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Experimental manipulation of dietary arsenic levels in great tit nestlings: Accumulation pattern and effects on growth, survival and plasma biochemistry☆

Pablo Sánchez-Virosta [a, b, *], Silvia Espín [a, b], Sandra Ruiz [a], Juha-Pekka Salminen [c], Antonio J. García-Fernández [b], Tapio Eeva [a]

[a] Department of Biology, University of Turku, Turku FI-20014, Finland
[b] Area of Toxicology, Department of Socio-Sanitary Sciences, University of Murcia, Campus de Espinardo, 30100 Murcia, Spain
[c] Laboratory of Organic Chemistry and Chemical Biology, Department of Chemistry, University of Turku, Turku FI-20014, Finland

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ABSTRACT

Arsenic (As) is a ubiquitous metalloid classified as one of the most hazardous substances, but information about its exposure and effects in free-living passerines is lacking. The aim of this study is to elucidate the effect of an As manipulation experiment on survival, growth and physiology of great tits (*Parus major*). Wild *P. major* nestlings inhabiting an unpolluted area were dosed with water, 0.2 or 1 $\mu g\ g^{-1}\ d^{-1}$ of sodium arsenite (Control, Low and High As groups), whereas those living in a metal-polluted area were dosed with water (Smelter group). Birds accumulated As in tissues (liver, bone and feathers) in a dose-dependent way. Nestlings exposed to 1 $\mu g\ g^{-1}\ d^{-1}$ of sodium arsenite showed reduced number of fledglings per successful nest, and those exposed to 0.2 $\mu g\ g^{-1}\ d^{-1}$ had reduced wing growth, which could have post-fledging consequences such as increased predation risk. These results suggest that the LOAEL for effects on nestling survival and development in great tits is likely equal to or below 1 $\mu g\ g^{-1}\ d^{-1}$. However, limited effects on the biochemical parameters evaluated were found. It has been shown that As may produce oxidative stress and tissue damage, so further research exploring this issue will be carried out in a future study.

1. Introduction

Arsenic (As) is a common component of the soil and is present in different rock types. Some industrial activities such as metallurgical processes and combustion of coal are important anthropogenic sources of As and other metals into the environment (Pacyna and Pacyna, 2001). In addition, some arsenical pesticides such as sodium arsenite have been widely used, although they are now prohibited in most countries (WHO, 2000). The Agency for toxic substances and disease registry (ATSDR), has ranked arsenic as the first compound in the Substance priority list 2015 based on its frequency, toxicity, and potential for human exposure (ATSDR, 2015), which points out the fact that As is of great toxicological concern.

Birds have been successfully used as biomonitoring tools of environmental pollution all over the world (Furness et al., 1993). However, the scientific community has prioritized studies on other elements such as lead (Pb), mercury (Hg) and cadmium (Cd) (e.g. Burger and Gochfeld, 2000; Scheuhammer, 1987), whereas very few (and correlative) field studies have assessed the effects of As in birds so far (Sánchez-Virosta et al., 2015). Moreover, in the wild, birds are generally exposed to a mixture of metals and other stressors. Thus, proving a relationship between a specific contaminant and its associated health effects is very challenging. In addition, long-term pollution may disturb biological communities, which may end up causing secondary effects on bird species due to changes in food availability and quality (Eeva et al., 1997). Arsenic is of particular concern for mammalian exposure and toxicity, however, it is not clear whether the same applies for wild birds. Therefore, As manipulation experiments providing environmentally-relevant levels are needed to explore the specific

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* Corresponding author. Area of Toxicology, Department of Socio-Sanitary Sciences, University of Murcia, Campus de Espinardo, 30100 Murcia, Spain.

E-mail addresses: pasanv@utu.fi, pablo.s.v@um.es (P. Sánchez-Virosta), sieslu@utu.fi, silvia.espin@um.es (S. Espín), srruiz@utu.fi (S. Ruiz), j-p.salminen@utu.fi (J.-P. Salminen), ajgf@um.es (A.J. García-Fernández), teeva@utu.fi (T. Eeva).

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effects of As on growth, survival and physiology in wild bird populations.

Experimental studies providing As compounds to bird species have mainly found developmental and reproductive effects (Sánchez-Virosta et al., 2015). Arsenic-treated mallard (*Anas platyrhynchos*) ducklings and zebra finches (*Taeniopygia guttata*) showed decreased weight gain and growth and reduced tarsus and wing length upon fledging (Albert et al., 2008a, 2008b; Camardese et al., 1990). Stanley et al. (1994) found that As altered mallard reproduction and ducklings' growth, decreased egg weight and produced eggshell thinning. Several physiological parameters, such as calcium (Ca), alkaline phosphatases (ALPs), vitamins D3 (cholecalciferol), E (tocopherol), K, A (retinol) and carotenoids are involved in different ways in nestling growth and development (Bügel, 2008; Chin and Ima-Nirwana, 2014; Cranenburg et al., 2007; Deeming and Pike, 2013; Espín et al., 2016a; Khazai et al., 2008; Zile, 2004). Regarding the effects of As in biochemistry, Albert et al. (2008a) suggested that its interaction with the mineral fraction of the bone may explain the effects on bone development, whereas Ortiz-Santaliestra et al. (2015) observed that As was associated with decreased retinol in plasma and increased creatine phosphokinase activity in Bonelli's eagle (*Aquila fasciata*) nestlings.

The main objective of this study is to explore if environmentally relevant As levels affect growth, survival and physiological biomarkers of great tits (*Parus major*). For this purpose, during the breeding season of 2015, nestlings were orally dosed with sodium arsenite daily (from day 3 to day 13 post-hatching) and were measured in terms of brood size, nestling survival, number of fledglings, body size and growth rate. Concentrations of As in feces of nestlings were analyzed to be used as indicators of As dietary intake, and a set of physiological biomarkers (hematocrit, vitamins, carotenoids and other biochemical parameters) that are expected to be potential indicators of health and/or As toxicity were measured in the blood. Dead nestlings were necropsied to investigate As accumulation in liver, bone and feathers. The responses to three experimental manipulations (Control, Low and High groups) carried out in a great tit population with low metal exposure levels are compared with those in a population breeding in the vicinity of a copper-nickel (Cu-Ni) smelter, an anthropogenic As source (Smelter group). Thus, we will be able to compare the effects of dietary As levels to those caused by exposure to a mixture of As and metals, other pollutants and potential associated resource limitations. Based on the developmental and reproductive effects reported in As-manipulation experiments, we hypothesize that As will interfere with one or several physiological parameters and decrease growth and survival.

2. Material and methods

2.1. Experimental set-up

The As-manipulation experiment was performed during the breeding season 2015 in the proximities of a Cu-Ni smelter in Harjavalta (61°20′ N, 22°10′ E), SW Finland. There is an accumulation of heavy metals (mainly Cu, Ni, Pb, Cd, As, and zinc, Zn) in the area of the smelter (polluted zone) as a result of present and previous emissions. Metal concentrations decrease with distance to the smelter. The study area is described in detail by Eeva and Lehikoinen (1995). The As manipulation was done on a great tit population using nest boxes placed in 11 different sites along the pollution gradient. The area was divided into the polluted and the unpolluted zone (0–2 km and 4.5–11 km, respectively, from the smelter). This study is part of a long-term (since 1991) follow-up of hole-breeding passerines in this area. The study sites have been selected to represent similar habitat, i.e. relatively barren pine dominated forests. Variation in tree species composition has been a very weak explanatory factor for clutch size or fledgling number, and no significant effects were found in our earlier studies (Eeva and Lehikoinen, 2000, 2013). On the other hand, long-term pollution has changed some habitat characteristics, like ground layer vegetation, which can be considered as one of the secondary effects of pollution. Tit population densities have been similar between study areas (Eeva and Lehikoinen, 2013). Details of the study species are given in Supplementay Material (Document S1).

There are ca. 500 nest boxes in the study area that were checked in April and then periodically to track the progress in the nest building. When newly hatched nestlings were found in a nest in the unpolluted area, the nest was assigned randomly either to the Control (distilled water), Low (0.2 $\mu g\ g^{-1}\ d^{-1}$ of liquid sodium arsenite), or High (1 $\mu g\ g^{-1}\ d^{-1}$ of liquid sodium arsenite) As-supplemented groups. In the polluted area, all the nests received distilled water (hereafter called Smelter group).

We aimed to provide environmentally relevant As doses in order to achieve As concentrations at which wild passerines are currently exposed at polluted sites. After preliminary trials of 8 $\mu g\ g^{-1}\ d^{-1}$ and 2 $\mu g\ g^{-1}\ d^{-1}$, we set 1 μg As $g^{-1}\ d^{-1}$ as the high treatment (hereafter called High As) corresponding to As exposure in relatively highly polluted areas, and another dose at 0.2 μg As $g^{-1}\ d^{-1}$ as the low treatment (hereafter called Low As). A more detailed explanation on the selection of these dosing levels is provided in Document S2. Sodium arsenite (Sigma S7400, Batch SLBH5736V, 98% pure) was used to prepare dilutions to dose the nestlings. Sodium arsenite was used because it is one of the most common trivalent inorganic As compounds (WHO, 2000) and because of its well-known toxic effects. Moreover, Moriarty et al. (2009) found that most of the As in terrestrial invertebrates is inorganic, with the proportion as arsenite versus arsenate varying by invertebrate type. Lepidoptera, particularly in larval form, is the main invertebrate group in the diet of great tits in our study area (Eeva et al., 2010). In this sense, Moriarty et al. (2009) showed that the As speciation in their study was 60% arsenite versus 34% arsenate in mature Lepidoptera and 29% arsenite versus 64% arsenate in larval Lepidoptera in contaminated zones, while larval Lepidoptera in the background zone had 55% arsenite and 45% arsenate. Two different dilutions of 100 μg As mL^{-1} and 20 μg As mL^{-1} in distilled water were used.

At 3 days of age (d3), we started providing As or distilled water daily for eleven days (until d13). We established d14 as the end of the experiment (last sampling and measurements) to avoid handling and dosing birds too close to the fledging date. Nestlings were dosed with increasing volumes of the corresponding treatment (Control, Low or High As) in order to receive the appropriate dose (0, 0.2 or 1 $\mu g\ g^{-1}\ d^{-1}$) according to their body mass. The volumes were provided orally with pipettes (from 50 to 170 μL from d3 to d13). For volume calculations, we used the long-term data on daily nestling body mass from nestlings of the same area. All nestlings from the same brood received the same treatment. In exceptional cases, if one sibling was clearly smaller than the other nestlings in the brood (around half weight), we provided half of the volume.

The ideal number of nests in the experiment was set at 60 (15 nests per treatment group: Control, Low As, High As and Smelter). Since some nests could fail later, we took a higher number of nests at the beginning of the experiment. In total, the experiment was carried out on 70 nests (16 Control nests, 17 Low As nests, 16 High As nests, and 21 Smelter nests) with a total of 400 nestlings dosed.

The study was approved by the Centre for Economic Development, Transport and the Environment, ELY Centre Southwest Finland (VARELY/593/2015) and the Animal Experiment Committee of the State Provincial Office of Southern Finland (ESAVI/11579/04.10.07/2014).