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INVESTIGATING STRESS AND IMMUNE STATUS OF JAPANESE QUAIL (COTURNIX JAPONICA) CHICKS EXPOSED TO EMERGING FLAME RETARDANTS

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Introduction

Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) and Dechlorane Plus (DP) are emerging flame retardants with increased detection in the environment and biota (1,2); however information on how exposure to these compounds affect avian wildlife is limited. Both compounds have been detected in bird eggs, indicating maternal transfer and exposure at vulnerable life stages (2,3). In ovo exposure to TDCIPP has been linked to disruption of steroid metabolism and compromised immune function (4,5), while early exposure to DP has showed no effects so far (6). Previous laboratory studies have focused on effects on poultry hatchlings and little attention has been devoted to later developmental stages. In this study, we have investigated possible dose-dependent and mixture effects of DP and TDCIPP on stress and immune status in Japanese quail (Coturnix japonica) chicks.

To evaluate the immune status of the chicks we chose to perform a phytohaemagglutinin (PHA) skin test. This is a sensitive, non-lethal variable measuring the proliferative response of circulating T lymphocytes when exposed to a mitogen (7). The swelling observed after injection is caused by accumulation of lymphocytes and macrophages at the injection site. To evaluate possible effects of chronic stress on immune responses, we measured the baseline concentration of corticosterone, the primary avian stress hormone (8).

Materials and methods

Fertilized Japanese quail eggs were injected into the yolk with vehiculum (lecithin, peanut oil and water) spiked with either DP (70:30, anti:syn), TDCIPP, or a mixture of DP and TDCIPP (MIX; 1:1). Three experimental doses were used, a low dose of 10 ng g-1 egg (LD), a middle-dose of 100 ng g-1 egg (MD) and a high-dose of 1000 ng g-1 egg (HD), resulting in 9 exposure groups. Injection volumes were 2 µg per gram egg. Two control groups were included, one with injection of vehiculum without contaminants, and one group without injection. Incubation started immediately after injection and lasted for 17 days. The eggs were incubated at 37.3 - 38°C and 60 - 50 % relative humidity. After incubation, the hatchlings (n = 101, divided into 11 groups) were transferred to home cages (max. 4 chicks per cage) and provided food and water ad libitum.

At the age of 10 days a PHA skin test was performed on all chicks according to Smits et al. (7). At the age of 14 days the chicks were killed by decapitation. Plasma samples were stored frozen in cryovials until corticosterone (CORT) analyses were performed according to Weisser et al. (9).

Statistical analyses were performed using RStudio. The CORT variable was ln transformed prior to analyses to obtain normality. A t-test revealed no significant difference between the non-injected and injected control groups for both CORT ($t = 0.73$, $p = 0.48$) and PHA ($t = -1.15$, $p = 0.27$), and the controls were pooled. PHA response was analyzed by linear models with exposure group and CORT as explanatory variables. CORT response was analyzed similarly. The most parsimonious models were selected based upon Akaikes information criterion with a correction for finite sample sizes (AICc) and models with ΔAICc > 2 are presented.

Results and discussion

All of the groups showed large variation in plasma concentration of CORT (Figure 1). The best model (ΔAICc = 0) in explaining this variation included only exposure group and accounted for 14 % of the total variation. The analysis of variance showed a significant difference between the groups ($F = 2.4$, p

= 0.02), however post hoc analyses revealed only a significant difference between MD TDCIPP and LD DP (diff. = -0.7, p = 0.04). The post hoc analyses revealed that the CORT concentrations measured in the exposure groups were not significantly different from the pooled controls.

Great variation within groups was also detected for the swelling response (Figure 2). The best model (ΔAICc = 0) in explaining this variation included exposure group and CORT, however it did not explain the variation well (adj.R2 = -0.04). The analysis of variance showed no significant differences between the groups (F = 0.7, p = 0.7) and no effect of CORT on the swelling response (F = 0.2, p = 0.6).

The large, but similar, variation within the groups, possibly due to the small sample size, made it hard to detect any significant differences in response to the PHA test or the corticosterone concentrations between the control and the exposed quail chicks. However, when investigating Figure 1, there is a slight, but non-significant, trend of higher corticosterone concentrations in the DP exposed chicks compared to the control and TDCIPP exposed chicks. This is interesting, as no studies have previously investigated an effect of DP on stress hormones.

A previous in ovo study with exposure to TDCIPP found dysregulation in genes related to immune function in chicken hatchlings (4). However, when challenging the immune system of the exposed quail chicks in this study, no effects were found when comparing control and exposure groups. This can indicate species differences, non-expression of the dysregulated genes or other confounding factors correcting the possible immune compromise. There was also no significant effect of corticosterone on the response to the PHA test, describing the possible variation in immune response caused by stress.
To summarize, in ovo exposure to TDCIPP and/or DP did not affect the PHA skin test response or plasma corticosterone concentrations when compared to controls. These results suggest that the selected flame retardants, at the studied concentrations, may not influence immune status by disruption of T-cell proliferation or suppression of immune function by chronic stress in nestling Japanese quails.

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References
1. Feo, M.L., Barón, E., Eljarrat, E. and Barceló, D. (2012) Anal Bioanal Chem; 404(9):2625–37.
2. Chen, D., Letcher, R.J. and Chu, S. (2012) J Chromatogr; 1220:169–74.
3. Barón, E., Máñez, M., Andreu, A.C., Sergio, F., Hiraldo, F., Eljarrat, E., et al. (2014) Environ; 68:118–26.
4. Farhat, A., Buick, J.K., Williams, A., Yauk, C.L., O'Brien, J.M., Crump, D., et al. (2014) Toxicol Appl Pharmacol; 275(2):104–12.
5. Farhat, A., Crump, D., Chiu, S., Williams, K.L., Letcher, R.J., Gauthier, L.T., et al. (2013) Toxicol Sci; 134(1):92–102.
6. Crump, D., Chiu, S., Gauthier, L.T., Hickey, N.J., Letcher, R.J. and Kennedy, S.W. (2011) Comp Biochem Physiol C Toxicol Pharmacol; 154(2):129–34.
7. Smits, J.E., Bortolotti, G.R. and Tella, J.L. (1999) Funct Ecol.;13:567–72.
8. Boonstra, R. (2004) Integr Comp Biol.; 108(44):95–108.
9. Weisser, J.J., Hansen, C.H., Poulsen, R.,Larsen, L.W., Cornett, C. and Styrishave, B. (2016) Anal Bioanal Chem; 1–13.

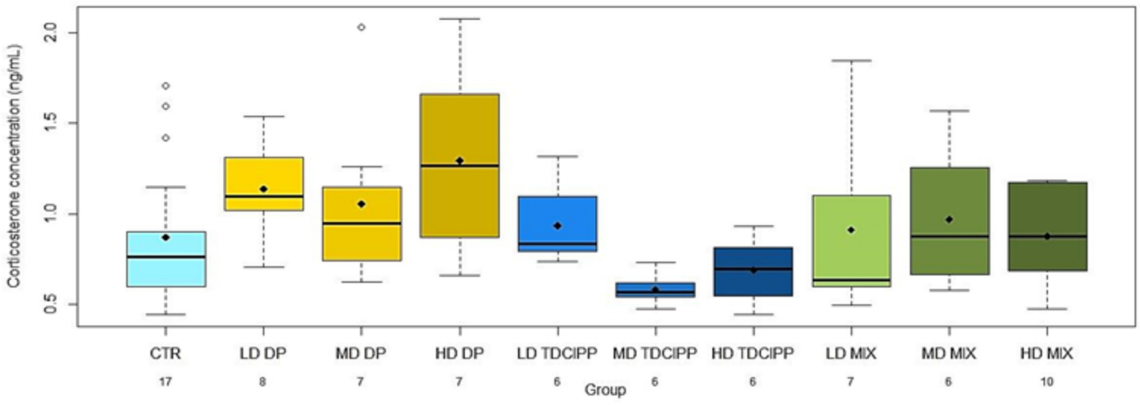

Figure 1: Boxplot showing the corticosterone concentration (ng/mL plasma) in the control and exposure groups. Sample sizes are indicated with numbers underneath the groups. The boxes represent the 25th-75th percentile, the whiskers represent the interquartile range, the lines are the median and the diamonds are the mean. Outliers are plotted individually, but included in the statistical analyses.

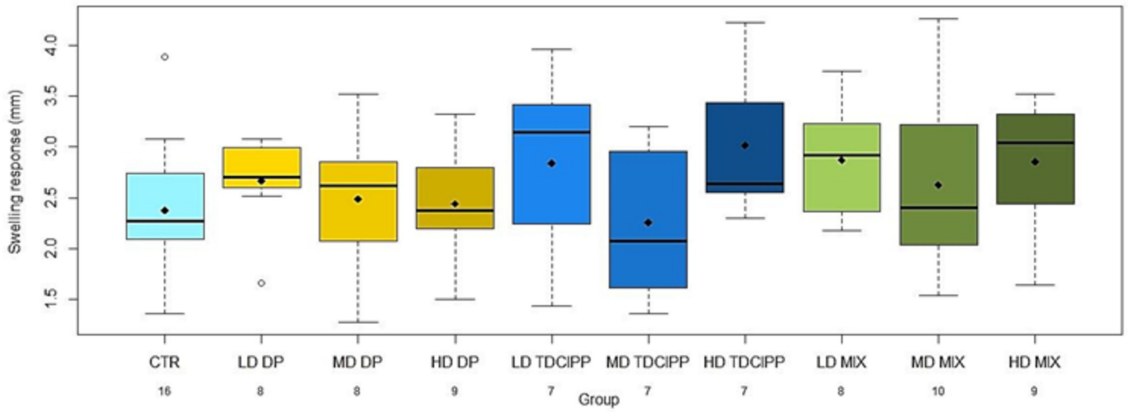

Figure 2: Boxplot of swelling response (mm) of the patagia within the control and exposure groups after the PHA skin test. Sample sizes are indicated with numbers underneath the groups. The boxes represent the 25th-75th percentile, the whiskers represent the interquartile range, the lines are the median and the diamonds are the mean. Outliers are plotted individually, but included in the statistical analyses.