

SUPPLEMENTARY MATERIAL

Genotoxicity and endocrine disruption potential of haloacetic acids in human placental and lung cells.

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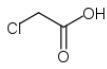
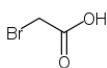
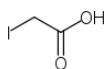
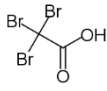
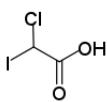
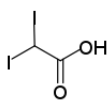
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Table S1. Main physical-chemical properties of the investigated haloacetic acids.

Acronym	Compound	Structure	CAS	Average mass (g/mol)	pKa ^a	log Kow ^b	Solubility (mol/L) ^b	Vapor pressure (mmHg) ^b
CAA	Chloroacetic acid		79-11-8	94.50	3.1	0.210 (0.220)	2.12 (8.51)	0.259 (0.065)
BAA	Bromoacetic acid		79-08-3	138.95	2.6	0.469 (0.410)	1.48 (12.6)	0.119 (0.118)
IAA	Iodoacetic acid		64-69-7	185.95	3.0	0.660	0.643	0.099
TBAA	Tribromoacetic acid		75-96-7	296.74	0.7	2.66	0.346 (0.674)	0.021
CIAA	Chloroiodoacetic acid		53715-09-6	220.39	2.3	1.10	1.15	0.002
DIAA	Diiodoacetic acid		598-89-0	311.85	2.3	2.21	0.172	0.015

^a ACE and JChem acidity and basicity calculator – www.chemicalize.com

^b CompTOx Chemicals Dashboard - Average predicted values (average experimental values) (Williams et al., 2017)

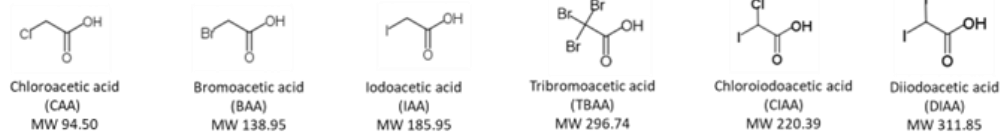
Table S2. Taqman gene expression assays for RT-PCR analysis and efficiency obtained from a pool of one replicate of each sample. The selected genes encode enzymes involved in steroidogenesis and are relatively highly expressed in JEG-3 placental cells.

Gene	Assay ID	Efficiency	Error	Slope	y intercept
<i>cyp19a1</i>	Hs00903413_m1	1.938	0.085	-3.479	36.9
<i>hsd3b1</i>	Hs00426435_m1	1.880	0.086	-3.647	38.9
<i>hsd17b1</i>	Hs00166219_g1	2.017	0.026	-3.283	36.4
<i>hsd17b7</i>	Hs04937189_g1	2.339	0.170	-2.709	37.0
<i>hsd17b12</i>	Hs00275054_m1	2.066	0.004	-3.173	34.4
<i>gadph</i>	Hs02786624_g1	2.016	0.003	-3.285	26.2

Table S3. Concentration of haloacetic acids that leads to a 50 % decrease in cell viability (EC_{50}) in JEG-3 cells (24 h of exposure), expressed in μM as mean \pm SD (n = 3).

	IAA	BAA	TBAA	CAA	DIAA	CIAA
AB	7.06 \pm 0.20	20.3 \pm 0.85	458 \pm 31.2	> 500	> 500	> 500
CFDA-AM	7.69 \pm 0.31	25.3 \pm 3.13	258 \pm 44.2	> 500	> 500	> 500

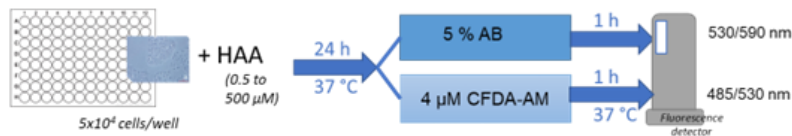
Molecular structures of HAAs tested



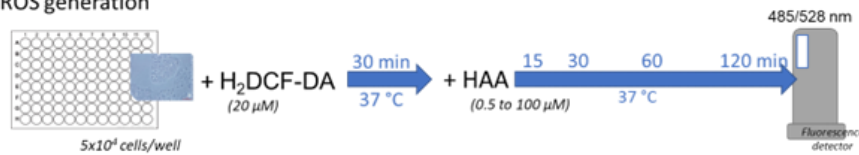
Experimental overview

Research objectives	Cell culture	Assays
Identify non-cytotoxic doses Dose-response information Endocrine disruption assessment	JEG-3 placental cells	Cell viability (AB and CFDA-AM dyes) ROS generation (H2DCF oxidized) P450 aromatase activity (³ H ₂ O released) Expression of steroidogenesis-involved genes
Genotoxicity assessment	A549 alveolar lung cells	Micronucleous test

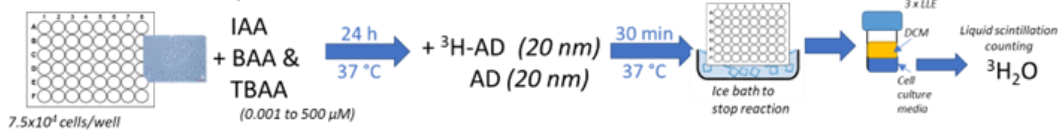
Cell viability



ROS generation



P450 aromatase activity



Expresion of steroidogenesis-involved genes



Micronucleus formation

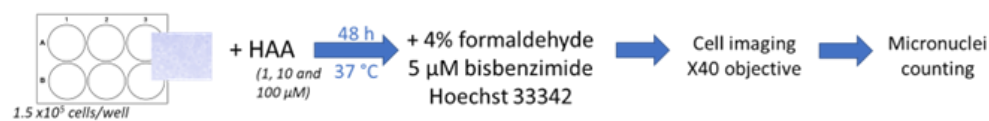


Figure S1. Experimental overview of the *in vitro* assays conducted.

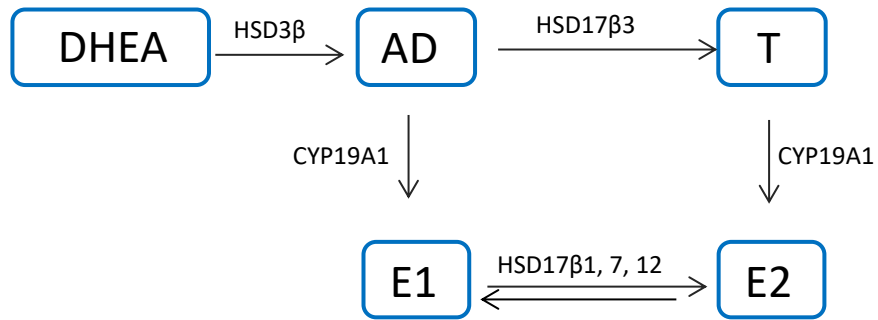


Figure S2. Summary of the steroid synthesis pathways investigated in JEG-3 placental cells (Karahoda et al., 2021; Samson et al., 2009).

References

- Karahoda, R., Kallol, S., Groessl, M., Ontsouka, E., Anderle, P., Fluck, C., Staud, F., & Albrecht, C. (2021). Revisiting Steroidogenic Pathways in the Human Placenta and Primary Human Trophoblast Cells. In *International Journal of Molecular Sciences* (Vol. 22, Issue 4). <https://doi.org/10.3390/ijms22041704>
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