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Organ weights and histopathology of double-crested cormorants (*Phalacrocorax auritus*) dosed orally or dermally with artificially weathered Mississippi Canyon 252 crude oil

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ABSTRACT

A series of toxicity tests were conducted to assess the effects of low to moderate exposure to artificially weathered Deepwater Horizon Mississippi Canyon 252 crude oil on representative avian species as part of the Natural Resource Damage Assessment. The present report summarizes effects of oral exposure (n = 26) of double-crested cormorants (DCCO; *Phalacrocorax auritus*) to 5 or 10 ml oil kg^{-1} day^{-1} for up to 21 days or dermal application (n = 25) of 13 ml oil to breast and back feathers every three days totaling 6 applications in 21 days on organ weights and histopathology. Absolute and relative kidney and liver weights were increased in birds exposed to oil. Additionally, gross and/or histopathologic lesions occurred in the kidney, heart, pancreas and thyroid. Clinically significant renal lesions in the orally dosed birds included squamous metaplasia and increased epithelial hypertrophy of the collecting ducts and renal tubules and mineralization in comparison to controls. Gross cardiac lesions including thin walls and flaccid musculature were documented in both orally and dermally dosed birds and myocardial fibrosis was found in low numbers of dermally dosed birds only. Cytoplasmic vacuolation of the exocrine pancreas was noted in orally dosed birds only. Thyroid follicular hyperplasia was increased in dermally dosed birds only possibly due to increased metabolism required to compensate damaged feather integrity and thermoregulate. Gastrointestinal ulceration was found in orally dosed birds only. There were no significant hepatic histopathologic lesions induced by either exposure route. Therefore, hepatic histopathology is likely not a good representation of oil-induced damage. Taken together, the results suggest that oral or dermal exposure of DCCOs to artificially weathered MC252 crude oil induced organ damage that could potentially affect survivability.

1. Introduction

During the Deepwater Horizon (DWH) oil spill in 2010, many live birds representing at least 93 species were found visibly oiled but not to the extent to cause immediate mortality (Deepwater Horizon Natural Resource Damage Assessment Trustees, 2016). It was of particular interest to examine the effects of these lower amounts of oil (less than 30% of body coverage) in order to assess avian injury as part of the DWH Mississippi Canyon 252 (MC252) Oil Spill Natural Resource Damage Assessment (NRDA).

Diving birds such as the double-crested cormorant (DCCO; *Phalacrocorax auritus*) are particularly susceptible to oil exposure

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during a spill because they dive through the water, and thus the oil, in order to feed (Szaro et al., 1978). They are therefore likely to be exposed not only dermally, but also by incidental ingestion through attempts to rid their feathers of oil through preening and possibly by ingesting oil through contaminated feedstuffs. In birds, anemia, disrupted feather function, hypothermia, respiratory distress, seizures, diarrhea, hepatic disease and renal disease have all been reported secondary to exposure to petroleum products (Mazet et al., 2002).

The two sets of data reported here address the effects of both oral and dermal oiling on the relevant oil exposure endpoints of organ weights and histopathology in the DCCO.

2. Methods

This study was performed under the authority of USFWS MBPO Federal Permit # MB019065-3, Mississippi and Alabama state (#8017) scientific collection permits, and Institutional Animal Care and Use Committee (IACUC) under NWRC protocol QA-2326. Cunningham et al. (2017, this issue) provides a detailed description of animal capture and handling for the experimental oral and dermal exposure of DCCO to DWH artificially weathered MC252 oil.

2.1. Oral dosing study

Captured DCCOs were randomly assigned to one of three treatment groups: a control group (n = 8, 7 male, 1 female) that was fed catfish that had been lightly anesthetized with MS222 and allowed to revive; a group dosed daily with up to 5 ml oil/kg bw/day through provision of oil-containing, lightly anesthetized catfish (n = 9, 6 male, 3 female); a group dosed daily with up to 10 ml oil/kg bw/day through provision of oil-containing, lightly anesthetized catfish as described below (n = 9, 7 male, 2 female).

2.2. Dermal dosing study

A total of 31 DCCO's were captured and retained in captivity. Birds were allowed to acclimate to captivity for a minimum of 21 days prior to initiation of the study. A total of 25 subadult DCCOs allocated to a control group (n = 12, 5 male, 7 female) and an exposed group (n = 13, 6 males, 7 females) were used in this trial. DCCOs were assigned to treatment groups based on the results of blood samples collected at the initiation of the three-week quarantine period. Complete blood count (CBC) values were used to ensure equal division of birds with potential health concerns between groups. DCCO's with monocyte counts greater than 2.0×10^9 cells/l were considered abnormal (severe monocytosis); and were divided between control (n = 4) and treatment (n = 3) groups. Additionally, a small oil spill took place one year prior to the study, not far from where 6 of the DCCOs were collected and were evenly distributed between groups. During the course of the trial, one bird from the control group and two birds from the treatment group died and were not replaced. Therefore, the final number of birds in the control and exposed group was 11 birds each to total 22 in the study. Oil on exposed birds (13 ml) and water on control birds (13 ml) was applied every three days through Day 15 of the trial (on Days 0, 3, 6, 9, 12, and 15). Detailed description of application is available in Cunningham et al. (2017).

2.3. Necropsy

The oral dosing study was terminated at 21 days of exposure and the dermal oiling study was terminated on days 21 and 22 of exposure. All members of the high dose group of the oral study were euthanized by day 18 for humane reasons and so the day 21 sampling was not performed. Final blood samples were obtained (Cunningham et al., 2017), then DCCOs were euthanized by cervical dislocation. Organs were exposed, photographed, then removed, weighed and preserved in 10% neutral buffered formalin for histological examination.

2.4. Histopathology

Tissues were paraffin embedded, sectioned at approximately 5 μm, affixed to glass microscope slides and stained with hematoxylin and eosin. Organs were examined by board certified veterinary pathologists in the oral (RES) and dermal (DRR) study. Lesions were graded using the scale 1 = minimal, 2 = mild, 3 = moderate, 4 = severe that both pathologists developed. Hepatic iron was assessed using standard hematoxylin and eosin staining and Prussian blue staining, which is specific for iron, using the same grading used for lesions (Khan and Nag, 1993) and was examined by RES only. Quality assurance was performed by a third boarded pathologist, Dr. Jennifer Brazzell, to insure consistent results.

3. Results

3.1. Mortality

Of the 26 adult, mixed-sex DCCO used in the oral dose study, 16 were euthanized on Day 21. A total of 10 treated DCCOs died or were euthanized within 17 days of the start of the study for humane reasons, including all 9 high dose animals. DCCO began exhibiting clinical signs such as anemia, abnormal feces, lethargy, and behavioral thermogenesis (shivering under a heat lamp) at a total dose of approximately 80 ml/kg and all were dead prior to 200 ml/kg total dose.

Of the 25 subadult, mixed-sex DCCO used in the dermal exposure study one control bird and two treated birds died prior to Day 21 of the study. DCCO began exhibiting clinical signs such as anemia, hematochezia, behavioral thermogenesis, decreased appetite, and lethargy by day 10. One exposed bird died with probable septicemia (underlying etiologic agent not identified). One exposed bird died with no significant lesions that could be assessed as a cause of death. A chronic, necrotizing granuloma was found at the heart base of the control bird at necropsy.

3.2. Absolute and relative organ weights

Absolute and relative kidney weights were significantly greater in orally and dermally dosed DCCOs compared to their respective controls (Fig. 1a and b). Absolute and relative liver weights were significantly greater in DCCOs orally dosed with 5 ml oil kg bw^{-1} day^{-1} and in DCCOs that were dermally oiled compared to controls (Fig. 2a and b). There was no significant difference in absolute or relative (expressed as % body weight) brain, heart or spleen weight in orally or dermally dosed DCCOs compared to controls.

3.3. Histopathology

A number of histological lesions were found in tissues of DCCOs dosed orally or dermally with artificially weathered MC252 oil (Table 1). Organ weights from both exposure groups are summarized in Table 2.

3.3.1. Oral dosing study

Gross pathologic findings in orally dosed birds included enlarged kidneys, hearts that had flaccid musculature, proventricular ulcerative lesions, intestinal edema, yellow bile, and large numbers of intestinal parasite numbers in the low dose group with no to few intestinal parasites in the high dose group. During necropsy of orally dosed birds, blood pooled in the cavities and did not clot after several minutes whereas blood did not pool in control birds. Gonad identification revealed 20 males and 6 females with immature gonads, divided as described in the materials and methods.

Inflammatory renal lesions were common in all groups; however,