The Toxic Cocktail: How Organochlorine Chemicals Disrupt Lipid Metabolism in Testicular Leydig Cells and Lead to Hormonal Disbalance

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Background & Study Aim

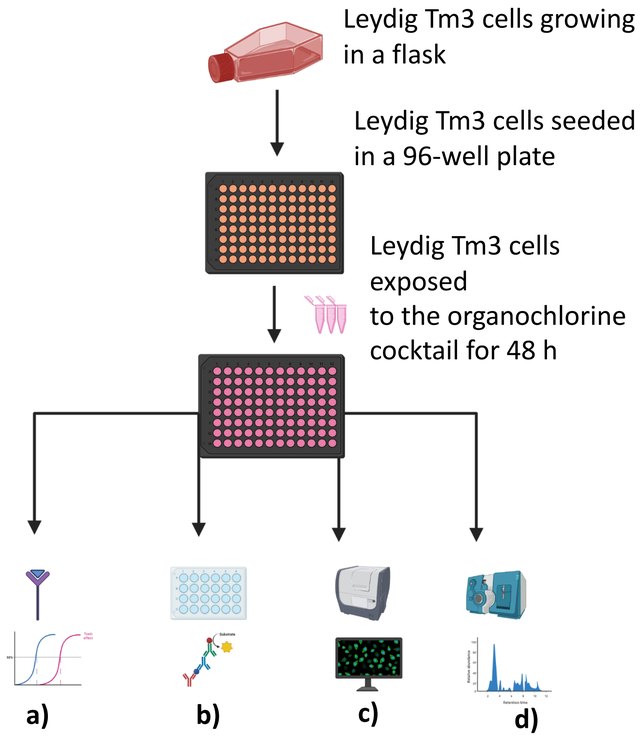
- **The infertility threat**: How exposure to real-life organochlorine chemical cocktails can contribute to male infertility **[1]**.
- **Fat-loving toxins:** Organochlorines' tendency to accumulate in fat tissues and impact male reproductive health **[2]**.
- **Arctic diet:** Organochlorines were found in the Inuit population residing in Greenland that consumes fat-rich animal tissues **[3]**.
- **Lipid imbalance:** Previous studies have shown that the organochlorine cocktail interferes with lipid homeostasis in males [4].
- **Lipid profiling:** Investigating the specific lipid species affected by the real-life organochlorine chemical cocktail in male reproductive cells.
- **Study Aim**: The study aimed to investigate the involved mechanisms behind the toxicity of organochlorine cocktail..

Organochlorine Cocktail

This mxture is selected from the previously conducted studies that showed soe link between exposure to this mixture and toxicity in leydig cells

Organochlorines	% Weight (w/v)
Polychlorinated biphenyls mixture	32.4
Technical chlordane	21.4
p, p- dichlorodiphenyldichloroethylene	19.3
p,p-dichlorodiphenylethane	6.8
Technical toxaphene	6.5
Aldrin	2.5
Dialdrin	2.5
1,2,3,4-Tetrachlorobenzene	0.9
Hexachlorobenzene	0.9
p,p- dichlorodiphenyldichloroethane	0.5
β-Hexachlorocyclohexane	0.4
Mirex	0.2
Lindane	0.2
Pentachlorobenzene	0.2

Methods



a) Receptor interaction assessment
b) Hormone measurement
c) Lipid accumulation
d) Lipid profiling

Results

a) The **organochlorine cocktail** of interest **interacted** strongly **with the androgen receptor (antagonist activity)**, weakly with the estrogen receptor.

b) The organochlorine cocktail of interest **caused hormonal disbalance** in Leydig TM3 cells

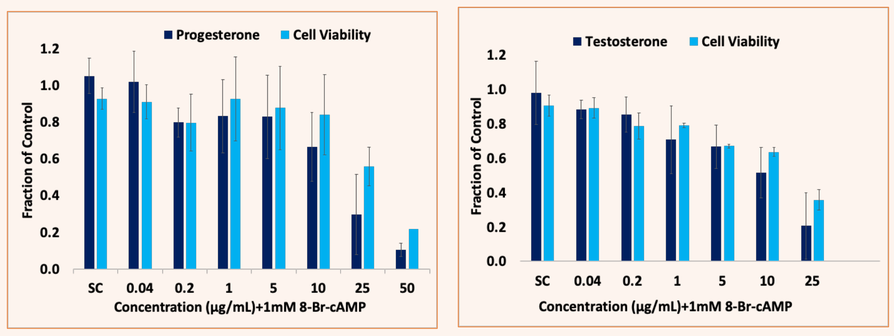


Figure 1: A concentration-dependent decline in progesterone and testosterone production by Leydig TM3 cells stimulated 8-Br-cAMP and exposed to the organochlorine cocktail for 48 h. 8-Br-cAMP, a membrane-permeable cAMP derivative; **SC**, solvent control (DMSO 0.1% v/v).

c) The organochlorine cocktail of interest **induced lipid accumulation** in Leydig TM3 cells leading to **lipotoxicity**

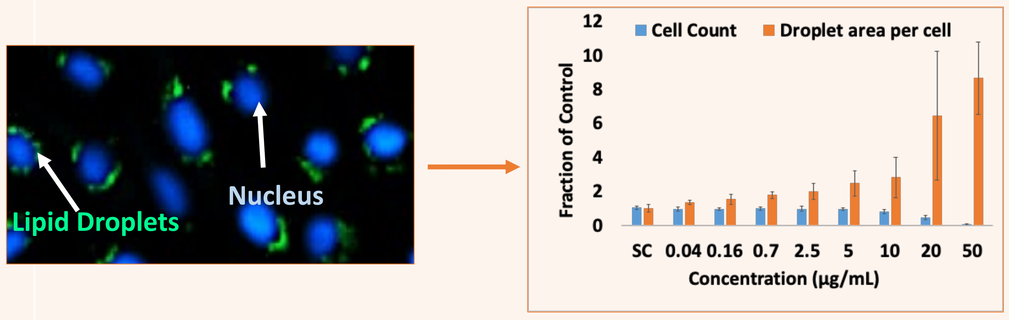


Figure 2: A concentration-dependent increase in lipid accumulation in Leydig TM3 cells exposed to the organochlorine mixture for 48 h accompanied by a decline in their number.

d) The organochlorine mixture of interest **affected lipid profiles** in Leydig TM3 cells differently compared to a lipotoxic drug, amiodarone (AMIOD), or fatty acid supplementation (OAPA).

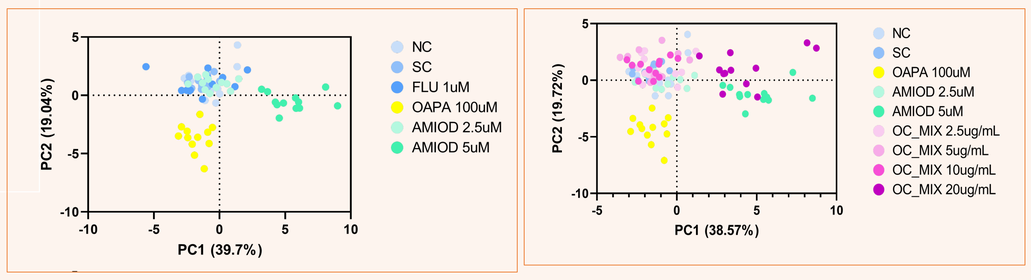


Figure 3: Principal component analysis (PCA).AMIOD, amiodarone (2.5 or 5 µM) – a lipotoxic drug; OC_MIX (2.5-20 µg/mL), organochlorine mixture of interest; FLU, flutamide (1 µM) – a prototypical antagonist of the androgen receptor; OAPA, a mixture of oleic and palmitic acids (2:1 ratio) (100 µM) – fatty acid supplementation.

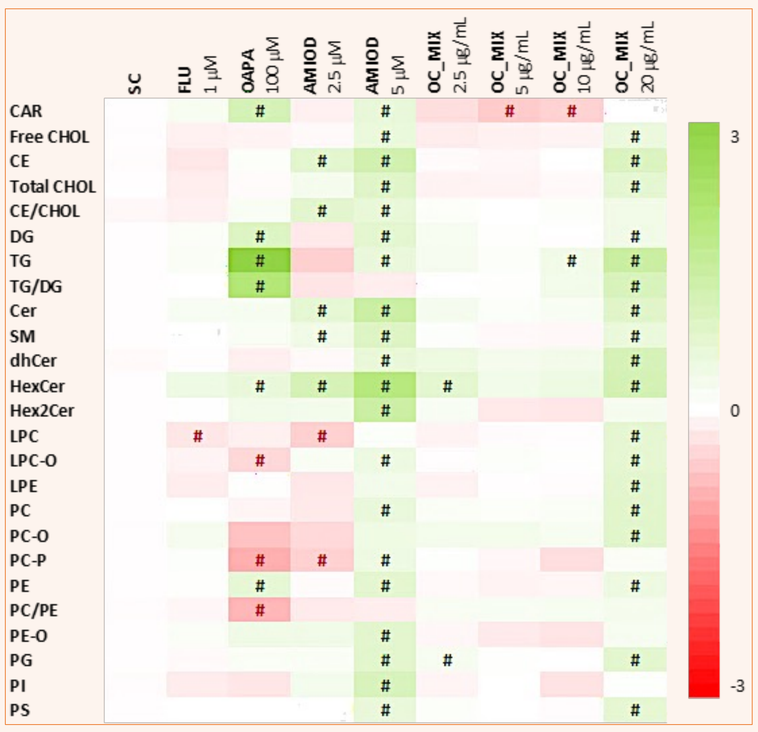
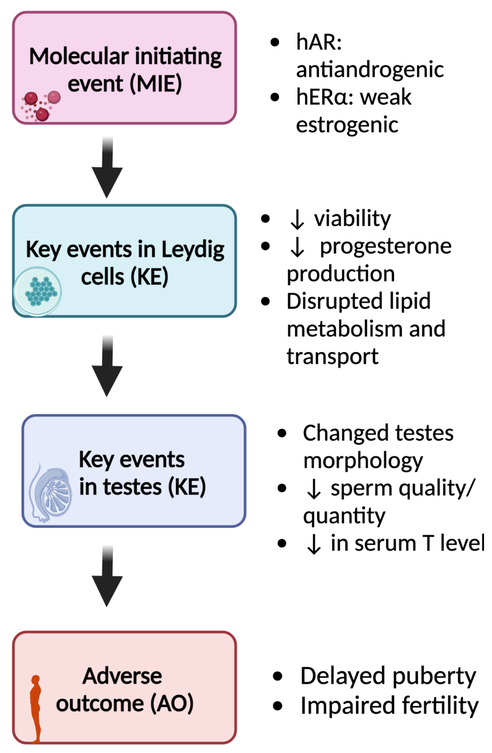


Figure 4: A heatmap representing the lipid profile in Leydig cells exposed to the mixture of interest. AMIOD, amiodarone (2.5 or 5 µM) – a lipotoxic drug; **OC_MIX** (2.5-20 µg/mL), organochlorine mixture of interest; **FLU,** flutamide (1 µM) – a prototypical antagonist of the androgen receptor; **OAPA**, a mixture of oleic and palmitic acids (2:1 ratio) (100 µM) – fatty acid supplementation.

- Almost all lipid classes were affected in the exposed cells to the organochlorine mixture including cholesterol.

Conclusions

- **The organochlorine chemical cocktail affected the functionality (hormone production, steroidogenesis) of immature Leydig Tm3 cells (Figure 1) by interfering with lipid metabolism leading to lipotoxicity (Figures 2-4).**
- **The organochlorine mixture affected differently lipid profile in Leydig TM3 cells compared to a lipotoxic drug, amiodarone, or fatty acid supplementation (OAPA) (Figures 3 & 4)**
- **Adverse outcome pathway integrating outcomes of our and previously published studies of the organochlorine mixture of interest:. It shows the AO in animal studies.**



- **The molecular initiating event is not clear, we excluded the role of the androgen receptor because a prototypical androgen receptor antagonist, flutamide, did not cause lipid accumulation and did not change significantly the lipid profile in Leydig TM3 cells (Figure 3).**

Recognized lipid classes: Carnitines (**CAR**), Cholesteryl ester (**CE**), Cholesterol (**CHOL**), Diglycerides (**DG**), Triglycerides **(TG**), Ceramides (**Cer**), Sphingomyelins (**SM**), Dihydroceramide (**dhCer**), Hexosylceramide (**HexCer**), Dihexosylceramides (**Hex2Cer**), Lysophosphatidylcholines (**LPC**), Alkyl ether-linkedlysophosphatidylcholines (**LPC-O**), Lysophosphatidylethanolamines (**LPE**), Phosphatidylcholines (**PC**), Alkyl ether-linked, Phosphatidylcholines (**PC-O**), Phosphatidylethanolamines **(PE**), Alkyl ether-linked, Phosphatidylethanolamines (**PE-O**), Phosphatidylgylcerols (**PG**), Phosphatidylinositols (**PI**), Phosphatidylserines (**PS**).

References: [1] Njembele (2014), Biol Reprod 118, 1; **[2]** Nebile (2010), Bull Environ Contam Toxicol 85, 97; **[3]** Muir (1992), Sci Total Environ 122, 75; [4] Enangue (2020), Int J Mol Sci 23, 3997.

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