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Evaluation of BDE-47 and -99 lipid modulating effects in HepG2 human carcinoma cells

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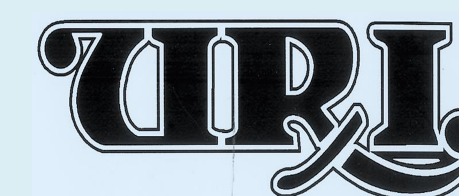


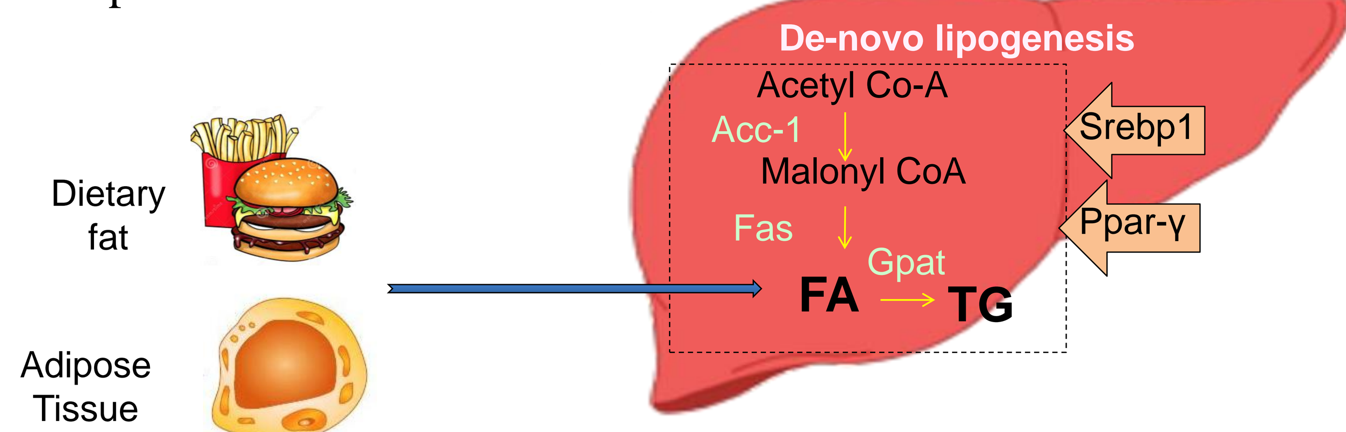
Background

Non-alcoholic fatty liver disease (NAFLD) is becoming a significantly more common problem in today's society, affecting up to 25% of people in the United States as reported by the American Liver Foundation. According to the American Association of the Study of Liver Diseases, NAFLD is the buildup of fat in the liver that is not caused by secondary factors such as alcohol consumption, hereditary disorders, or the use of steatogenic medication such as amioderone. A liver is considered fatty when 5-10% of the liver's weight is fat. The progression of NAFLD can lead to cirrhosis, liver cancer, or liver failure. Risk factors for NAFLD include obesity, type II diabetes mellitus, hypertriglyceridemia, hypercholesterolemia, age, gender, and ethnicity. In addition, there are examples of toxicant-induced liver disease in occupationally exposed workers, suggesting that the environment may also be a risk factor for the development of NAFLD. This study aims to determine whether direct exposure to environmental compounds cause fatty liver using cultured liver carcinoma cells.

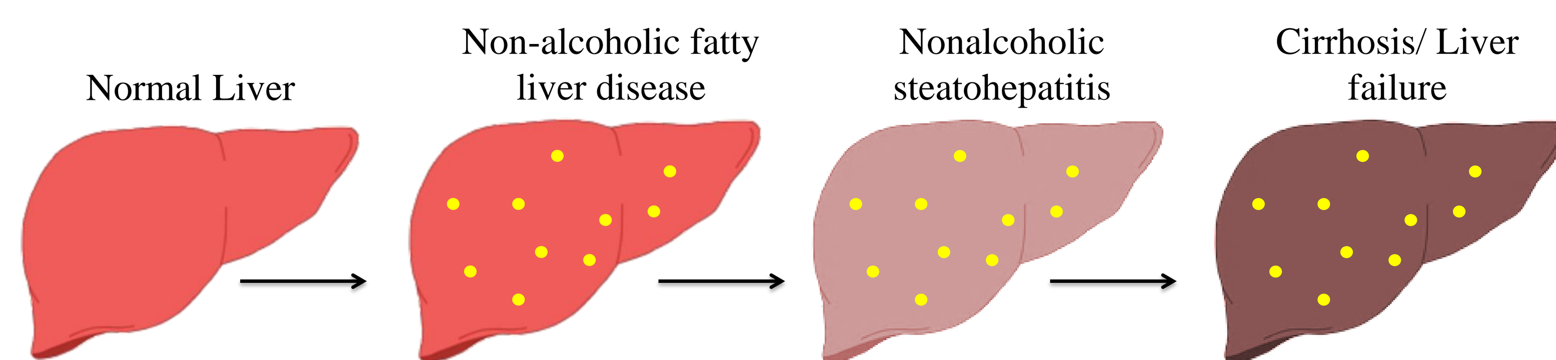
Introduction

BDE-47 (2,2′,4,4′-tetra-bromodiphenyl ether) is a brominated flame retardant used in a wide variety of consumer products such as polyurethane foam, which is used in furniture and car upholstery, packaging and electronic equipment . BDE-47 is released into the environment by manufacturers and by the products themselves and can be ingested or inhaled and then stored in the liver as lipids. The pentaBDE congener that is usually predominant in environmental media is BDE-99 (2,2′,4,4′,5-penta-bromodiphenyl ether). BDE-99 is a brominated flame retardant chemical and is released into the environment. PentaBDEs are thought to be distributed through the human body and found in adipose tissues, blood, liver, and maternal milk. My hypothesis is that BDE-47 and BDE-99 will increase the total lipid content in cultured HepG2 liver carcinoma cells.

Hepatic Lipid Homeostasis



Hepatic Steatosis Progression



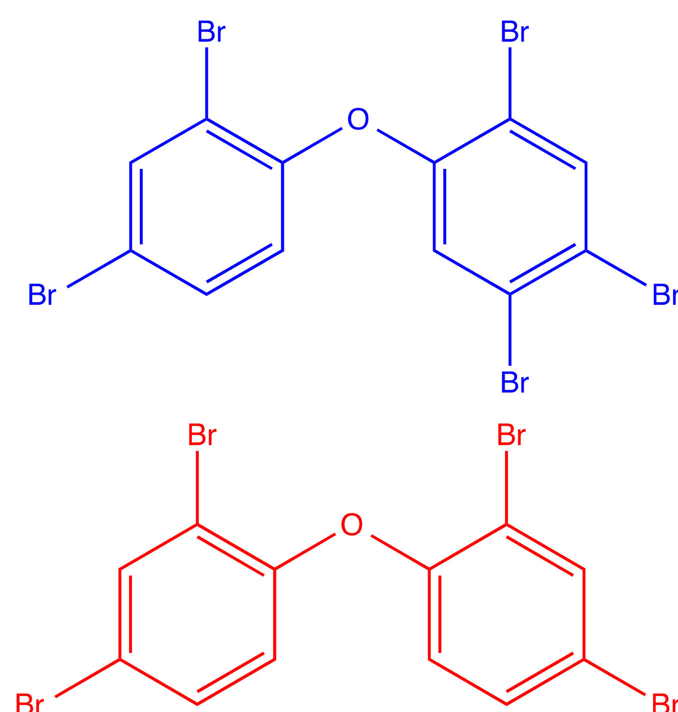
Pentabromodiphenyl Ethers

BDE-99 (2,2′,4,4′,5-penta-bromodiphenyl ether)

- Abundance: 45-49%

BDE-47 (2,2′,4,4′-tetra-bromodiphenyl ether)

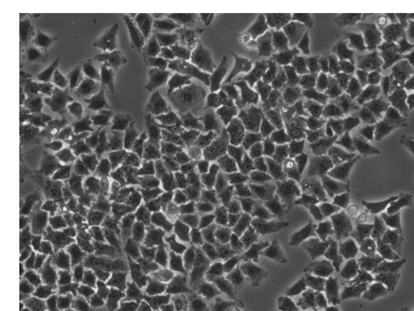
- Abundance: 38-42%



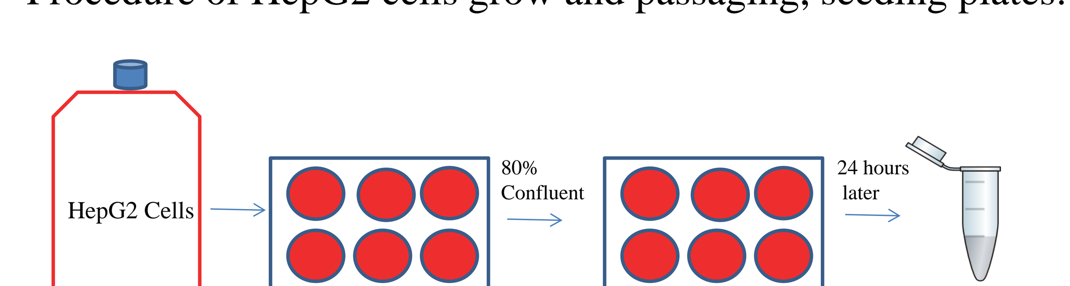
Methods and Materials

HepG2 Cells:

HepG2 cells are a human liver carcinoma cell line derived from a 15-year old Caucasian male . Hepg2 cells are a good in vitro model system for this study because they have morphological and functional differentiation which accurately represents human liver cells.

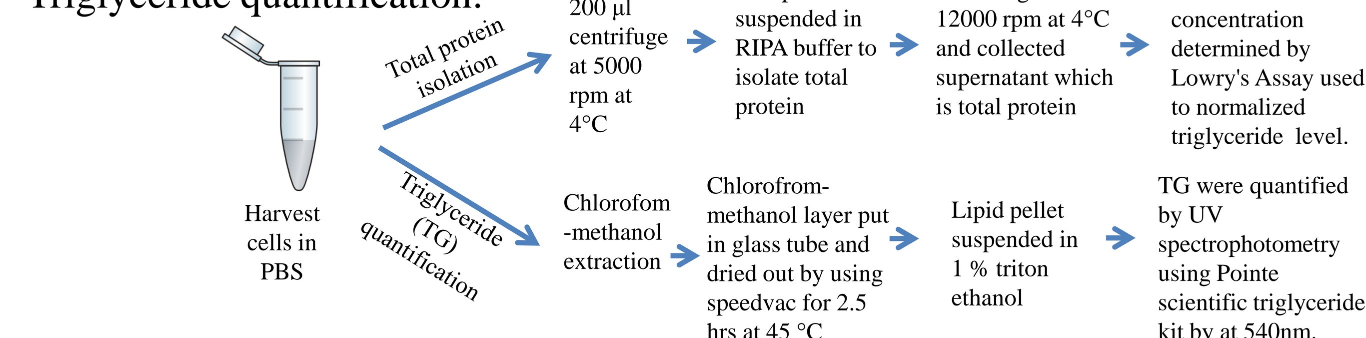


Procedure of HepG2 cells grow and passaging, seeding plates:



Compounds	Final concentration	Working stock (1000X)	Sstock solution /ml of media
DMSO	0.1%	100%	1ul
Oleic Acid	750 um	3 mM	250 ul
BDE 47	0.1um (100nm)	100 um	1ul
	1 um	1 mM	1ul
	5 um	5 mM	1ul
	25 um	25mM	1ul
BDE 99	0.1um (100nm)	100 um	1ul
	1 um	1 mM	1ul
	5 um	5 mM	1ul
	20 um	20mM	1ul

Triglyceride quantification:



Oil Red O (ORO):

After 24hrs of BDE treatment , cells were fixed with 10% formalin and stained with Oil Red O working solution made in isopropranalol. After washing with water, cell plates were observed under a phase contrast microscope to aquire images. For ORO quantification ORO was extracted by using 100% isopropanol and measured absorbance by UV spectrophotometry at 500nm.

Gene Expression:

After treatment with BDEs, RNA was extracted from the cells using Trizol Reagent. mRNA was reverse transcribed to cDNA and the relative abundance of transcripts related to lipid transport and synthesis was measured via qPCR. The PCR primers were optimized for measurement of multiple genes involved in lipid homeostasis.

Data/Results

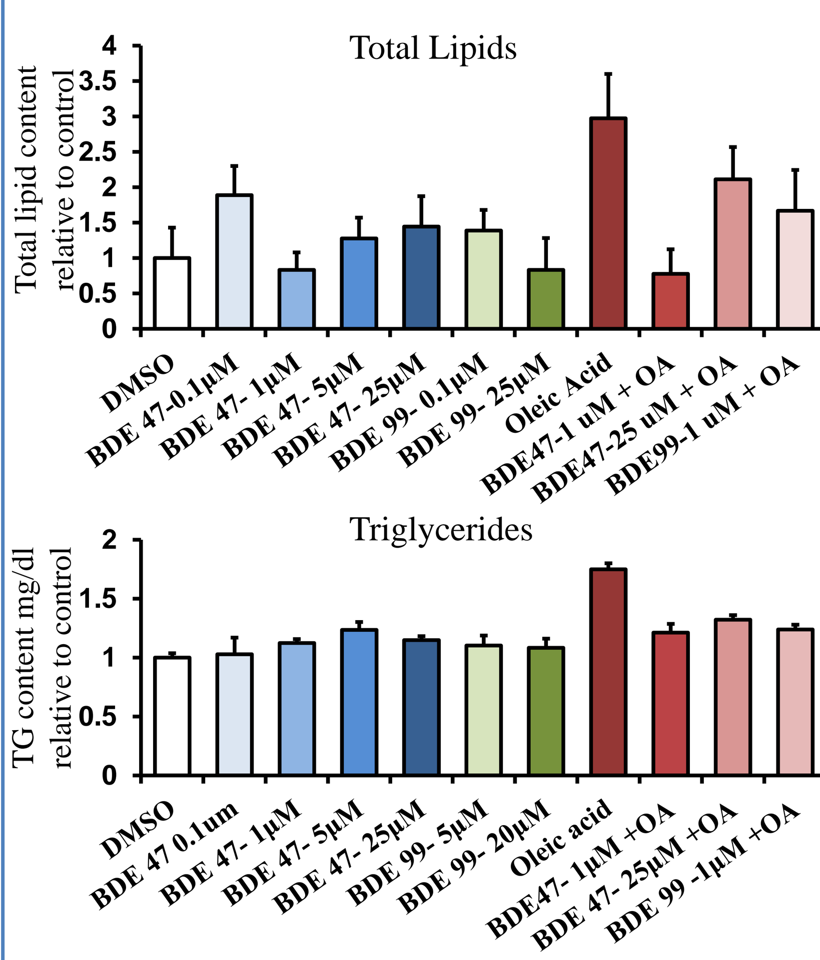


Figure 1: A. Total lipid accumulation in HepG2 cells was measured after treatment of vehicle or different doses of BDE-47 and 99 along with oleic acid. After 24hrs of treatment total lipids were extracted and dried in a glass tube using a speedvac. The weight of the lipid pellet was noted. Total lipid content is represented as normalized to control group and one-way ANOVA followed by Dunnett's test were applied to compare treatment exposure versus control group.

B. Triglycerides from the solubilized lipid pellet were quantified by calorimetric methods. The triglyceride content is represented as normalized to control group and one way ANOVA followed by Dunnett's test were applied to compare BDEs exposed versus control group.

Oleic acid co-treatment increased lipid content. 0.1 mM BDE 47 increased lipid content in HepG2 cells. In agreement with total lipids, TG levels also increased with 5mM BDE 47 treatment. Oleic acid co-treatment increased TG content in 1 and 25mM BDE 47 and 1mM BDE 99.

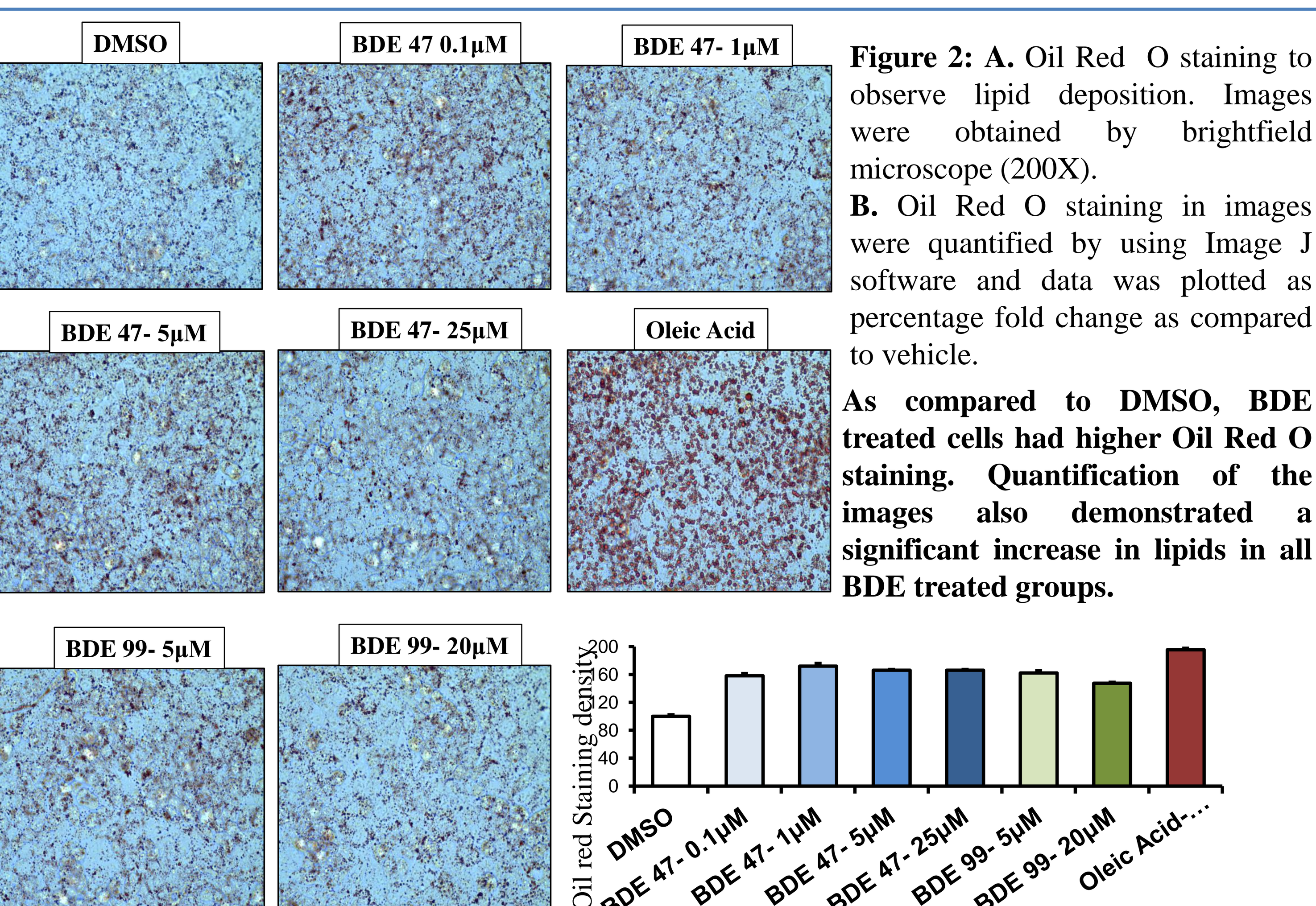


Figure 2: A. Oil Red O staining to observe lipid deposition. Images were obtained by brightfield microscope (200X).
B. Oil Red O staining in images were quantified by using Image J software and data was plotted as percentage fold change as compared to vehicle.

As compared to DMSO, BDE treated cells had higher Oil Red O staining. Quantification of the images also demonstrated a significant increase in lipids in all BDE treated groups.

Data/Results

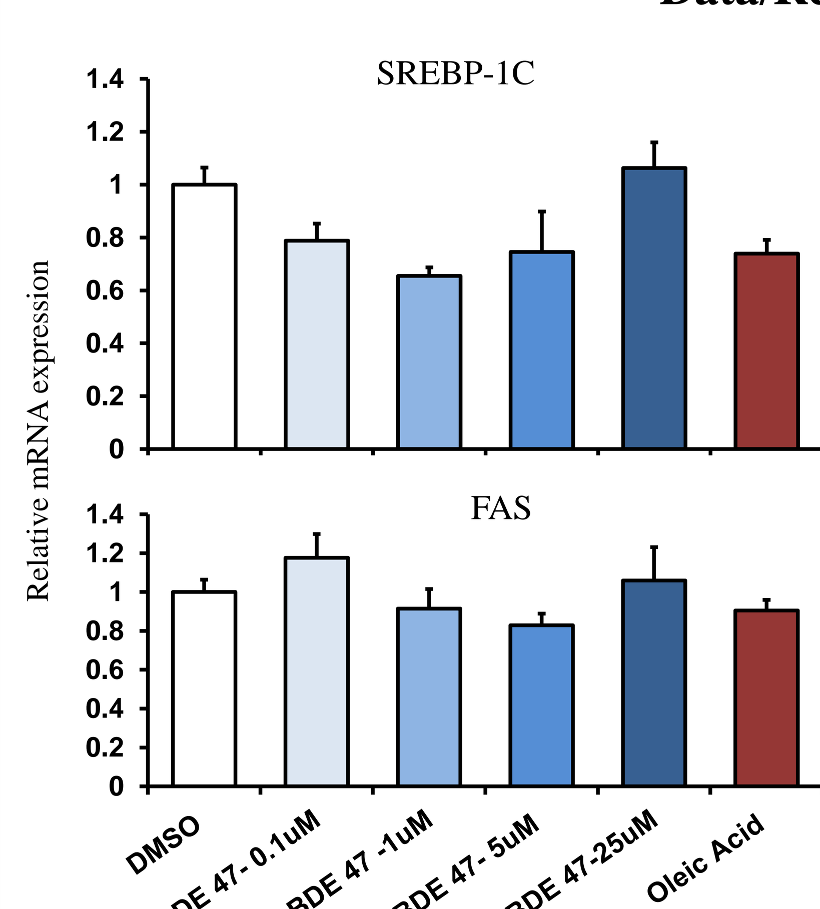
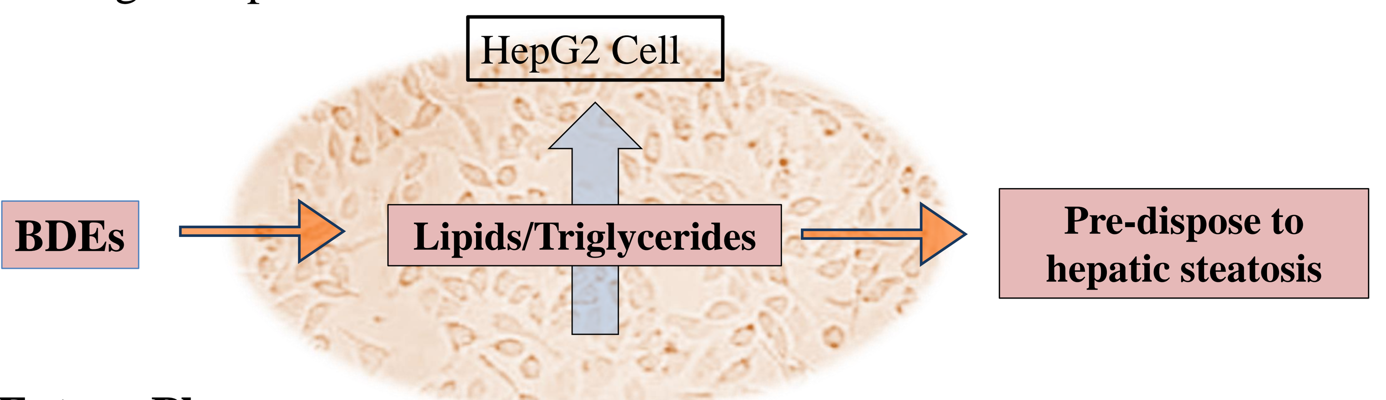


Figure 3: Messenger RNA expression of sterol regulatory element binding protein 1c (Srebp1c) and fatty acid synthase (Fas) HePG2 cells. Data is represented as normalized to GAPDH (houekeeping gene). Differences between groups were determined by one-way ANOVA. Asterisk (*) represents significant difference in BDEs treatment as compared to vehicle ((P≤ 0.05).

None of the BDE exposure levels were able to induce the lipogenic target gene expression in HepG2 cells. Unexpectedly, 1mM BDE 47 decreased SREBP-1C mRNA expression.

Conclusion

BDE exposure may increase the risk of non-alcoholic fatty liver disease. BDE 47 showed the most prominent effects on HepG2 cells leading to hepatic steatosis.



Future Plan

Future research will be conducted on the mechanism of BDE-induced non-alcoholic fatty liver disease. We will be researching other genes that may be involved in BDE-induced non-alcoholic fatty liver disease through the use of Real-time PCR. We will also be using different time frames for BDE treatment.

Acknowledgements

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