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Stress response, biotransformation effort, and immunotoxicity in captive birds exposed to inhaled benzene, toluene, nitrogen dioxide, and sulfur dioxide ☆

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ABSTRACT

In the oil sands of Alberta, Canada, toxicology research has largely neglected the effects of air contaminants on biota. Captive Japanese quail (*Coturnix c. japonica*) and American kestrels (*Falco sparverius*) were exposed to mixtures of volatile organic compounds and oxidizing agents (benzene, toluene, NO_2 and SO_2) in a whole-body inhalation chamber, to test for toxicological responses. Hepatic biotransformation measured through 7-ethoxyresorufin-*O*-dealkylase (EROD) tended to be increased in exposed kestrels ($p=0.06$) but not in quail ($p=0.15$). Plasma corticosterone was increased in the low dose group for quail on the final day of exposure ($p=0.0001$), and midway through the exposure period in exposed kestrels ($p=0.04$). For both species, there was no alteration of T and B-cell responses, immune organ mass, or histology of immune organs ($p > 0.05$). This study provides baseline information valuable to complement toxicology studies and provides a better understanding of potential health effects on wild avifauna.

1. Introduction

The Canadian oil sands are in the boreal forest ecozones of the Athabasca, Peace River and Cold Lake regions of Alberta. Whereas the development of the oil sands is an important economic driver for Alberta (Government of Alberta, 2013) such development has generated widespread environmental and human health concerns (Gosselin et al., 2010).

Measuring the activity of mixed function oxidases (MFOs) has become a standard means for determining xenobiotic exposure in wild animals including birds (Melancon, 1996; Custer et al., 2001). This is a surrogate measure to indicate the animal's effort to detoxify foreign substances through biotransformation. One of the components of this enzymatic system, the cytochrome P450 (CYP 450) isozymes, is often used to determine exposure to hydrocarbon-related compounds (Rattner and Eastin, 1981). For example, the induction of CYP 4501A1, measured by the 7-ethoxyresorufin-*O*-dealkylase (EROD) assay (Walker, 1998), is considered a biomarker of exposure to polycyclic aromatic hydrocarbons (Whyte et al., 2000). The EROD assay, mainly done with hepatic microsomes, requires euthanasia of the animals. However, since CYP 450 activity has been detected in endothelial cells of several tissues, including the integument (Ben-David et al., 2001), growing feathers of birds having active vasculature, might be a tissue in which contaminant exposure can be detected non-lethally (Melancon et al., 2006).

Aquatic contaminants typical of the oil sands region, such as naphthenic acids (NAs) and polycyclic aromatic hydrocarbons (PAHs), have induced hepatic EROD in fish (Colavecchia et al., 2007; Tetreault et al., 2003), wood frogs (Hersikorn and Smits 2011), and birds including tree swallows (Smits et al., 2000, Gentes et al., 2006) and ducks (Gurney et al., 2005). Similarly, immunotoxic effects, including decreased circulating leukocytes and decreased antibody response to a bacterial pathogen, have been described in fish (Rainbow trout *Oncorhynchus mykiss*) exposed *in situ* to oil sands processed waters (OSPW) and experimentally to NAs (Leclair et al., 2013; McNeill et al., 2012).

In addition to contaminants in OSPW, airborne contaminants are emitted from oil sands operations. Among the major air pollutants are sulfur dioxide (SO_2), nitrogen dioxide (NO_2), and volatile organic compounds (VOCs) (Gosselin et al., 2010). However,

☆ Adult American kestrels are more sensitive than juvenile Japanese quail to contaminants in emissions from oil sands extraction and processing.

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there is limited information on the immunotoxic and stress-mediated effects from air borne contaminants on biota compared to on-going aquatic toxicology research. Using birds to study the effects of air pollutants is advantageous because of their uniquely sensitive respiratory anatomy and physiology (Brown et al., 1997).

Therefore, an experimental inhalation study was designed to test the hypothesis that exposure to inhaled benzene, toluene, NO_2 and SO_2, would induce hepatic EROD and alter the stress and immune responses of captive Japanese quail and American kestrels. In addition, we hypothesized that EROD induction would be detectable in feather tissue (growing feathers).

2. Materials and methods

2.1. Animals and housing conditions

2.1.1. Japanese quail

Using protocols approved by the Animal Care Committee of the University of Calgary, the dosing trial was conducted with 36 male, juvenile (3-wk old) Japanese quail purchased from a local breeder (Byrconn Dev. Inc. Edmonton, AB, Canada.). The birds were individually identified with color leg bands and randomly allocated into 3 groups (control, low and high; $n=12$/group). The birds were kept in stacked cages (6 birds/cage) in a room with controlled ventilation, temperature (21–23 °C), and light cycle (12-h light/dark). Water was offered *ad libitum* in glass containers and they were fed unmedicated turkey starter *ad libitum*. After 1-wk acclimation period, pre-dosing blood samples ($\leq 1\%$ of their body weight) were collected through jugular venipuncture using heparinized syringes and needles (25 g). Blood samples were taken at the beginning, middle and end of the exposure trial and used to determine CORT levels and antibody responses.

2.1.2. American kestrels

In a separate trial, 20 (17 females and 3 males), adult (3–7 years old) American kestrels were obtained from a breeding colony (avian science conservation center, McGill university). The birds were individually identified with fluorescent color and aluminum leg bands and randomly allocated into 2 groups (control and exposed, $n=10$/group) except for the three males that were allocated to the control group because they were required by another facility after this study. The birds were housed together in an indoor flight room (7 m widex5 m highx3 m long) with controlled ventilation, temperature (21–23 °C), and light cycle (12-h. light/dark); the same conditions as used with the quail. The room had wood shavings covering the floor, a perch (1-in. diameter rope) across the room at 1.8 m high, two feeding platforms, and water baths.

The birds were fed 1.5 frozen/thawed cockerels/bird/day that were soaked in warm water for 30 min prior to feeding. After a 2-wk acclimation period, pre-dosing blood samples were collected as for the quail. To minimize stress, the room lights were dimmed to very low levels for cleaning, feeding and for capturing the birds to transport them to the inhalation chamber. Blood samples were taken from the birds at the beginning, middle and end of the exposure trial and the plasma was used to determine CORT and antibody responses.

2.2. Experimental design

2.2.1. Inhalation chamber

We used a whole body, avian-specific inhalation chamber (Olsgard and Smits 2008) with the following modifications (Fig. 1). To prevent corrosion of the chamber's components, the copper piping was replaced with polytetrafluoroethylene tubing (0.5-in. diameter) and the copper fittings were replaced by stainless steel fittings (Calgary Valve and Fittings Ltd., Calgary, AB, Canada). The gas mixtures and the hydrocarbon-free air (for controls) were released from pre-filled aluminum and steel cylinders (Praxair Inc., Canada, Mississauga, ON, Canada; concentration accuracy $\pm 5\%$, manufacturer guarantee), except for the low dose of NO_2 that was diluted by mixing this gas with air into a 3-in. widex22-inch long polyvinyl chloride (PVC) tube. All the gas lines merged into a 4-piece, stainless steel fitting and from there, the gas mixture passed through a flow meter (Matheson Tri-gas®, Inc., Basking Ridge, NJ, USA), attached to a bubbler humidifier (Salter Labs, Arvin, CA, USA). The gas mixture then flowed through two stainless steel chambers each with six individual animal holding units (straight 2.6-in. diameter PCV tubes with interior perches). After passing through the animal holding units, the gas mixture flowed through a carbon-activated filter and into the room's exhaust and scavenger system. To fit the quail morphology, the straight PCV tubes were replaced by "S"-shaped PCV tubes (4-in. widex13-in. long), with flat stainless steel mesh perches (Fig. 2).

2.2.2. Gas mixture and rationale for dose selection

The low dose for quail and the only dose of contaminant gas mixture for the kestrels were based on air contaminant data recorded at monitoring stations located throughout the oil sands regions from the Wood Buffalo Environmental Association (WBEA) during 2010. Specifically, the 1-h maximum concentrations that were recorded at each station were averaged, and multiplied by a safety factor of 100 (Merril, 2003) for benzene and toluene since similar concentrations have been used before on kestrels without evidence of morbidity (Olsgard et al., 2008). For NO_2 and SO_2 there were limited references on which to base the concentrations without lethal effects. The closest concentrations (averaged maximum concentration recorded in a 6-month period near a coal-fired power plant) were 0.28 ppm for NO_2 and 0.5 ppm SO_2. Therefore, for the quail, the 1-h maximum concentrations recorded at each station for NO_2 and SO_2 were averaged and multiplied by a factor of 10. For the kestrels this safety factor was increased to 100 since no mortalities or overt health effects were seen in the quail.

For the quail, the control group was dosed with hydrocarbon-free air; the low dose group with a combination of 0.6 ppm benzene, 1 ppm toluene, 0.2 ppm NO_2 and 0.5 ppm SO_2; and the high dose group with 100 times the concentration of the low dose group (Merril, 2003). For the kestrels, the control group was dosed with air and the exposed group with 0.6 ppm benzene, 1 ppm toluene, 2 ppm NO_2 and 5.6 ppm SO_2.

2.2.3. Dosing protocol and exposure times

The studies were conducted in October–November (quail) and November–December (kestrels) 2011. The birds were transported from their cages/flight room into the inhalation chamber where the control group was exposed first, followed by the low, and then high dose groups. The chamber was flushed for 5–7 min with hydrocarbon-free air between dosing. Once the birds were inside the chamber, the lids of the individual holding units were tightly closed and the gas cylinders were opened with a total controlled flow of 10 L per min (L/min). The flow rate was calculated using avian specific air requirements (Brown et al., 1997) and by adapting the kestrel's specific requirements. The minimum flow rate suitable for kestrels is 3.5 L/min, which maintained normal body temperature (Olsgard et al., 2008) and 6.5 L/min for quail. As a safety precaution, these flow rates were increased to 10 L/min (Brown et al., 1997).

The chamber was tested for leaks every day of exposure with a non-corrosive liquid at joints (Snoop® Liquid Leak Detector, Swagelok, Calgary, AB, Canada). The official exposure time started once equilibrium was reached inside the chamber (~20 min after