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Effect of oral exposure to artificially weathered Deepwater Horizon crude oil on blood chemistries, hepatic antioxidant enzyme activities, organ weights and histopathology in western sandpipers (*Calidris mauri*)

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ABSTRACT

Shorebirds were among birds exposed to Mississippi Canyon 252 (MC252) crude oil during the 2010 Deep Water Horizon (DWH) oil spill in the Gulf of Mexico. The western sandpiper (*Calidris mauri*) was chosen as one of four species for initial oral dosing studies conducted under Phase 2 of the avian toxicity studies for the DWH Natural Resource Damage Assessment (NRDA). Thirty western sandpipers were assigned to one of three treatment groups, 10 birds per group. The control group was sham gavaged and the treatment groups were gavaged with 1 or 5 mL oil kg bw^{-1} daily for 20 days. Periodic blood samples for hemoglobin measurements were collected during the trial. A final blood sample used to determine hemoglobin concentration in addition to complete blood counts, plasma clinical chemistries, haptoglobin concentration and plasma electrophoresis was collected when birds were euthanized and necropsied on day 21. Tissues were removed, weighed and processed for subsequent histopathological evaluation. There were numerical decreases in hemoglobin concentrations in oil-dosed birds over the 21-day trial, but values were not significantly different compared to controls on day 21. There were no significant differences between controls and oiled birds in complete blood counts, plasma chemistries, haptoglobin concentration, and plasma electrophoresis endpoints. Of the hepatic oxidative stress endpoints assessed, the total antioxidant capacity assessment (Trolox equivalents) for the control group was lower compared to the 1 mL oil kg bw^{-1} group. Absolute liver weights in the 5 mL oil kg bw^{-1} group were significantly greater compared to controls. While not conclusive, the numerical decrease in hemoglobin concentration and significant increase in absolute liver weight are consistent with exposure to oil. Histological changes in the adrenal gland could be considered a non-specific indicator of stress resulting from exposure to oil. It is possible that the quantity of oil absorbed was not sufficient to induce clearly evident hemolytic anemia or that the western sandpiper is relatively insensitive to ingested oil.

1. Introduction

Shorebirds were among birds exposed to Mississippi Canyon 252 (MC252) crude oil during the 2010 Deep Water Horizon (DWH) oil spill in the Gulf of Mexico. The western sandpiper (*Calidris mauri*) was chosen as one of four species for initial oral dosing studies conducted under Phase 2 of the avian toxicity studies for the DWH Natural Resource Damage assessment (NRDA). Details about the DWH oil spill and the DWH NRDA are given in Bursian et al. (2017). The western sandpiper (WESA) was chosen as a test species because it is often found in the Gulf of Mexico during the winter months and during migration (Nebel et al., 2002) and thus is representative of other migratory shorebirds that might have been exposed to MC252 crude oil, its small size is applicable to methods used in metabolism and flight performance studies that are included in the Phase 2 avian toxicity studies, and because researchers at the University of Western Ontario have success-

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fully used this species for laboratory metabolism and flight studies in the past.

A preliminary oral toxicity study conducted with WESAs followed similar protocols described in the literature (Leighton, 1986) as well as recommendations from an expert panel. Birds were gavaged once with a 1:1 mixture of artificially weathered MC252 oil and mealworm slurry that provided a dose of 20 mL oil kg body weight^{-1} (bw^{-1}) or on four consecutive days at daily doses of 10 or 20 mL oil kg bw^{-1}. Results of this initial oral toxicity trial indicated few effects characteristic of oil exposure that include anemia, decreased nutrient absorption, altered stress response, and decreased immune function (Szaro et al., 1978; Leighton et al., 1985; Leighton, 1985, 1986, 1993; Peakall et al., 1989). More detailed information about the effects of oil exposure in birds can be found in Bursian et al. (2017). Rapid clearance of the oil administered by gavage (20–30 min) was assumed to have prevented sufficient polycyclic aromatic hydrocarbon (PAH) absorption to cause oil toxicity as indicated by no significant decrease in packed cell volume (PCV).

The present oral toxicity study incorporated modifications of the dosing methods used in the initial oral toxicity study to better emulate field conditions and potentially increase PAH absorption. The daily oil doses were decreased from 10 and 20 mL kg bw^{-1} to 1 and 5 mL kg bw^{-1}. The number of consecutive days that dosing occurred increased from four to 20 to prolong the duration of exposure and extend the time over which endpoints indicative of exposure to oil were evaluated. The present study was undertaken to contribute to the definition of the appropriate dose of oil to be administered in order to induce hemolytic anemia in these birds.

2. Materials and methods

2.1. Study approval

This study was reviewed and approved by the University of Western Ontario's Council on Animal Care (approval number 2012-027).

2.2. Western sandpiper capture, housing and diet

Thirty adult, mixed-sex WESAs that had previously been held at the University of Western Ontario's Advanced Facility for Avian Research (AFAR) were used in this study. These birds were captured in Delta, British Columbia, Canada using mist nets in July 2012 under the guidelines of the University of Western Ontario's Council on Animal Care and according to permit CA-0256 from the Canadian Wildlife Service. Prior to the study, WESAs were maintained in one of the specialized 2.4 × 3.7 m shorebird rooms at AFAR under 12:12 light conditions at approximately 19 °C. Birds were fed ad libitum a diet of 80% Mazuri Waterfowl Starter and 20% Purina Aquamax Fingerling Starter 300. The diet was supplemented with approximately 50 mealworms per 20 birds every other day.

One week prior to study initiation (March 1, 2013), birds were transferred into a large holding room under the same photoperiod and temperature conditions. Dividers were placed in the room to create six 0.9 × 1.8 × 1.8 m corrals. There were two corrals per treatment group with five birds in each corral. Birds remained in their respective corrals throughout the trial. Their diet remained unchanged.

2.3. Toxicant, treatments and dosing

The toxicant was artificially weathered-MC252 oil collected on July 26, 2010 during the DWH spill and artificially weathered (batch #: B030112, TDI-Brooks International, College Station, TX) as described in Forth et al. (2016) prior to receipt for use in the study. Western sandpipers were randomly chosen for the oral toxicity study and assigned to one of three treatment groups: a control group of 10 birds that were sham gavaged twice daily for 20 days; a group of 10 birds that were gavaged daily with 1 mL oil kg bw^{-1} for 20 days; and a group of 10 birds that were gavaged daily with 5 mL oil kg bw^{-1} for 20 days. Birds were weighed daily prior to dosing to standardize the dose across individuals. A mealworm homogenate was prepared at the ratio of six mealworms per 2 mL water placed in a 12 mL polypropylene tube and homogenized with an Omni 2000 (Omni International) variable speed tissue homogenizer. This mixture was centrifuged at 2000*g* in a Galaxy Mini microcentrifuge (VWR International) to remove cuticle particles that would clog the gavage needle. The dosed birds were gavaged with a 1:1 oil/mealworm slurry. For each bird, *the appropriate volume of oil was combined with the appropriate volume of mealworm homogenate* in a microcentrifuge tube and the mixture thoroughly vortexed for 30 s. Half the daily dose was administered initially, and the second half was administered an hour later. The single dose of oil/mealworm slurry administered to the birds was calculated as bw (kg) × 0.5 (mL kg bw^{-1}) × 2 for the 1 mL kg bw^{-1} dose group and bw (kg) × 2.5 (mL kg bw^{-1}) × 2 in the 5 mL kg bw^{-1} dose group. The oil/mealworm slurry was administered to a manually restrained bird through a 5.08-cm, 20-gauge stainless steel gavage needle attached to a 1-mL Luer lock glass syringe. Dosing of birds began on March 7, 2013.

2.4. Blood collection

Adult WESAs generally weigh from 22 to 35 g and blood samples taken during the study were cumulatively limited to 10% of the bird's total blood volume. Approximately 75 µl of blood was collected from all birds two days prior to dosing, and on days 8 and 15 in a heparinized microhematocrit tube after pricking the brachial vein with a 27-gauge needle. This blood was used for hemoglobin measurements (VetScan iStat analyzer; Abaxis Medical Diagnostics).

2.5. Necropsy

Birds were necropsied on day 21 following collection of as much blood as possible from the brachial vein as described above. Birds were then euthanized by cervical dislocation. Blood collected at necropsy was used to determine hemoglobin concentration, and complete blood count (CBC). In addition, plasma clinical chemistries (VetScan VS2 analyzer; Abaxis Medical Diagnostics) were analyzed using an avian/reptilian or liver profile rotor. Plasma haptoglobin concentration and plasma electrophoresis were analyzed by the University of Miami's Avian and Wildlife Laboratory when sufficient blood was available.

Upon exposure of the body cavity, all organs were assessed grossly for abnormalities, which, if present, were documented by digital images. The liver and brain were removed and weighed to the nearest 0.1 g. The liver was sectioned into five 50 mg samples that were placed in individual cryovials and frozen in liquid nitrogen for subsequent determination of oxidative damage. A sixth liver sample was frozen in liquid nitrogen for subsequent determination of cytochrome P450 (CYP) activity (Alexander et al., 2017). The remaining portion of liver was placed in 10% neutral buffered formalin for subsequent histopathology. The gastrointestinal (GI) tract, spleen, kidneys, heart, lung and adrenal glands were also placed in 10% neutral buffered formalin for subsequent histopathology.

2.6. Assessment of hepatic oxidative damage

Oxidative damage in liver was assessed on liver homogenates prepared from the individual liver subsamples described in 2.5. Total, oxidized and reduced glutathione (TGSH, GSSG, and RGSH, respectively), malondialdehyde +4-hydroxylalkenals (MDA), and total antioxidant power (Trolox), were assayed according to (Pritsos et al. 2017).

2.7. Histopathology

Histopathology was performed by a board certified veterinary pathologist (Zoo/Exotic Pathology Service), using standard paraffin