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Hepatic Porphyrin Patterns in Birds as a Promising Measure of Effect and Bioavailability of PCBs and other HAHs in Water and Sediments

Glen A. Fox, Sean W. Kennedy, and Suzanne Trudeau, Canadian Wildlife Service, National Wildlife Research Centre, Hull, QU, K1A 0H3; Christine A. Bishop, Canadian Wildlife Service, Environmental Conservation Branch, P.O. Box 5050, Burlington, ON L7R 4A6; and Mark Wayland, Canadian Wildlife Service, Prairie and Northern Wildlife Research Centre, 115 Perimeter Road, Saskatoon, SK., S7N 0X4.

Introduction

In susceptible bird species, chronic dietary exposure to polychlorinated biphenyls (PCBs)^{1) 2)}, hexachlorobenzene (HCB)³⁾, 2,3,7,8- TCDD⁴⁾, some chlorinated benzenes⁵⁾ and octachlorostyrene⁶⁾ results in the derangement of the heme biosynthetic pathway leading to the accumulation of uroporphyrin, hepata-, hexa-, and penta-carboxylporphyrins, collectively known as highly carboxylated porphyrins or HCPs. We have used measurements of the concentration of the individual HCPs to detect derangements in this essential biosynthetic process and therefore as a biological marker of effect in wild birds⁷⁾.

Uroporphyrins were the only HCPs detected (maximum 22 pmol/g) in 78 livers of 8 species of birds consuming relatively contaminated diets of fish and grains⁷⁾.

Uroporphyrins (≤ 24 pmol/g) were the only porphyrins detected in livers of all 61 adult Herring Gulls (*Larus argentatus*) collected from colonies on Canada's Atlantic coast; heptacarboxylporphyrins were infrequently detected, hexacarboxylporphyrins were detected in only one individual, and hexa- and penta-carboxylporphyrins were not detected. PCB concentrations in the livers of these, when pooled by colony, were all < 10 ppm.

We interpret these data as indicating that uroporphyrin concentrations in the liver of birds are normally less than 25 pmol/g and that hexa- and penta-carboxylporphyrins are very rarely detected in normal individuals. On this basis, we regard uroporphyrin concentrations of > 50 pmol/g and the presence of hexa- and penta-carboxylporphyrins as an indication of significant disruption of the heme biosynthetic pathway.

In this paper we extend our observations to prefledgling Herring Gulls and Double-crested Cormorant (*Phalacrocorax auritus*) and to prefledglings of the insectivorous Tree Swallow (*Tachycineta bicolor*). The Tree Swallow is a small, widely distributed 'songbird' that feeds over wetlands on emergent insects and its numbers can be manipulated by the introduction of suitable nest boxes.

Experimental Methods

Adult Herring Gulls were trapped on their nests during incubation and 21-day and 28-day old gull and chicks were captured by hand. Sixteen-day old Tree Swallow nestlings were collected from nest boxes erected in shoreline wetlands throughout the Great Lakes, and

from reference areas in the prairie provinces and Atlantic Coast. All were sacrificed by decapitation, and their livers removed and frozen in liquid nitrogen.

Highly carboxylated porphyrin concentrations in subsamples of liver were measured using the HPLC method of Kennedy and James⁸⁾ or Kennedy et al.⁹⁾ (pre-1990). PCB concentrations in gull livers, pooled by colony, or whole bodies of sibling Tree Swallow nestlings were determined in the laboratories of the National Wildlife Research Centre, Hull, as described in Peakall et al.¹⁰⁾.

Results and Discussion

Fish-eating birds

Unlike Herring Gulls from the Atlantic coast, the concentration of uroporphyrins ranged from 11 to 166 pmole/g liver in 136 adult Herring Gulls collected from 11 Great Lakes colonies and one colony in Northern Lake Winnipeg in 1991 (colony medians = 18 - 77 pmol/g). Heptacarboxylporphyrins were detected in 87% of individuals (50-100% in individual colonies), hexacarboxylporphyrins in 35% of individuals (5 - 100% in individual colonies), and pentacarboxylporphyrins in 4% of individuals (in 3 of 12 colonies at 0-12%). The total HCP concentrations ranged from 11 to 354 pmol/g liver, while colony medians ranged from 22 to 132 pmol/g. Hexacarboxylporphyrins were detected (≥ 4 pmol/g) in $>50\%$ of individuals in three locations; Hamilton Harbour (70%), lower Green Bay (80%), and Saginaw Bay (100%), all International Joint Commission-designated Areas of Concern. PCB concentrations in the livers of these gulls, when pooled by colony, ranged from 5 to 24 ppm.

Six of 12 Herring Gull chicks (less than one week old) collected from Scotch Bonnet Island in eastern Lake Ontario in 1973-74 had hepatic HCP concentrations ≤ 30 pmol/g, while the remaining individuals had concentrations of 55, 77, 94, 120, 570 and 850 pmol/g¹¹⁾. The latter value is the highest we have recorded to date in Herring Gulls of any age, and is much higher than the maximum (370 pmol/g) recorded in the adults collected in the same colony in 1974⁷⁾. Contaminant residues have declined markedly since 1974¹²⁻¹⁴⁾, and in 1991, the highest HCP concentration detected in 21-day old chicks (n=46) and 28-day old chicks (n=83) from nine Great Lakes colonies was 61 and 104 pmol/g, respectively. Both these maximums were in chicks from the Hamilton Harbour colony. The colony medians ranged from 11 to 16 pmol/g of uroporphyrins and 11 to 21 pmol/g of HCPs in 21-day old chicks from four colonies, and 7 to 14 pmol/g (all uroporphyrins) in 28-day old chicks from nine colonies. In the 21-day old chicks from Hamilton Harbour, heptacarboxylporphyrins were detected in 70%, hexacarboxylporphyrins in 50%, and pentacarboxylporphyrins in 5%. Similarly, hexacarboxylporphyrins were detected in 33% of 21-day old chicks from eastern Lake Ontario colonies in 1991, but not from colonies in Saginaw Bay, in Lake Superior or Lake Winnipeg. Hexacarboxylporphyrins were detected in only 2% of 28-day old chicks (n=103) from 11 colonies, in individuals from Hamilton Harbour and upper Green Bay. We believe that the presence of hexacarboxylporphyrins is an indicator of disruption of the heme biosynthetic pathway in young Herring Gulls, in the absence of elevated HCP concentrations. We wonder if porphyrin levels decline with age in gull chicks, and whether the optimal time to sample might be at hatching.

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Uroporphyrin (<4 to 23.5 pmol/g) was the only HCP detected in 21- and 28-day old Double-crested cormorant chicks (n=57) from colonies in northern Saskatchewan, eastern Lake Ontario, and upper Green Bay in 1991. There was little variation in uroporphyrin concentrations within these collections (medians = <4 to 5 pmol/g). This species may be less sensitive to the porphyrinogenic effect chemicals than Herring Gulls.

Insectivorous birds

To our knowledge, the only insectivorous bird species in which liver porphyrin concentrations have been reported is the Starling (*Sturnus vulgaris*). In 15-day old starling nestlings, uroporphyrin concentrations were at or below our detection level of 4 pmol/g, and hepta-, hexa-, and penta-carboxylporphyrin concentrations were lower still ¹⁵.

Tree Swallows feed on emergent insects and therefore provide a measure of sediment contamination, and the transfer of these contaminants to terrestrial food chains. We have limited data for this species from relatively pristine sites. Uroporphyrin and HCP values are somewhat higher than we have seen in other species. In 16-day old nestlings collected from nest boxes along the Wapiti River in Northern Alberta, the median uroporphyrin concentration was 31 pmol/g, and hexa- and penta-carboxylporphyrins were detected in 8%, and 4%, respectively of the 26 individuals. The highest uroporphyrin concentrations measured were 59 and 101 pmol/g liver. The median uroporphyrin concentration was 27 pmol/g and hexa- and penta-carboxylporphyrins were detected in 85% and 14% respectively in 47 individuals collected from nest boxes along the North Saskatchewan River, near Prince Albert. Here the highest uroporphyrin concentrations measured were 68 and 383 pmol/g liver, and four individuals had HCP concentrations >100 pmol/g. In 16-day old nestlings collected from nest boxes located in a relatively pristine marsh in central Ontario, uroporphyrin concentrations ranged from 9 to 36 pmol/g liver and hexa- and penta-carboxylporphyrins were detected in 40% and 0% of individuals.

Quite a different picture emerged when we examined Tree Swallows nesting in Great Lakes wetlands. Uroporphyrin concentrations in the livers of nestlings from boxes located on a tributary to Lake Erie ranged from 4 to 23 pmol/g and hexacarboxylporphyrins were detected in 75% of the individuals. Uroporphyrin concentrations in 7 of 8 individuals collected from a harbour on Lake Erie ranged from 5 to 57 pmol/g liver (the remaining liver contained 128 pmole/g), but hexacarboxylporphyrins were detected in only one (12%). This is in sharp contrast to 20 swallow chicks collected from Hamilton Harbour and Toronto Island on the Lake Ontario shoreline, in which individuals had uroporphyrin concentrations of 112, 183, 281, 289, 339, 516, and 795 pmol/g liver. Significant amounts of hexa- and penta-carboxylporphyrins were detected in most individuals from Toronto Island. The maximum HCP concentration of 930 pmol/g liver in the liver of one individual from Hamilton Harbour is the highest concentration we have measured in any species to date, while the 602 pmol/g in the liver from the highest individual in the Toronto Island collection is the third highest. Clearly, these nestling Tree Swallows fed emergent insects from Lake Ontario have a more severe porphyria than is seen currently in

fish-eating adult or pre fledgling Herring gulls from colonies in Hamilton Harbour, on Scotch Bonnet Island or elsewhere in the Great Lakes.

We have recently shown that extracts of porphyric Herring Gull livers are porphyrinogenic in a chick embryo hepatocyte bioassay, and that this porphyrinogenicity is the result of certain mono-ortho substituted PCB congeners ¹⁶⁾. A similar study is required before we can confirm the identity of the chemicals responsible for the porphyria observed in Tree Swallows fed emergent insects from some Great Lakes wetlands. Mean PCB concentrations in whole bodies of Tree Swallows from Lake Ontario wetlands were < 1 ppm, and eggs contained < 2 ppm. If PCBs are responsible for porphyria in Tree Swallows, we would expect very severe is the porphyria in nestlings of Tree Swallows from sites along the Hudson River where some young swallows contain more than 50 ppm of PCBs ¹⁷⁾, and that this porphyria might affect their health. Porphyria may occur in adult Tree Swallows at locations where it is seen in pre fledglings.

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Dioxin '97, Indianapolis, Indiana, USA

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