Determining the Toxicity of Cosmetic Products Using Spermatozoa as a Biological Screening Model

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1. Introduction

- **Dibutyl phthalate** (**DBP**) is used in cosmetic products as a perfume solvent and fixative as well as plasticizer in nail polish and fingernail extensions.
- DBP is absorbed through the skin .
- Studies have investigated the toxicity of DBP and the chemical has been associated with birth defects, alteration of fatty acids and degeneration in liver cells as well as reproductive toxicity (Dominique, 2007).
- Exposure to DBP has been shown to induce oxidative stress (OS) (Zhou D. et al, 2010).
- When male albino rats were treated with oral doses of DBP for 15 consecutive days, OS was induced which resulted in a decrease in sperm endpoints such as motility and concentration (Aly H. A. et al, 2015).
- The American Chemistry Council declares that DBP is not harmful and safe to use ***but*** DBP is banned in Europe.
- Further research is needed to determine toxicity of DBP to human cells.
- Using spermatozoa as a biological screening model to predict effect on other human cells.

2. Rationale

Our rationale is to use spermatozoa as a biological screening model to determine the toxicity of cosmetic products that contain DBP.

Why Sperm?
- Cost effective
- Easily accessible
- Easy to measure endpoints
- Does not require animal testing

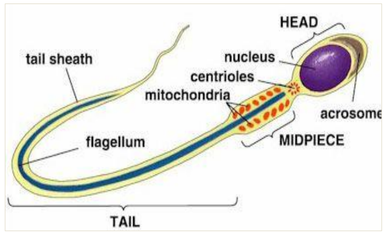

Figure 1 . Components of sperm cell with normal morphology

3. Hypothesis

We hypothesize that cosmetic products which contain DBP are toxic to sperm and will negatively affect sperm parameters.

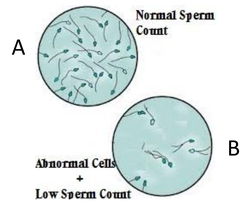

Figure 2. Hypothesized effect of DBP. A is a normal sperm sample with a high number of motile and morphologically normal spermatozoa. B is a sperm sample after the addition of DBP with a low sperm concentration and reduced motility as well a high number of abnormal spermatozoa.

4. Experimental Methods

Sperm samples will be collected from healthy donors. The macroscopic and the microscopic evaluation will be conducted. Sperm preparation by density gradient will be performed to obtain a highly motile and morphologically normal sperm fraction. All the sperm samples will then be pooled. The pooled sample will be divided into 5 vials. One vial will remain untouched (the control), 2 vials will be subjected to nail polish containing DBP and 2 vials will be exposed to DBP-free nail polish. Sperm motility, concentration, vitality, osmolarity, TUNEL and Lipid peroxidation (LPO) measurements will be taken for all the samples at 0hr, 3hr and 24hr of incubation time at 37 degrees.

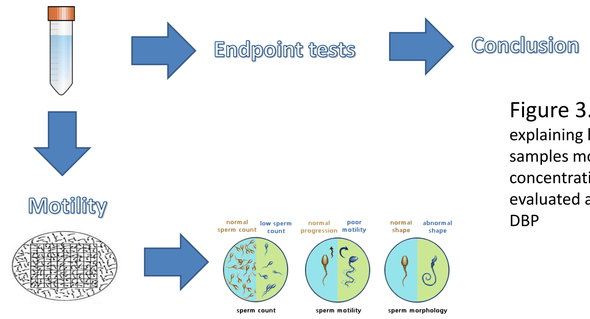

Figure 3. A diagram explaining how the sperm samples motility and concentration will be evaluated after exposure to DBP

5. Discussion

• If DBP reduces sperm motility, concentration and vitality, then DBP can also be implicated in reduced fertilization capacity of the sperm.

• Determining the toxicity of DBP to spermatozoa is just a model by which we can predict how DBP can affect other cells in the body. Spermatozoa are being used because they are a more feasible method of research. For example, if DBP causes LPO or DNA fragmentation in a sperm cell, the chemical can also cause LPO or DNA damage in a skin cell. In other words, the results of our experiment have wider implications than just establishing whether DBP harms sperm cells.

• The primary limitation of our study is that sperm cells are being used as a biological screening model and the chemical could act differently when interacting with different kinds of cells.

7. Bibliography

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6. Anticipated Results

DBP is associated with testicular toxicity (Zhou. D. et al, 2010). Exposing spermatozoa to DBP will result in lower sperm motility and concentration. This decrease will be associated with decreases in sperm membrane integrity (indicated through vitality measurements).

Figure 4. An example of a vitality reading as seen through a microscope. A is a normal health sperm while 8 is stained red thus indicating that its sperm membrane has been damaged.

The number of stained spermatozoa in the vitality reading will increase with more exposure to DBP.

Mechanisms by which DBP can impair sperm membrane function is:

• Lipid Peroxidation

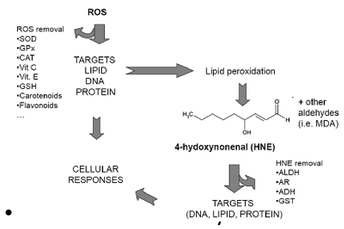

Figure 5. LPO is the result of a free radical chain reaction where reactive oxygen species steal electrons from lipids in the phospholipid bi layer membranes of cells. This results in cell membrane damage.

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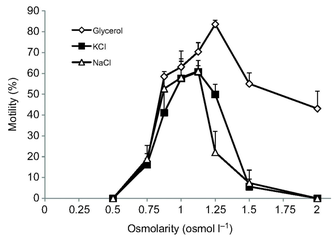

Figure 6. The graph shows a clear correlation between high osmolarity readings and reduced sperm motility. As the osmolarity increases, sperm motility increases until a threshold is surpassed at which point the cell uptakes too much water and bursts.

• DBP can further impair normal sperm function through DNA Fragmentation as measured using the TUNEL Assay.

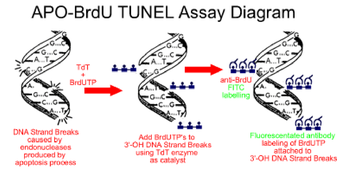

Figure 7. The TUNEL assay is a method to identify DNA damage that is a result of apoptotic signalling pathways. Terminal deoxynucleotidyl transferase is used to identify nicks in DNA strands and in turn the enzyme catalyses the addition of dUTP's which are labelled with a marker.