apoptosis in MCF-7 cells may occur only at higher concentrations. The residual organic compounds in treated PW, such as benzene, toluene, and xylene (BTX), might induce apoptosis in human cell lines. A study reported that exposure to BTX significantly increased the apoptosis rate in a concentration-dependent manner. Specifically, benzene, toluene and xylenes at their $IC_{20}$ concentrations (3.40 mM for benzene, 1.14 mM for toluene, and 0.36 mM for xylene) induced apoptosis in promyelocytic leukemia HL-60 cell line, erythromyeloblastoid leukemia K562 cell line and leukemic monocyte lymphoma U937 cell line (Sarma et al., 2011). The results demonstrated that no apoptosis occurred. Although several BTEX compounds previously reported to induce apoptotic responses in similar cell lines were detected, their concentrations in this study appeared insufficient to elicit a significant apoptotic response in the MCF-7 cell line.

### 3.5. Cell oxidative stress after PW exposure

Nitric oxide (NO) is an important physiological messenger and effector molecule in many biological systems (Tuteja et al., 2004). NO has been demonstrated to be a crucial molecule in the regulation of

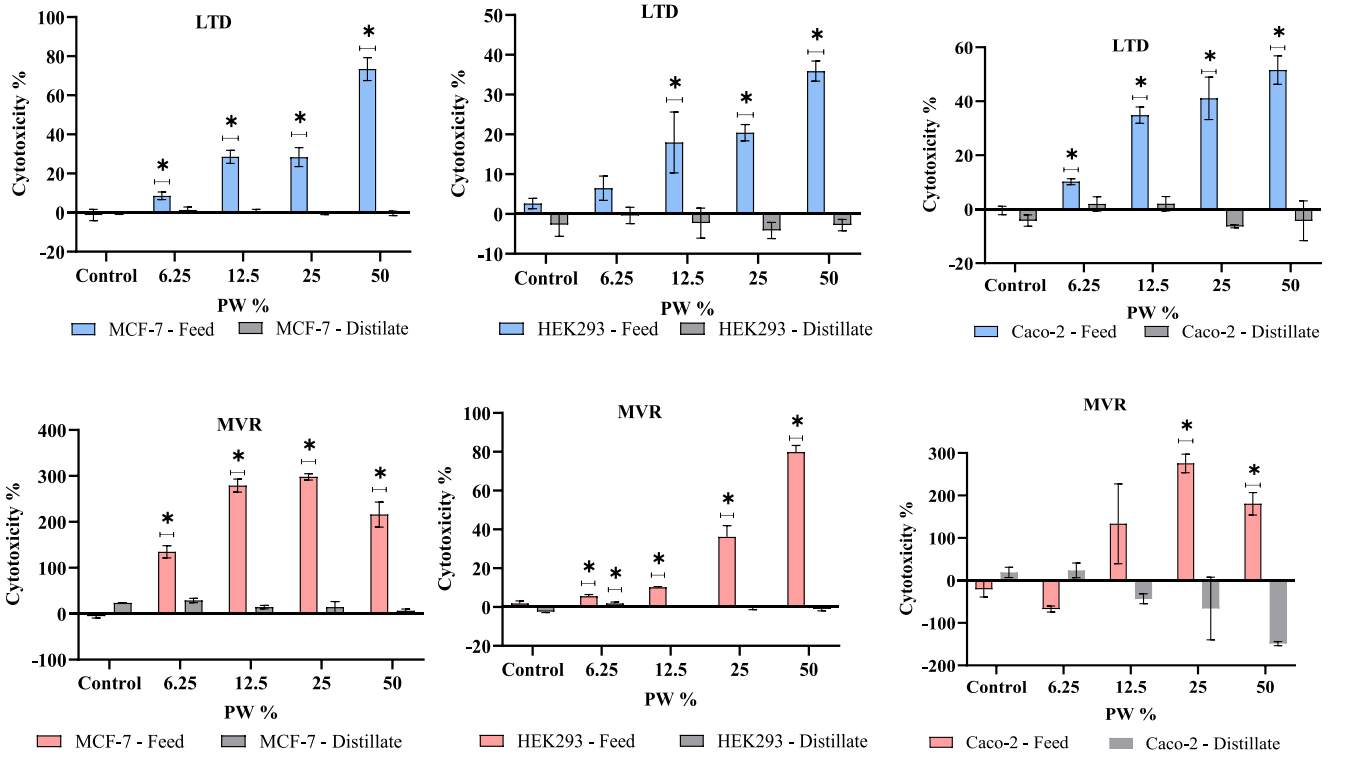


**Fig. 3.** MCF-7, HEK293 & Caco-2 cytotoxicity after exposure to feed and distillate from LTD and MVR units based on membrane damage from LDH Assay. The * represents the statistical difference (P-value < 0.05) in contrast to the control.

acute and chronic inflammation and host defense mechanisms (Tripathi et al., 2007). In this study, nitrite was measured to determine the cell response to PW since it is a primary breakdown product of nitric oxide. Exposure to feed PW in the two systems caused HEK293, Caco-2, and MCF-7 cell lines to undergo significant cell inflammation or cell oxidative stress ($P < 0.05$). The distillate and GAC+zeolite-treated PW did not show high nitrite concentrations compared to the control, indicating no significant cell inflammation or oxidative stress (Fig. 4). High ammonia concentrations have been reported to cause oxidative stress in cell lines when concentration is equal to or greater than 5 mM, leading to cellular inhibition due to a substantial increase in reactive oxygen species (ROS) levels (Wang et al., 2018). At 50 % dilution, feed PW samples from the LTD and MVR systems contained ammonia concentrations of 21.8 mM (305.5 mg/L) and 5 mM (70 mg/L), respectively. These elevated ammonia levels in feed PW suggest that ammonia may be one of the contributing factors to the oxidative responses observed in the cell lines. Oxidative stress can damage cellular DNA, as well as impair proteins and lipids, thereby initiating a genotoxic stress response (Cooke et al., 2003). In addition to ammonia, oxidative stress in human cell lines can be caused by other constituents present in the feed PW, such as PAHs. A study reported that exposure to anthracene solutions at concentrations of 7 μM or higher altered the redox balance in a non-tumorigenic human breast epithelial cell line (MCF-10A), inducing oxidative stress and leading to reduced cell viability and proliferation (Mardirosian et al., 2024). Similarly, another study assessing oxidative stress in rainbow trout cells after exposure to PW from the North Sea revealed PAHs and alkylphenols (APs) were the primary contributors to oxidative stress. The PW extracts contained PAH concentrations ranging from 38.9 to 480.8 μg/L and AP concentrations between 9.1 and 636 μg/L. Within this range, an increase in intracellular ROS was observed, indicating significant oxidative stress in the rainbow trout cells (Farmen et al., 2010). Because PAH concentrations in the feed PW samples exceeded previously reported levels, their potential contribution to the observed oxidative stress cannot be entirely excluded. To better understand the role of PAHs in oxidative stress, future research should evaluate PAH-induced oxidative responses in human cell lines at concentrations representative of those found in both raw and treated PW effluents.

### 3.6. Effects of PW on the activation of AhR

Fig. 5 shows the expression of CYP genes in MCF-7 after exposure to PW. According to the results, gene CYP1A1 was upregulated in both distillates due to the AhR activation. CYP1B1 was also upregulated in LTD distillate. The expression of the CYP1A1 gene in cells exposed to both feed PW samples was downregulated. Exposure to 80 % PW for 24 h has impaired the growth of the MCF-7 cell lines and triggered multiple stress responses in MCF-7 cells, which may have influenced CYP1A1 expression despite the presence of xenobiotics capable of activating the AhR pathway. Factors such as elevated salinity, the presence of heavy metals, and the cumulative or synergistic effects of various constituents may suppress AhR-mediated transcriptional activity, masking the potential activating effects of certain organics. That's why the other study used 6.25 % raw PW to evaluate the activation of AhR by the untreated PW (Van Houghton et al., 2024). Following thermal distillation, an upregulation of AhR-responsive genes was observed, likely due to the reduced concentration of non-organic stressors. The lower levels of salinity, metals, and other constituents in the distillate may have allowed residual organic compounds to activate the AhR pathway. In line with our findings, another study assessed the expression of CYP1A1 and CYP1B1 following exposure to treated PW with a membrane bioreactor and reported that the membrane permeate induced greater gene upregulation than the feed sample (Van Houghton et al., 2024).

It was also found that exposure of Rainbow Trout (*Oncorhynchus mykiss*) to hydraulic fracturing flowback and PW resulted in significant induction of ethoxyresorufin-O-deethylase (EROD) activity in both liver and gill tissues (He et al., 2017b). EROD is an enzyme within the cytochrome P450 (CYP) 1A1 family that plays a role in metabolizing xenobiotics. Compared to the control, the GAC+zeolite post-treated PW has almost unchanged expression ($P > 0.05$). Since activation of the AhR regulates the expression of CYP1A1 and CYP1B1, the results suggest that GAC+zeolite post-treatment effectively removed organic compounds capable of activating AhR. According to Table 1, the target analysis also showed that the concentration of aromatic compounds decreased to below the detection limit, which explains the unchanged CYP1A1 and CYP1B1 expression. Due to the complexity of PW composition, the target analysis does not include all the compounds in raw and treated PW. It is also important to consider that certain AhR-activating compounds may not have been detected through targeted chemical analyses. These could include unknown organics, compounds below the method detection limit, or compounds excluded from the analytical scope. As such, target chemical characterization alone may underestimate the full spectrum of biologically active compounds influencing AhR activity. A previous study using non-target analysis on PW revealed that, after treatment with photocatalytic MD (PMD) and vacuum MD (VMD), the PW still contained constituents with potential toxicity (Delanka-Pedige et al., 2024). The sensitivity of AhR activation by aromatic compounds in PW was also demonstrated in another study. While the *D. magna* $EC_{50}$

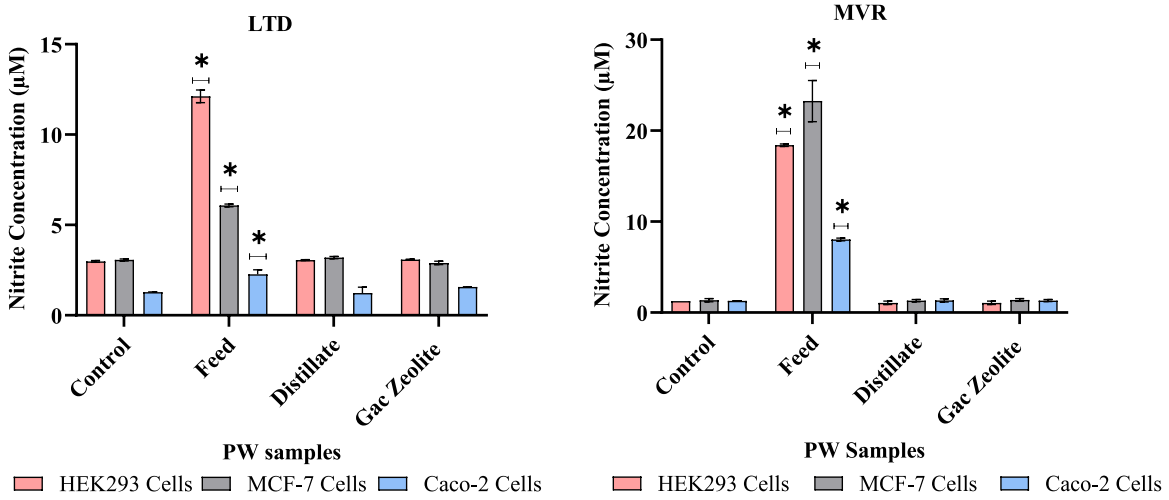


**Fig. 4.** Nitrite concentration was evaluated after exposure to 50 % feed, distillate, and distillate with GAC, followed by zeolite post-treatment PW. The * represents the statistical difference (P-value < 0.05) in contrast to the control.

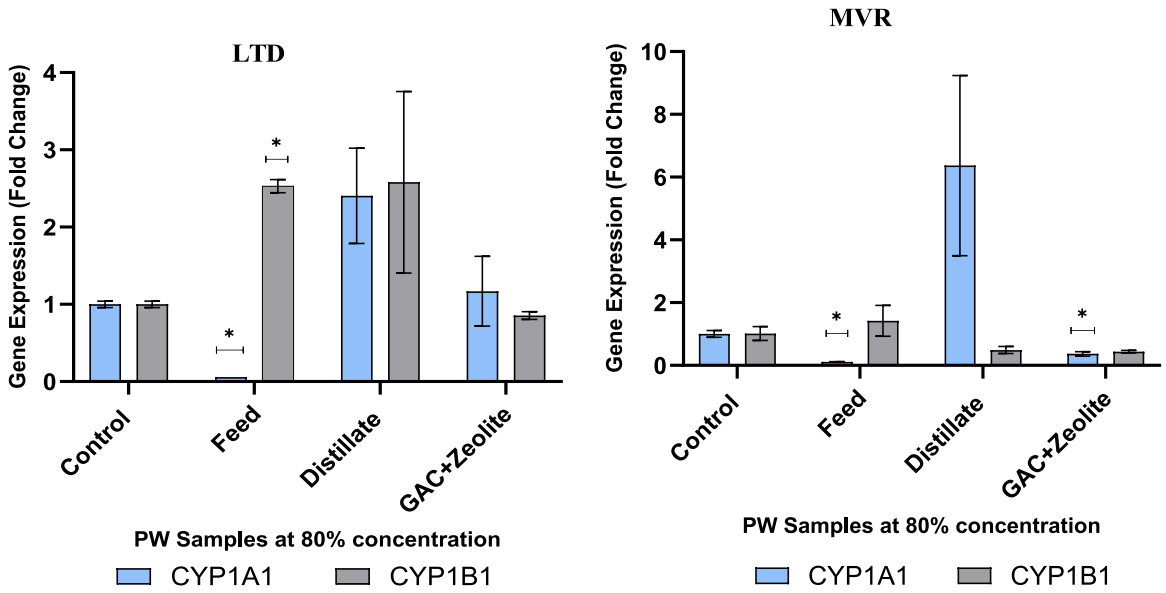


**Fig. 5.** Gene Expression of CYP1A1 and CYP1B1 using MCF-7 cell line after 24 h exposure to 80 % PW. The * represents the statistical difference (P_value < 0.05) in contrast to the control.

test did not capture the toxicity of the PW from conventionally drilled wells in the Denver-Julesburg Basin, the AhR receptor in proprietary mammalian cells was activated by other undetected chemicals, rather than the 12 PAHs tested (Wiltse et al., 2025). The assays for AhR inactivation could also be used as a complement for target analysis to indicate the risk of unknown aromatic compounds in the treated PW.

### 3.7. Endocrine disruption after exposure to PW

Some compounds in PW are classified as endocrine-disrupting compounds (EDCs), which can interfere with the endocrine system and potentially lead to adverse developmental, reproductive, neurological, and immune effects (He et al., 2010). Specifically, these EDCs interact with hormone receptors, mimicking hormone activity and altering the functions of the endocrine system to affect human and animal health adversely (Schilirò et al., 2011).

E-Screen assay uses a cell proliferation mechanism with estrogen receptors in MCF-7 cell lines to determine the estrogenic activity in the PW samples. Fig. 6 shows that the proliferative effect (PE) of 80 % feed PW was higher than that of the treated PW and even exceeded the response induced by the positive control (estradiol, E2). These results suggest that the 80 % feed PW contained estrogen analogs at concentrations sufficient to promote estrogen-mediated proliferation in MCF-7 cells. The 80 % distillate also showed a significant increase in cell proliferation ($p < 0.05$), indicating the presence of residual EDCs capable of activating estrogen receptors. Compared to the other effluents, the post-treated PW resulted in comparable or reduced cell proliferation relative to the control, suggesting the absence of compounds causing

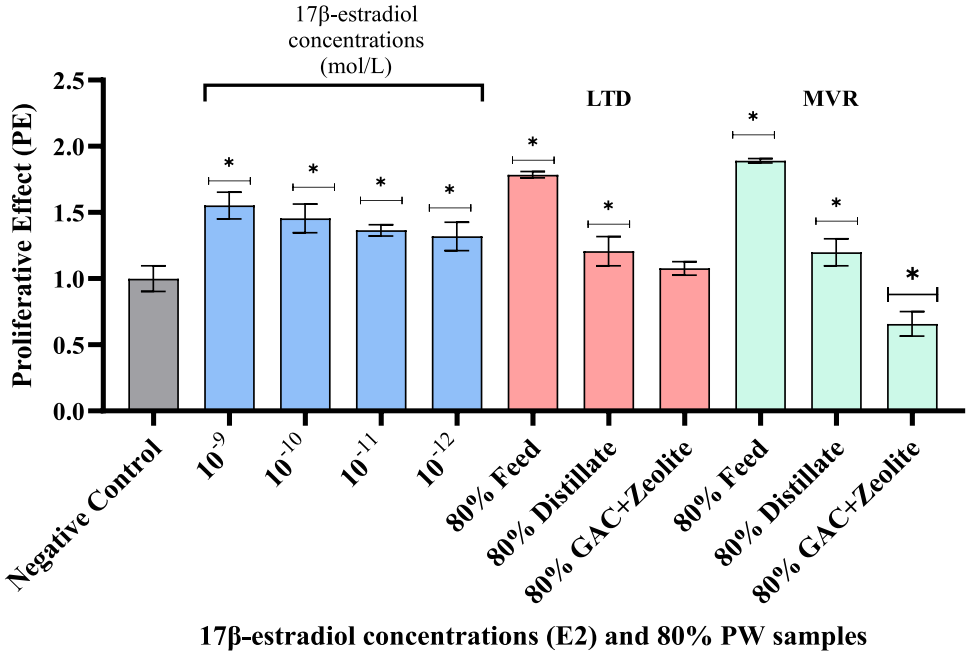


**Fig. 6.** The proliferative effect induced in MCF-7 cell lines by 17β-estradiol (E2), 50 % and 80 % feed, distillate, and GAC+zeolite treated PW. The * represents the statistical difference (P_value < 0.05) compared with the negative control.

estrogenicity in post-treated PW. While various PW constituents may have influenced the observed outcomes, the estrogenic activity detected in the feed and distillate is likely linked to organic compounds identified in the samples, including TPH, BTEX, and several PAHs (e.g., naphthalene, fluorene, phenanthrene, anthracene), all of which are known to interfere with sex hormone regulation (Kuppusamy et al., 2020). Estrogenic activity could also be induced by exposure to heavy metals. Choe et al. (2003) reported that exposure to antimony chloride, cadmium chloride, lithium hydroxide, barium chloride, and chromium chloride induced high endocrine-disrupting activity at concentrations of 1 μM. Specifically, this corresponded to 0.121 mg/L for antimony (Sb), 0.00694 mg/L for lithium (Li), 0.137 mg/L for barium (Ba), and 0.052 mg/L for chromium (Cr) (Choe et al., 2003). In our study, the feed PW from the LTD and MVR systems contained lithium at concentrations of 30.8 and 25.9 mg/L, and barium at 5.9 and 31.0 mg/L, respectively. These values exceeded the concentrations reported by Choe et al. (2003) to cause endocrine-disrupting activity, which may have contributed to the elevated estrogenic responses observed. Following thermal distillation and post-treatment, heavy metal concentrations were reduced to levels well below those known to induce estrogenic activity.

Several *in vivo* studies also show the endocrine-disrupting effect of untreated PW. After exposure of water flea *D. magna* to flowback and PW, 0.04 % PW resulted in decreased reproduction, delayed first brood, and changed gene expression in xenobiotic metabolism and moulting (Blewett et al., 2017). Rainbow Trout (*Oncorhynchus mykiss*) was also used as a model organism to test the impact of PW on it. It was found that exposure to PW resulted in elevated gene expression in biotransformation, oxidative stress, and endocrine disruption (He et al., 2017b).

Ammonia and VOCs have been identified as major contaminants in thermal distillate from the conventional thermal process (Tarazona et al., 2024a). Membrane distillation with hydrophobic membranes can be operated at lower temperatures, which allows water vapor to pass through while blocking liquid water and dissolved salts. However, ammonia and VOCs can still partially pass the membrane and enter the permeate (Chen et al., 2023; Delanka-Pedige et al., 2024). In addition to integration with other technologies (e.g., activated carbon adsorption and RO filtration) for pretreatment or post-treatment, functional MD membranes targeted for VOC removal were developed by integrating a dense, selective layer, catalytic properties, and electrochemistry with the hydrophobic membrane (Zhang et al., 2024).

## 4. Conclusion

This study evaluated the performance of two thermal pilot systems in reducing the cytotoxicity of PW from the Permian Basin. Exposure to feed PW significantly reduced cell viability and induced cell inflammatory responses. No significant effects on cell viability, apoptosis, or oxidative stress were observed in any of the three cell lines exposed to the thermal distillate. However, residual PAHs present in the distillate caused the activation of the AhR. The expression of CYP1A1 and CYP1B1 was not altered following exposure to post-treated PW, suggesting GAC+zeolite post-treatment effectively removed AhR-activating compounds. The E-Screen assay showed that estrogenic activity was induced in 80 % feed PW and 80 % distillate samples, indicating the presence of potent estrogenic compounds. No increased estrogenicity was observed after post-treatment, suggesting a substantial reduction in estrogenic compounds. In summary, thermally distilled PW, further polished with GAC and zeolite, did not elicit adverse cellular responses related to cell viability, membrane integrity, oxidative stress, apoptosis, or endocrine activity. These findings suggest that, with appropriate treatment, PW can be suitable for safe discharge or beneficial reuse. Further development of thermal distillation and post-treatment technologies is warranted to mitigate potential VOCs carryover and ensure consistent product water quality.

## CRediT authorship contribution statement

**Mike Hightower:** Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Huiyao Wang:** Writing – review & editing, Investigation, Funding acquisition, Formal analysis. **Pei Xu:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Yanyan Zhang:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Senuri Wijekoon:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Yeinner Tarazona:** Writing – review & editing, Data curation.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Pei Xu, Yanyan Zhang, and Huiyao Wang report financial support was provided by US Bureau of Reclamation. Yanyan Zhang, Pei Xu, Huiyao Wang and Mike Hightower report financial support was provided by New Mexico Produced Water Research Consortium. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgment

This work was financially supported by the U.S. Bureau of Reclamation [Agreement No. R22AC00428] and the New Mexico Produced Water Research Consortium. The authors thank Circle Verde and Crystal Clearwater Resources for providing the water samples and operational data from the pilot-scale thermal distillation unit. The views presented in this manuscript are the personal views of the authors and do not reflect those of the New Mexico Environment Department, the Bureau of Reclamation, New Mexico State University, or the New Mexico Produced Water Research Consortium.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2025.118726.

## Data availability

Data will be made available on request.

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