Impacts of Embryonic Exposure to PCBs on Heart Development in Japanese Quail

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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)

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
  - Few PCB mixtures studied
  - Rarely address ventricular wall thinning and involved mechanisms

Photo by Picturesofbirds

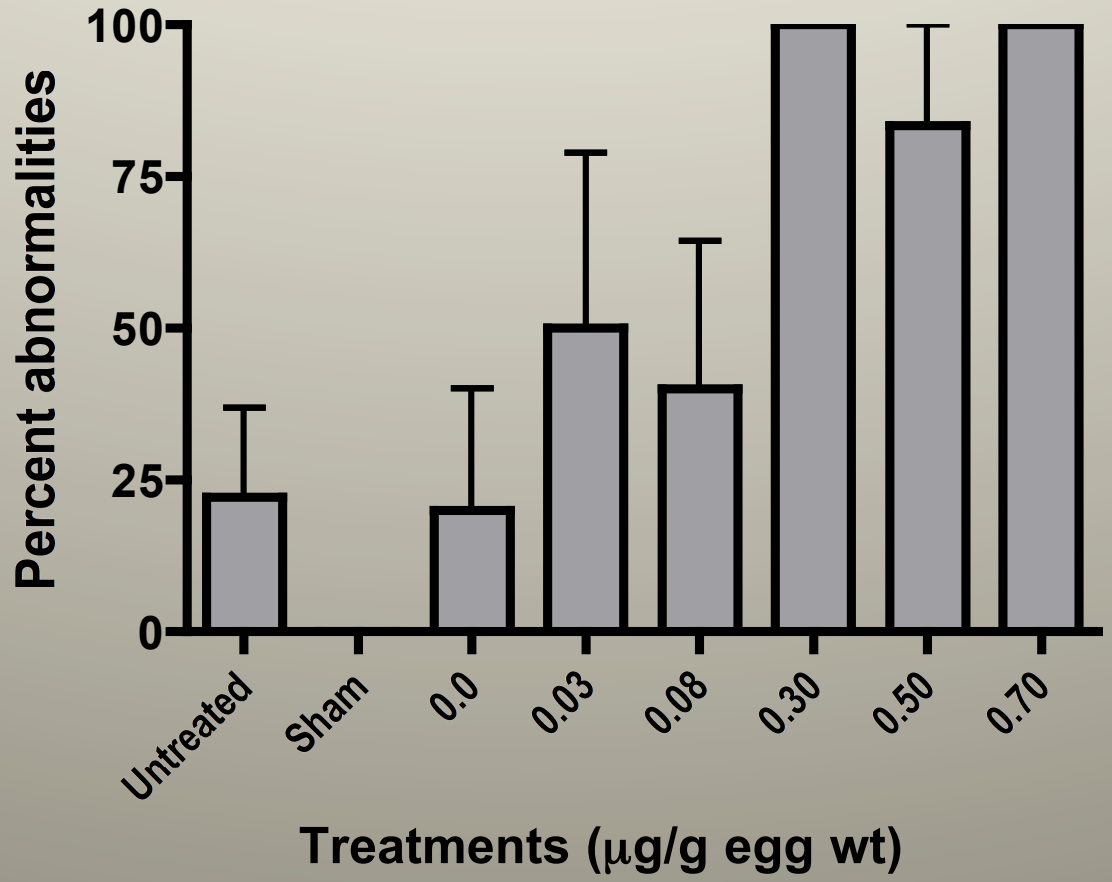
Photo by Rebecca Craig

Comparing Potential Adverse Effects

- Field studies in tree swallows revealed heart impacts from PCB 77 exposure (Carro et al., 2009 SETAC Presentation)
- Chick embryos showed dose-related heart abnormalities, particularly loss of ventricular wall compact layer (Carro et al., ETC 32 (6): 1317-24; 1325-31)
- Comparison of mixtures (58 congeners=Sandpiper Mix; 66 congeners=Tree Swallow Mix) with PCB 77, PCB 126, estradiol and vehicles in laboratory model (Japanese quail) under controlled conditions to reveal adverse effects
- study specific impacts, in this study—effects on cardiac development

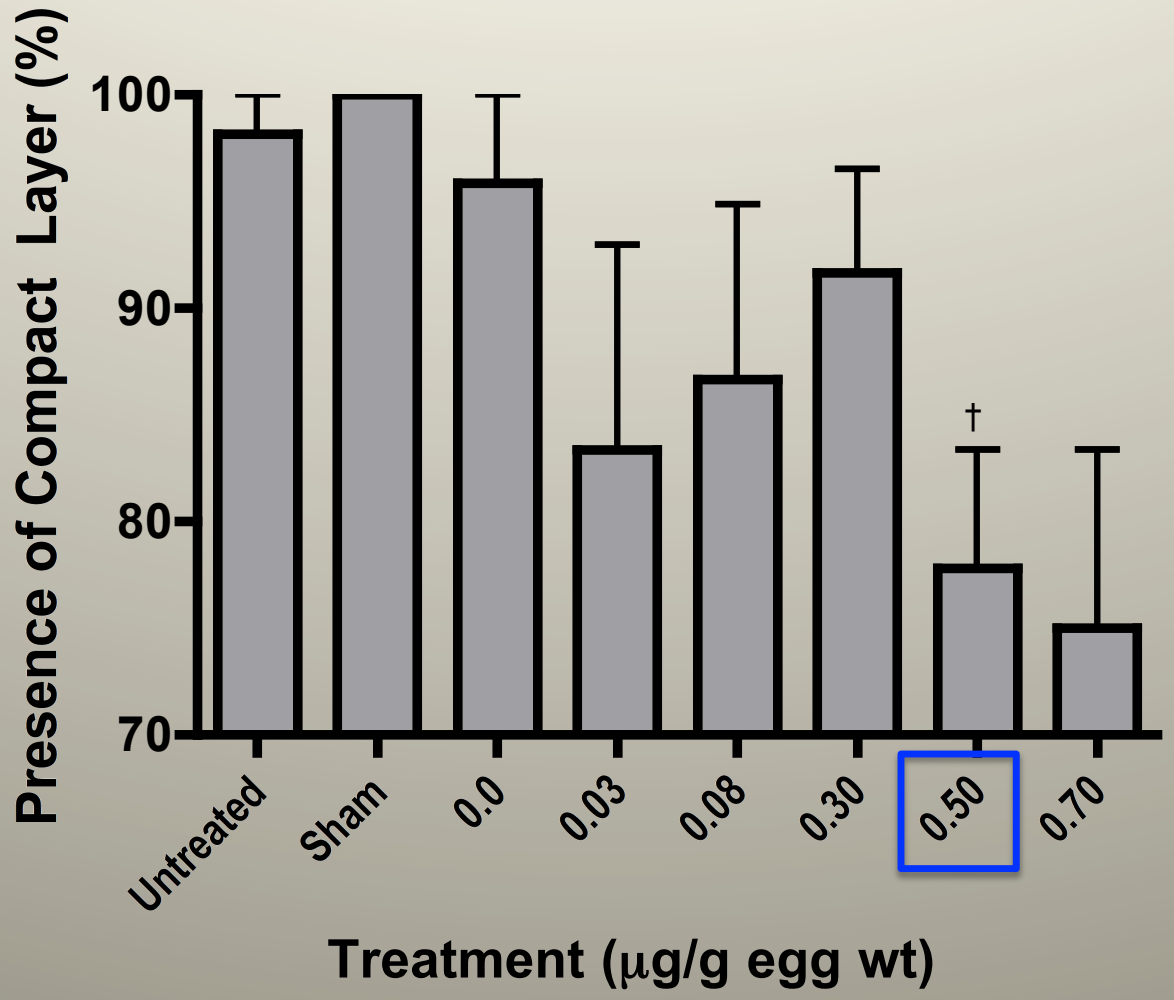
Heart Effects of PCB Mix: Tiffany Carro's Studies

Incidence of Cardiomyopathies Increased in PCB Treatments Compared to Controls



From Carro et al., 2013

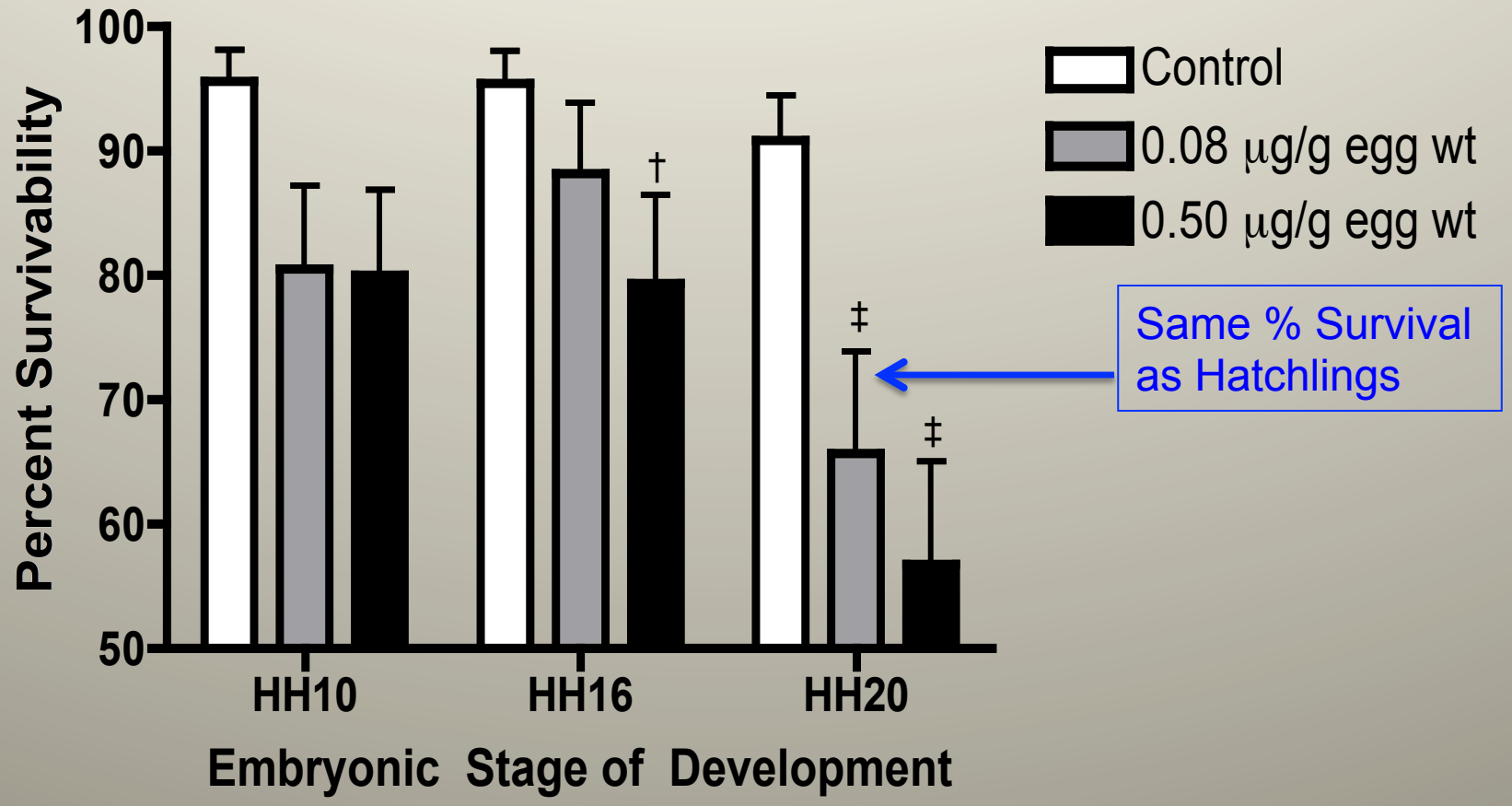
Ventricular Wall Compact Layer was Absent in Chicken Embryos Exposed to the PCB Mix



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

From Carro et al., 2013

Percent Survival Decreased Across PCB Treatments at HH16 and HH20 in Chicken Embryos

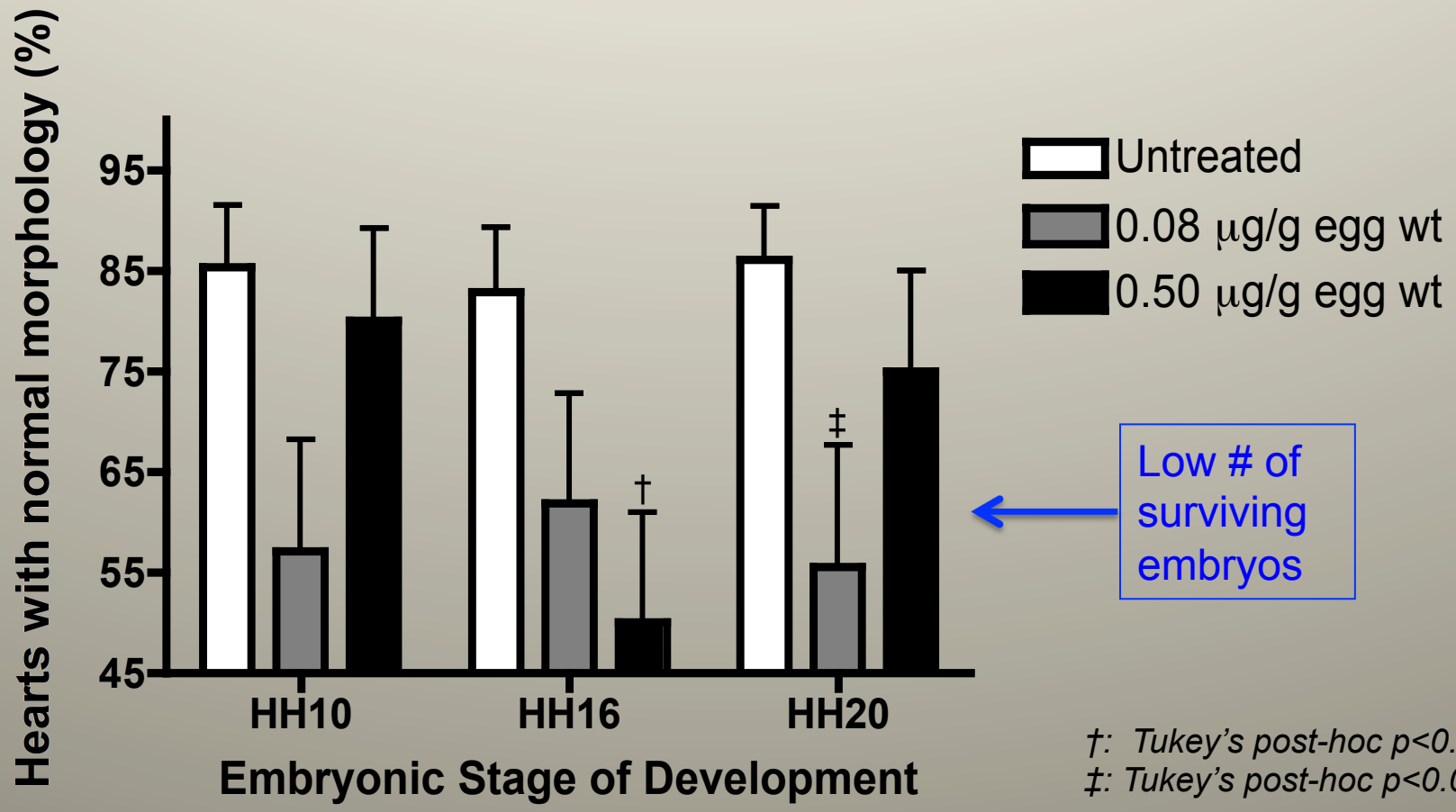


†: Tukey's post-hoc p<0.05
‡: Tukey's post-hoc p<0.05

From Carro et al., 2013

Early Effects of PCB Mix on Chick Embryos

Incidence of Cardiomyopathies Increased in PCB Treatments

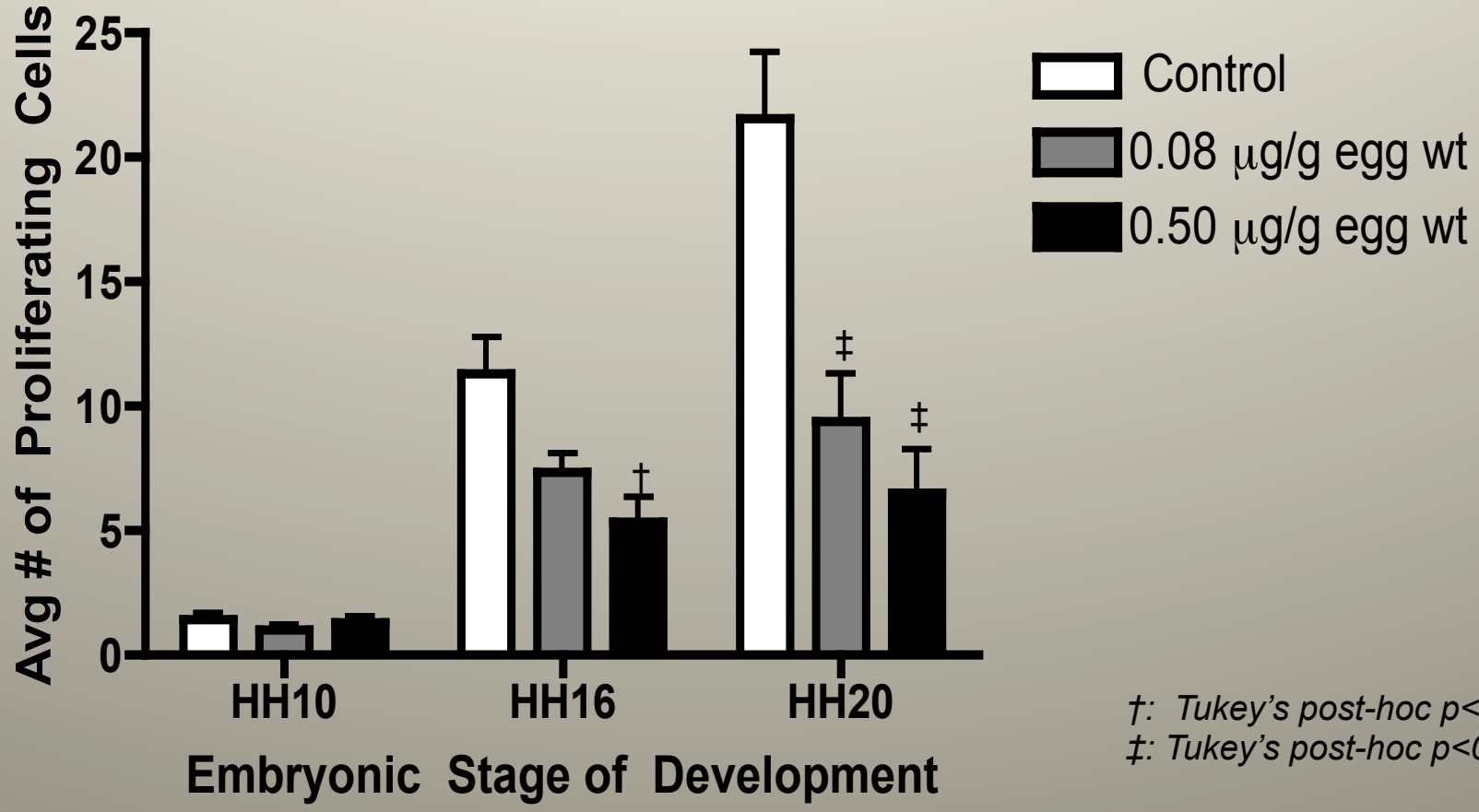


†: Tukey's post-hoc p<0.05
‡: Tukey's post-hoc p<0.05

From Carro et al., 2013

PCBs Decreased Proliferation

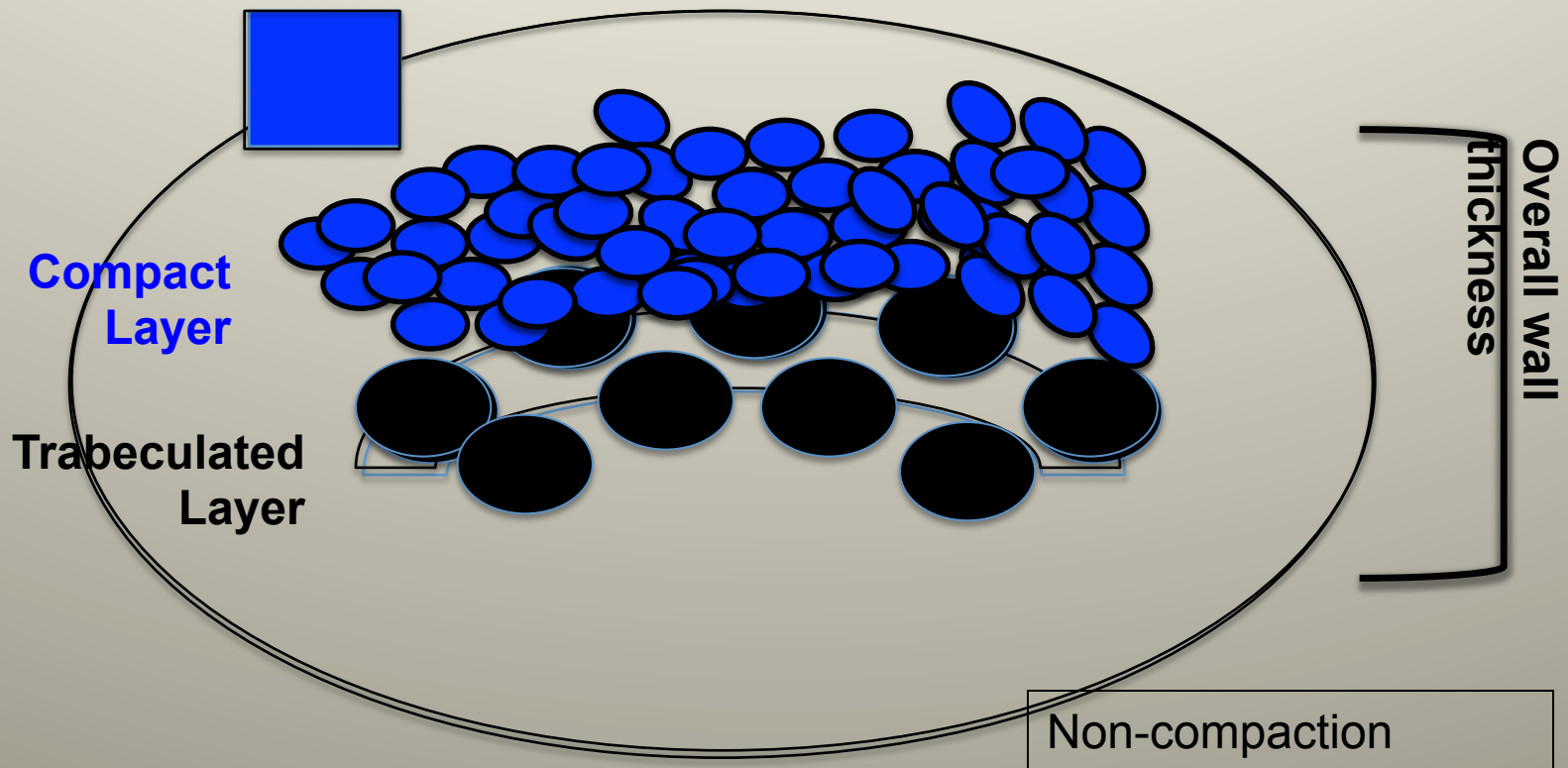
Rates of Proliferating Cardiomyocytes Decreased Across PCB Treatments



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

From Carro et al., 2013

Most Common Abnormality Identified was Non-Compaction of the Ventricular Wall

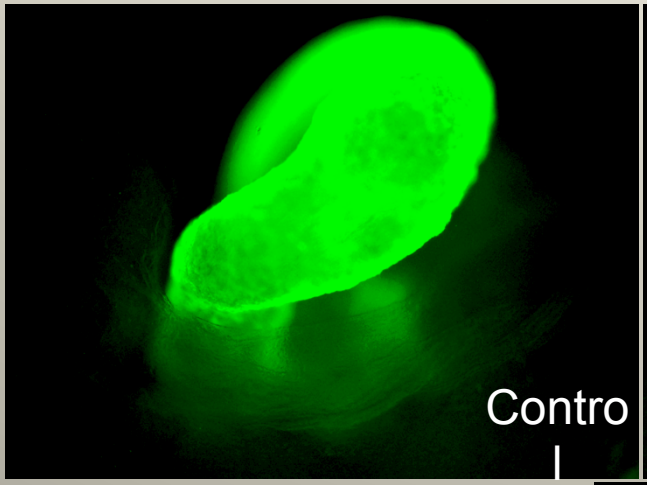


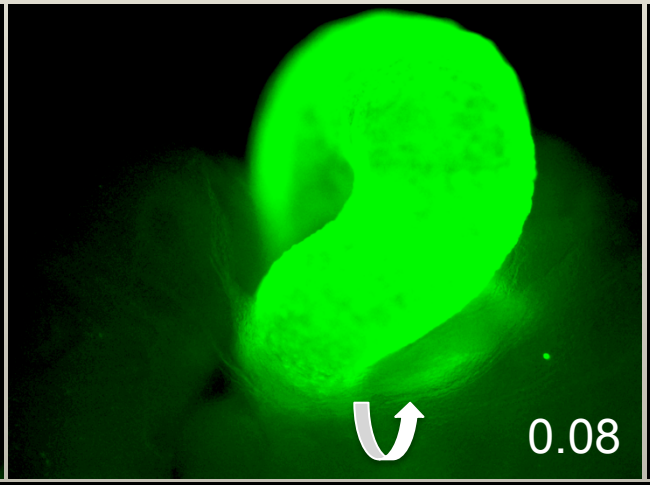
Non-compaction causes:
-cardiac arrhythmia
-systemic embolism
-myocardial infarction

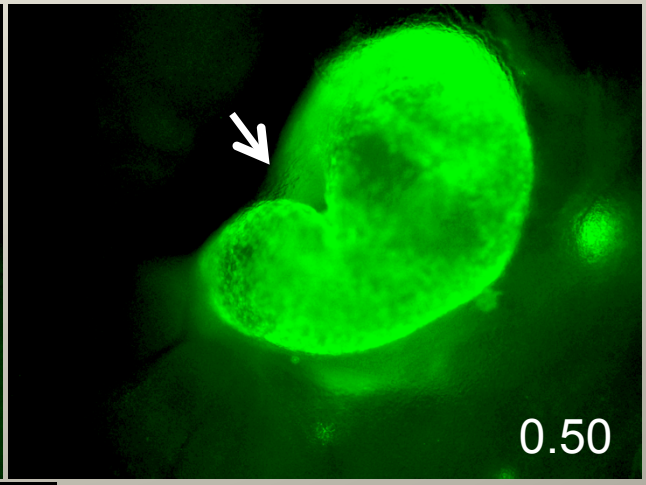
Brief description of ventricular wall formation

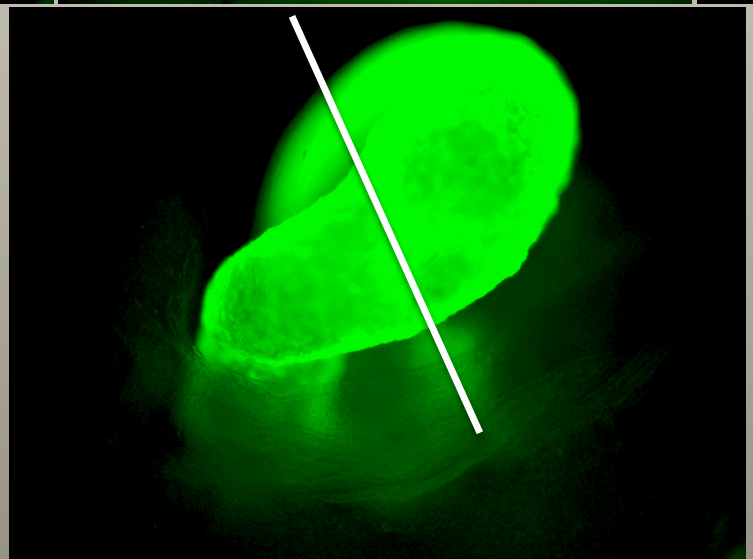
Exp 2: Results

VMHC Whole-mount Immunohistochemistry at HH20 shows Cardiomyopathies in Embryos Exposed to the PCB mix (0.08 or 0.50 µg/g egg wt SD)



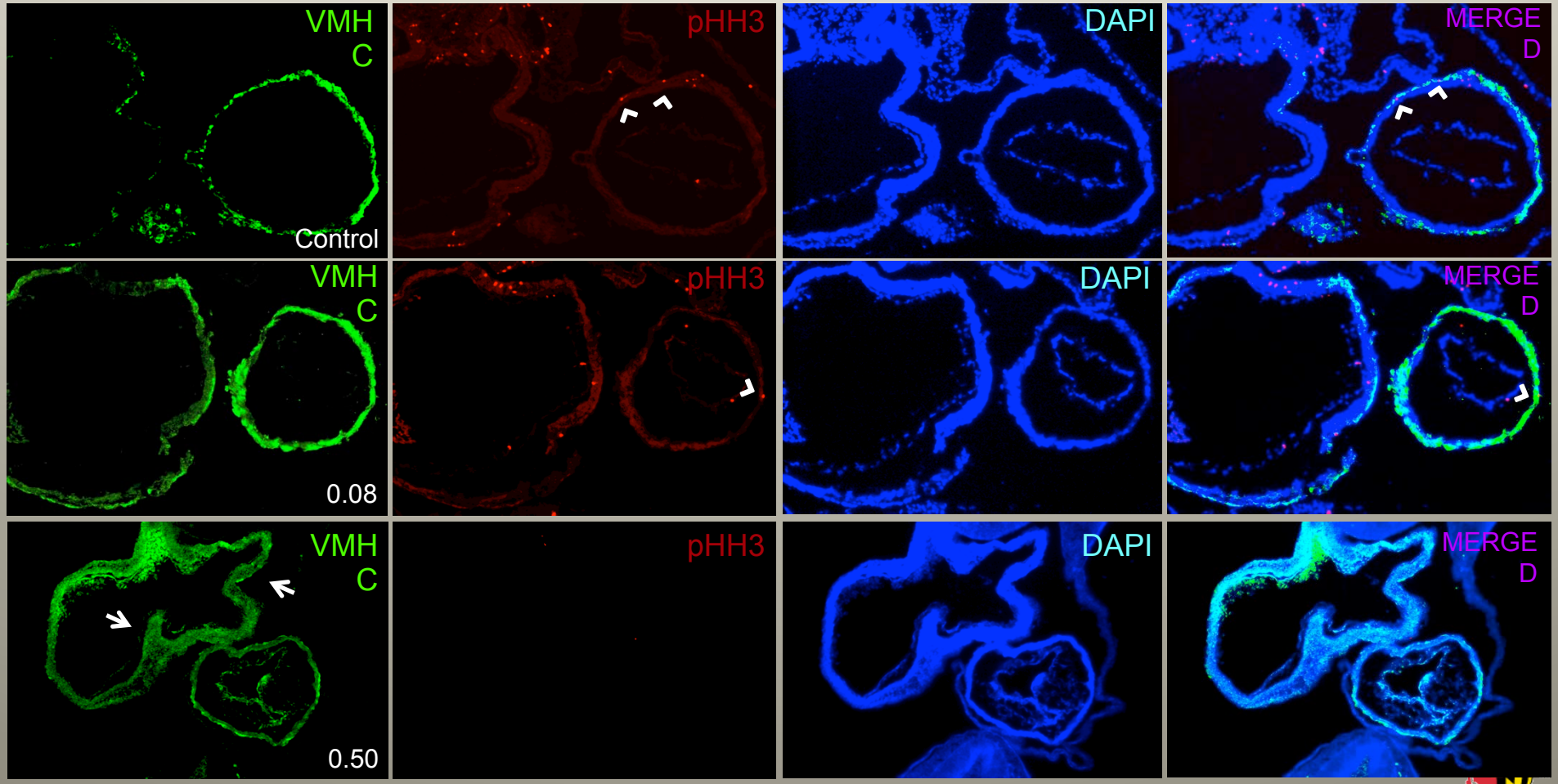






Overexposed for Projector

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



PCB mixtures:

- Based on profile of PCBs measured in spotted sandpiper or tree swallow eggs collected at the Upper Hudson River, NY in 2004
- 58 or 66 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; see http://www.fws.gov/contaminants/restorationplans/HudsonRiver/docs/Trustee_USGS_Avian_Egg_Injection_Studies_Dosing_Solutions_final.pdf http://www.fws.gov/contaminants/restorationplans/HudsonRiver/docs/58CongenerReportREVISED.pdf
- Contains 9 dioxin-like PCBs 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

Experimental Design

n=30/treatment group

Doses of PCBs (w/w) for Japanese quail egg injections.

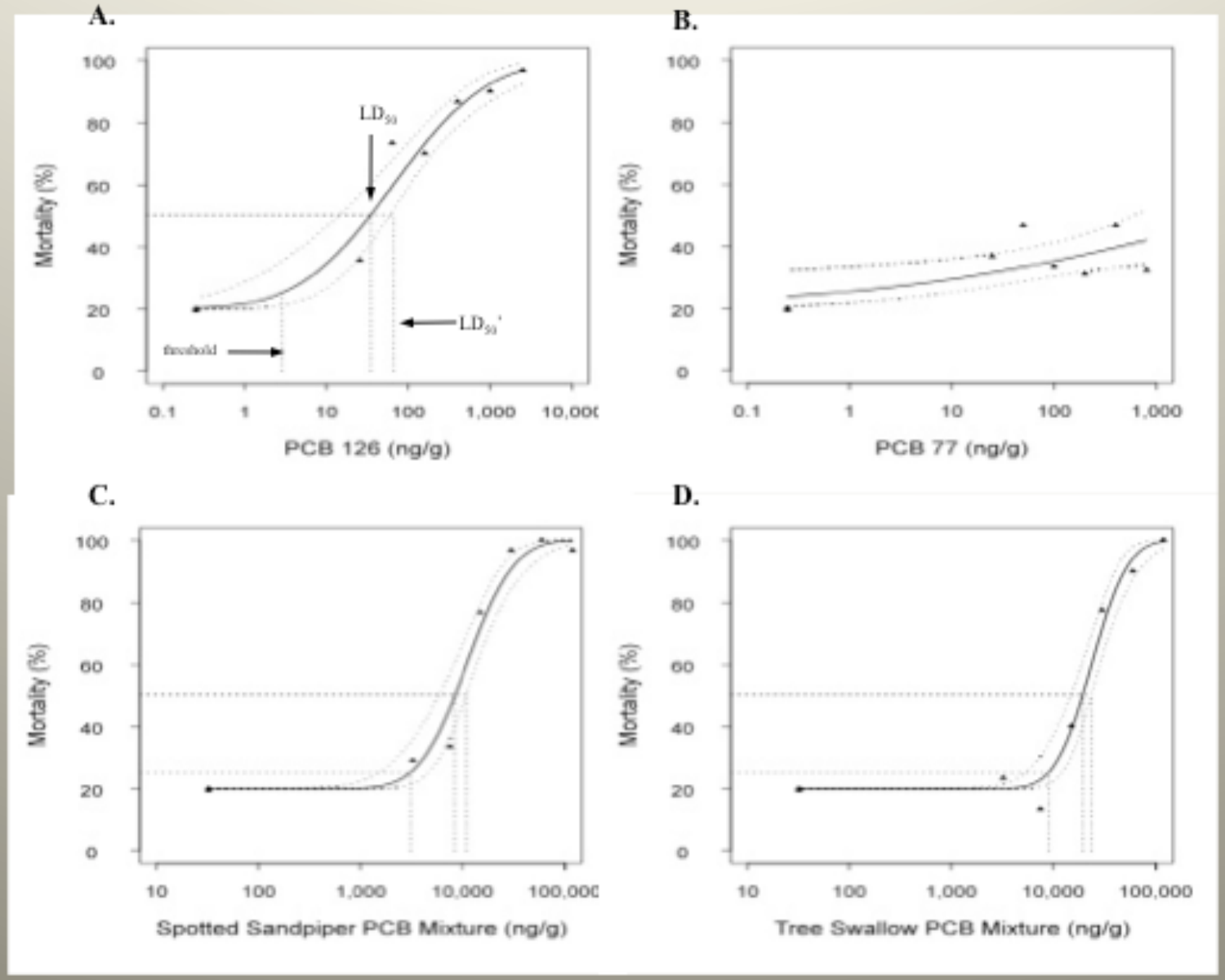
treatments			
untreated (UNT) sham (SHAM) vehicle (VEH; charcoal stripped corn oil)			
PCB 126 (ng/g)	PCB 77 (ng/g)	TRES* PCB mix (µg/g)	SPSA** PCB mix (µg/g)
2500	800	120	120
1000	400	60	60
400	200	30	30
160	100	15	15
64	50	7.5	7.5
25.6	25	3.75	3.75

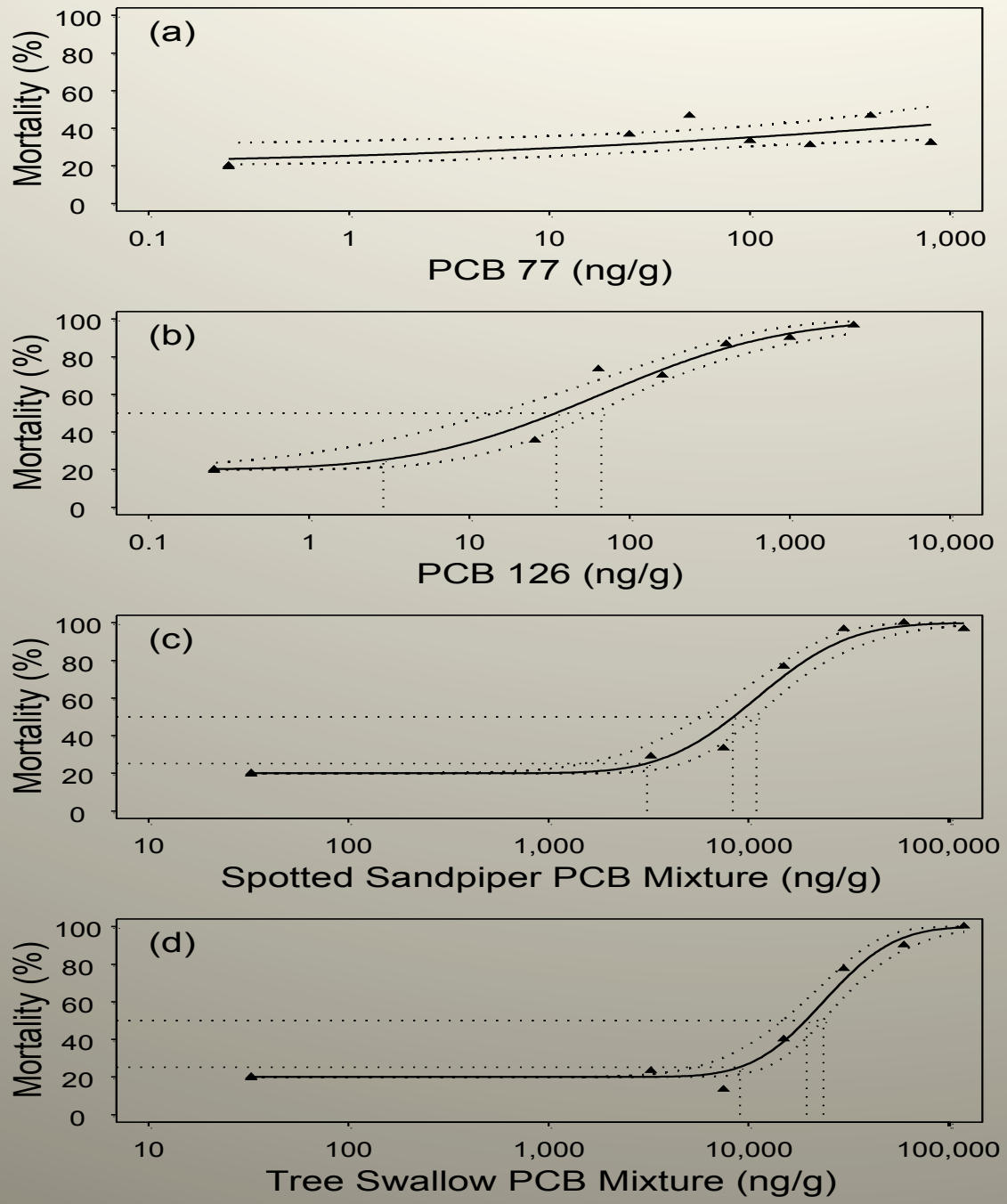
** TRES: tree swallow; **SPSA: spotted sandpiper*

Mortality During Development

Treatment (µg/g egg)	Timing of mortality					
	ED0-4	ED4-7	ED7-10	ED10-13	ED13-16	hatch
PCB126						
0.0256	10.00	16.67	20.00	23.33	26.67	36.67
0.064	6.67	16.67	43.33	70.00	70.00	73.33
0.16	3.33	46.67	66.67	66.67	66.67	70.00
0.40	6.67	60.00	86.67	86.67	86.67	86.67
1.0	13.33	60.00	86.67	90.00	90.00	90.00
2.5	0.00	63.33	93.33	93.33	96.67	96.67
PCB 77						
0.025	16.67	16.67	30.00	30.00	33.33	36.67
0.05	13.33	13.33	20.00	20.00	26.67	46.67
0.1	20.00	20.00	20.00	23.33	30.00	33.33
0.2	10.00	16.67	20.00	20.00	26.67	30.00
0.4	13.33	16.67	16.67	26.67	26.67	46.67
0.8	20.00	20.00	20.00	26.67	30.00	33.33
tree swallow mix						
3.75	6.67	6.67	6.67	16.67	20.00	26.67
7.5	3.33	10.00	10.00	13.33	13.33	13.33
15.0	10.00	10.00	23.33	26.67	26.67	40.00
30.0	3.33	16.67	53.33	63.33	73.33	80.00
60.0	10.00	26.67	66.67	76.67	80.00	90.00
120.0	6.67	36.67	90.00	96.67	100.00	100.00
spotted sandpiper mix						
3.75	10.00	13.33	13.33	16.67	20.00	26.67
7.5	10.00	16.67	26.67	30.00	33.33	33.33
15.0	13.33	20.00	46.67	53.33	56.67	76.67
30.0	13.33	46.67	86.67	93.33	93.33	96.67
60.0	6.67	40.00	96.67	100.00	100.00	100.00
120.0	13.33	40.00	96.67	96.67	96.67	96.67
controls						
UNT	13.33	13.33	13.33	13.33	13.33	20.00
SHAM	6.67	6.67	10.00	13.33	13.33	20.00
VEH	0.00	0.00	3.33	10.00	10.00	16.67

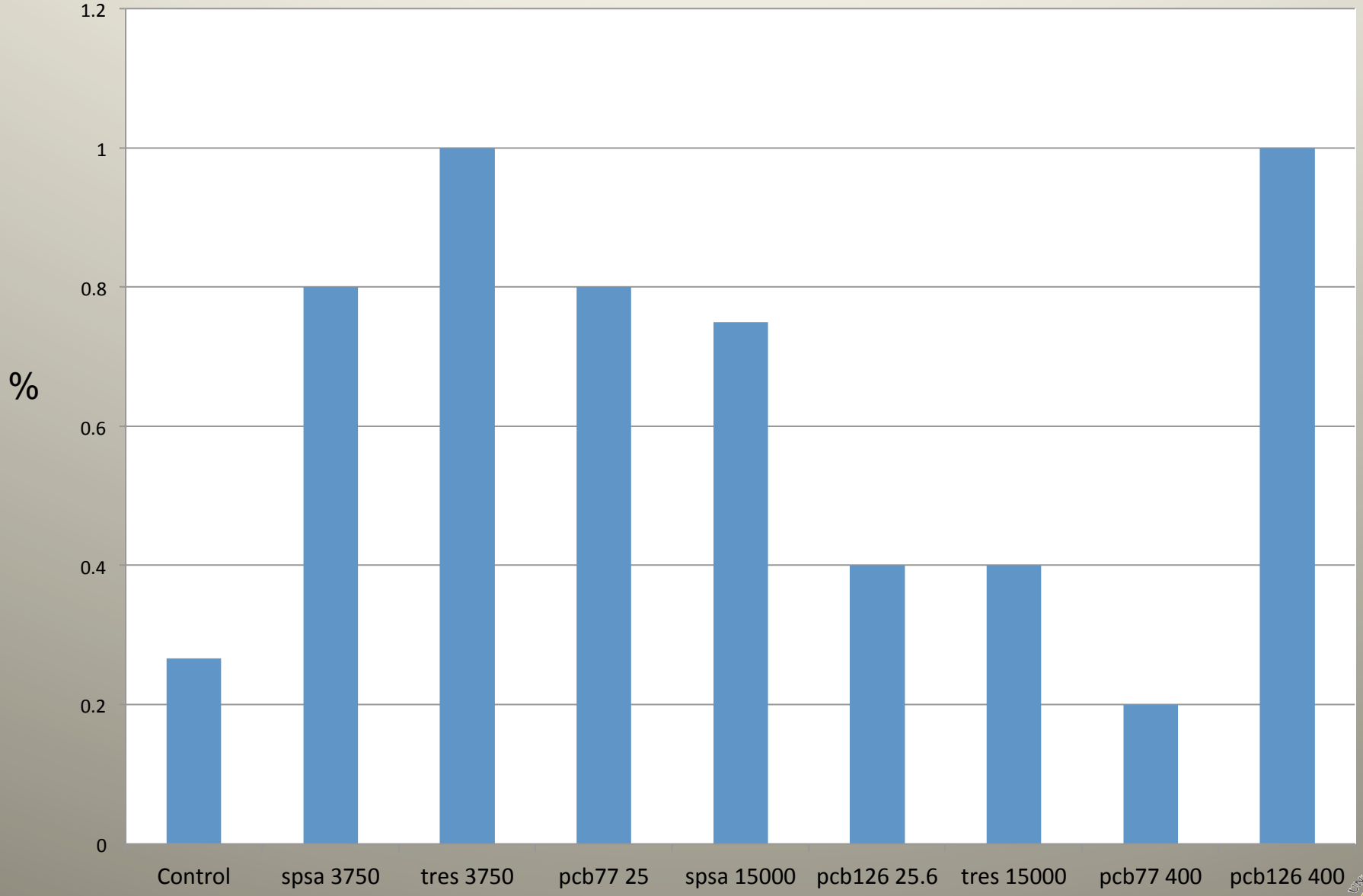
Lethality Dose-Response Curves from Japanese Quail Study



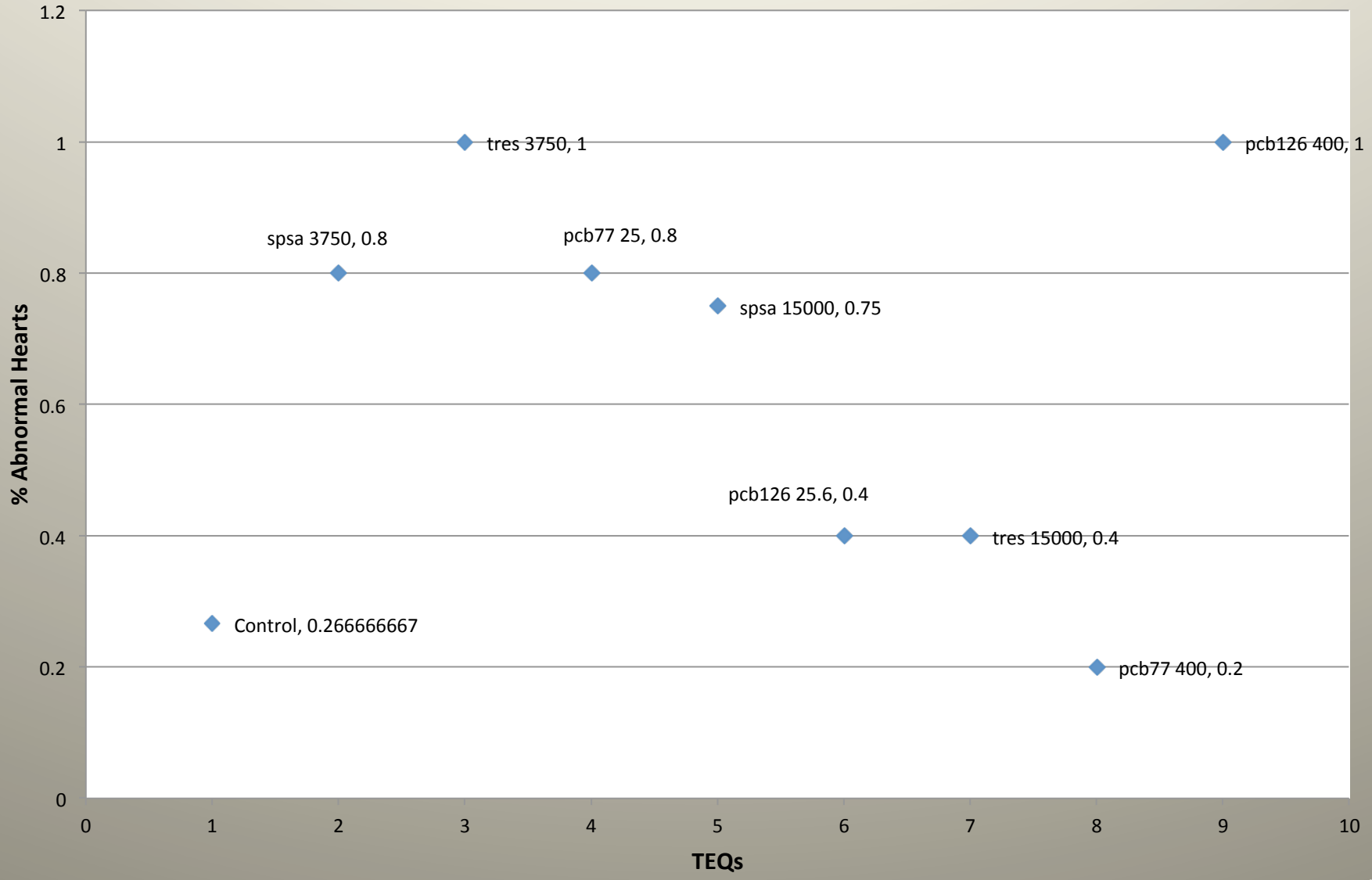


Lethality curves: Probit Analysis provide comparisons of embryonic effects of the PCBs, either separately or as mixes.

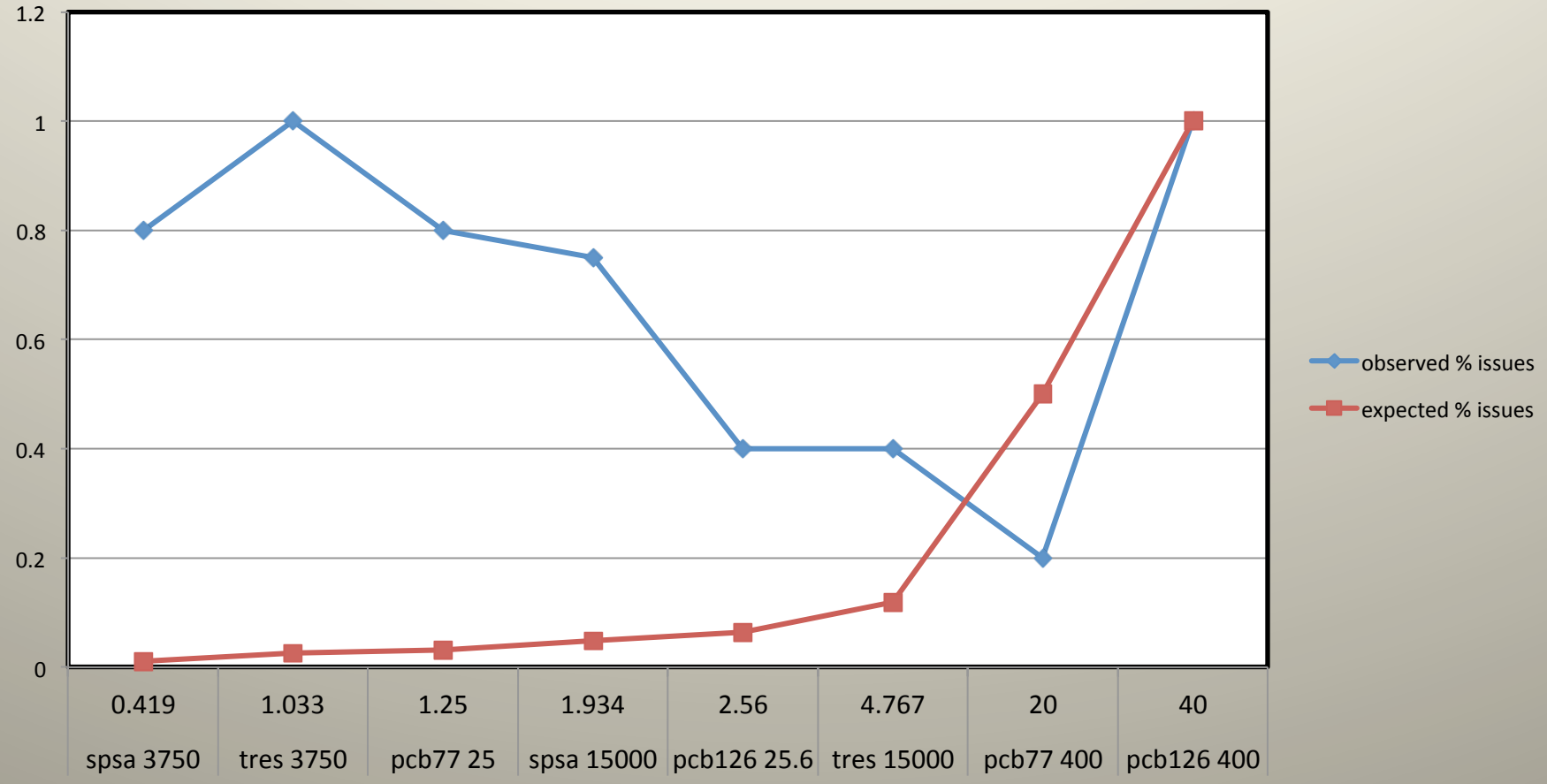
% of Birds Having Multiple Abnormalities



% Individuals with Multiple Abnormalities



Comparison of TEQ Expected versus Observed with Treatments



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