Triclosan-induced embryotoxicity in the yellow-legged gull

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1. Introduction

Triclosan (TCS) is a chemical compound extensively used as synthetic and antimicrobial agent in a wide range of personal care products for more than 40 years [1]. After human use, TCS enters the sewage system, where it is treated in wastewater treatment plants. Because of its hydrophobic nature, TCS is retained in sewage sludge and may be transfered to agricultural soils [2]. Therefore, the wastewater effluent discharges to surface waters, together with the application of biosolids in agriculture, contributes to the contamination of aquatic and terrestrial ecosystems [3]. Despite there is evidence showing TCS accumulation in some vegetal and animal species [2,4], information on the presence and the toxicity of this compound in birds is very limited. Seabirds are highly exposed to environmental contamination because of their ecological habits, high trophic position in the food webs, and relative long life-span [5]. Since their eggs incorporate maternal lipophilic pollutants [5], seabirds have been proposed as a non-invasive tool to assess the levels of environmental contaminants and their potential adverse effects on the offspring. To date, information on the contamination of bird eggs by emerging pollutants, such as TCS, and their toxicity to developing embryos is completely lacking. Thus, the aim of the present study was to explore, through *in ovo* injection, the potential embryotoxicity of TCS in the yellow-legged gull (*Larus michahellis*). We injected 150 ng/g egg weight of TCS into the egg yolk. To check for the reliability of the injection method, we quantified the TCS concentration in the yolk of unincubated eggs and, to assess its transfer to the embryo from incubated eggs, we measured TCS in residual yolk and in the liver and brain. The effects on embryo morphology, oxidative stress and genetic damage in embryo liver were investigated. In detail, the amount of pro-oxidant species (i.e. ROS), activity of defense enzymes (SOD, CAT, GST), the levels of lipid peroxidation (LPO) were assessed as biomarkers of oxidative stress, and the levels of DNA fragmentation as genetic damage endpoint.

2. Materials and methods

We performed a field study in a large breeding colony on an island in the Comacchio lagoon (NE Italy). We injected 150 ng TCS/g egg weight into the egg yolk according to a within-clutch design, i.e. both control and TCS-treated groups of eggs were established within the same clutch. We arbitrarily decided the dose to be injected because no data of its presence in the yellow-legged gull eggs are currently available. To verify that the injection procedure was correctly performed and that the amount of TCS injected was as close as possible to the nominal concentration, 15 unincubated eggs from 5 nests were collected on the same day they were laid and injected. Eggs from other 20 nests were instead collected when any sign of imminent hatching occurred (i.e. 'cracking stage'). The eggs were frozen at -20 °C until the dissection of embryos and the analysis of the effects of TCS on morphological and biochemical endpoints were performed.

TCS concentration in the yolk from unincubated eggs, residual yolk sac, embryo liver and brain were measured by using gas chromatography (GC) coupled to mass spectrometry (MS) in electron capture negative ionization mode (ECNI). The extraction of TCS from yolk, liver and brain samples was performed according to Poma et al. (2016) [6], with slight modifications.

Body mass, tarsus length, head size, and liver and brain weight were used as morphometric endpoints. Biochemical effects were investigated by a suite of biomarkers performed on liver homogenates. ROS levels were measured by using a dichlorofluorescein-diacetate (DCFH-DA) fluorimetric method. The activity of SOD, CAT and GST was determined through a spectrophotometric method. Lipid peroxidation was measured according to the TBARS method, while DNA fragmentation was assessed by the DNA precipitation assay. Detailed methods of all biomarkers are reported in Parolini et al. (2013) [7].

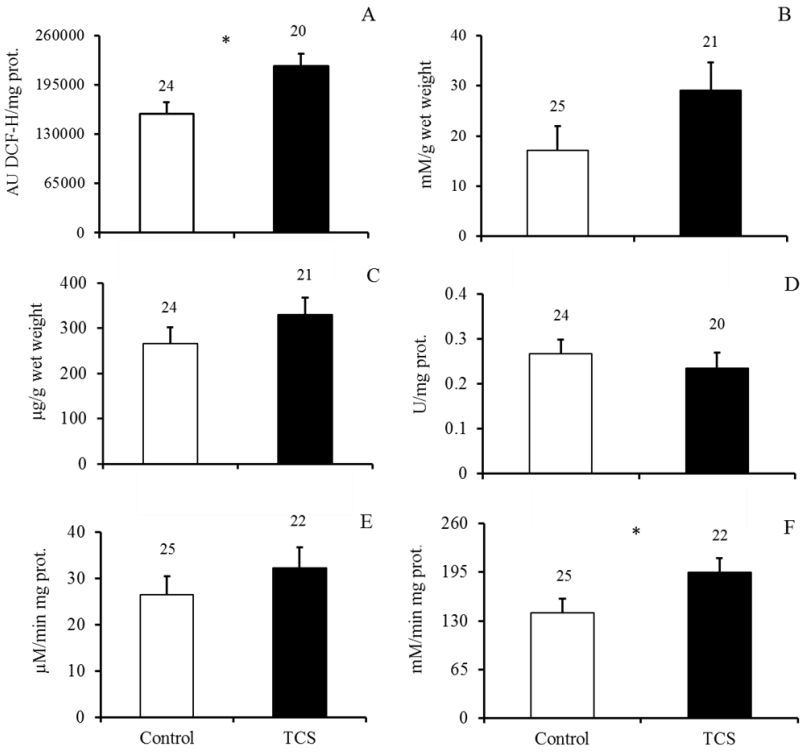

Figure 1 Estimated marginal means (+ SE) of: A) the amount of oxidant species (ROS), B) levels of lipid peroxidation (LPO), C) levels of DNA fragmentation, D) SOD, E) CAT and F) GST enzyme activities of embryos. Sample sizes are reported. Significant differences between TCS-treated and control embryos are indicated by the asterisk (*P<0.05).

The effect of TCS injection on embryo morphology and biomarkers of oxidative stress and genetic damage was analysed in linear mixed models, including egg treatment, embryo sex and egg laying order as fixed factors, as well as their two-way interaction terms. Clutch identity was included in the models as a random effect. When the interaction terms were non-significant, they were removed from the model in a single step. In the model of embryo morphological traits, we included the mass of the egg at the time of laying as a covariate. All statistical analyses were performed using SAS 9.3 PROC MIXED.

3. Results and discussion

The mean (±SD) TCS concentration in the yolk from unincubated eggs was 158.9 ± 35.3 ng/g wet weight and it was close to the nominal injected concentration, confirming the reliability of our treatment. A limited amount of TCS was found in residual yolk sac of eggs soon before hatching (2.9 ± 1.1 ng/g wet weight), suggesting that this compound was transferred to the embryo during early development. Yet, TCS was detected only in low concentrations in embryo liver (2.3 ± 1.1 ng/g wet weight) and brain (0.2 ± 0.1 ng/g wet weight). TCS treatment did not significantly affect embryo morphological traits, with the exception of a marginally non-significant effect ($F_{1,23}$=4.15, P=0.053) on head size. However, TCS injection induced notable biochemical effects on embryos. A significant activation of GST was found in the liver of treated embryos compared to controls, suggesting that phase II enzymes act to detoxify and excrete this lipophilic chemical. The intense metabolic activity caused by TCS detoxification processes significantly increased the hepatic ROS levels in embryos from TCS-injected eggs with respect to controls ($F_{1,23}$=10.50, P=0.004). The lack of activation of SOD and CAT (P>0.342 in both cases) suggested that the embryo cannot efficiently counterbalance the increase of ROS, leading to a disproportion of the oxidative status and to an oxidative stress situation. Thus, the excess of TCS-induced ROS caused an increase, even if marginally non-significant, of both lipid peroxidation ($F_{1,31}$=4.03, P=0.053) and DNA fragmentation ($F_{1,24}$=3.73, P=0.066) in liver from embryos developd in treated eggs compared to controls (Figure 1).

4. Conclusions

The present study showed, for the first time in any wild bird species, that TCS can negatively affect the phenotype of bird offspring, inducing an oxidative stress situation that lead to the onset of oxidative and genetic damage. Our findings suggest that TCS might be a threath for birds because early-life effects can result in serious consequences to diverse life-history traits. For this reason, further studies are necessary to shed light on the toxicity and long-term consequences due to TCS exposure in birds.

5. References

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