# PHTHALATE EXPOSURE AFFECTS INTERCELLULAR CONNECTIVITY AND CALCIUM HANDLING OF HUMAN STEM CELL-DERIVED CARDIOMYOCYTES

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## INTRODUCTION

Diethylhexyl phthalate (DEHP) is a widely used plasticizer, that imparts flexibility to rigid polyvinyl chloride (PVC) materials (Latini, 2004). Since it is not covalently bound to PVC, DEHP can leach out upon contact with blood, serum, and saline solutions. Critically ill neonates, hemodialysis patients, and patients undergoing blood transfusion are at a heightened risk of exposure to DEHP (Jaegar, 2005).

## AIM

To assess the effects of DEHP on intercellular connectivity and calcium handling in human stem cell derived cardio-myocytes (hESC-CMs).

## MATERIALS AND METHODS

**Differentiation:** Human stem cell-derived cardiomyocytes (hESC-CM) were prepared using an established Activin-A/BMP4 protocol . Cells were genetically modified to express an endogenous calcium sensor (gCAMP3).

**Culture/Treatment:** hESC-CMs were cultured in control (supplemented with 0.1% DMSO) or DEHP (50 μg/mL) RPMI media for 72 hrs.

**Imaging:** A Zeiss LSM 510 confocal system was used to monitor calcium transients and immunofluorescence using appropriate excitation/emission filters.

**Immunohistochemistry:** hESC-CM were fixed, permeabilized, and labeled with α-actinin and connexin-43. Cy3 and Cy5 secondary antibodies were used, and DAPI stain for nuclei.

**Statistical Analysis:** All values expressed as mean ± SEM, with $p<0.05$ considered statistically significant (determined by Students t-test; Graphpad Prism).

## RESULTS

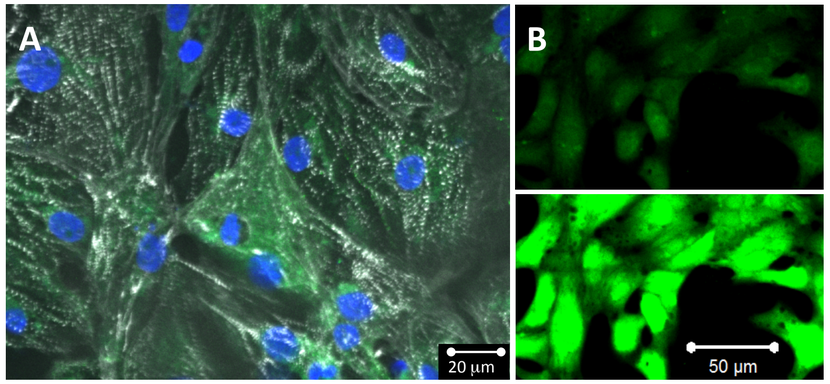


**Figure 1**. (A) Striated α-actinin staining. (B) GCaMP-3 calcium indicator.

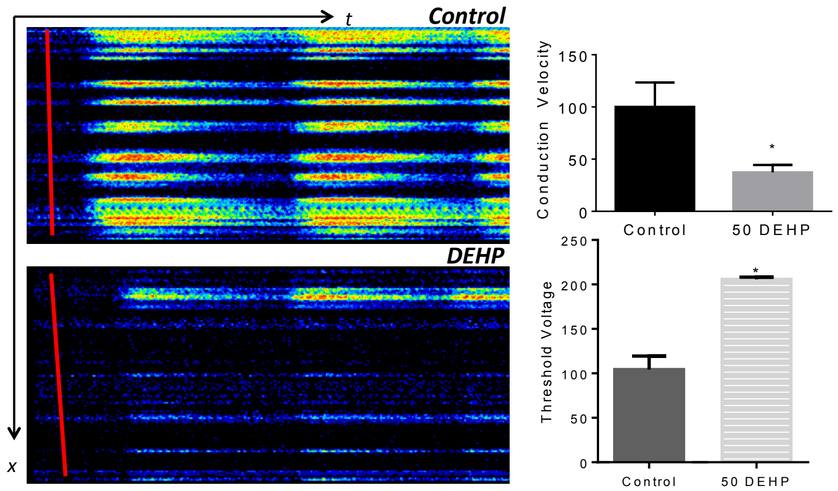


**Figure 2**. DEHP slows conduction velocity and increases threshold voltage.

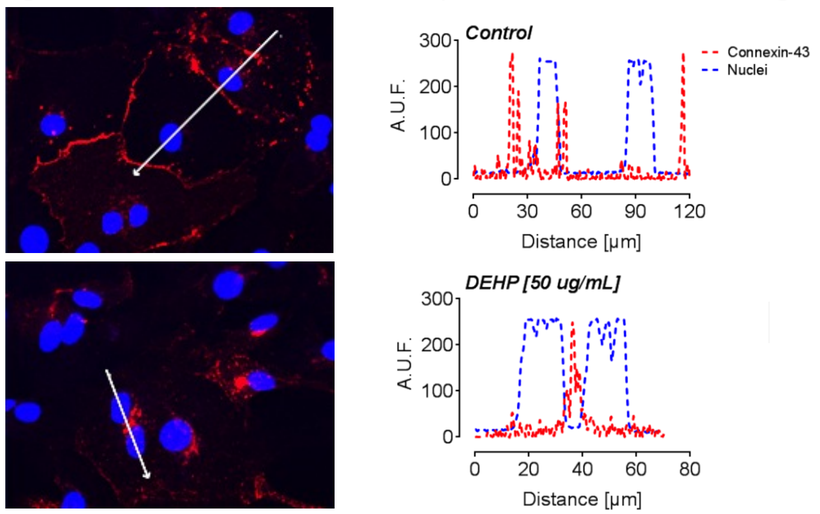


**Figure 3**. DEHP treatment causes localization of connexin-43 in perinuclear regions compared to the cell membrane in control cells.

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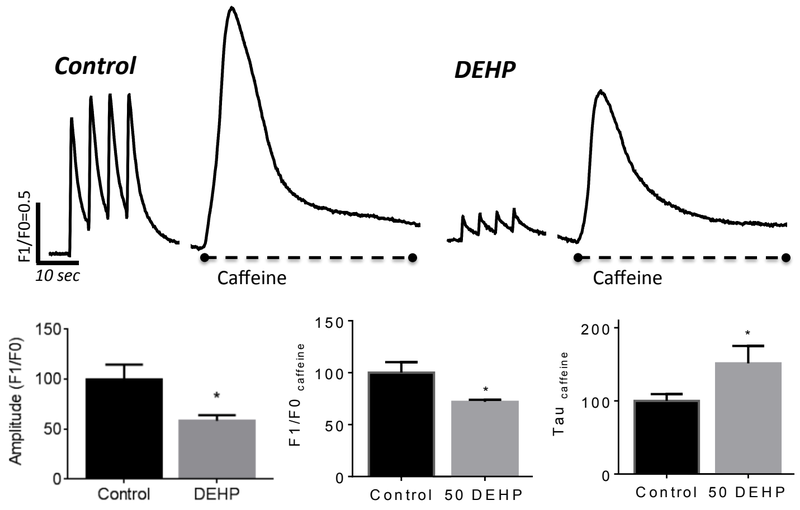


**Figure 4**. DEHP modifies calcium transient morphology; decreases amplitude, total SR load, and increases decay constant.

## DISCUSSION

- DEHP causes asynchronous cell beating, decreased conduction velocity, and higher threshold voltage due to post-translational modification in connexin-43.
- Intracellular calcium handling is a regulator of cardiac function.
- Modifications in $Ca^{2+}$transient morphology likely due to calsequestrin or SERCA activity.

## FUTURE DIRECTION

- Compare and contrast the effects of DEHP and PPARα agonist (Wy-14,643) on hESC-CMs
- Investigate genetic and post-translational modifications in gene/protein expression
- Examine the effect of DEHP on the whole heart (electrical conduction, mechanical function)

## ACKNOWLEDGEMENTS

We gratefully acknowledge the Luther Rice Fellowship, Harlan Trust, and NIH (K99ES023477 NGP).

References available upon request.