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学 位 論 文 要 旨
Dissertation Summary

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論　文　名：Effects of exposure to tris (2-chloroethyl) phosphate (TCEP) on *ex ovo* chicken embryos
(Dissertation Title)

Tris (2-chloroethyl) phosphate (TCEP) is one of the pervasive organophosphorus flame retardants (OPFRs) that has been commercially used in polyurethane foam, textiles, and furniture to delay the spread of fire after ignition. TCEP has been detected in the atmosphere, dust, river water, and tissues and eggs of wild birds. However, there are few studies regarding the toxic effects of TCEP on avian embryos. *In* in ovo exposure test of avian embryogenesis, *in situ* observation has been difficult because avian embryos develop within non-transparent eggshells. A novel shell-less incubation system for chicken embryos that enables visualization *in situ* of the development of chicken embryos was developed in 2014. This study thus aimed to investigate the phenotypes and transcriptome effects of TCEP exposure in *ex ovo* chicken.

Fertilized chicken (*Gallus gallus domesticus*) eggs were treated with 50, 250, and 500 nmol/g egg of TCEP (TCEP-L, -M, -H, respectively) or DMSO (vehicle control) at the beginning of incubation.

Survival rates started to be greatly reduced from day 3 of incubation and were significantly decreased until day 9 in TCEP-M and TCEP-H groups. Morphological biometrics, the heart rate, and total extraembryonic blood vessel length were significantly decreased from days 3 to 9 in TCEP-exposed groups. These results suggested that TCEP exposure induced developmental delay and cardiovascular dysfunction in chicken embryos. In transcriptome analysis, the cardiac development transcription factor NKX2-5 and the cardiac conduction-related gene CACNA1G were downregulated in the day five heart. Gene expression of MYLs, a cardiac contraction-related gene, and RYR2, a calcium transport-related gene, was decreased in the day nine heart. It was suggested that the downregulation of these genes induced a decrease in heart rate. In vitelline membranes with reduced vessel length, genes associated with VEGF signaling were downregulated, which may

trigger vasculogenesis and angiogenesis inhibition. SNAI2, a transcription factor for epithelial-mesenchymal transition, and TBXT, a mesoderm marker gene, were downregulated during the gastrulation of chicken embryos by TCEP exposure. Therefore, it was suggested that TCEP inhibits mesoderm differentiation and causes cardiovascular failure in chicken embryos.

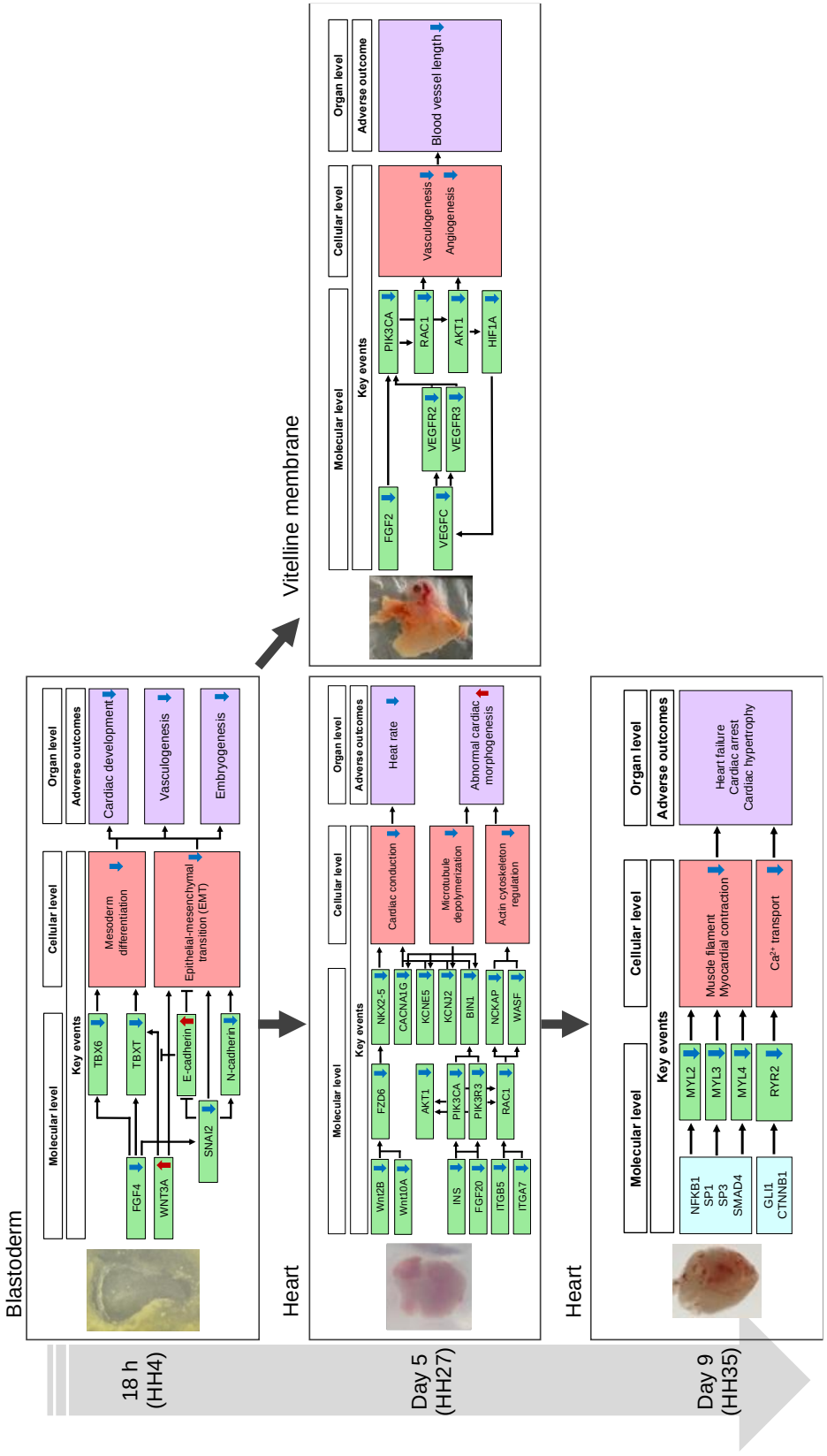

Fig. Proposed chronological adverse outcome pathways (AOPs) for the toxicity of TCEP in 18 h, day 5, and day 9 chicken embryos.