Integrated perspective of adverse cellular effects of water-accommodated and other oil fractions using Cell-Sensitivity Distribution as a New Approach Methodology for risk analysis.

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Supplementary material

Supplementary table 1: Data selected from published works involving hydrocarbon effects on cell culture.

Cell line/ type of primary cell culture	Species	Contaminant	Effect	LOEC (mg/L)	Reference
NCI-H358 bronchio-alveolar carcinoma cells	human	PAH	genotoxicity	0.0008	https://doi.org/10.1016/j.toxlet.2020.03.007
Hep3B hepatoblastoma cells	human	PAH	genotoxicity	0.002	https://doi.org/10.1016/j.toxlet.2020.03.007
Bewo placental carcinoma cells	human	PAH	viability	0.005	https://doi.org/10.1016/j.toxlet.2017.08.002
LS174T colorectal adenocarcinoma cells	human	PAH	genotoxicity	0.006	https://doi.org/10.1016/j.toxlet.2020.03.007
MDA-MB-231 breast cancer cells	human	WAF	clonogenicity	0.01	present study

JEG-3 choriocarcinoma cells	human	PAH	viability	0.04	https://doi.org/10.1016/j.toxlet.2017.08.002
MCF-10A mammary epithelial cells	human	WAF	clonogenicity	0.07	present study
BEAS-2B bronchial epithelial cells	human	PAH	apoptosis	0.1	https://doi.org/10.1016/j.ecoenv.2023.115308
Fibroblasts	whale	WAF	genotoxicity	0.1	https://doi.org/10.1016/j.cbpc.2017.10.009
Coelomocytes	earthworm	PAH	viability	0.1	https://doi.org/10.1007/s11356-020-10049-y
Jurkat T lymphocytes	human	WAF	cytotoxicity	0.12	https://doi.org/10.1016/j.ecoenv.2011.08.012
Hemocytes	mussel	WAF	viability	0.125	https://doi.org/10.1016/j.scitotenv.2019.03.187
MCF-10A mammary epithelial cells	human	WAF	cytotoxicity	0.15	present study
HepG2 hepatocellular carcinoma cells	human	WAF	proliferation	0.18	https://doi.org/10.1016/j.ecoenv.2011.08.012
HepG2 hepatocellular carcinoma cells	human	PAH	growth	0.3	https://doi.org/10.1016/j.tiv.2005.08.005
WRB K3 bronchial epithelial cells	rat	PAH	clonogenicity	0.5	https://doi.org/10.1016/0378-4274(94)90011-6
NL-20 bronchial epithelial cells	human	PAH	genotoxicity	0.55	https://doi.org/10.1016/j.tiv.2019.104645
NL-20 bronchial epithelial cells	human	PAH	apoptosis	0.55	https://doi.org/10.1016/j.tiv.2019.104645
ES-D3 embryonic multipotent stem cells	mouse	PAH	differentiation	1	https://doi.org/10.1016/j.toxlet.2019.08.001
HBEC bronchial epithelial cells	human	PAH	genotoxicity	1	https://doi.org/10.1016/j.envpol.2018.12.092
MCF-10A mammary epithelial cells	human	PAH	clonogenicity	1.56	present study
Fibroblasts	whale	WAF	genotoxicity	2	https://doi.org/10.1016/j.cbpc.2017.10.009

BEAS-2B bronchial epithelial cells	human	PAH	cytotoxicity	2	https://doi.org/10.1016/j.atmosenv.2014.04.003
BEAS-2B bronchial epithelial cells	human	PAH	secretion	2	https://doi.org/10.1016/j.atmosenv.2014.04.003
Fibroblasts	human	BTEX	cytotoxicity	2.1	https://doi.org/10.1016/S0167-4889(02)00271-9
Skin fibroblasts	whale	PAH	clonogenicity	2.5	https://doi.org/10.1016/j.marenvres.2006.04.016
Testis fibroblasts	whale	PAH	clonogenicity	2.5	https://doi.org/10.1016/j.marenvres.2006.04.016
Lung fibroblasts	whale	PAH	clonogenicity	2.5	https://doi.org/10.1016/j.marenvres.2006.04.016
M3E3/C3 lung epithelial cells	hamster	PAH	clonogenicity	2.5	https://doi.org/10.1016/0378-4274(94)90011-6
TK6 human lymphoblasts	human	PAH	viability	2.56	https://doi.org/10.1093/toxsci/kfs012
A549 lung adenocarcinoma cells	human	BTEX	cytotoxicity	3.1	https://doi.org/10.1016/S0167-4889(02)00271-9
Fibroblasts	whale	WAF	clonogenicity	3.9	https://doi.org/10.1016/j.cbpc.2017.10.009
HepG2 hepatocellular carcinoma cells	human	BTEX	cytotoxicity	4.1	https://doi.org/10.1016/S0167-4889(02)00271-9
MCF-10A mammary epithelial cells	human	PAH	cytotoxicity	6.25	present study
HepG2 hepatocellular carcinoma cells	human	PAH	cytotoxicity	30	https://doi.org/10.1016/j.tiv.2005.08.005
Hep3B hepatoblastoma cells	human	PAH	clonogenicity	30	https://doi.org/10.1016/j.toxlet.2020.03.007
Alveolar macrophages	rabbit	Alkane	viability	54	https://doi.org/10.1007/BF00316205
Alveolar macrophages	rat	BTEX	viability	85	https://doi.org/10.1007/BF00316205
Alveolar macrophages	rabbit	BTEX	viability	85	https://doi.org/10.1007/BF00316205
Alveolar macrophages	rat	Alkane	viability	107	https://doi.org/10.1007/BF00316205

A549 lung adenocarcinoma cells	human	BTEX	cytotoxicity	500	https://doi.org/10.1016/j.tiv.2022.105409
LL24 lung fibroblasts	human	BTEX	viability	2125	https://doi.org/10.1016/j.chemosphere.2015.01.058
A549 lung adenocarcinoma cells	human	BTEX	viability	4250	https://doi.org/10.1016/j.chemosphere.2015.01.058
RTgill-W1 gill epithelial cells	rainbow trout	PAH	cytotoxicity	6400	https://doi.org/10.1016/S0300-483X(98)00031-6
HBEC bronchial epithelial cells	human	PAH	cytotoxicity	10000	https://doi.org/10.1016/j.envpol.2018.12.092

LOEC: Lowest observed effect concentrations associated with each biological endpoint indicated as “Effect” in the previous column, calculated as mg/L from the respective reported units; Oil fractions: WAF: water-accommodated fraction of oil; PAH: polycyclic aromatic hydrocarbons; BTEX: monocyclic aromatic hydrocarbons benzene, toluene, ethylbenzene, and xylene.

Supplementary table 2: Published data on water and biota PAH concentrations used to estimate EBWR .

BIOTA (µg/Kg)	WATER (µg/L)	EBWR (L/Kg)	log (EBWR)	References
50,51	0,09091	555,604444	2,744765711	https://doi.org/10.1016/j.marpolbul.2024.117343
56,86	0,1315	432,3954373	2,635881103	https://doi.org/10.1016/j.marpolbul.2024.117343
2040	0,09091	22439,7756	4,35101851	https://doi.org/10.1016/j.marpolbul.2024.117343
3390	0,09091	37289,6271	4,57158804	https://doi.org/10.1016/j.marpolbul.2024.117343
1390	0,09091	15289,8471	4,184403142	https://doi.org/10.1016/j.marpolbul.2024.117343
718	0,09091	7897,921021	3,897512786	https://doi.org/10.1016/j.marpolbul.2024.117343
1648,6	0,0764	21578,53403	4,334021937	https://doi.org/10.1007/s11356-023-29365-0
3,6	0,0136	264,7058824	2,422763592	https://doi.org/10.1007/s11356-023-29365-0
1984	0,0712	27865,16854	4,445061674	https://doi.org/10.1016/j.marpolbul.2025.118381
5,4	0,0204	264,7058824	2,422763592	https://doi.org/10.1016/j.marpolbul.2025.118381
5,2	0,006	866,6666667	2,937852093	https://doi.org/10.1016/j.scitotenv.2024.173043
6	0,0074	810,8108108	2,908919531	https://doi.org/10.1016/j.scitotenv.2024.173043
6,2	0,0078	794,8717949	2,900297087	https://doi.org/10.1016/j.scitotenv.2024.173043
10,5	0,0068	1544,117647	3,188680386	https://doi.org/10.1016/j.scitotenv.2024.173043
119,5	0,0258	4631,782946	3,665748199	https://doi.org/10.1016/j.scitotenv.2024.173043
23	0,016	1437,5	3,157607853	https://doi.org/10.1016/j.scitotenv.2024.173043
124,6	0,0258	4829,457364	3,683898336	https://doi.org/10.1016/j.scitotenv.2024.173043
23,6	0,0174	1356,321839	3,132362755	https://doi.org/10.1016/j.scitotenv.2024.173043
61,2	0,0145	4220,689655	3,62538342	https://doi.org/10.1016/j.scitotenv.2024.173043
19,6	0,0148	1324,324324	3,121994356	https://doi.org/10.1016/j.scitotenv.2024.173043
39	0,0162	2407,407407	3,381549592	https://doi.org/10.1016/j.scitotenv.2024.173043

13	0,0116	1120,689655	3,049485363	https://doi.org/10.1016/j.scitotenv.2024.173043
48	0,0102	4705,882353	3,672641066	https://doi.org/10.1016/j.scitotenv.2024.173043
23	0,0094	2446,808511	3,388599982	https://doi.org/10.1016/j.scitotenv.2024.173043
21,6	0,0215	1004,651163	3,002015291	https://doi.org/10.1016/j.scitotenv.2024.173043
12	0,0088	1363,636364	3,134698574	https://doi.org/10.1016/j.scitotenv.2024.173043
21,6	0,009	2400	3,380211242	https://doi.org/10.1016/j.scitotenv.2024.173043
9	0,0087	1034,482759	3,014723257	https://doi.org/10.1016/j.scitotenv.2024.173043
8	0,012	666,6666667	2,823908741	https://doi.org/10.1016/j.scitotenv.2024.173043
7,5	0,0118	635,5932203	2,803179256	https://doi.org/10.1016/j.scitotenv.2024.173043
10	0,014	714,2857143	2,853871964	https://doi.org/10.1016/j.scitotenv.2024.173043
10,5	0,0108	972,2222222	2,987765544	https://doi.org/10.1016/j.scitotenv.2024.173043
11	0,0185	594,5945946	2,774220957	https://doi.org/10.1016/j.scitotenv.2024.173043
230	0,001	230000	5,361727836	https://doi.org/10.3389/fmars.2023.1085858
133,66	0,05	2673,2	3,427031452	https://doi.org/10.3389/fmars.2023.1085858
178,31	0,113	1577,964602	3,198097257	https://doi.org/10.3389/fmars.2023.1085858
176,65	0,041	4308,536585	3,634329785	https://doi.org/10.3389/fmars.2023.1085858
205	0,009	22777,77778	4,357511352	https://doi.org/10.3389/fmars.2023.1085858
162,63	0,074	2197,702703	3,341968942	https://doi.org/10.3389/fmars.2023.1085858

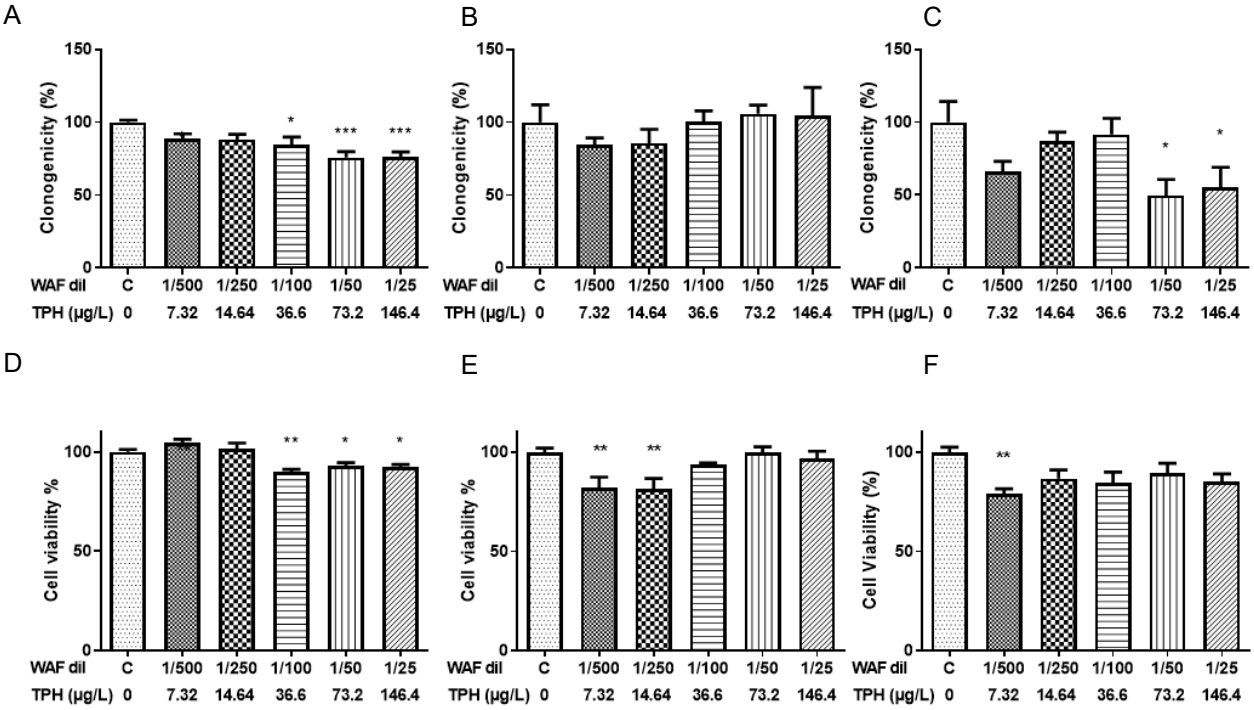
EBWR: Environmental Biota/Water Ratios . The descriptive parameters for the log EBWR distribution were: mean log EBWR =3,328558323 ; SD mean = 0,6156695708 ; all values expressed in L/kg, for water-biota

Supplementary table 3: Individual PAH profiles recorded in ambient water samples collected from previously published data.

	Norway Sea (ref. x)			(ref. y)	Mexico Gulf (ref. z)**			(ref. w)
PAH (rings)/ Frequency	W discharge	seawater	mussels	Bay W	W(2010)	(2011)	Terminos Lagoon***	[4]TPH fractions
1 ring								(+)
Naphtalene (2)	22,1	0	1,4					(+++)
Methyl-Naphtalenes (2)	70,4 (92.5)*	54,1 (54.1)*	26.0 (27.4)*	2,4				
Phenanthrene (3)	1,6	2,3	4,3				+++	(++)
Methyl-Phenanthrenes (3)	3,8 (5.4)*	24,8 (27.1)*	54,1 (58.4)*	4,3				
Methyl-Dibenzothiophenes (3)		11,9	6,3					
Anthracene (3)								
Fluorene (3)		2,1		7,6				
Fluoranthene (4)		1,6		8,6			+	
Benzo-a-Anthracene (4)				5,7	+			
Chrysene (4)		1,4		25,7	+			
Pyrene (4)				24,7			++	
Benzo-b-Fluoranthene (5)					+	+		
Indeno-123cd-Pyrene (6)		0		6,7	+			

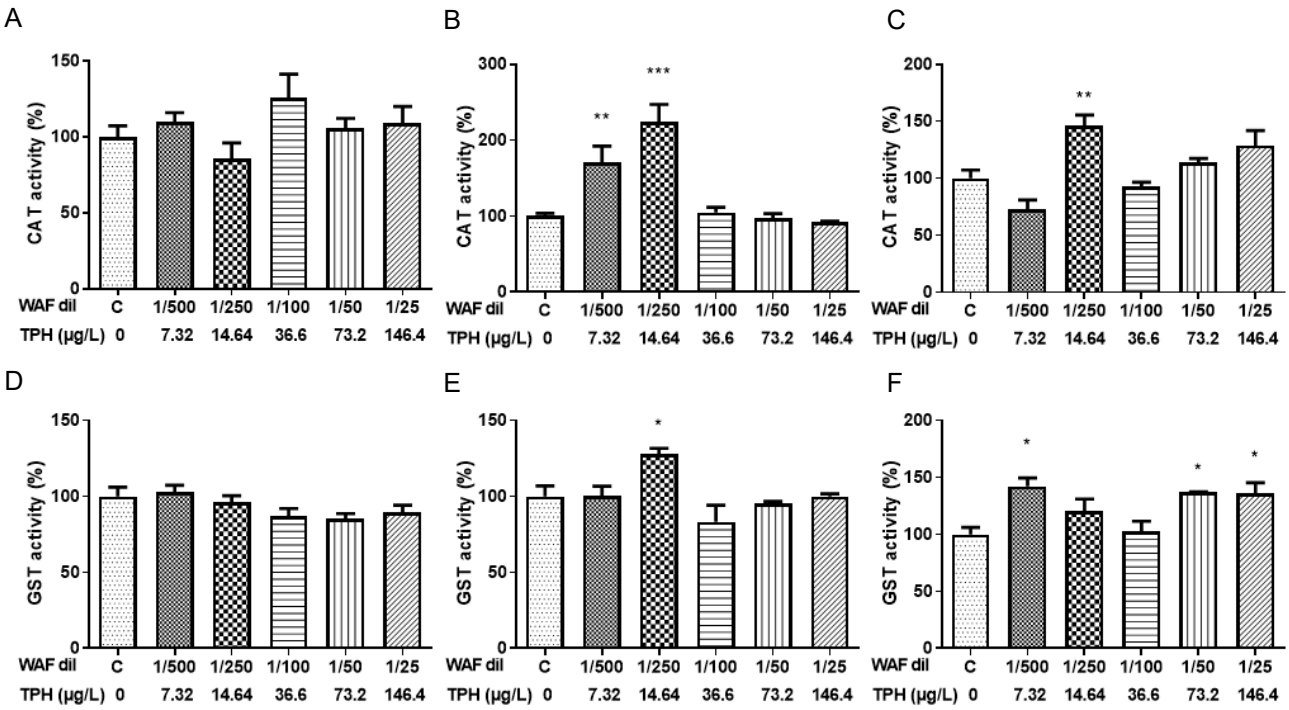
Ref x: https://doi.org/10.1016/j.scitotenv.2024.173043 , Ref y: https://doi.org/10.1007/s00244-018-0538-6 , Ref z: https://doi.org/10.1016/j.marpolbul.2024.117343, Ref w: https://doi.org/10.1016/j.jhazmat.2010.03.124 * Total percentage of the primary PAH, ** Main PAH detected, *** PAH in increasing order, 4 Main aromatic fractions found in TPH in water samples

Supplementary Figure 1



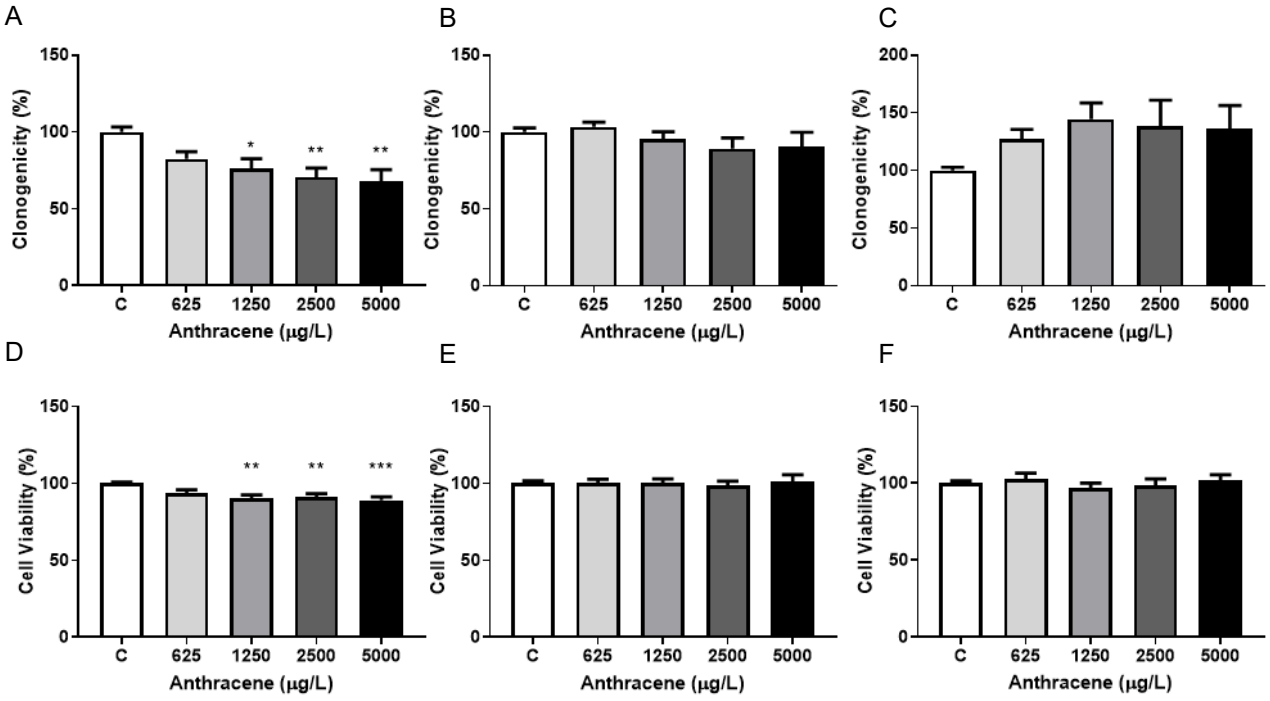
Supplementary Figure 1. Effects of WAF on clonogenic capability and cellular viability. MCF-10A (A and D), MCF-7 (B and E) and MDA-MB-231 (C and F) cells were treated with WAF dilutions (WAF dil) : 0, 1/500, 1/250, 1/100, 1/50, 1/25 corresponding to total petroleum hydrocarbon concentrations (TPH) of 7.32, 14.64, 36.6, 73.2 and 146.4 µg/L, respectively) for 7 days and the number of colonies with more than 50 cells was assessed to evaluate clonogenicity, or for 72 h followed by MTT assay to study cell viability. *p<0.05, **p<0.01, ***p<0.001. One-way ANOVA and Dunnet's post hoc test. Results are expressed as a percentage of control. n= 3 independent experiments.

Supplementary Figure 2



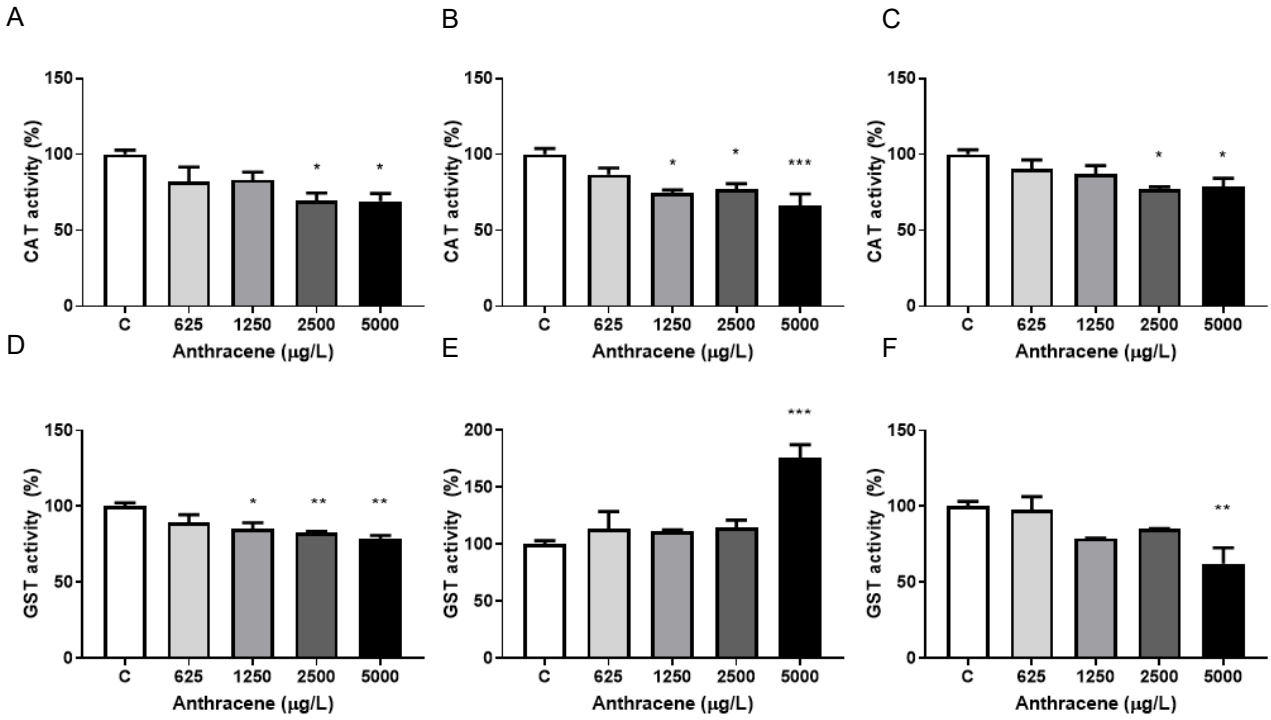
Supplementary Figure 2. Effects of WAF on catalase (CAT) and glutathione S-transferase (GST) activity. MCF-10A (A and D), MCF-7 (B and E) and MDA-MB-231 (C and F) cells were treated for 72h with WAF dilutions (WAF dil): 0, 1/500, 1/250, 1/100, 1/50, 1/25 corresponding to total petroleum hydrocarbon concentrations (TPH) of 7.32, 14.64, 36.6, 73.2 and 146.4 µg/L, respectively, and CAT (A, B, C) or GST (D, E, F) activity was evaluated. *p<0.05, **p<0.01, ***p<0.001. One-way ANOVA and Dunnet's post hoc test. Results are expressed as a percentage of control. n= 3 independent experiments.

Supplementary Figure 3



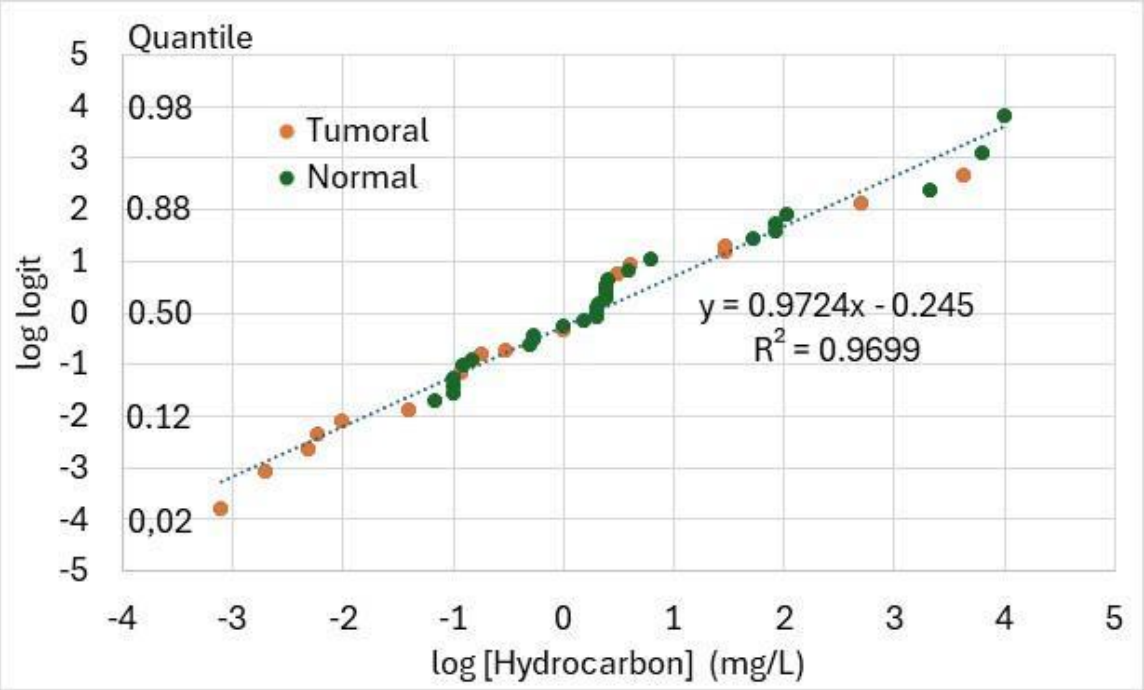
Supplementary Figure 3. Effects of anthracene (ANT) on clonogenic capability and cellular viability. MCF-10A (A and D), MCF-7 (B and E) and MDA-MB-231 (C and F) cells were treated with ANT (0, 625, 1250, 2500, 5000 µg/L) for 7 days and the number of colonies with more than 50 cells was assessed to evaluate clonogenicity, or for 72 h and then MTT assay was performed to study cell viability. *p<0.05, **p<0.01, ***p<0.001. One-way ANOVA and Dunnet's post hoc test. Results are expressed as a percentage of control. n= 3 independent experiments.

Supplementary Figure 4



Supplementary Figure 4. Effects of ANT on catalase (CAT) and glutathione S-transferase (GST) activity. MCF-10A (A and D), MCF-7 (B and E) and MDA-MB-231 (C and F) cells were treated for 72h with ANT (0, 625, 1250, 2500, 5000 µg/L) and CAT (A, B, C) or GST (D, E, F) activity was evaluated. *p<0.05, **p<0.01, ***p<0.001. One-way ANOVA and Dunnet's post hoc test. Results are expressed as a percentage of control. n= 3 independent experiments.

Supplementary Figure 5



Supplementary Figure 5. Comparison of tumorigenic vs. non-tumorigenic cells in the integrated CSD. The collected data was grouped according to the actions on tumorigenic or non-tumorigenic cells. Quantile values corresponding to log logit scale are shown.