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Evidence of environmental transfer of tebuconazole to the eggs in the house sparrow (*Passer domesticus*): An experimental study

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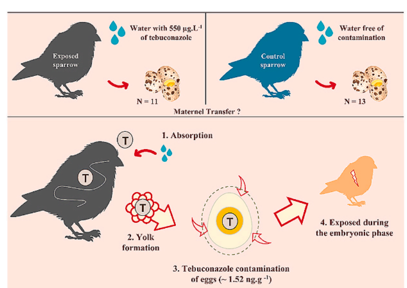
HIGHLIGHTS

- We exposed adult house sparrows to environmental concentrations of tebuconazole and collected their eggs.
- Tebuconazole was found in the eggs of exposed sparrows, demonstrating egg contamination.
- This contamination represent an early exposure route during chick embryonic development.

GRAPHICAL ABSTRACT



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ABSTRACT

Triazole compounds are among the most widely used fungicides in agroecosystems to protect crops from potential fungal diseases. Many farmland birds spend a significant part of their life cycle in agroecosystems, which may chronically expose them to pesticides. We experimentally tested whether exposure to environmental concentrations of tebuconazole could induce a contamination of the eggs in an agroecosystem sentinel species, the house sparrow (*Passer domesticus*). Wild-caught adult sparrows were maintained in captivity and exposed (exposed group) or not (control group) for seven months to tebuconazole through drinking water. Eggs were opportunistically collected for the determination of tebuconazole concentration by Liquid Chromatography coupled to tandem Mass Spectrometry in eggs. We found that eggs from exposed parents all contained tebuconazole with a mean concentration of 1.52 ng g^{-1} dry weight. In eggs from control parents, the tebuconazole concentration was below the limit of quantification (0.23 ng g^{-1} dry weight) for 11 out of 13 eggs. Thus, our study demonstrates for the first time that environmental exposure of female birds to tebuconazole can translate into egg contamination by this fungicide.

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1. Introduction

Since the 1960s, the intensification of agricultural practices has been identified as a major cause of decline for birds, especially farmland species (Donald et al., 2001; Stanton et al., 2018). In addition to habitat loss and fragmentation, the massive use of pesticides has also been suggested to be involved in these population declines (*e.g.,* reviewed by Stanton et al., 2018). Indeed, about 2 million tonnes of pesticides are used each year worldwide to protect crops from potential diseases and pests (Ali et al., 2021) and they are commonly detected in wildlife, including birds (De Souza et al., 2020; Montaigu and Goulson, 2020). This contamination can result either from a direct exposure of tissues to the pesticides (*i.e.,* inhalation or *trans*-cutaneous absorption, Vyas et al., 2007) or from an oral exposure through the consumption of water, seeds, or any other contaminated food (Lopez-Antia et al., 2016; Fernández-Vizcaíno et al., 2022). Due to these multiple routes of exposure, pesticides are detectable in the blood, organs, feathers, and faeces of birds (Van Drooge et al., 2008; Abbasi et al., 2016; Mateo et al., 2016; Rial-Berriel et al., 2020; Esther et al., 2022) indicating a contamination of the organisms.

Since, many farmland birds spend a significant part of their life cycle in agroecosystems, they may be chronically exposed to pesticides (Bro et al., 2015). Such a chronic exposure to pesticides can affect reproduction (*e.g.,* Lopez-Antia et al., 2013) and potentially the development of the progeny (Garcês et al., 2020; Ortiz-Santaliestra et al., 2020), especially if the embryo is exposed to pesticides. Indeed, a few field studies have reported the presence of pesticides in eggs, demonstrating a potential exposure for the embryos (Bro et al., 2015, 2016; Corcellas et al., 2017; Venugopal et al., 2020). Although some pesticides seem to be able to penetrate the eggshell and chorionic membrane (Bro et al., 2015), they can also be transferred from the mother to the egg during vitellogenesis (*e.g.,* persistent organic pollutants, Verreault et al., 2006; Kitulagodage et al., 2011; Ruuskanen et al., 2020; Jouanneau et al., 2021).

Among pesticides, the family of triazole fungicides is of primary concern because of its ubiquitous use in agroecosystems (Ribas e Ribas et al., 2016). For example, tebuconazole is the most widely used triazole fungicide in the world and it is detected in the majority of environmental compartments, such as air (Désert et al., 2018), soil (Kalogridi et al., 2014), and water (Kahle et al., 2008). Importantly, it has been convincingly demonstrated that tebuconazole is the most frequently ingested pesticide by farmland bird species (Lopez-Antia et al., 2016; Fernández-Vizcaíno et al., 2022). In addition, this fungicide is highly suspected to affect reproductive output (Lopez-Antia et al., 2021), to disrupt physiological systems (Bellot et al., 2022), and to act as an endocrine disruptor during development (Robinson et al., 2012; Li et al., 2019), including in birds (Fernández-Vizcaíno et al., 2020). However, and surprisingly, very little data is currently available regarding the potential contamination of eggs by triazoles in agroecosystems.

A recent avian study showed that tebuconazole is rapidly absorbed, distributed to the different tissues (liver, brain, kidneys, and muscles), metabolised, and then excreted (Gross et al., 2020). Because of the short half-life of tebuconazole in tissues (Konwick et al., 2006; Gross et al., 2020), the bioaccumulation and biomagnification risk of tebuconazole, and therefore the possibility of transfer of tebuconazole to the egg, are estimated to be low. However, some authors suggest that contamination of eggs could indeed occur because some contaminants would not be retained egg by the mother and might therefore be easily transferred to the egg during vitellogenesis (Kadokami et al., 2004; Verreault et al., 2006). A past report from the Food and Agriculture Organization (FAO) suggests that tebuconazole may be transferred to eggs because radio-labeled tebuconazole was found in multiple tissues, including eggs, after oral administration in laying hens (Ecker and Weber, 1991; Unpublished; Beedle and Ying, 2002, Unpublished). To our knowledge, data are however really scarce and no published and available study has experimentally evaluated whether tebuconazole can be transferred from the mother to the egg in birds.

In this study, we experimentally tested whether parental exposure to tebuconazole could result in egg contamination by this fungicide in a sentinel bird species of agroecosystems, the house sparrow (*Passer domesticus*). Captive sparrows were chronically exposed to tebuconazole through drinking water (exposed group) or not (control group) during the pre-breeding and breeding season as tebuconazole is used during these periods in several agroecosystems (vineyards, orchards, vegetable farming). During this experiment we opportunistically collected abandoned eggs to test whether the eggs of exposed females contained tebuconazole.

2. Materials & method

2.1. Study species and experimental design

This study was conducted on captive adult house sparrows. A total of 32 adult sparrows were used in this experiment ($N_{females} = 16$ and $N_{males} = 16$). All sparrows were maintained in captivity in 6 similar outdoor aviaries (length: 4 m, width: 3 m, height: 3 m). Each aviary was equipped with artificial shelter boxes (*i.e.,* one box per bird) and a shrub. The sparrows were fed *ad libitum* with a mixture of commercial seeds, millet on the stack, sand and salt/minerals blocks. In all aviaries, water was dispensed through drinking trough and changed every week. Of the 6 aviaries, three were exposed to tebuconazole (exposed group) and the three others were not exposed to tebuconazole (control group). The sex ratio was balanced in each aviary and the birds were divided by treatment type as follows (Aviary A: 6 controls; Aviary B: 6 exposed; Aviary C: 4 controls; Aviary D: 6 exposed; Aviary E: 6 controls; Aviary F: 4 exposed). Exposed individuals were treated with tebuconazole through their drinking water. In the three exposed aviaries, tebuconazole (Sigma-Aldrich, CAS. No: 107,534-96-3, purity: ≥ 98.0%) was diluted with tap water using a precision balance (±0.0001 g) to reach a concentration of 550 $\mu g.L^{-1}$. The NOAEL (no observed adverse effect level) of tebuconazole in birds is estimated to be 5.8 mg kg^{-1} bw day^{-1} (*Colinus virginianus*, http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/610.htm), while the exposure by our treatment is estimated to be much lower (*i.e.,* 0.165 mg kg^{-1} bw day^{-1}), because a bird drinks approximately 30 percent of its body weight per day (Bartholomew and Cade, 1963). This exposure concentration was also chosen because it has been shown to result in realistic plasma contamination (*i.e.,* similar to the plasma contamination of wild birds living in vineyards, Bellot et al., 2022). In three control aviaries, control individuals had access to regular tap drinking water without tebuconazole. The concentration of tebuconazole in the drinking water of the exposed and control groups was analysed to confirm an exposure concentration of 550 $\mu g.L^{-1}$ in the exposed individuals and a tebuconazole-free water for the control individuals (by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS, Qualyse laboratory, La Rochelle, France)). Exposure to tebuconazole began in December 2020 until July 2021 (7 months). This exposure period was chosen because tebuconazole can be applied in winter but also during the spring-summer period. For example, it can be used on vines from 7 to 8 leaves until bunch closure against mildew or powdery mildew corresponding to the sparrow breeding period. During this breeding period (May to July), sparrows formed pairs and laid eggs, some of which were abandoned by the parents without being incubated. These eggs were collected opportunistically and stored at - 20 °C until chemical analysis. A total of 24 eggs from 18 clutches were recovered (11 eggs from 8 clutches for the 'exposed parents' group and 13 eggs from 10 clutches for the 'control parents' group). In a few cases, 2 or 3 eggs from the same clutch were collected (3 eggs: 2 clutches; 2 eggs: 2 clutches, 1 egg: 14 clutches). This opportunistic collection of abandoned eggs was chosen for ethical reasons, and it is unlikely to lead to any bias because control and exposed sparrows showed similar rates of egg abandonment. The concentration of tebuconazole was determined in exposed and control eggs (albumen and yolk, without shell). The limit of quantification (LOQ) was 0.23 ng g^{-1} dry weight and the limit of detection (LOD) was 0.075 ng g^{-1} dry weight. Pesticides were determined by LC-MS/MS in Paris, France. The protocol for the determination of pesticides is detailed in the supplementary material.

2.2. Statistical analysis

All statistical analyses were performed in Rstudio (R version 4.0.3). When several eggs from the same clutch were collected, the concentrations of tebuconazole of all the eggs were averaged to avoid pseudoreplication (N = 8 clutches for the exposed group; N = 10 clutches for the control group). In order to test the effect of the parental exposure to tebuconazole (exposed vs. control group) on the quantification or not of tebuconazole in the collected clutches, a GLM (Generalized Linear Model) was performed with a binomial distribution and a logit link function (dependent binary variable: contaminated or uncontaminated clutch; dependent factor: exposed or control). In addition, to test the effect of the experimental treatment (exposed vs. control) on the concentration of tebuconazole found in the collected clutches, a non-parametric test was used with the NADA package (Peto-Prentice test, Shoari and Dubé, 2018). This test allowed us to consider censored values, *i.e.,* below the limit of quantification (N = 8). The censored values were replaced by LOQ/2 (*i.e.,* 0.115 ng g^{-1}).

3. Results & discussion

Tebuconazole was quantified in all the clutches of exposed females (Fig. 1) and the number of quantifiable samples was significantly higher for clutches from exposed females (exposed group) compared to those from control females ($\chi^2 = 14.7$, df = 1, $p < 0.001$). Indeed, 80% of the clutches in the control group were below the LOQ while all clutches in the exposed group were above the LOQ. Overall, the concentration of tebuconazole in sparrow eggs was significantly affected by the experimental treatment with the levels of tebuconazole in the eggs of exposed parents being significantly higher than those in the eggs of control parents ($\chi^2 = 17.8$, df = 1, $p < 0.001$, Fig. 1).

In this study, we demonstrated for the first time that chronic exposure to tebuconazole in breeding sparrows results into egg contamination, demonstrating that tebuconazole can be transferred from the environment to the egg in birds. This result may appear surprising because of the rapid biotransformation potential of tebuconazole in tissues. Indeed, previous studies have investigated the toxicokinetic of tebuconazole in birds and they have shown that tebuconazole has a high clearance rate (Konwick et al., 2006; Gross et al., 2020) and a moderate lipophilicity (EFSA – European Food Safety Authority, 2014). Accordingly, Gross et al. (2020) did not show any bioaccumulation potential of tebuconazole in the Japanese quail even after repeated oral contamination with low or high doses of tebuconazole (3.08 or 9.24 μg per day respectively) for 10 consecutive days and the possibility of maternal transfer of this compound to the egg was not favoured (Lopez-Antia et al., 2021). However, this fungicide is considered to be relatively lipophilic with an octanol/water partition coefficient (log Kow) of 3.7 (EFSA – European Food Safety Authority, 2014), and some studies suggest that maternal transfer may be favoured by low-Kow and/or less persistent compounds as these would be weakly recalcitrant in maternal tissues and therefore potentially more easily transferred into the egg (Kadokami et al., 2004; Verreault et al., 2006). A few studies have measured the levels of pesticides including azoles in eggs (Gómez-Ramírez et al., 2014; Bro et al., 2015, 2016; Corcellas et al., 2017). Interestingly, Bro et al. (2016) found relatively high concentrations of four triazoles in the eggs of wild grey partridges (*e.g.,* 21 ng g^{-1} cyproconazole, 13 ng g^{-1} difenoconazole or tebuconazole <10 ng g^{-1}, on whole eggs by gas chromatography-tandem mass spectrometry). These concentrations can be 10 times higher than those found in our experiment. However, the route of contamination remained an open question and such high concentrations may result from direct transfer from the environment to the egg of these ground-nesting birds during spraying of pesticides (Bro et al., 2016). Because tebuconazole was found in the eggs of exposed sparrows, our study suggests that tebuconazole may be transferred from the environment into the egg through the ingestion of tebuconazole by the mother. Importantly, this demonstrates that the embryo can be exposed to tebuconazole when it is used in the environment (*i.e.,* on agricultural land).

In our study, the contamination of the eggs by tebuconazole may have been amplified by the chronic exposure to this fungicide (*i.e.,* several months before egg-laying). Indeed, continuous exposure to tebuconazole may have resulted in oxidative damage, and as a result in hepatotoxicity in exposed sparrows (Ferreira et al., 2010; Yang et al., 2018). Such hepatotoxicity may have led to a lower ability of the liver to metabolize the parent compound, resulting in an accumulation of

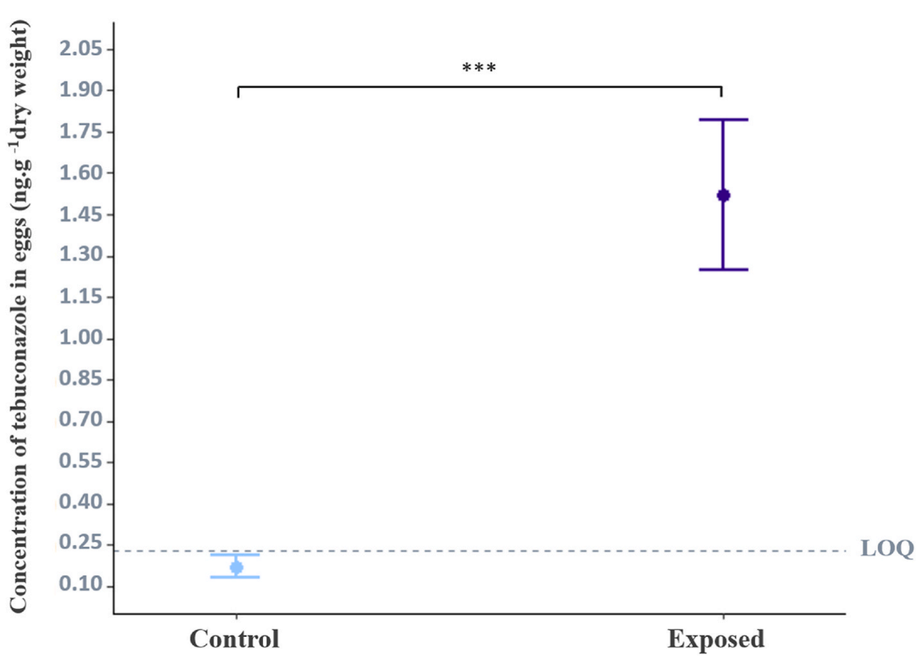

Fig. 1. Tebuconazole concentration of sparrow eggs (ng g^{-1} dry weight) according to the experimental treatment (control parents, N = 13 eggs or exposed parents, N = 11 eggs). The eggs of control or exposed parents are shown in light and dark blue, respectively. Tebuconazole concentrations were significantly different between the two groups ('***' < 0.001). Means ± SE are presented. The dotted line represents the analytical limit of quantification (LOQ = 0,23 ng g^{-1} dry weight). Detected concentrations that were below the LOQ were graphically replaced by the LOQ/2 (= 0.115 ng g^{-1} dry weight).

tebuconazole in the mother tissues. Similarly, a chronic exposure to tebuconazole may also lead to the saturation of phase I and II enzymes or xenobiotic transporters, and this may lead to a reduced biotransformation of tebuconazole in the mother tissues (Drouillard and Norstrom, 2001). Finally, it cannot be excluded that tebuconazole may interfere at different levels with the induction and/or repression of xenobiotic biotransformation pathways (*e.g.*, Yang et al., 2018), leading to accumulation of the parent substance or increased toxicity.

In this study, we exposed individuals chronically, including the breeding and egg-laying season. Although this exposure mimics a realistic environmental scenario (farmland birds are repeatedly exposed to azole compounds for several months in agroecosystems, especially in vineyards, orchards or vegetable crops), it did not allow us to fully understand the kinetic of the maternal transfer of tebuconazole into the egg. More specifically, it is unclear whether tebuconazole is transmitted from mother to egg by maternal tissue or more directly by the intestinal route. Although the presence of tebuconazole in the plasma of exposed parents has been confirmed (Bellot et al., 2022), it would be interesting to conduct a study with radiolabelled tebuconazole to follow its kinetics during the laying period. Furthermore, we were not able to test to what extent and for how long the maternal transfer of tebuconazole into the egg persists after exposure of the female to this triazole because females were continuously exposed to tebuconazole in our study. It would therefore be interesting to expose birds during the pre-breeding only and to subsequently test whether tebuconazole or its metabolites can be found in maternal tissues and into eggs during the laying period. This would allow us to test whether the maternal transfer to the eggs is directly reversible after stopping the exposure or whether these effects persist until the complete elimination of tebuconazole in the maternal tissues or in the digestive system. Future studies should also measure the metabolites of tebuconazole in eggs to know the mixture of compounds to which the embryo is likely to be exposed. This is of primary importance because the presence of xenobiotics during embryonic development could cause deleterious effects on the developing chick (Lopez-Antia et al., 2013, 2015; Garcês et al., 2020; Ortiz-Santaliestra et al., 2020; Ruuskanen et al., 2020). For example, Ortiz-Santaliestra et al. (2020) showed that experimental spraying of red-legged partridge eggs with tebuconazole at environmental concentrations resulted in a reduction in chick survival of around 26%, as well as physiological effects such as increased levels of alkaline phosphatase activity. These potential effects need to be further explored, particularly with regard to long-term effects that persist into adulthood.

Author statement

Bellot Pauline: Conceptualization, Data curation, Formal analysis, Writing – Original Draft, Visualization. **Brischoux François**: Conceptualization, Validation, Writing – Review & Editing, Funding acquisition. **Fritsch Clémentine**: Writing – Review & Editing, Funding acquisition. **Goutte Aurélie:** Methodology, Validation, Writing – Review & Editing. **Alliot Fabrice:** Methodology, Validation, Writing – Review & Editing. **Rocchi Steffi**: Writing – Review & Editing. **Angelier Frédéric**: Conceptualization, Validation, Ressources, Writing – Original Draft, Writing – Review & Editing, Supervision, Funding acquisition.

Ethical statement

All applicable institutional and/or national guidelines for the care and use of animals were followed. This work was approved by the French authorities (COMETHEA ethic committee and Ministère de L'Enseignement Superieur, de la Recherche et de L'innovation) under permit #APAFIS#12918–2018,010,515,574,796.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2022.136469.

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