Levels of a Possible Naturally-Produced Bromo/Chloro Compound in Bird Eggs from the Great Lakes, Atlantic, and Pacific Coastal Areas

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Introduction

In 1988, an unknown halogenated compound was found at relatively high levels in a Leach's storm petrel egg sample (1). Subsequent work on a bald eagle liver sample demonstrated that a related series of organohalogens were present, rather than just a single compound. The series consists of four hexahalogenated compounds, $C_{10}H_6N_2Br_3Cl_3$ to $C_{10}H_6N_2Br_6$. The major congener was identified as $C_{10}H_6N_2Br_4Cl_2$.

An extensive literature search revealed no previous mention of such a compound. However, there exists a vast number of naturally-produced compounds that are highly halogenated (2). The majority of these are produced by marine organisms, and range structurally from simple halogenated alkanes to complex halogenated aromatic heterocycles. Some of these compounds are known to contain both bromine and chlorine, rather than just one type of halogen atom.

The major congener $C_{10}H_6N_2Br_4Cl_2$ was isolated from bald eagle liver and quantitated using GC-FID. Absolute and relative (to CB-153) concentrations of $C_{10}H_6N_2Br_4Cl_2$ in bird eggs were determined. The egg samples were obtained from the Great Lakes, Pacific, and Atlantic coastal areas.

Material and Methods

(I) Isolation of $C_{10}H_6N_2Br_4Cl_2$. Two 12 g samples of bald eagle liver were each combined with 145 g of activated Na_2SO_4 and Soxhlet extracted with 1:1 dichloromethane/hexane for 17 hours. The lipids were removed from the extract according to the GPC method used by Letcher *et al.* (3). The organohalogens in the extract were then

fractionated on a Florisil column (4). The combined F2 fractions from both samples were reduced to 390 μL.

(II) Quantitation of $C_{10}H_6N_2Br_4Cl_2$ in eagle liver extract. The amount of $C_{10}H_6N_2Br_4Cl_2$ in the extract was determined using GC-FID. It has been shown that FID relative carbon weight response factors are almost completely independent of molecular structure (5). This behaviour allows for the quantitation of a compound of unknown structure by FID using other organic compounds as standards. The following compounds were used as standards in the quantitation of $C_{10}H_6N_2Br_4Cl_2$: nicotine, hexachlorobenzene, heptachlor epoxide, pyrene, 2,2',4,4',5,5'-hexachlorobiphenyl, 2,3,3,4',5,5',6-heptachlorobiphenyl, mirex, t-chlordane, 2-(p-tolyl)-pyridine, α,α,α-tribromoquinaldine, 1,3-dibromoadamantane, and 2,3,3',4,4',5,6-heptabromodiphenyl ether. The concentration of $C_{10}H_6N_2Br_4Cl_2$ was calculated using the mean of the average carbon weight response factor for each of the standards.

(III) Sample analysis. Egg samples of species representing offshore surface feeders, offshore subsurface feeders, and inshore omnivores (Table 1) from the Great Lakes, Atlantic, and Pacific Oceans were obtained from the Specimen Bank at the National Wildlife Research Centre. Each sample of homogenized egg contents (5 g) was combined with Na_2SO_4 (50 g) and spiked with $^{13}C_1$-heptachlor epoxide recovery standard. The mixture was wet packed into a glass column and extracted with 220 mL of 1:1 dichloromethane/hexane. Lipid content of the extract was determined gravimetrically before lipid removal by GPC. The lipid-free extract was then ran through a Florisil column. The same GPC and Florisil methods were used during the sample analysis as with the isolation of $C_{10}H_6N_2Br_4Cl_2$. The F1 fraction was analyzed for CB-153 using a method similar to the one outlined in Norstrom *et al.* (4). The F2 and F3 fractions were combined and analyzed for $C_{10}H_6N_2Br_4Cl_2$ using GC-ECNI/MS in the SIM mode (m/z=79/81). The bald eagle liver extract analyzed in *(II)* was used as the $C_{10}H_6N_2Br_4Cl_2$ standard.

Results and Discussion

Quantitation of $C_{10}H_6N_2Br_4Cl_2$ in eagle liver extract. The carbon weight response factors for all 12 standard compounds were similar (mean 1888 ± 165). These results conform to those obtained by Yieru *et al.* (5). This consistency in carbon weight response factors allowed the $C_{10}H_6N_2Br_4Cl_2$ to be quantitated using the structurally dissimilar standards. Its concentration in the eagle liver extract was found to be 160 ± 20 ng/μL.

Levels of $C_{10}H_6N_2Br_4Cl_2$ in egg samples. All recoveries of internal standard were above 70%, therefore no corrections were made to the absolute and relative (to CB-153) concentrations listed in Table 1. CB-153 is a major bioaccumulating organohalogen, and was used in this case to normalize the levels of $C_{10}H_6N_2Br_4Cl_2$ to take into account the overall degree of contamination of the various samples. This normalization allowed samples less contaminated by organohalogens to be directly compared to highly contaminated samples.

There are two main trends observed in the results. The first is related to geographical distribution. Levels of $C_{10}H_6N_2Br_4Cl_2$ were only detected in marine samples; the compound was not seen in any of the Great Lakes samples. In addition, the absolute and relative concentrations of $C_{10}H_6N_2Br_4Cl_2$ were significantly higher (p=0.037) in the Pacific samples than in the Atlantic samples. The absolute and relative concentrations in the Pacific samples also varied much more (140-1.8 ppb, 2.1-0.043) than for the Atlantic samples (4.8-0.6 ppb, 0.097-0.0088).

This geographical trend is also apparent when only the results for the same or ecologically similar species are compared. For Leach's storm petrels, the Pacific samples had approximately 25 times higher absolute and relative concentrations of $C_{10}H_6N_2Br_4Cl_2$ than the Atlantic samples.

With the other two groups of species this trend is less pronounced. Between the rhinoceros auklets from the Pacific and the Atlantic puffins from the Atlantic coast the differences between absolute and relative concentrations were reduced to 1.2 and 1.4 times respectively. Finally, the glaucous-winged gulls on the Pacific coast had an absolute concentration of $C_{10}H_6N_2Br_4Cl_2$ roughly 5.1 times higher, and a relative concentration 11 times higher than the herring gulls on the Atlantic coast.

The second trend involves the feeding behaviour of the species studied. The species were chosen to represent offshore surface feeders, offshore subsurface feeders, and inshore omnivores; their classifications as such are listed in Table 1. Even though Leach's storm-petrels feed on vertically migrant fish and crustaceans, they were classified as surface feeders because their diet also includes fish, crustaceans, cephalopods, and other soft-bodied animals which exist near the surface. The relative amounts of each food item in the diet varies with geography and seasonal availability (6).

It was found that the offshore surface feeders from both the Pacific and Atlantic coasts had the highest absolute and relative concentration of $C_{10}H_6N_2Br_4Cl_2$ compared to both the offshore subsurface feeders and inshore omnivores. There was no similar prominent difference in absolute and relative concentrations between offshore subsurface feeders and inshore omnivores.

The source of $C_{10}H_6N_2Br_4Cl_2$ is not yet known. However, the two observed trends suggest that the presence of $C_{10}H_6N_2Br_4Cl_2$ is strictly a marine phenomenon and that it predominantly occurs in the surface layer. The absence of $C_{10}H_6N_2Br_4Cl_2$ from the Great Lakes also implies that atmospheric transport does not play a role in the distribution of the compound. These observations do not exclude a natural source for $C_{10}H_6N_2Br_4Cl_2$.

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References

1. Elliot, J.E.; Noble, D.G.; Norstrom, R.J.; Whitehead, P.E.; Simon, M.; Pearce, P.A.; Peakall, D.B. *Patterns and Trends of Organic Contaminants in Canadian Seabird Eggs,*

1968-90. In: *Persistant Polluants in Marine Ecosystems*; Walker, C.H.; Livingstone, D.R. Eds. Pergamon Press: **1992**; pp 181-194.

2. Gribble, G.W. Herz, W., Kirby, G. W., Moore, R. E., Steglich, W., and Tamm, Ch. editors. Naturally Occurring Organohalogen Compounds - A Comprehensive Survey. **1996**, 68. ISBN 3-211-82702-7886.

3. Letcher, R.J.; Norstrom, R.J. *Anal.Chem.* **1995**, *67*, 4155.

4. Norstrom, R.J.; Simon, M.; Muir, D.C.G.; Schweinsburg, R.E. *Environ.Sci.Technol.* **1988**, *22*, 1063.

5. Yieru, H.; Qingyu, O.; Weile, Y. *Anal.Chem.* **1990**, *62*, 2063.

6. Huntington, C.E.; Butler, R.G.; Mauck, R.A. *The Birds of North America* **1996**, *233*, 1.

Table 1. Concentrations of $C_{10}H_6N_2Br_4Cl_2$ relative to PCB-153 in various egg samples.

Sample collection location[a]	Ocean / Great Lakes	Number of eggs in pool	Collection date	$C_{10}H_6N_2Br_4Cl_2$ (ng/g sample, wet weight)	Ratio of $C_{10}H_6N_2Br_4Cl_2$ to CB-153
Offshore Surface Feeders					
LSP, Storm Isl., BC	Pacific	6	02/07/94	140	2.1
LSP, Cleland Isl., BC	Pacific	3	30/06/94	120	0.96
ALB, Sand Isl., Pac. Ocean	Pacific	10	03/12/93	32	0.34
LSP, Gull Isl., NF	Atlantic	10	22/06/96	4.6	0.097
LSP, Kent Isl., NB	Atlantic	10	16/06/96	4.8	0.029
Offshore Subsurface Feeders					
RA, Cleland Isl., BC	Pacific	5	29/04/90	3.1	0.050
RA, Storm Isl., BC	Pacific	5	04/05/90	1.8	0.043
AP, Gull Island, NF	Atlantic	10	15/06/96	1.7	0.048
AP, Machias Seal Isl., NB	Atlantic	10	19/05/96	2.5	0.017
Inshore Omnivores					
GWG, Five Finger Rocks, BC	Pacific	10	28/06/90	5.6	0.10
HG, Silver Isl., L. Superior	Great Lakes	7	18/05/96	nd	---------
HG, Middle Isl., L. Erie	Great Lakes	13	28/04/96	nd	---------
HG, Snake Isl., L. Ontario	Great Lakes	13	26/04/96	nd	---------
HG, Chantry Isl., L. Huron	Great Lakes	13	23/04/96	nd	---------
HG, Big Sister Isl., L. Michigan	Great Lakes	13	03/05/96	nd	---------
HG, Sable Isl., NS	Atlantic	10	13/05/96	1.6	0.0096
HG, Gull Isl., NF	Atlantic	4	20/05/96	0.61	0.0088

[a]LSP = Leach's storm-petrel, RA = rhinoceros auklet, GWG = glaucous-winged gull, ALB = black-footed albatross, HG = herring gull, AP = Atlantic puffin.
nd = not detected