Effects of Embryonic Exposure to PCBs on Heart Development in Avian Laboratory Species

Tiffany Carro, Karen M. Dean,
Leah D. Baltos, Mary Ann Ottinger
Department of Animal and Avian Sciences
University of Maryland
College Park, MD

Why are PCBs a Concern?

- EPA : PCBs & Adverse Health Effects
  - Mortality at high exposure levels
  - Carcinogenicity
  - Impaired immune response function
  - Impaired reproductive fitness
  - Impaired nervous system function
  - Endocrine disruption
  - Possible cardiovascular impairment
  - Prenatal development not well understood

Photo courtesy of J. Weiderhorn

- PCBs banned from manufacture, processing, distribution in 1979
- 1980: EPA established the Comprehensive Environmental Response and Liability Act (CERCLA)

Assessing Potential Adverse Effects

- Understand the dynamics of the ecology and specifically the habitat and needs of selected species—use sentinel species if possible
- Comparative laboratory trials under controlled conditions to reveal mechanisms and impact
- study specific impacts, in this study—effects on cardiac development

PCB mixtures:

- Based on profile of PCBs measured in spotted sandpiper or tree swallow eggs collected at the Upper Hudson River, NY in 2004 (N=3)
  - 58 or 64 different congeners in injection mixture
    - 49 of highest ranked congeners on a mass basis; represented 95% of the total PCB content in the sandpiper eggs on a mass basis
    - 9 dioxin-like PCBs added because of toxicological importance
    - All congeners in mixture at relative proportion to levels in sandpiper egg sample
    - Excluded non dioxin-like congeners that cumulatively constituted <5% of the mass

Studies Assessing the Cardiovascular System

- Wildlife studies (DeWitt *et al*., 2006)
  - Limited number of studies
  - Large sample sizes needed
  - Variability in age
- Laboratory studies
  (Walker & Catron, 2000; Henshel & Sparks, 2006; Kopf & Walker, 2009)
  - Only studied most biologically toxic PCB congeners
  - No PCB mixtures studied
  - Sampling did not address ventricular wall thinning

Photo by Picturesofbirds

Photo by Rebecca Craig

- Introduction
- Experiment 1
  - The effects of a 58 congener PCB mixture on cardiac development following *in ovo* exposure in the domestic chick hatchling
- Experiment 2
  - The effects of a 58 congener PCB mixture on cardiac development, at critical stages of embryonic heart formation, following *in ovo* exposure in the domestic chick

Exp 1: Materials & Methods

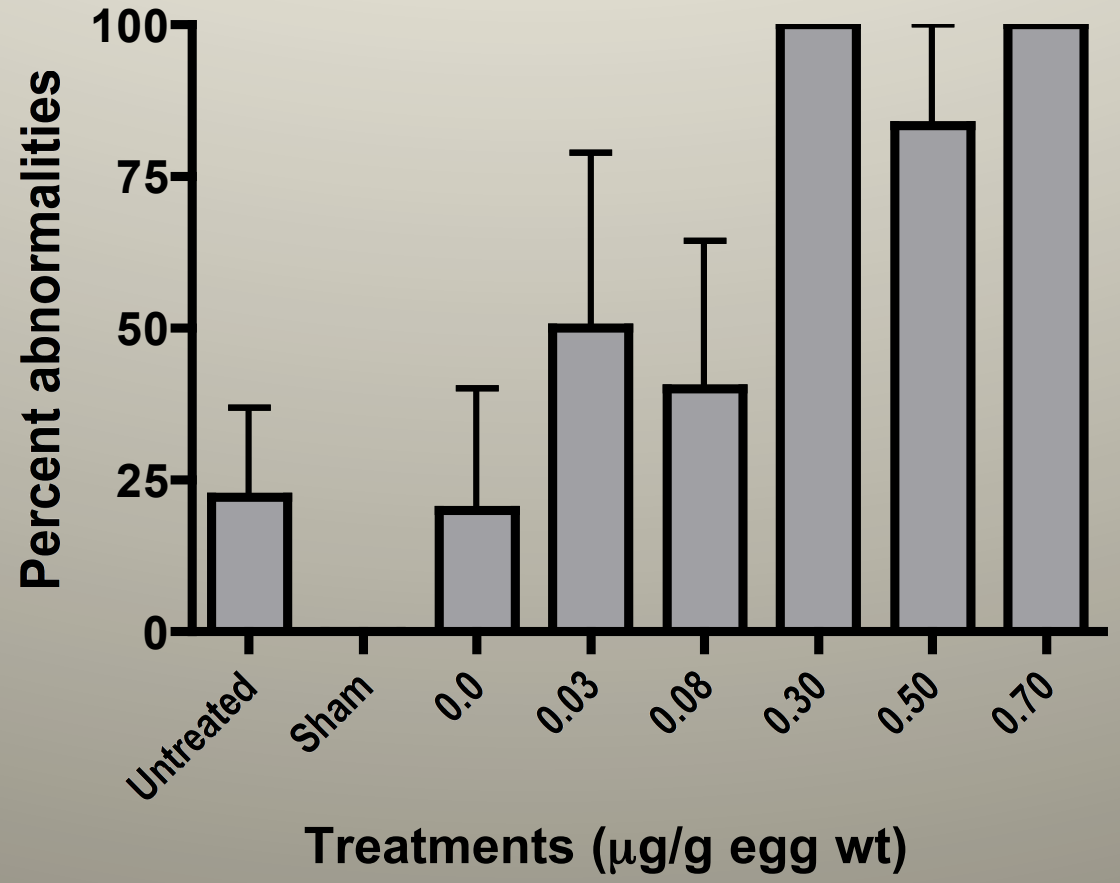
- 58 PCB congeners mixed in proportion to Upper Hudson River egg contaminant analysis
  - Solubilized in corn oil vehicle
- Injected at embryonic day (ED) 0 into albumen
- Treatments: untreated, sham, 0.00, 0.03, 0.08, 0.30, 0.50, 0.70, and µg/g egg wt
  - TEQ: 0.004-0.266 ng/g
- Sample size per treatment (n=20)
- Monitored heart rate at intervals throughout incubation
  - Avian buddy apparatus

Exp 1: Materials & Methods

- Hearts collected at hatch, hearts analyzed for cardiomyopathies using histology techniques
  - Analysis performed at 4 morphological layers
- Statistical analysis
  - Infertile eggs removed from analysis
  - Hatch success & heart rate
    - Tukey's post-hoc significance criteria: $p<0.05$
  - Cardiomyopathies & Compact Layer Presence: subset of samples (n=5 PCB treatments; n=10 untreated/baseline)
    - Cardiomyopathies: Chi sq analysis
    - Compact layer scoring: 3 layers of heart
      - (Tukey's post-hoc significance criteria: $p<0.05$)

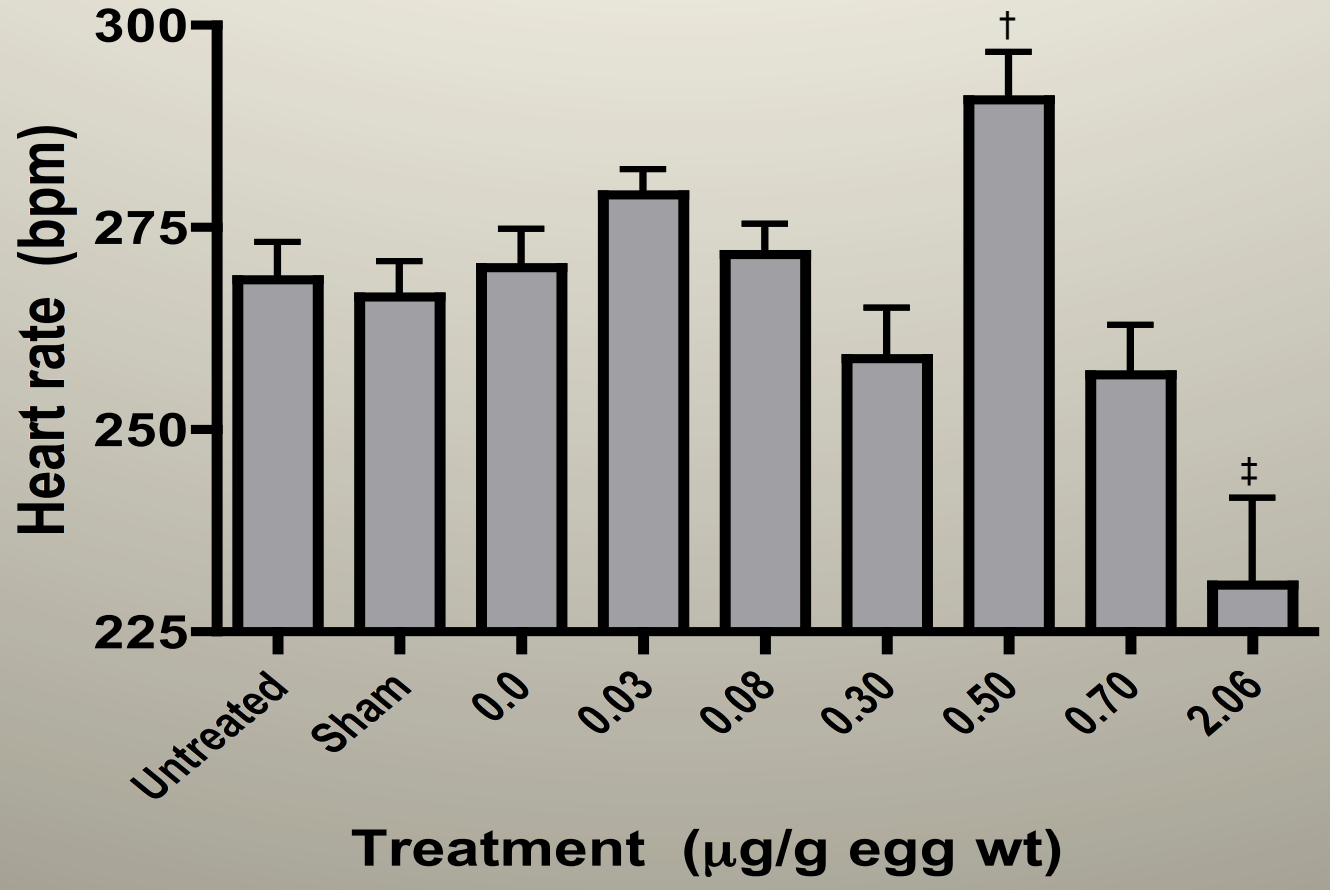
Heart Effects of PCB Mix:

Incidence of Cardiomyopathies Increased in PCB Treatments Compared to Controls



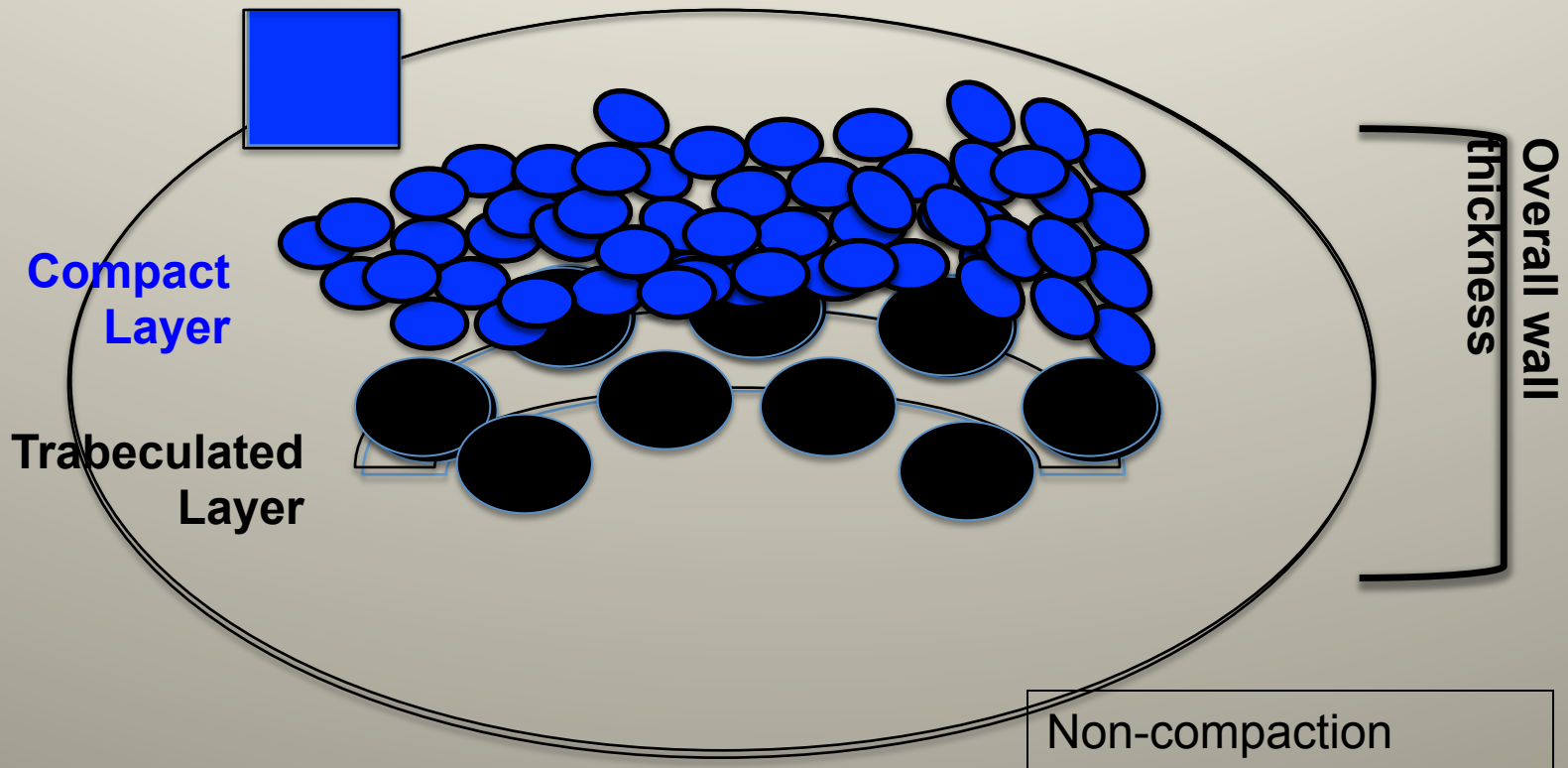
From Carro et al., 2012 dissertation

Embryos Exposed to PCB Mix Exhibited Cardiac Arrhythmia at Embryonic Day 14



Tukey's post-hoc criteria: *p<0.05*
(† & ‡ separately compared to controls)

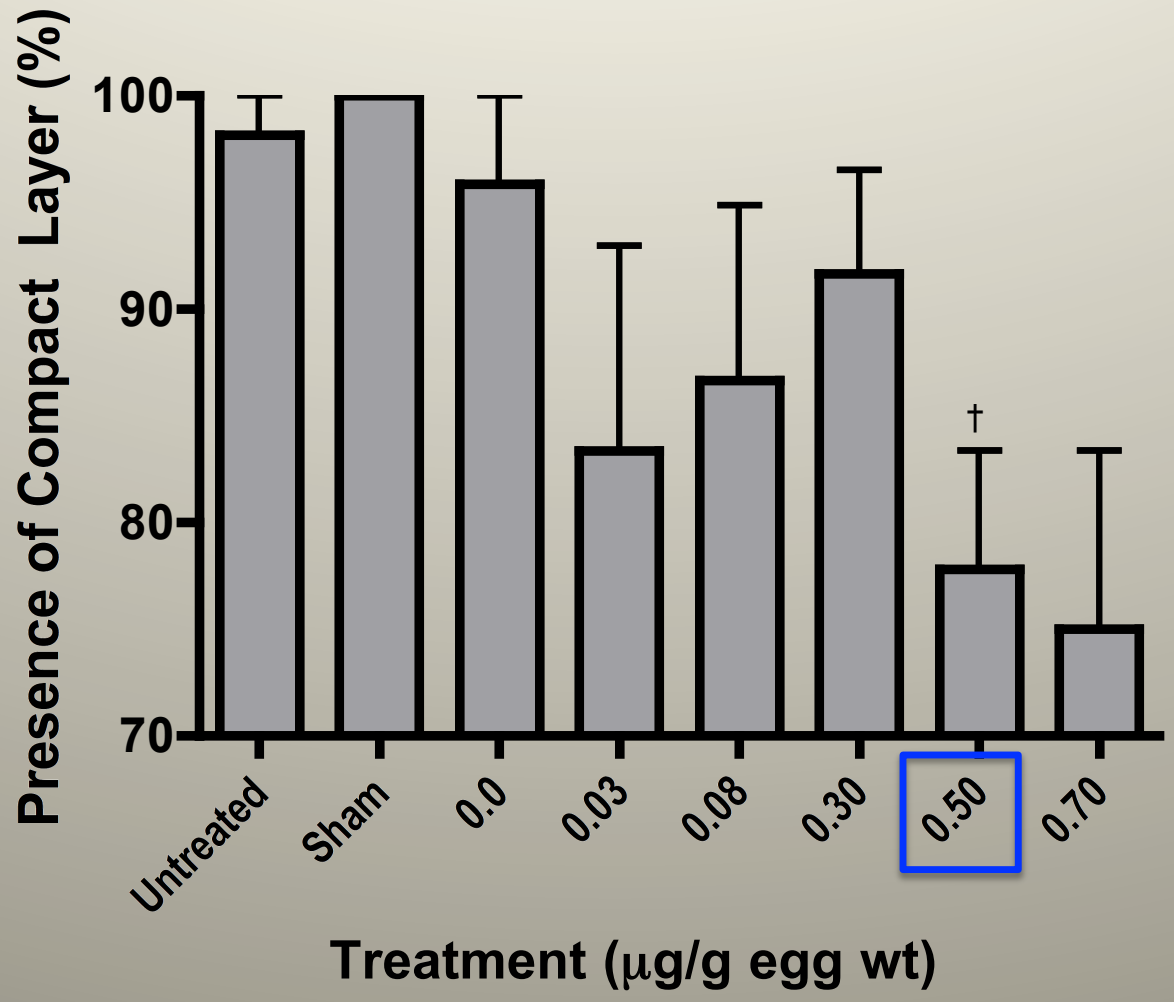
Exp 1: Results – Most Common Abnormality Identified was Non-Compaction of the Ventricular Wall



Non-compaction causes:
-cardiac arrhythmia
-systemic embolism
-myocardial infarction

Brief description of ventricular wall formation

Ventricular Wall Compact Layer was Absent in Embryos Exposed to the PCB Mix



†: Tukey's post-hoc (compared to controls) *p<0.05*

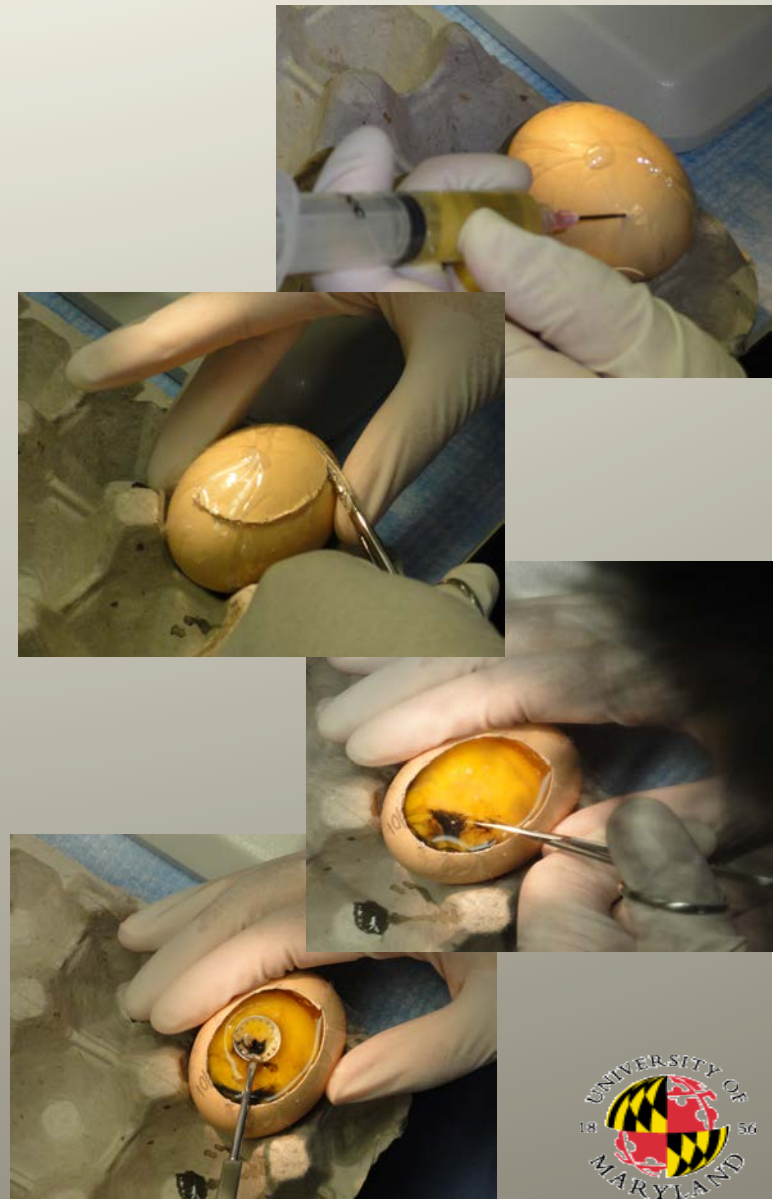
Percent of Tree swallow hatchling hearts with Compact Layer absences following *in ovo* exposure to PCB 77.

Treatment	(n)	# hearts w/ intact CL	% hearts with CL deformities
Untreated	15	13	13.33
Vehicle	11	10	9.09
100 ng/g*	14	8	42.86
1000 ng/g*	15	4	73.33
UHR e. e.	8	8	0.00

Chi square ($p<0.001$)

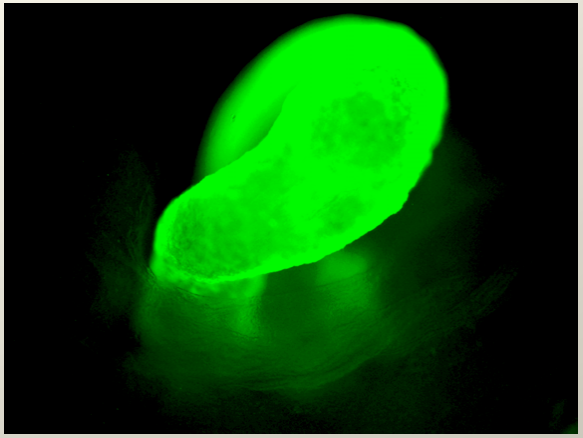
Early Embryo Experiment (Exp. 2): Materials & Methods

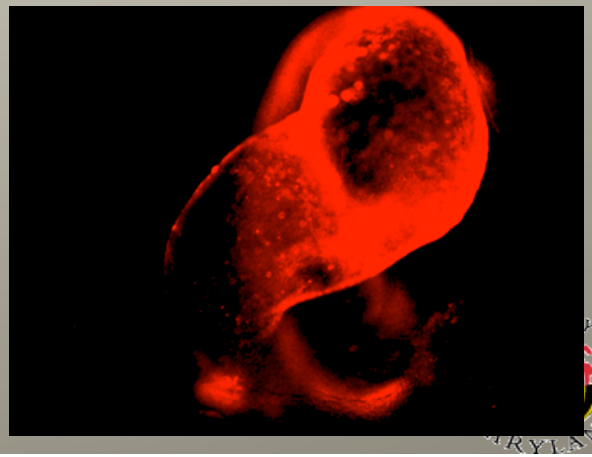
- Previous PCB mix used at two dose concentrations
  - Criteria: above/below LD_{50} & LOAEL
- Treatments: untreated, 0.00, 0.08, and 0.50 µg/g egg wt
- Sample size (n=40)
  - Increased (n) compared to hatchling study
- Collections at three stages (HH)
  - HH10: heart tube formation
  - HH16: heart looping & A/V differentiation
  - HH20: Proliferation and further A/V differentiation



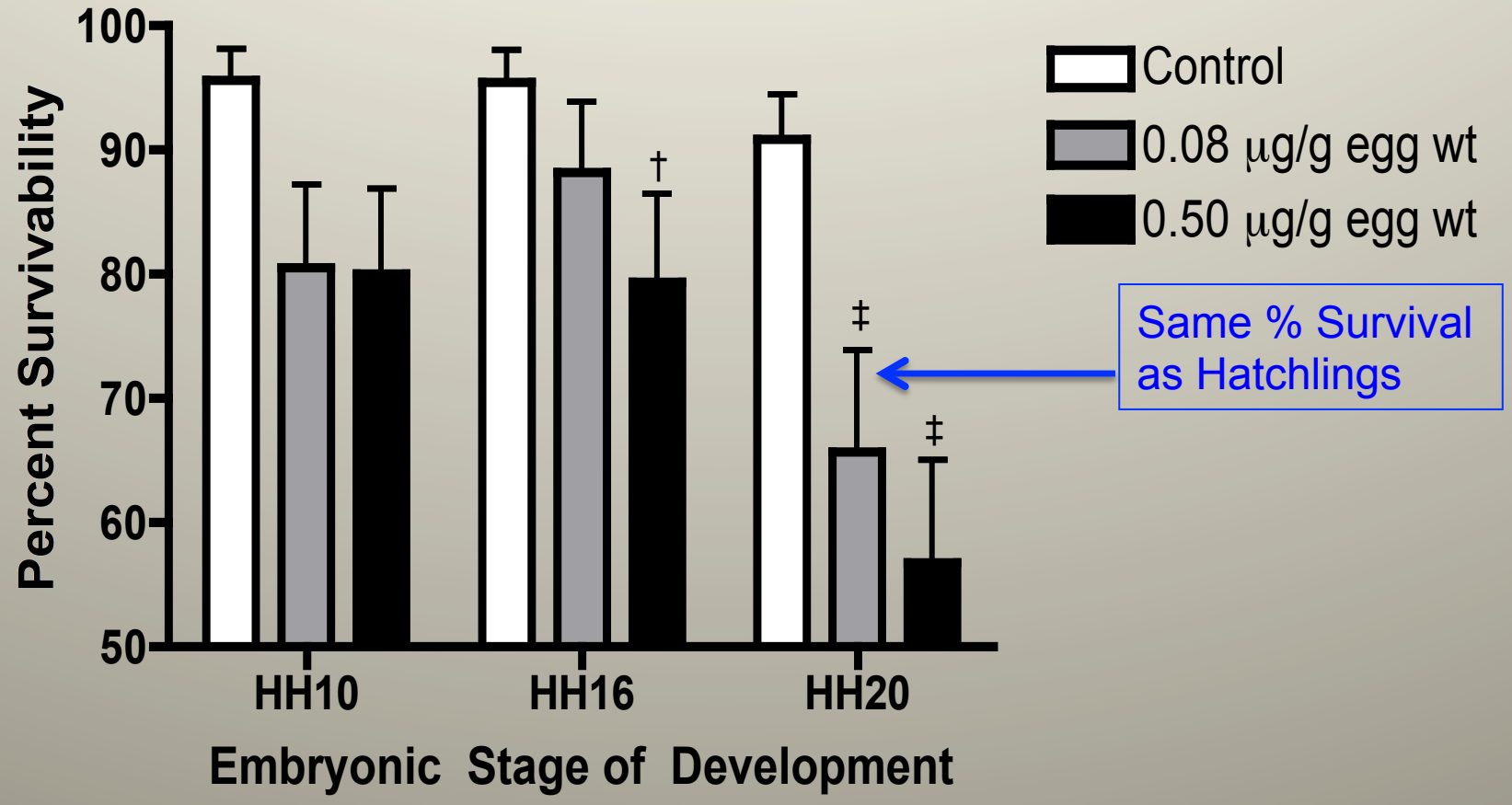
Methods continued

- Hearts analyzed for cardiomyopathies (w.m./ sections) & immunohistochemistry of two proteins
  - Ventricular myosin heavy chain (VMHC) ~220 kDa
  - Titin (Connexin in mammals) ~ 3,000 kDa
  - (10 samples per protein harvested)
- Morphological measurements, proliferation immunohistochemistry (phospho-histone-H3), apoptosis assay performed on sections
- Statistical Analysis:
  - Untreated and Vehicle combined as control group (did not differ)
  - One way ANOVA, Tukey's post-hoc test utilized for analysis (*significance criteria $p<0.05$*)
    - Hatch success/cardiomyopathy analysis used complete sample set
    - Molecular assays ($n \geq 7$ embryos)
      - Apoptosis assay ($n \geq 4$ embryos)
      - Sections analyzed across serial cross-sections (≥ 8)





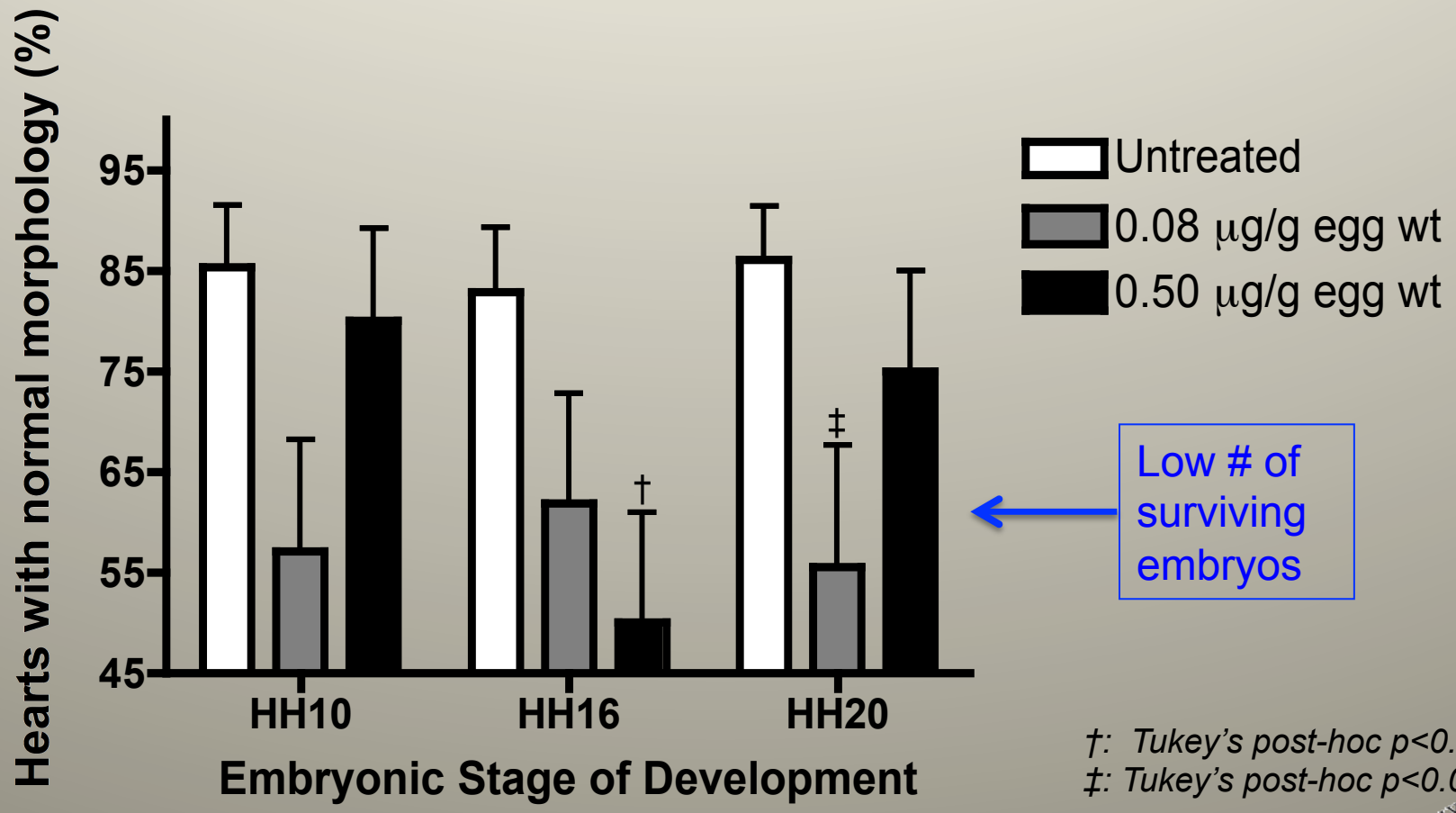
Percent Survival Decreased Across PCB Treatments at HH16 and HH20



†: Tukey's post-hoc p<0.05
‡: Tukey's post-hoc p<0.05

Exp 2: Results

Incidence of Cardiomyopathies Increased in PCB Treatments

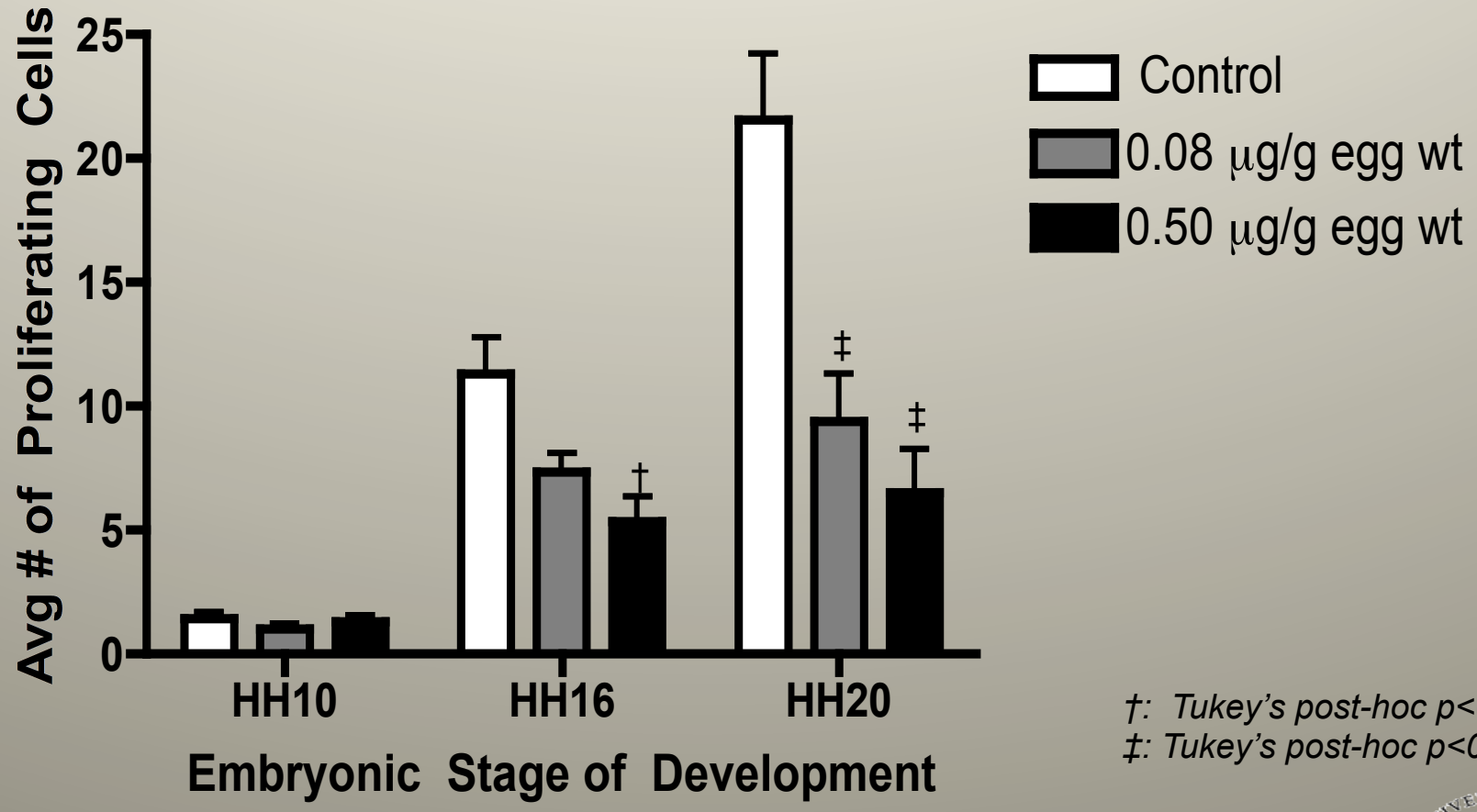


Low # of surviving embryos

†: Tukey's post-hoc p<0.05
‡: Tukey's post-hoc p<0.05

Exp 2: Results

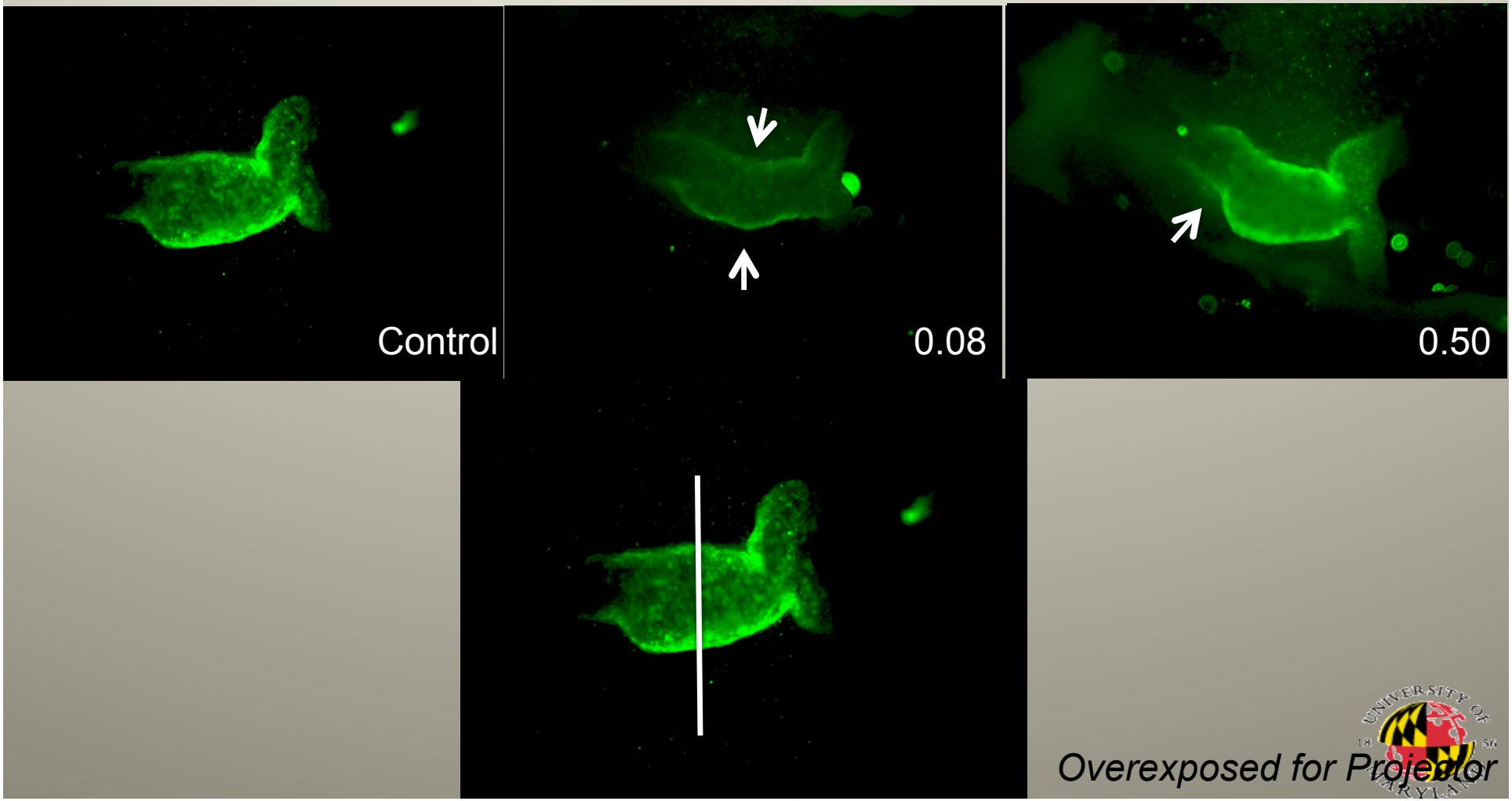
Rates of Proliferating Cardiomyocytes Decreased Across PCB Treatments



†: Tukey's post-hoc p<0.05
‡: Tukey's post-hoc p<0.05

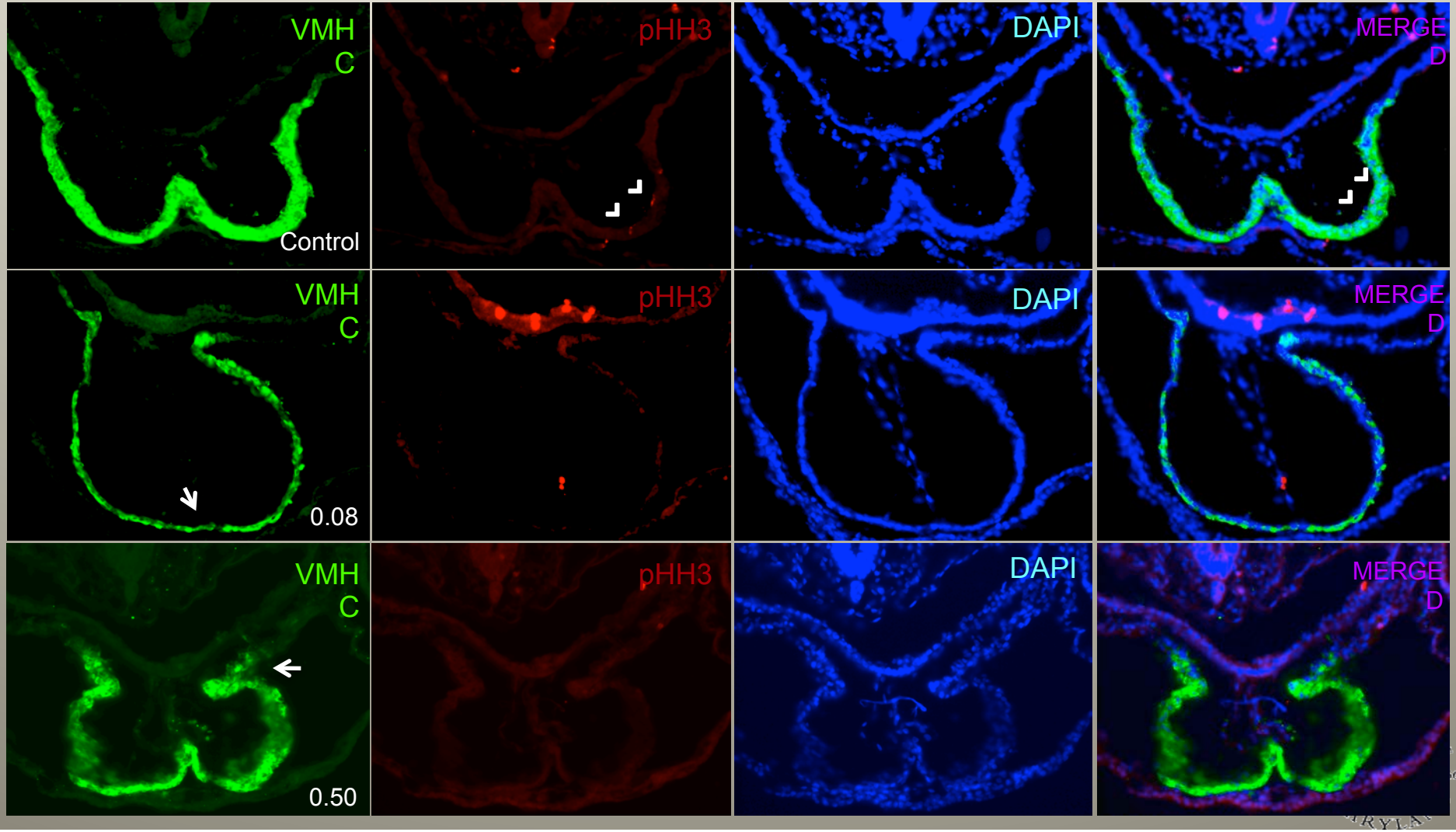
Exp 2: Results

VMHC Whole-mount Immunohistochemistry at HH10 shows Cardiomyopathies in Embryos Exposed to the PCB mix (NSD)



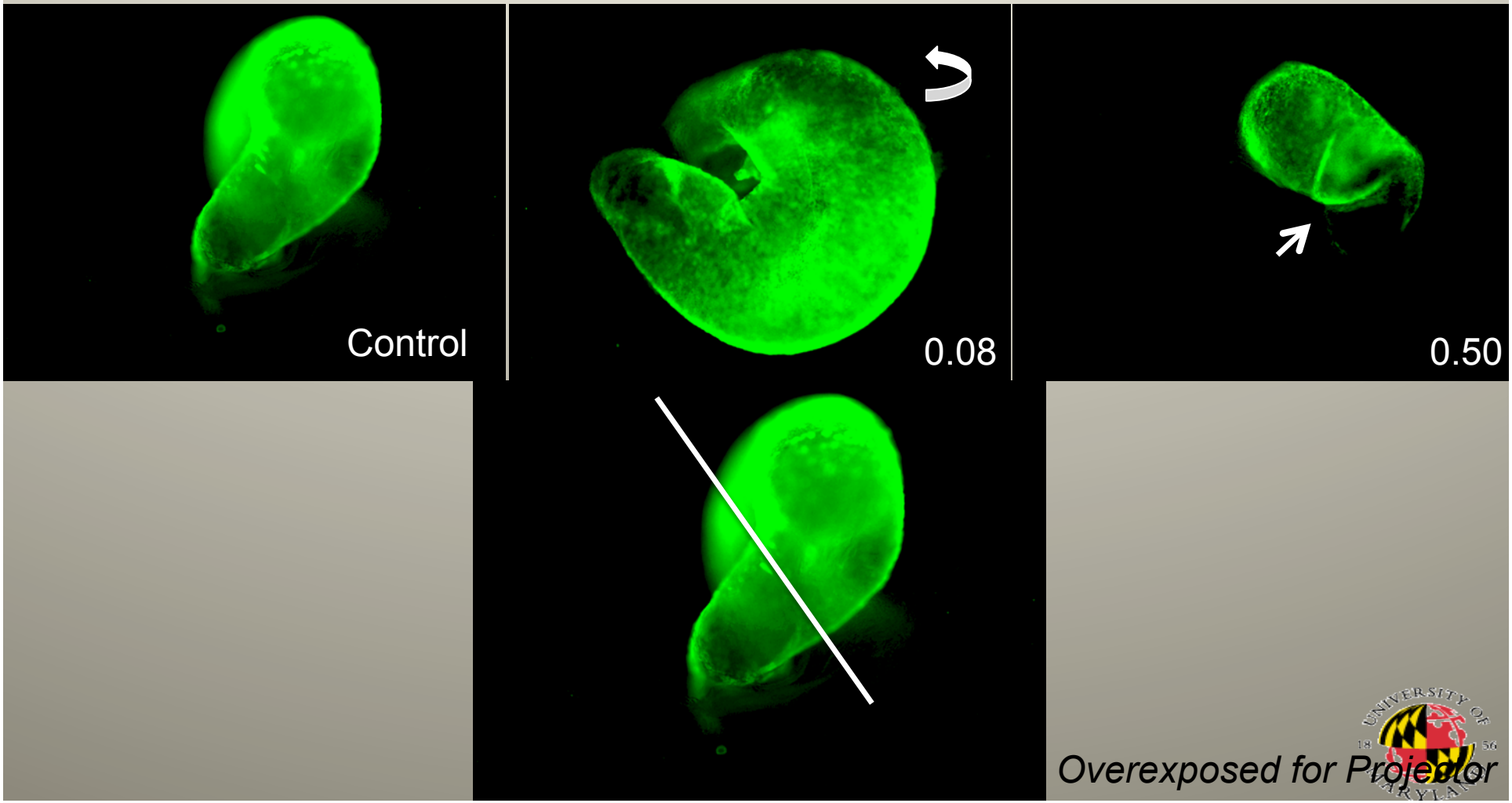
Exp 2: Results

VMHC and pHH3 Immunohistochemistry of Heart Sections at HH10 shows Cardiomyopathies in Embryos Exposed to the PCB mix (NSD)



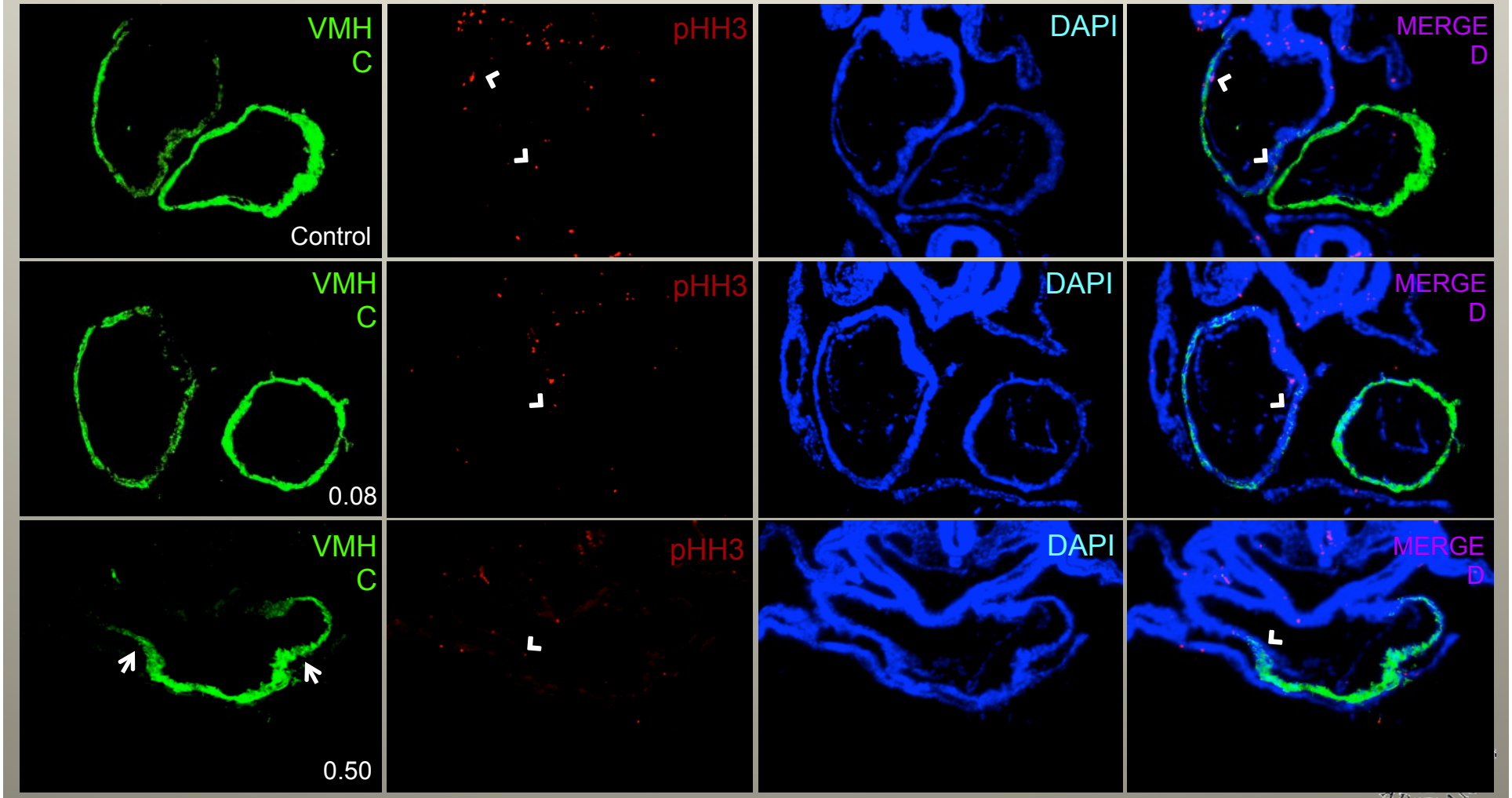
Exp 2: Results

VMHC Whole-mount Immunohistochemistry at HH16 shows Cardiomyopathies in Embryos Exposed to the PCB mix (0.50 µg/g egg



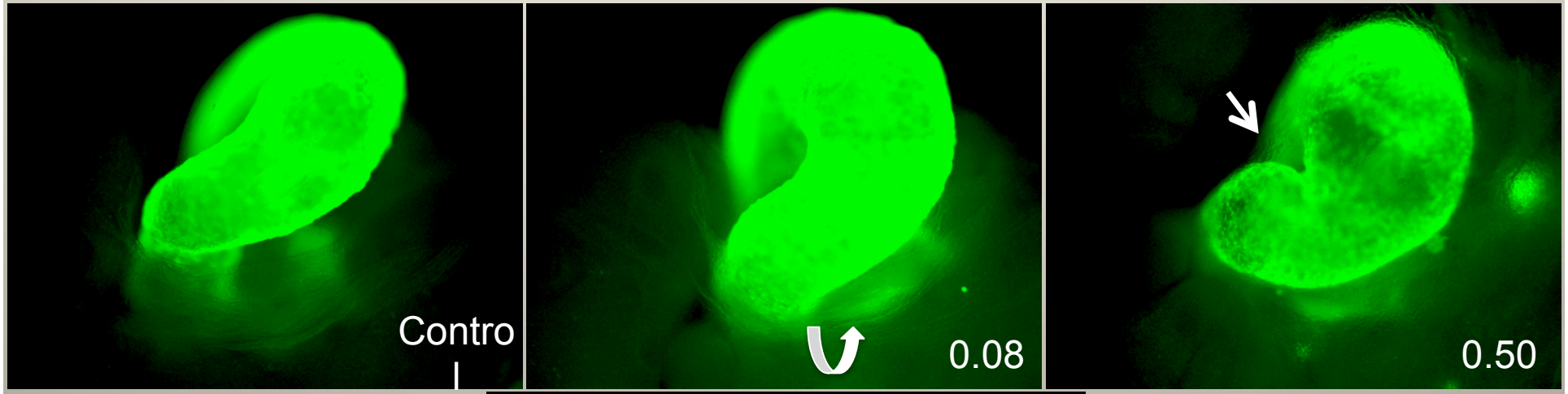
Exp 2: Results

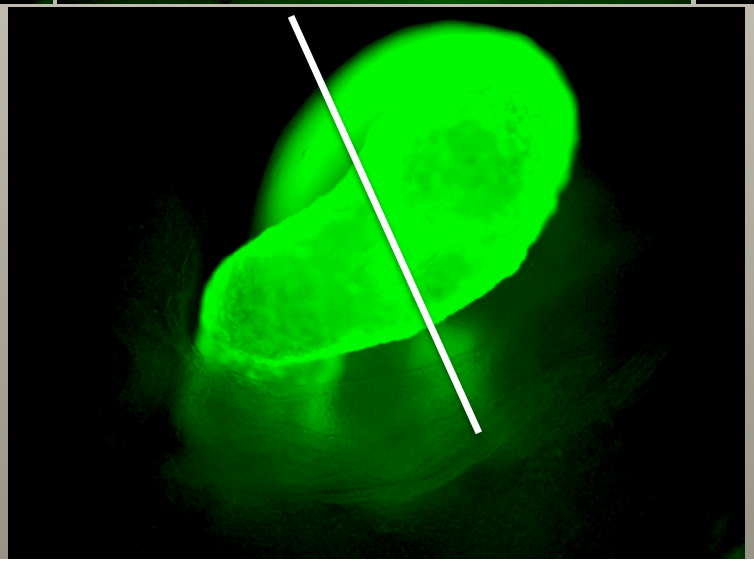
VMHC and pHH3 Immunohistochemistry of Heart Sections at HH16 shows Cardiomyopathies and Reduced Proliferation Rates in Embryos Exposed to the PCB mix (0.50 µg/g egg wt SD)



Exp 2: Results

VMHC Whole-mount Immunohistochemistry at HH20 shows Cardiomyopathies in Embryos Exposed to the PCB mix (0.08 µg/g egg

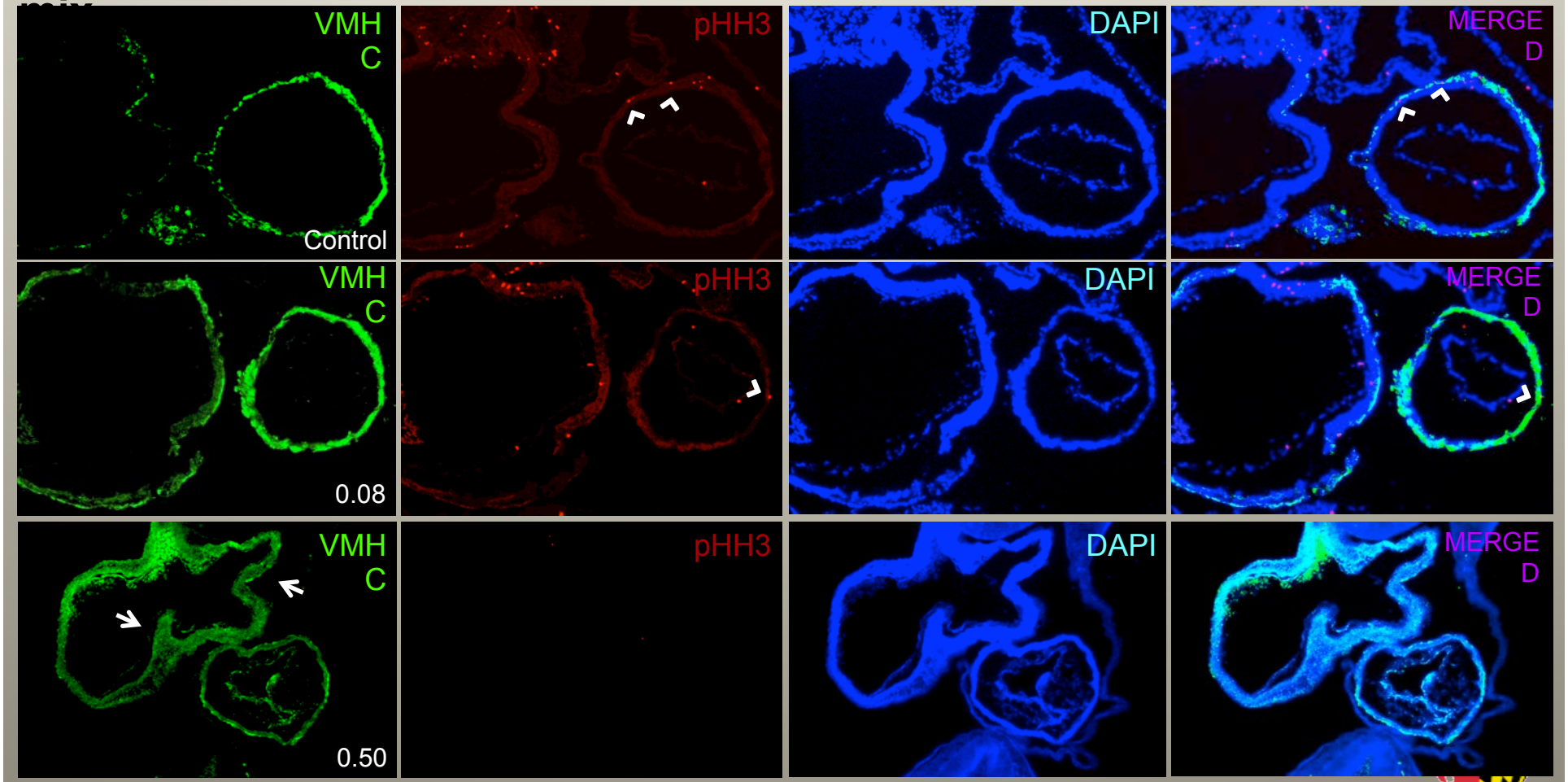




Overexposed for Projector

Exp 2: Results

VMHC and pHH3 Immunohistochemistry of Heart Sections at HH20 shows Cardiomyopathies (0.08 µg/g egg wt SD) and Reduced Proliferation Rates (both [PCB mix] SD) in Embryos Exposed to the PCB mix



Studies in Birds

Mary Ann Ottinger, PhD
Karen Dean, PhD
Emma Lavoie , PhD
Mahmoud Abdelnabi, PhD
Allegra Marcell
Nichola Thompson
Sean Madden
John French, PhD

Graduate students:
Meredith Bohannon
Leah Carpenter
Tiffany Carro
Kara Duffy
Karen Eisenreich
Karen Lohnes
Moira McKernan
Anna Schlappal

Undergraduates:
Ashley Barton
Vaneesha Bradford
Cassipoeia Cutchis
Kimya Davani
Linda Huang
Krisztina Larson
Laurie Mann
Enrico Proctor
Susannah Reed
Marci Strauss
H. Kasen Whitehouse

This work has been supported by EPA grants # R826134010 (Star Grant) and R-82877801; Battelle contract for EPA-EDSTAC validation studies, NRI #92-37203 and NSF #9817024; MAES, University of Maryland, College Park; Fish and Wildlife Service and Hudson River NRDA Trustees. The conclusions and opinions presented here are those of the authors, they do not represent the official position of any of the funding agencies, the Hudson River Trustees, or the United States.