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IN OVO TRANSFORMATION OF TWO EMERGING FLAME RETARDANTS IN JAPANESE QUAIL (COTURNIX JAPONICA)

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Introduction

Flame retardants (FRs) are commonly found in consumer products to reduce their flammability and risk of fire. Strict regulations on the use of FRs in electrical and electronical equipment have forced industries to switch to new and unrestricted alternatives, two of which are dechlorane plus (DP) and tris(1,3-dichloro-2-propyl)phosphate (TDCIPP). Both compounds are additive and thus not chemically bound to the product, making them very susceptible to leakage into the environment. Detection of these FRs in the environment has increased in the recent decade (1), including detections in bird eggs (2–4). This provides evidence for maternal transfer and raises questions on the metabolizing capacity of avian embryos.

Until now, information on exposure and metabolism of DP and TDCIPP during the embryonic development of birds is very scarce (5) and has mainly been investigated in cell cultures (7,10). This study shows the metabolic process of these two FRs in avian embryos during the course of the development, by using Japanese quails as a model organism.

Material and methods

Japanese quail eggs were injected with DP (syn- and anti-isomer, 30:70) or TDCIPP. A dose of 1000 ng/g egg of the compound was injected into the yolk sac using a vehiculum (6) of water and peanut oil, emulsified with lecithin. Eggs were injected at embryonic day (ED) zero. A 0.6 mm hole was made in the shell with a sterile dentist drill, without damaging the outer shell membrane. The solution was then injected using a Hamilton syringe and disposable needles (0.5 x 25 mm). Eggs were incubated for 17 days at 37.5 °C and 50 – 70 % humidity and were turned continuously by an automatic egg-turning device.

Each day one egg per treatment was taken randomly from the incubator and stored at -20 ºC until analysis. When opening the eggs for analysis, eggs treated with TDCIPP were checked for the developmental stage of the embryo. This determination was not done for eggs treated with DP. Chemical analysis was performed by gas chromatography-mass spectrometry for DP and by liquid chromatography-tandem mass spectrometry for TDCIPP and its metabolite bis(1,3-dichloro-2-propyl)phosphate (BDCIPP).

Results and discussion

TDCIPP and its metabolite BDCIPP

During embryonic development, concentrations of TDCIPP (median: 617 ng/g egg) decreased from 1085 ng/g egg, with a maximal rate of 6.78 ng/g egg per hour at ED10 (251 hours, Fig. 1). At the end of the incubation period (ED16), TDCIPP was no longer quantifiable. The rapid transformation of this compound in Japanese quail embryos is in accordance with a previous study in chicken embryos, in which TDCIPP was almost completely depleted by ED19 (5).

Concentrations of BDCIPP (median: 102 ng/g egg) increased with a maximal rate of 0.97 ng/g egg per hour at ED9 (214 hours, Fig. 1) to 205 ng/g egg at ED14, indicating that TDCIPP was transformed into BDCIPP, but not at the same rate.

A complete (1:1) conversion of TDCIPP to BDCIPP within 36 hours was previously found in chicken embryonic hepatocytes (CEH) (7). At the initiation of embryonic development (until ED6) and in the undeveloped egg, the conversion of TDCIPP to BDCIPP was close to 1:1. When the embryo became

observable (ED6), this was no longer the case, suggesting that BDCIPP was further transformed after ED6 (148 hours). After ED14, the concentration of BDCIPP found in the most developed egg was 75.6 ng/g egg. This sudden drop in concentration could indicate a second dechlorination reaction in the last embryonic stage, leading to a metabolite that was not targeted for analysis.

Formation of BDCIPP prior to ED6 indicates chemical hydrolysis in addition to metabolism (Fig. 1), in contrast to what was previously found in CEHs (7). However, the transformation of TDCIPP to BDCIPP was shown to be more extensive when an embryo became observable.

BDCIPP was also found to be the major metabolite of TDCIPP in urine of rats (8). To a lesser extent, mono(1,3-dichloro-2-propyl)phosphate (MDCIPP) and 1,3-dichloro-2-propanol were also identified (8). Also in human liver fractions, BDCIPP was the major metabolite, followed by the glutathione conjugate of TDCIPP (9). Other detected metabolites were hydroxylated forms of both TDCIPP and BDCIPP and hydrocarboxylated TDCIPP, resulting from phase I metabolism (9).

Dechlorane plus

Concentrations of DP were found to be stable around a median of 951.6 (min - max: 507 - 1249) ng/g egg during the entire embryonic development (Fig. 2). The absence of a transformation process is in accordance with previous in vitro and in vivo studies on birds (10,11). However, degradation products of DP have been detected in the environment, including wildlife and bird eggs (1,2,12), suggesting bioaccumulation and maternal transfer of these metabolites. These have been identified as the monoadduct of DP (DPMA) and two dechlorinated products, decachloropentacyclooctadecadiene (aCl10DP) and undecachloropentacyclooctadecadiene (aCl11DP). These latter were also detected in our samples, but not confirmed.

Conclusions

This experiment confirms that TDCIPP is rapidly metabolized by the developing avian embryo to BDCIPP and other metabolites. By ED16, TDCIPP was no longer quantifiable. Even though the transformation was partly explained by chemical degradation, it is clear that the major part of the transformation occurred when an embryo was observed. Embryos were not able to metabolize DP, as the concentration during incubation stayed around the injected dose (1000 ng/g egg). The presence of DP during the entire embryonic development, as well as the metabolites of TDCIPP, warrants further investigation on potential effects in birds.

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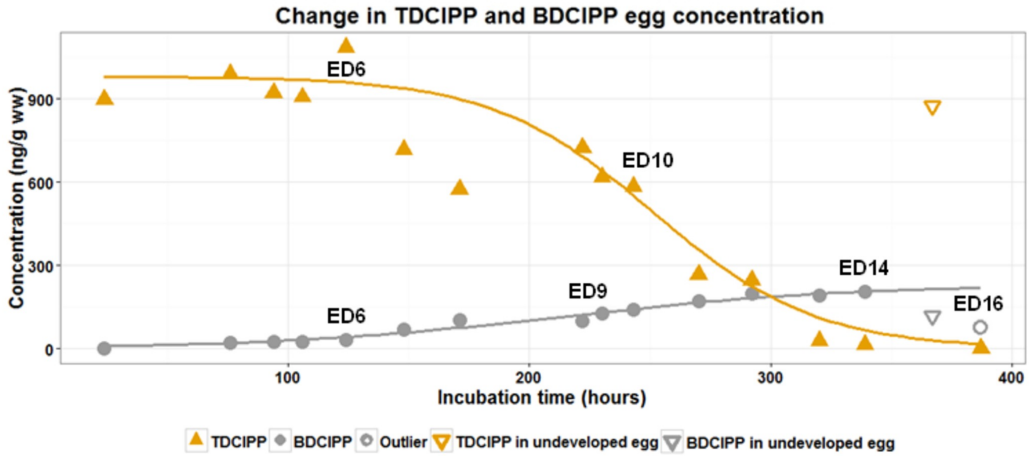

Figure 1: Metabolization of TDCIPP (median: 617 ng/g egg, range: 1 - 1085 ng/g egg) and formation of BDCIPP (median: 102 ng/g egg, range: 2 - 205 ng/g egg) in Japanese quail eggs during 16 days of incubation. The open triangles represent concentrations in an undeveloped egg. The open circle represents BDCIPP concentration in an embryo in the last stage of development (ED16). These outliers were not included in the regression lines.

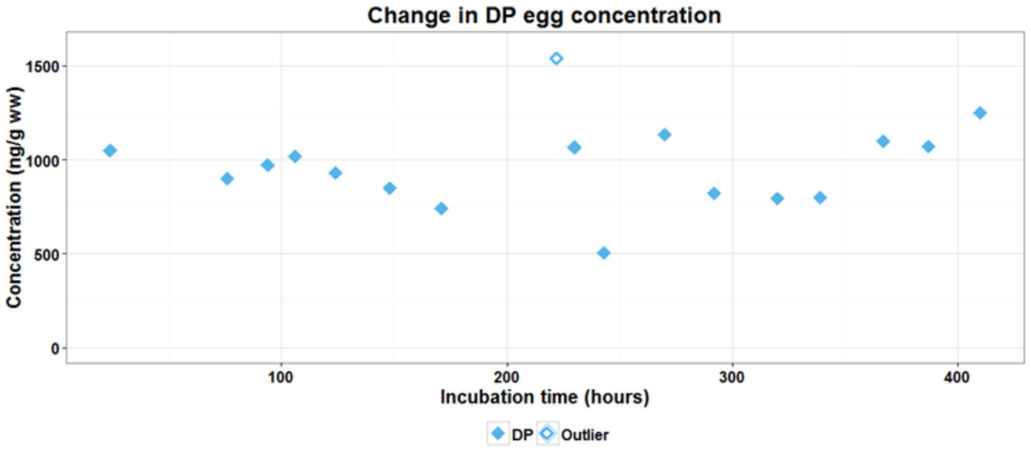

Figure 2: DP concentration (median: 952 ng/g egg, range: 507 - 1249 ng/g egg) in Japanese quail eggs during 17 days (410 hours) of incubation. One outlier was determined based on boxplot and QQ-plot.