Effects of an environmentally relevant PCB mixture on embryonic heart development at HH20 in *Gallus domesticus* (Domestic Chicken)

T. Carro and M.A. Ottinger

Department of Animal and Avian Sciences
University of Maryland, College Park MD 20742 USA

**INTRODUCTION**

Polychlorinated biphenyls (PCBs) are synthetically made chemicals that consist of biphenyl rings with one to ten chlorine substitutions. A previous study in line eels demonstrated that embryonic exposure to a single PCB congener did not affect survivability, but did affect heart morphology in hatchlings. To examine whether PCBs adversely affected heart development in vivo, an environmentally relevant 58-congener PCB mixture was administered to broiler chicken embryos. This study showed that the PCB mixture decreased survivability, heart development, and produced a variety of cardiomyopathies in hatchling hearts. The effects noted in this hatchling study suggested a disruption of protein essential in heart muscle formation during embryonic heart development. To explore this possibility, chicken embryos were exposed to a low and high concentration of the PCB mixture and collected at three significant stages of chick heart development. Hearts were analyzed for abnormalities, proliferative and apoptotic changes, and spatio-temporal distribution of important heart proteins.

**SPECIFIC AIMS**

- Determine if PCB exposure affects heart morphology at critical stages of embryonic heart development in the domestic chick.
- Determine if PCB exposure affects rates of proliferation and apoptosis in the heart field at a critical stage of heart development.
- Determine if spatio-temporal expression of ventricular myosin heavy chain and Titin proteins are affected following PCB exposure in vivo.

**MATERIALS & METHODS**

Broiler chicken (Gallus domesticus) eggs were obtained from an Maryland eastern shore hatchery, cleaned, and transported to the University of Maryland, College Park. Eggs were sorted by weight and randomly assigned to treatment groups so that the range and mean of body weight was as close as possible between groups. Broiler chicken eggs were obtained from a hatchery located in Maryland along the Hudson River and used for egg injections. The eggs were randomly assigned to treatment groups so that the range and mean of body weight was as close as possible between groups. Treatment groups were untreated control, 0.0 µg/g egg wt, lowPCB dose (0.08 µg/g egg wt), and a highPCB dose (0.12 µg/g egg wt). The PCB mixture was administered by the air space using a micropipette. The air space was developed through a small hole drilled into the side of the egg. A micropipette was used to deposit the volume into the albumen; the hole was sealed with paraffin wax and the egg placed in an incubator set at 37°C, 55-65% humidity.

**RESULTS**

- Developmental exposure to an environmentally relevant 58-congener PCB mixture affects survivability in both the low dose and high dose treatment (Chi square: p<0.001, Figure 1).
- Exposure to the 58 congener PCB mixture increased heart abnormalities in the low dose treatment in the surviving embryos (Chi square: p<0.05, Figure 2).
- Proportion of cells decreased in the heart field in embryos exposed to the 58 congener PCB mixture, with a significant effect at the high dose (Tukey’s p<0.001, Figure 3).
- Exposure to the PCB mixture did not affect apoptosis of cells in the heart field.

**DISCUSSION**

- Exposure to the 58 congener PCB mixture affected survivability at both the low dose and high dose treatments (Figure 1). In a previous study using the same doses, survivability at hatch increased at the low dose treatment but was not significantly different associated with the small sample size (Carro, 2009).
- The results of this study demonstrated that there was an increase in heart abnormalities in both PCB treatment groups when compared to the controls at embryonic HH20, with a greater percent of abnormalities at the low dose (Figure 2). It can be assumed that the most severe cardiomyopathies will result in embryonic death, while chicks may survive less severe abnormalities (Kirby, 1995). This may account for the increased abnormalities seen in viable embryos at the low dose (HH20). Proliferation decreased significantly in the heart field at HH20 (Figures 3 & 5), while no difference in apoptosis was found. At HH20, proliferation of the cardiomyocytes is essential in creating proper heart wall morphology. This lack of proliferation might account for the abnormalities observed in hatchling hearts (Carro, 2009). No difference was observed in the spatio-temporal expression of VMHC and Titin proteins, two heart specific proteins essential in the proper formation of the heart at HH20. The analysis of these proteins did not show any differences in spatio-temporal expression; however potential follow-up should consider pHH3 expression or additional heart specific proteins (Figures 4 & 5). In conclusion, the major effect of PCB exposure was a decreased proliferation in the heart field.

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**REFERENCES**


**FIGURES**

Figure 1: Percent survivability of fertile broiler chicken embryos at HH20 (72hrs) of embryonic development.

Figure 2: Percent abnormalities in hearts of fertile broiler chicken embryos at HH20 (72hrs) of embryonic development.

Figure 3: Average number of proliferating cells in broiler chick embryonic heart field at HH20 (72 hours) of embryonic development.

Figure 4: Immunohistochemistry of HH20 chicken embryonic heart sections (14m). GFP-labeled Ventricular Myosin Heavy Chain (VMHC) protein, DS-Red-labeled anti-phosphohistoneH3 (pHH3), DAPI nuclear staining (blue).

Figure 5: Immunohistochemistry of HH20 chicken embryonic heart sections (14m). GFP-labeled Ventricular Myosin Heavy Chain (VMHC) protein, DS-Red-labeled anti-phosphohistoneH3 (pHH3), DAPI nuclear staining (blue).