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DNA Strand Length and EROD Activity in Relation to Two Screening Measures of Genotoxic Exposure in Great Lakes Herring Gulls

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Abstract. We collected tissues from herring gulls (*Larus argentatus*) nesting within and outside of the Great Lakes basin. Genotoxin exposure was assessed as fluorescent aromatic compounds (FACs) in bile and SOS Chromotest-inducing activity in muscle extracts. We determined whether these exposures were associated with decreased erythrocyte DNA strand length and/or induction of hepatic ethoxyresorufin-*O*-deethylase (EROD) activity. FACs were detected in all bile samples. Most muscle extracts produced a positive or marginal SOS response in the presence of S9. SOS induction potentials were strongly associated with dietary trophic level. The median molecular length of DNA isolated from erythrocytes for 14 of 17 adult and 10 of 11 prefledgling collections was reduced compared to the modal class for their respective age group suggesting widespread DNA damage. DNA damage was greatest in gulls from Saginaw Bay, Lake Huron. Median EROD activity in both adults and prefledglings from remote locations was significantly lower than that of gulls from the lower Great Lakes and was not associated with concentrations of benzo[*a*]pyrene (B[a]P)-like FACs. Our results indicate Great Lakes herring gulls were exposed to genotoxins and Ah-receptor activating agents in biologically significant concentrations in the early 1990s. These agents appear to be persistent bioaccumulative compounds and/or their metabolites.

Keywords: DNA strand length, EROD, SOS Chromotest, FACs, herring gulls, Great Lakes

Introduction

Wildlife residing in the Great Lakes basin is exposed to a wide variety of point sources of industrial pollution, many of which are genotoxic. Genotoxic chemicals have the capacity to affect the structural integrity of DNA and/or the fidelity of its biological expression, potentially resulting in carcinogenesis, decreased reproductive success, teratogenesis, and altered genotypic diversity with potential for population level and

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transgenerational consequences (Anderson et al., 1994; White et al., 1999; Schoen et al., 2002).

In the late 1960s and 1970s, hatching success of Herring Gulls (*Larus argentatus*) in some Great Lakes colonies was reduced, and deformities were observed in chicks of some fish-eating birds (Grasman et al., 1998). Ellenton and McPherson (1983) collected and incubated herring gull eggs from five Great Lakes colonies and a 'clean' reference site in 1981 and did not find significant differences among colonies in the incidence of sister chromatid exchanges in 7-day embryos. However, extracts prepared from the same clutches all produced sister chromatid exchanges and chromosome aberrations in Chinese hamster ovary (CHO) cells in culture but failed to produce point mutations in the Salmonella/mammalian microsome (Ames) assay (Ellenton et al., 1983). These observations suggested that herring gulls were exposed to biologically significant amounts of genotoxins. More recently, Yauk et al. (2000) found a significantly increased incidence of germline mutations in minisatelite DNA (tandem repeat DNA sequences) from herring gull families from Great Lakes colonies located near large steel mills operating coking facilities relative to colonies in rural settings removed from point sources of contamination.

The structural diversity of genotoxic agents makes their chemical identification and measurement in environmental media very complex. Analytical techniques fail to provide insight into the biological hazards associated with complex mixtures as they interact with, or are acted upon by, various environmental pathways (Tice, 1995) or on the impacts of compounds that are rapidly metabolized and excreted (Arcand-Hoy and Metcalfe, 1999). Screening methods have been introduced to detect the presence of certain classes of chemicals in environmental media, tissue extracts and body fluids at reduced costs while bioassays allow the detection of various forms of genotoxic *activity*. Such approaches are highly suited for environmental biomonitoring for genotoxicity.

Polycyclic aromatic hydrocarbons (PAHs) are a major class of genotoxic chemicals commonly found in organisms living in contaminated environments. Ingested PAHs are rapidly converted to water-soluble metabolites and excreted in bile. Krahn et al. (1984) developed a relatively simple procedure to measure fluorescent aromatic compounds (FACs) as a semiquantitative estimate of the relative concentrations of PAH metabolites in fish bile. This method has been used in studies of brown bullheads (*Ameiurus nebulosus*) in the Great Lakes (Maccubbin et al., 1988; Johnston and Baumann, 1989; Leadly et al., 1999). Broman et al. (1990) reported that the gall bladder and bile contained the highest PAH concentrations in common eiders (*Somateria mollissima*) suggesting this procedure would be applicable for monitoring PAH exposure in birds. Some PAHs, especially those containing more than three aromatic rings, induce the CYP1A family by an Ah-receptor dependent mechanism and increase aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-*O*-deethylase (EROD) activity. Induction of CYP1A often leads to the formation of electrophilic reactive metabolites which result in adduct formation, the initial biochemical lesion thought to be involved in genotoxicity and carcinogenicity. There was a strong association between EROD activity and CYP1A protein concentration in 47 adult herring gulls (Kennedy et al., 2003). Cytochrome P450 1A1 induction, measured as EROD activity, reflects Ah receptor activation and is thus an effective biomarker of both exposure and potential toxicity.

Assays for detecting the effects of PAHs and other genotoxins include a variety of *in vitro* bioassays of genotoxic potency of contaminated environmental media and assays of the DNA integrity of indigenous organisms living *in situ* in contaminated environments. The SOS Chromotest, a sensitive, quantitative *in vitro* bioassay, has been used to measure some forms of genotoxic activity of unidentified chemicals and metabolites including those accumulated in, or bound to, river sediments (White et al., 1998a), bivalve molluscs (White et al., 1997), and fish and macroinvetebrates (White et al., 1998b) from the St. Lawrence and Saguenay Rivers. Induction of this error-prone DNA repair pathway, or "SOS response" (Walker, 1984), in *Escherichia coli* occurs in response to a wide range of DNA damage scenarios (Quillaret et al., 1982). *In situ* assays used to detect effects of PAHs and complex mixtures of genotoxins include measurements of DNA integrity including sister chromatid exchanges,

chromosome aberrations, unscheduled DNA synthesis, variability in DNA content, micronuclei, and DNA adducts. Another measure of DNA integrity is the relative length of nuclear DNA strands extracted from erythrocytes and separated under alkaline conditions using the agarose gel electrophoresis technique (Theodorakis et al., 1994).

In an effort to assess exposure and potential genotoxic effects of PAHs and other persistent contaminants in Great Lakes food chains in the early 1990s, we collected bile, erythrocytes, liver and muscle from individual herring gulls nesting in numerous colonies located within and outside of the Great Lakes basin as part of a comprehensive health assessment (Fox et al., 2002). We assessed exposure to genotoxins using bile FACs and the SOS Chromotest bioassay and determined whether these exposures were associated with decreased erythrocyte DNA strand length and/or induction of hepatic EROD activity in these piscivorous birds.

Study area and methods

Under Federal permit, we trapped 200 adult gulls on their nests in 16 colonies and captured 101 prefledglings by hand in 11 colonies. The locations of these colonies and their identifiers are illustrated in Figure 1. STR, HAM, FTG, SAG, KID, JFB and THU are in IJC-designated areas of concern (AOCs), and GNT is near the Nipigon Bay AOC. With the exceptions of the collections from STR, DBL, and MON (1992), and MSI, MAR, CAR, THU and JFB (1993), the collections were made in 1991.

Gulls were weighed, measured, and bled from the brachial vein. Heparinized blood was immediately centrifuged and the plasma and pelleted erythrocytes were aliquoted into cryogenic vials and quickly immersed in liquid nitrogen. Gulls were then decapitated with a guillotine and allowed to bleed out. Gender was determined by gonadal examination. The bile duct was clamped with a hemostat, and the gall bladder excised and

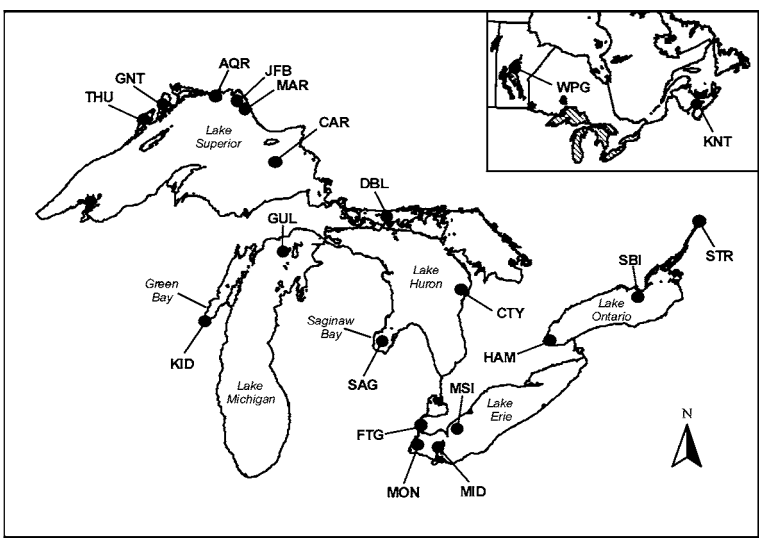

Figure 1. Collection locations and their identifiers. In the Great Lakes, we sampled in colonies on Strachan Island (STR) west of Cornwall in the St. Lawrence River; Scotch Bonnet Island (SBI) and Hamilton Harbour (HAM) in Lake Ontario; in Lake Erie's western basin at Monroe (MON), Middle Sister (MSI), and Middle (MID) Islands; Fighting Island (FTG) in the Detroit River; in Lake Huron on Double Island (DBL) in the North Channel, on Chantry Island (CTY), and on the Confined Disposal Facility (adults) and Little Charity Island (prefledglings) in Saginaw Bay (SAG); in Lake Michigan on Big Gull Island (GUL), and near the mouth of the Fox River on Kidney Island (KID) in lower Green Bay; on the north shore of Lake Superior on Skin Island near Marathon (MAR), an unnamed Island (AQR) east of the mouth of the Aquasabon River east of Terrace Bay, Cody Island in Jackfish Bay (JFB) east of Terrace Bay**,** Granite Island (GNT) in Black Bay, Mutton Island near Thunder Bay (THU), and remote Caribou Island (CAR) in the offshore waters. Reference colonies (insert) were on an unnamed island (WPG) in the north basin of Lake Winnipeg, near Grand Rapids, MB, and on Kent Island (KNT), off Grand Manan, NB in the Bay of Fundy.

punctured in a small weighing boat. The bile was transferred to a small cryogenic vial and frozen in liquid nitrogen. Within 10 min of the bird's death the entire left lobe of the liver was wrapped in high density polyethylene film, placed in 20 ml linear polyethylene scintillation vials and preserved in liquid nitrogen for isolation of microsomes. A 4–5 g portion of the right lobe was placed in an acetone and hexane-rinsed glass jar for organochlorine analysis and transported to the lab on wet ice before freezing at –20 °C. The left kidney was placed in a nitric acid-washed polyethylene vial for preservation in liquid nitrogen prior to metals determinations. The entire breast muscle was excised, placed in a polyethylene bag and transported to the lab on wet ice before freezing at −20 °C. Various other tissues were collected and appropriately preserved for biochemical and histological studies.

The microsomal fraction was isolated from a homogenate of the entire left lobe of the liver using the gel filtration method of Pyykkö (1983). The EROD catalytic activity of the microsomal preparations was determined in triplicate using the CWS Biomarker Laboratory's protocol based on the method of Pohl and Fouts (1980) and protein concentrations were determined according to Peterson's (1977) simplification of Lowry's method. The immunodetectable CYP1A protein concentration in microsomes was determined by SDS-PAGE for a subset of individuals (see Kennedy et al., 2003 for details).

We assessed DNA integrity by quantifying the relative length of nuclear DNA strands isolated from the packed erythrocytes using the agarose gel electrophoresis technique of Theodorakis et al. (1994). Methods for controlling DNA shearing during isolation, controlling the temperature, and recirculation of the buffer to dissipate pH gradients at the electrodes during electrophoresis were as described in Theodorakis et al. (1999). Under alkaline conditions (pH = 12), the double helix of the DNA molecule unwinds resulting in single strands of DNA. The midpoint of the DNA size distribution in the gel was determined by densitometry, and reported as the median molecular length (MML) for that DNA sample. The MML is inversely proportional to the number of strand breaks.

We estimated relative exposure to PAHs by measuring FACs in bile using a HPLC-fluorescence technique adapted from Krahn et al. (1984). The density of the bile was determined and a 1 in 10 dilution in distilled water was injected onto a 4.6 mm × 25 cm reverse phase 5 μm Ultrasphere ODS column (Beckman) equipped with a 4.6 mm × 3 cm 5 μm C18 ODS Spheri-5 guard column (Brownlee) and a Varian 9019 solvent delivery system and 9100 autosampler, controlled by a Varian LC Star system. The column temperature was maintained at 35 ± 1 °C. Samples were eluted with acetic acid/water 5 μl/l (solvent A) and acetonitrile (solvent B) using a linear gradient as follows: 100% A to 100% B in 15 min, 10 min at 100% B, 1 min to return to 100% A, and 4 min at 100% A to reequilibrate at a flow rate of 1.0 ml/min. Fluorescence of the eluate was measured with a Perkin-Elmer LC 240 dual channel detector at wavelength pairs $280_{ex}/340_{em}$ and $380_{ex}/430_{em}$ nm, for naphthalene (NPH)- and benzo[*a*]pyrene (B[*a*]P)-like compounds, respectively. Integrated areas of peaks eluting from 7 to 26 minutes were summed and the area sum was converted to NPH- and B[*a*]P-EQuivalents (ng/g wet weight); the amount of NPH or B[*a*]P that would be present if the integrated area were attributed only to that compound based on identical chromatography of B[*a*]P and NPH standard solutions.

Five to 7 g of breast muscle from each individual was used to create pools of adults and prefledglings from 12 and 8 collections, respectively. The 20 pooled homogenates (25 g) were lyophilizied overnight, and ground to a fine powder with anhydrous sodium sulfate in a glass mortar and pestle. Tissues were Soxhlet extracted with 300 ml of dichloromethane for 18 h. Extracts were rotary evaporated to ~2 ml, placed in preweighed amber glass vials, and reduced under a gentle stream of nitrogen until only lipid remained. The residue was re-dissolved in dichloromethane and lipids and extractable organics separated via gel permeation chromatography on Bio-Beads SX-3 (see White et al., 1997 for details). The collected eluates were reduced via rotary evaporation and the solvent exchanged for 200 μl of dimethyl sulfoxide in preparation for SOS Chromotest analysis. The automated SOS Chromotest procedure, described in detail in White et al. (1996, 1997), employed *E. coli* tester strain PQ 37. The strain possesses an *SfiA-lacZ* fusion that places the

production of B-galactosidase under the control of the SOS response (the error-prone DNA repair pathway).

Constituitive alkaline phosphatase expression provides a simultaneous measure of bacteriostatic effects. Each extract, assayed without knowledge of sample origin, was tested both in the presence and absence of 1% v/v postmitochondrial supernatant (S9 fraction) obtained from Aroclor 1254-induced rat liver (Molecular Toxicology Products Inc., Boone, SC). Genotoxicity results were qualitatively categorized as: negative, marginal, significant positive, or erratic. Two measures of genotoxic potency were calculated for each sample that elicited positive or marginal responses: SOS Response Inducing Potency (SOSIP), the initial slope of the concentration response-curve, and Threshold Concentration Value (TCV), the concentration, inferred from the fitted concentration-response function, that is required to elicit a response equal to the upper 95% confidence limit of the solvent control. 2-aminoanthracene (2-AA) and 4-nitroquinoline-1-oxide (4-NQO) were used as positive controls. Mean SOSIP for 2-AA was 1.256 (SD = 0.256, n = 21). Mean SOSIP for 4-NQO was 55.422 (SD = 12.509, n = 33). The upper 95% confidence limit for the solvent control (DMSO) is 1.061 (SD = 0.058, n = 108). Lipid content values were used to convert SOSIP and TCV values to effect units per equivalent mg of extractable lipid.

Organochlorine contaminants and 42 PCB congeners, 5 nonortho PCB congeners, and all 2,3,7,8-substituted polychlorinated dibenzo-*p*-dioxin (PCDD) and dibenzofuran (PCDF) congeners (in site pools of liver homogenate), and Hg, Cd, Pb, and Se (in site pools of kidney homogenate) were measured in the analytical service laboratory of the Canadian Wildlife Service's National Wildlife Research Centre, Hull, QC.. 2,3,7,8-tetrachloro-*p*-dioxin equivalents (TEQs) were calculated using the WHO TEFs for birds. Thirty trace elements were measured by ICP-MS in pooled adult kidney homogenates by Fenwick Laboratories Ltd. (Halifax, NS) (see Fox et al., 2002 for details). Extracts were also prepared from a subset of individual livers and analyzed for organochlorine contaminants and PCB congeners as above. These extracts were also assayed for TCDD-EQ concentrations using the Chicken Embryo Hepatocyte bioassay (see Kennedy et al., 2003 for details).

Relative trophic level was calculated using muscle $\delta^{15}N$ measurements, adjusted for interlake differences in baseline $\delta^{15}N$ signatures (details in Fox et al., 2002). Stable-nitrogen ($\delta^{15}N$) isotope signatures were determined in approximately 1 g samples of breast muscle using the methodology of Schmutz and Hobson (1998).

Statistics

With the exception of EROD activity, not all individuals were analyzed for all parameters. The data reported here are restricted to the common set of 160 individuals to insure statistical validity. Most data were not normally distributed or consistently so. Consequently, the non-parametric Kruskal–Wallis ANOVA (H) followed by Dunn's Test for pairwise comparisons, Mann Whitney U-test, Kolmogorov–Smirnov two-sample test (D), and Spearman's correlation (r_s) were used to explore the untransformed data and test hypotheses. The data were also transformed ($\log_{10}$ except for MML which required square root transformation) to meet the assumptions of linear regression. The degree of animal to animal heterogeneity in EROD induction and MML within collections was assessed as dispersion (variance ÷ mean) (Tice, 1995). Correlation analysis followed by stepwise linear regression was used to identify exposure-response associations between pooled tissue concentrations of various contaminants (as measured above and published in Fox et al., 2002) and responses including SOS Chromotest potency measures, and the medians for EROD activity and MML using the transformed variables. SigmaStat (SPSS Inc., Chicago, IL) statistical software was used for statistical analyses.

Results

Genotoxin exposure

FACs

Detectable biliary FACs concentrations were found in all 136 adults from 16 colonies (Table 1). NAP-EQs varied by a factor of 276× among all individuals and from 2.7 to 64× within the 13

Table 1. Concentrations of naphthalene- and benzo[*a*]pyrene-like FACs in the bile of adult herring gulls, reported as NPH- and B[*a*]P-EQs. (ng/g wet weight of bile)

			NPH-EQs			B[*a*]P-EQs			
Colony	Year	*N*	Median	Mean	Range	Median	Mean	Range	NPH/B[*a*]P
WPG	1991	12	8120	13,023	659–42,168	124	153	10–698	65
THU	1993	2	54,270	54,270	24,614–83,927	846	846	643–1049	64
AQR	1991	10	32,986	40,279	12,577–100,225	428	572	131–1557	77
GNT	1991	10	22,657	32,207	9148–135,433	344	1089	95–6105	66
MAR	1993	3	15,071	14,826	13,772–15,689	476	534	457–668	32
CAR	1993	4	6021	6852	3869–11,496	294	312	195–464	20
GUL	1991	10	11,336	17,032	8672–57,576	170	215	87–546	67
KID	1991	10	48,500	52,275	18,867–132,342	115	136	0–310	422
SAG	1991	6	28,419	39,635	19,061–75,238	392	499	114–1121	72
CTY	1991	10	7100	16,055	1788–65,475	283	555	105–1925	25
FTG	1991	9	43,594	60,289	12,776–18,1954	362	466	141–1549	120
MSI	1993	8	27,601	36,548	17,136–87,350	250	348	134–1113	110
MID	1991	10	28,162	33,666	8488–65,066	162	200	56–560	174
HAM	1991	8	25,437	26,865	11,465–48,373	151	303	71–409	168
SBI	1991	8	17,483	25,644	5954–61,611	360	457	182–993	49
KNT	1991	13	4344	4870	2651–9472	122	144	23–302	35

colonies where five or more bile samples were analyzed. The median NAP-EQ concentration varied from 4344 μg/g (KNT) to 48,500 μg/g (KID), or ~11×. Among-individual variation for B[*a*]P-EQs was greater (>6105×), and ranged from 5.4× to >310× within the 13 colonies. The median B[*a*]P-EQ concentration varied from 115 μg/g (KID) to 428 μg/g (AQR), or 3.7×.

There was significant among-colony variation for both NPH-EQs ($H_{16} = 71.07$, $p < 0.001$) and B[*a*]P-EQs ($H_{16} = 60.82$, $p < 0.001$ K–W test). With the exception of the GNT colony, concentrations of NPH-EQs were lowest in gulls from remote colonies (KNT, CAR, WPG, GUL) and highest in gulls from KID in lower Green Bay and FTG in the Detroit River. NPH-EQs in bile from KNT and KID differed significantly (Dunn's test). The highest individual concentrations of NPH-EQs were measured in gulls from FTG, KID, and GNT. There were no significant differences in B[*a*]P-EQs among sites ($p > 0.05$, Dunn's test). Surprisingly, the colony in lower Green Bay (KID) had the lowest biliary concentration of B[*a*]P-EQs, followed by remote colonies (KNT, CAR, WPG). The highest individual concentrations of B[*a*]P-EQs were measured in gulls from GNT, CTY, AQR, and FTG.

The within-colony variability in NPH- and B[*a*]P-EQs were not significantly correlated ($r_s^{13} = 0.434$, $p > 0.05$) suggesting that their sources differ. According to Krahn et al. (1986a,b), B[*a*]P-like PAHs predominate in pyrolytic sources, while NPH-like PAHs predominate in petroleum sources. NPH/B[*a*]P varied significantly among sites ($H_{16} = 55.92$, $p < 0.001$, K–W test) suggesting that the relative importance of pyrolytic versus petroleum sources varies among sites (Table 1). Petroleum-related sources were of greater importance at the industrialized sites on the lower Great Lakes (KID, MID, and HAM) than in the more remote sites in Lake Superior (CAR, MAR) and the Bay of Fundy (KNT). The NPH/B[*a*]P ratio for KID was significantly higher than MAR, CAR and KNT ($p < 0.05$, Dunn's test).

SOS Chromotest

The results of the SOS Chromotest for the dichloromethane extracts of breast muscle pools are summarized in Table 2. Of the 20 extracts, only one (SAG prefledglings) failed to induce a response that exceeded the solvent control. Two extracts, both from adults (SBI and FTG), gave positive responses (exceeded the solvent control at >2 concentrations) in the absence of S9 but the responses were erratic (not strictly dose-related). The extract from adults from MID gave a marginal response (exceeded the solvent control at <3 concentrations) in the absence of S9. Seven extracts from adults gave a positive response and 9 (4

Table 2. SOS Chromotest response for dichloromethane extracts of pooled herring gull breast muscle collected in 1991

Colony	Age	*N*	Response	S9	SOSIP	TCV	Trophic level	SOSIP/TL
WPG	Ad	10	Pos	+	5.63	0.00978	3.7	1.52
AQR	Ad	8	Pos	+	2.62	0.01984	3.6	0.73
GNT	Ad	10	Mar[a]	–	–	–	3.4	–
GUL	Ad	10	Pos	+	11.89	0.00383	4.5	2.64
KID	Ad	10	Pos[b]	+	–	–	4.7	–
SAG	Ad	8	Mar[b]	+	–	–	5.0	–
CTY	Ad	10	Pos	+	5.30	0.00761	3.4	1.56
FTG	Ad	8	Mar	+	1.13	0.0704	2.1	0.54
MID	Ad	10	Mar[c]	–	8.19	0.01507	4.7	1.74
HAM	Ad	10	Pos	+	5.23	0.00689	3.0	1.74
SBI	Ad	8	Pos[c]	+	4.28	0.02189	3.3	1.30
KNT	Ad	10	Mar	+	2.04	0.06350	2.6	0.78
WPG	Pf	10	Mar	+	2.99	0.03496	–	0.81[d]
AQR	Pf	8	Mar[a]	+	–	–	–	–
GUL	Pf	10	Mar[a]	–	–	–	–	–
SAG	Pf	10	Neg	+/–	–	–	–	–
CTY	Pf	10	Mar[a]	–	–	–	–	–
HAM	Pf	7	Mar	+	5.00	0.02941	–	1.67[d]
SBI	Pf	8	Mar	+	4.68	0.01493	–	1.42[d]
KNT	Pf	10	Mar	+	4.18	0.02912	–	1.61[d]

SOSIP and TCV calculated on per mg lipid basis. The relative Trophic level was based on the mean $\delta^{15}N$ signature for breast muscle.
[a]Slope of concentration-response negative.
[b]Erratic concentration-response prevented calculation of SOSIP and TCV.
[c]Extract highly toxic.
[d]Calculated using adult trophic level.

from adults, 5 from prefledglings) gave a marginal response in the presence of S9. The S9 mix increased the SOS response in 9 extracts and appeared to decrease it in 3. The extracts from SBI (L. Ontario) and MID (L. Erie) were highly toxic to the tester bacterium.

It was possible to calculate SOSIPs for 9 of 12 adult extracts and 4 of 8 prefledgling extracts. The SOSIPs for adult extracts ranged from 11.89 (GUL in northern L. Michigan) to 1.13 (FTG in the Detroit R.), a factor of 10.5×. For prefledglings, the SOSIPs ranged from 5.00 (HAM in L. Ontario) to 2.29 (WPG in L. Winnipeg), a factor of 1.7×. The SOSIPs for prefledglings were greater than adults at SBI and KNT (in Bay of Fundy), similar at HAM, and less than adults at WPG. Variation in TCV was 16.6× in adults and 2.3× in prefledglings.

On a colony basis, SOSIP was significantly correlated only with trophic level in adults (Table 3). A stepwise linear regression model ($R^2 = 0.94$) selected (1) trophic level (2) DDE, and (3) TEQs for adults but rejected all variables when adults and prefledglings were combined (Table 4). TCV was significantly correlated with PCDDs in adults, and PCDDs, DDE, and PCBs in adults and prefledglings combined (Table 3). However, only PCDDs were selected in a stepwise linear regression in adults ($R^2 = 0.44$) and adults and prefledglings combined ($R^2 = 0.50$).

Responses to exposure

DNA strand length

The median length of DNA strands isolated from erythrocytes of herring gulls ranged from 1.72 to 36.4 kilobases (kb) in 160 adults and 1.34 to 46.5 kb in 101 prefledglings (Tables 5 and 6). There was also significant spatial variation among colonies in median MML (Table 5) for both adults ($H_{16} = 30.305$, $p = 0.016$, K–W test) and prefledglings ($H_{10} = 27.50$, $p = 0.002$, K–W test). Strand length distributions differed significantly between the pooled adults and prefledglings ($p < 0.01$, K-S 2-sample test). However, in none of the eight colonies where both adults and prefledglings were collected in the same year, did the

Table 3. Spearman's Rank correlations between contaminant measures and SOS Chromotest activity, MML, and EROD activity in adult (Ad) and prefledgling (Pf) herring gulls

Y	X	Age	r_s	*n*	*p*
SOSIP	Trophic level	Ad	+0.867	9	0.000
TCV	PCDDs	Ad	−0.695	9	0.030
	PCDDs	Ad + Pf	−0.758	13	0.002
	DDE	Ad + Pf	−0.643	13	0.017
	PCBs	Ad + Pf	−0.610	13	0.025
MML	PCDDs	Pf	−0.656	11	0.026
	Hg	Pf	−0.618	11	0.039
√MML	PCDDs	Ad	−0.651	12	0.020
	NPH-EQs	Ad	−0.623	12	0.028
	Pb	Ad	−0.601	12	0.036
	Trophic level	Ad	−0.571	12	0.048
EROD	NPH-EQs	Ad	0.378	135	0.000
	B[*a*]P-EQs	Ad	0.168	135	0.052
EROD	PCDDs	Ad	0.627	12	0.026
	NPH-EQs	Ad	0.587	12	0.042
	Pb	Ad	0.699	12	0.010
EROD	PCDDs	Pf	0.624	11	0.035
EROD	PCDDs	Ad + Pf	0.749	23	0.000
	Cd	Ad + Pf	0.590	23	0.003
	PCBs	Ad + Pf	0.562	23	0.005
	TEQs	Ad + Pf	0.516	23	0.012
	Hg	Ad + Pf	0.464	23	0.026
	DDE	Ad + Pf	0.459	23	0.027

Based on non-transformed data (with the exception of √MML).

Table 4. Significant stepwise linear regressions of contaminant measures in relation to MML, SOS Chromotest and EROD activity in adult (Ad) and prefledgling (Pf) herring gulls

Y	X	Age (*n*)	R^2	*p*	DF	SEE	Power
SOSIP	1.Trophic level	Ad (12)	0.793	0.001	1	0.153	
	2. DDE		0.891	0.020	2	0.120	
	3. TEQs		0.944	0.081	3	0.094	0.999
TCV	1. PCDDs	Ad (12)	0.440	0.051	1	0.290	0.449
	1. PCDDs	Ad + Pf (23)	0.504	0.007	1	0.254	0.801
MML	1. PCDDs	Ad (12)	0.751	0.000	1	0.041	
	3. Se		0.972	0.000	3	0.016	
	4. SOSIP		0.991	0.000	4	0.011	
	5. TEQs		0.999	0.004	5	0.005	
	6. PCBs		0.999	0.081	5	0.003	1.000
	1. Hg	Pf (11)	0.387	0.041	1	0.094	0.540
√MML	1. NPH-EQs	Ad (12)	0.302	0.064	1	1.538	0.457
	1. Hg	Pf (11)	0.404	0.036	1	0.468	0.564
	1. PCBs	Ad + Pf (23)	0.793	0.000	1	0.394	0.994
EROD	1. NPH-EQs	Ad (134)	0.157	0.000	1	0.359	0.998
	1. B[*a*]P-EQs	Ad (29)	0.377	0.000	1	0.522	
	2. PCB 118		0.554	0.003	2	0.450	0.999
	1. PCDDs	Ad (12)	0.350	0.043	1	0.240	0.532
	1. PCDDs	Pf (11)	0.482	0.018	1	0.267	0.678
	1. PCDDs	Ad + Pf (23)	0.514	0.001	1	0.268	
	2. Cd		0.625	0.025	2	0.241	0.998

All regressions passed tests for normality and constant variance. SSE = Standard error of the estimate.

Table 5. DNA strand length (MML) and EROD activity in adult herring gulls

			MML (kb)				EROD activity (pmol/min/mg protein)			
Colony	Year	*N*	Median	Mean	Range	Dispersion	Median	Mean	Range	Dispersion
WPG	1991	10	27.38	26.67	13.26–5.37	2.142	40.7	45.3	11.4–118.3	19.046
THU	1993	10	26.78	22.94	4.58–6.12	4.773	124.6	130.3	52.9–217.5	23.351
AQR	1991	8	26.62	23.97	15.56–0.08	1.489	248.2	312.9	173–616	75.732
AQR	1993	10	30.00	31.00	27.10–6.3	0.326	145.1	115.6	21–208	36.010
GNT	1991	10	24.95	24.19	16.18–0.69	1.210	155.7	195.2	38.5–391	95.677
MAR	1993	10	25.82	23.81	12.09–1.25	1.887	125.6	134.4	92.1–194	10.057
CAR	1993	10	24.54	24.94	17.14–33.42	1.274	66.1	75.9	4.5–205	71.797
GUL	1991	10	29.55	27.82	15.24–32.78	1.038	213.4	225.3	122–376	40.693
KID	1991	10	26.45	26.28	17.30–34.98	1.553	330.4	329.6	209–483	25.781
SAG	1991	9	18.19	17.63	1.72–29.20	5.055	335.4	328.2	180–501	34.469
CTY	1991	10	20.53	18.86	4.54–32.73	4.579	263.2	372.7	165–1077	195.87
FTG	1991	8	25.11	23.91	11.83–31.42	2.012	289.9	310.8	198–470	85.823
MSI	1993	7	28.53	27.72	17.46–35.29	1.305	87.5	132.7	58–295	55.195
MID	1991	10	18.22	19.01	5.76–30.95	3.514	108.9	123.1	87–184	10.314
HAM	1991	10	27.08	25.73	13.90–33.79	2.080	338.9	438.2	199–849	132.681
SBI	1991	8	25.16	23.08	9.77–36.43	2.977	234.2	233.3	69–427	43.503
KNT	1991	10	18.65	19.71	9.69–30.63	3.207	88.1	113.8	14–237	33.132

Table 6. DNA strand length (MML) and EROD activity in prefledgling herring gulls

			MML (kb)				EROD activity (pmol/min/mg protein)			
Colony	Year	*N*	Median	Mean	Range	Dispersion	Median	Mean	Range	Dispersion
WPG	1991	10	17.60	17.96	1.77–38.80	8.458	24.4	25.8	12.0–42	3.576
AQR	1991	8	24.36	23.53	16.38–31.75	1.391	156.8	155.7	52–293	40.733
GUL	1991	10	25.30	26.29	16.42–35.38	0.973	32.4	57.6	6.5–214	71.095
DBL	1993	9	25.5	21.04	14.50–30.99	4.781	28.0	42.1	17–159	49.437
SAG	1991	10	12.54	11.68	1.72–27.76	5.778	80.3	79.0	25–139	20.384
CTY	1991	10	20.91	23.12	13.61–40.14	2.967	174.6	194.1	38–525	100.966
MON	1993	9	22.41	21.46	11.41–26.33	0.969	99.0	101.7	30–179	23.151
HAM	1991	7	15.87	18.01	7.70–30.18	4.859	253.5	299.4	125–653	108.423
SBI	1991	8	15.69	16.82	10.38–30.18	2.431	69.3	82.7	24–190	30.282
STR	1991	10	14.98	14.52	1.34–30.99	6.170	111.8	181.7	30–839	315.285
KNT	1991	10	27.26	28.04	13.70–46.53	3.571	33.6	32.7	15–53	4.701

MML distribution of adults differ significantly from prefledglings (K-S 2 sample test, $p > 0.10$).

With the exception of the JFB collection, the distribution of MMLs in most collections was bi- or multi-phasic, suggesting some degree of structural damage had occurred at most sites in both age groups, including both reference sites. Among colonies, dispersion varied from 0.3 (JFB) to 5.0 (SAG) or a factor of 15.5× for adults and from 1.0 (MON) to 8.5 (WPG) or a factor of 8.7× for prefledglings. Dispersion was quite similar in adults and prefledglings at JFB, GUL, SBI, and KNT but was 2× higher in CTY adults, and 2× and 4× higher in HAM and WPG prefledglings, respectively ($p = 0.057$, MWU).

The median MML for the JFB collection was significantly greater than that for the SAG, CTY, MID and KNT collections (Tukey test). We assume the modal class of the frequency distribution represents the most "normal" MML. The modal class for the 160 adults was 28+ to 30 kb, and for the 101 prefledglings 26+ to 28 kb, (261 individuals pooled was 26+ to 28 kb). The median MML for adults from two of 17 collections (GUL in

L. Michigan and MSI in L. Erie) fall within this "normal" adult value, while one (JFB in L. Superior) exceeds it, and the remaining 14 are lower than the modal class, the lowest (MID in L. Erie and SAG in Saginaw Bay) by 37%. In the case of the prefledglings, only the prefledgling median from KNT falls within the modal class while 10 of 11 were lower, the lowest (SAG) by 54%.

In 124 adults in which both MML and FACs were measured, there were no associations of biological significance, and in 31 adults which were analyzed individually for contaminants, MML was not correlated with TPCBs, PCBs 105 and 118, HCB, DDE, mirex, *t*-Nonochlor or chick embryo hepatocyte-derived TCDD-Eqs. Similarly, there were no significant correlations on a colony basis (Table 3). However, a stepwise linear regression model ($R^2 = 0.999$) selected (1) PCDDs, (2) Se, (3) SOSIP, and (4) TEQs (Table 4). In prefledglings, MML was negatively correlated with PCDDs and Hg (Table 3) and a stepwise linear regression model ($R^2 = 0.39$) selected Hg (Table 4). In adults √MML was negatively correlated with PCDDs, NPH-EQs, Pb, and trophic level (Table 3). Stepwise linear regression models selected NPH-EQs ($R^2 = 0.30$) for adults, Hg ($R^2 = 0.40$) for prefledlings, and PCBs ($R^2 = 0.79$) when adults and prefledglings were combined (Table 4).

EROD activity

There was significant spatial variation in EROD activity (Table 5) among colonies for adult gulls ($H_{16} = 100.4$, $p < 0.001$, K–W test). Overall EROD activity varied by a factor of 239× among all adults, and 8.3× among colony medians. Within Great Lakes colonies, EROD activity varied by a factor of 16× among individuals, and 5.1× among colony medians. Within colonies, dispersion varied from a low of 10.057 (MAR in L. Superior) to a high of 185.87 at CTY (in L. Huron) or by a factor of 19.5×.

Median EROD activity in adults from both WPG (in L. Winnipeg) and CAR (in L. Superior) was significantly lower than that of HAM in L. Ontario), KID in Green Bay, L. Michigan, SAG in Saginaw Bay, FTG in the Detroit River, CTY in Lake Huron, and AQR in Lake Superior. WPG was also significantly lower than SBI in L. Ontario and GUL in L. Michigan. Median EROD activity in adults from KNT in the Bay of Fundy was significantly lower than HAM, KID, and SAG, while JFB in L. Superior and MID in L. Erie were both significantly lower than HAM and KID ($p < 0.05$, Dunn's test).

EROD activity varied by a factor of 129× among all prefledglings (Table 6), and 10.5× among colony medians ($H_{10} = 57.3$, $p < 0.001$, K–W test). Median EROD activity in prefledglings from WPG, KNT, and DBL (in L. Huron) was significantly lower than HAM and CTY. WPG was also significantly lower than AQR, SBI and STR in St. Lawrence R. ($p < 0.05$, Dunn's test). The median activity in prefledglings from GUL was significantly lower than those from HAM. Within colonies, dispersion in prefledglings varied from 3.6 (WPG) and 4.7 (KNT) to 108.4 (HAM) and 315.3 (STR) or by a factor of 88.2×. In the eight colonies where both adults and prefledglings were collected, the median EROD activities for the adults were all greater than those for the prefledglings ($p = 0.038$, MWU), while dispersion in EROD activity was greater in adults than prefledglings in all colonies but GUL in L. Michigan ($p = 0.038$, MWU). EROD activity did not differ with gender.

In 134 adults in which both EROD activity and FACs were measured there was a significant association with NPH-EQs (Table 3). However, in a linear regression, NPH-EQs only accounted for 16% of the variability in EROD activity ($p < 0.001$). There was a significant correlation between log CYP1A and log NPH-EQs ($R^2 = 0.278$, $n = 29$, $p = 0.003$), but not log B[*a*]P-EQs. For these 29 adult individuals, a stepwise linear regression model ($R^2 = 0.55$) selected (1) B[*a*]P-EQs and (2) PCB 118 (Table 4). However, there was no measure of PCDD concentration for these individuals. On a colony basis, there were significant correlations with PCDDs, NPH-EQs, and Pb in adults, PCDDs in prefledglings, and PCDDs, Cd, PCBs, TEQs, Hg, and DDE when adults and prefledglings were combined (Table 3), but not trophic level or any SOS measure. Stepwise linear regression models selected (1) PCDDs ($R^2 = 0.35$) in adults, PCDDs ($R^2 = 0.48$), in prefledglings and (1) PCDDS, (2) Cd ($R^2 = 0.63$) adults and prefledglings combined (Table 4).

Discussion

Herring gulls are exposed to genotoxins

We detected FACs in the bile of all herring gulls and a positive or marginal SOS response in 75% of their muscle pools, suggesting widespread chronic exposure to genotoxic chemicals. Biliary concentrations of B[*a*]P-EQs measured in adult herring gulls from this study were in the same range as those measured in the bile of English sole from Puget Sound, WA in the early 1980s. NPH-EQs in gulls were 5–10× higher (Krahn et al., 1984, 1987). Bile from sole with liver lesions had significantly higher B[*a*]P-EQs, but not NAP-EQs, than fish without such lesions (Krahn et al., 1984; Landahl et al., 1990). The B[*a*]P-EQs in herring gulls were lower than those in brown bullheads caught in tributaries to the lower Great Lakes in the 1980s which showed a high incidence of hepatic neoplasms (Maccubbin et al., 1988; Johnston and Baumann, 1989). However, they were in the same range as those measured in bile of brown bullheads collected at three PAH-contaminated sites and two reference sites in Lakes Erie and Ontario in 1994 (Arcand-Hoy and Metcalfe, 1999; Leadly et al., 1999). The bullheads from the contaminated sites had an increased incidence of hepatic alterations and neoplasms. None of the 20 gull breast muscle extracts produced a consistently positive SOS response in the absence of S9 mix, however marginal or erratic responses were obtained for 15 (75%). In previous analyses of feral aquatic biota, 85% of tissue extracts from bivalves (*Mya* sp., and *Mytilus* sp.) collected in the Saguenay Fjord produced positive responses (White et al., 1997) and 70% of fish and 88% of invertebrate extracts from the St. Lawrence and Saguenay Rivers produced positive, marginal, or erratic responses in the absence of S9 (White et al.,1998b). Responses from gull extracts were often increased in the presence of S9, suggesting that at least some of the genotoxic agents required exogenous metabolic activation and implying that this tissue is incapable of creating the genotoxic metabolite on its own. White and Rasmussen (1996) found a strong relationship between SOS genotoxic potency and (i) mutagenic potency for 256 direct acting and 112 S9-activated substances, and (ii) carcinogenic potency of 56 substances.

Considering their apparent exposure, the absence of lesions in herring gulls suggests a lower sensitivity to PAHs or their metabolites, differences in the metabolites produced, or a greater repair capacity than English sole or brown bullheads. Alternatively, when taken with the finding of Ellenton and McPherson (1983) of the absence of between-colony differences in the incidence of sister chromatid exchanges in 7-day herring gull embryos from six colonies collected in 1981, they may indicate selection for resistant genotypes at contaminated sites. This has been reported for populations of the estuarine fish *Fundulus heteroclitus* exposed to contaminated sediments (Meyer et al., 2002; Nacci et al., 2002).

Origin of the exposures and nature of the genotoxins

The highest SOSIPs (GUL in L. Michigan and MID in L. Erie) were associated with a diet of piscivorous fish, while the lowest (FTG in the Detroit R.) was associated with a diet of molluscivorous fish, molluscs, and garbage (Fox et al., 2002), reflecting a strong association with trophic level. Positive or marginal SOS responses were obtained for both adults and prefledglings from HAM and SBI in L. Ontario, WPG in L. Winnipeg, and KNT in the Bay of Fundy, suggesting that the SOS-inducing agents were present and biologically available in the local diet/environment. In contrast, positive SOS responses were obtained for adults but not prefledglings from GUL in L. Michigan, AQR in L. Superior, and CTY in L. Huron. This suggests that either exposure did not occur in the vicinity of these colonies (i.e. the SOS-inducing agent was imported), or the SOS-inducing agent had a low bioavailability, and was accumulated over several years. If we assume that adults and prefledglings (fed by adults) are feeding at the same trophic level, then the similarity between the trophic level-normalized SOSIP in adults and prefledglings from HAM and SBI is consistent with local exposure to highly bioavailable genotoxins. In contrast, the markedly higher normalized SOSIP in adults than prefledglings from WPG and lack of response in prefledglings from GUL, AQR, and CTY suggests low local bioavailability. The markedly higher normalized SOSIP in prefledglings than adults from KNT suggest *in ovo*

transfer of the genotoxins and /or greater fish consumption.

A recent study of PCB accumulation in the food web of Lake Michigan's Grand Traverse Bay (near GUL) found that the slope of the regression between $\delta^{15}N$ and PCBs was greater than that reported for Lakes Superior and Ontario, indicating greater biomagnification at each trophic level (Stapleton et al., 2001). De Vault et al. (1996) suggest that the invasion of Lake Michigan by the predaceous cladoceran *Bythrotrephes cederstroemi* in the early 1980s lengthened the food chain and increased the accumulation in planktonivorous fish. Thus, the relatively high SOSIP for adult gulls from GUL is likely a reflection of greater bioaccumulation rather than some unique source or compound. Similarly, the unexpectedly low SOSIP for gulls from FTG in the Detroit R. may be a result of a unique diet. The positive SOS responses for extracts from gulls from KNT in the Bay of Fundy and WPG in L. Winnipeg were unexpected, based on their remote geographic location, associated industries, and degree of conventional contamination. However, the surface area of the Bay of Fundy is similar to, and that of Lake Winnipeg greater than, Lake Ontario making them highly susceptible to atmospheric deposition. Over 85% of the loadings of PCDDs, B[*a*]P, and Pb to Lakes Superior, Michigan and Huron are estimated to be attributable to atmospheric deposition (IAQAB, 2003). The estimates are much lower for Lakes Erie and Ontario, and for PCBs. B[*a*]P-EQ concentrations were greatest in bile from gulls from the Lake Superior colonies.

The strong association between SOSIP and trophic level suggests that the SOS-inducing genotoxins were biomagnified. Broman et al. (1990) found PAH concentrations decreased with increasing trophic levels in a seston $\rightarrow$ blue mussel (M*yitilus edulis*) $\rightarrow$ common eider (*Somateria mollissima*) food chain in the Baltic, suggesting PAHs are biodiminished. This was also reported by White et al. (1998b). PCDDs, DDE, and PCBs were associated with TCV in adults and prefledglings, but only PCDDs had explanatory significance in stepwise linear regression models. A regression model incorporating trophic level and DDE explained 89% of the variance in SOSIP in adults.

The requirement for metabolic activation to induce SOS activity and the correlation of SOS activity with DDE and PCBs are both consistent with the involvement of cytochrome P450-activated metabolites such as tissue-bound hydroxy or methylsulphone metabolites of PCBs and DDT derivatives. Hydroxy metabolites of some lower chlorinated PCBs are known to be recombinogens (Butterworth et al., 1995). PCDDs may induce those cytochrome P450 isoforms responsible for the metabolic activation. Sinkevicius and Lekevicius (1990) found DDT, its metabolite DDE, and Dicofol (OH-DDT) to be potent inducers of chromosome aberrations in bone marrow cells of Black-headed gulls (*Larus ridibundus*). Significant concentrations of OH-PCBs, which are selectively retained in the blood, and other chlorinated phenolic compounds have recently been measured in the plasma of fish from Lakes Ontario and Superior (Campbell et al., 2003) and the Detroit River (Li et al., 2003), and from plasma of herring gulls from many of these Great Lakes colonies (Fox and Sandau, unpublished).

Current concentrations of DDE measured in livers of herring gulls from this study were greatest in adults from SBI in Lake Ontario and prefleglings from GUL in northern Lake Michigan. Historically, concentrations of DDE have been highest in Lake Ontario and Lake Michigan colonies, reflecting the intense historical applications of DDT to row crops and fruit growing areas surrounding these lakes. Similarly, large amounts of DDT were applied in the forests of New Brunswick (which drain into the Bay of Fundy) to control defoliating lepidopteran larvae between 1952 and 1965. The extract prepared by Ellenton et al. (1983) from eggs collected in a Lake Ontario colony in 1981 was more potent in producing chromosome aberrations in CHO cells than extracts from 5 other colonies (ONT > KNT = CTY > SAG > FTG = SUP). Eggs from this colony had high concentrations of DDE, PCBs, TCDD, and hexachlorobenzene (Ellenton et al., 1983). Ellenton et al. (1983) speculate "the relatively high DDE content of the extract may account for this extract causing chromosome aberrations at lower doses than the extracts from other colonies". It is noteworthy that gull breast muscle extracts from Lake Ontario (HAM, SBI both ages), Lake Michigan

(GUL adults), and the Bay of Fundy (KNT both ages) collected 10 years later were relatively genotoxic in the SOS Chromotest bioassay, whereas the extract from Saginaw Bay (SAG both ages) was not. The spatial and temporal consistency of these data sets using two different tissues, different but non-destructive and non-discriminatory extraction methods, and very different measures of genotoxicity is remarkable. When coupled with published experimental evidence that DDT and its metabolites are potent inducers of chromosome aberrations in another gull species (Sinkevicius and Lekevicius, 1990), they provide additional support for the involvement of DDE in the genotoxicity of our extracts.

Compounds which fluoresce at a particular wavelength pair include the parent and its alkylated derivatives, their metabolites, and N-, S-, and O-containing compounds with the same aromatic ring structure (Krahn et al., 1984). The 290/335 nm wavelength pair detects 2- and 3-ring PAHs from petroleum sources including naphthalenes, fluorene, and phenanthrene. Also detected by this wavelength pair are biphenols, dibenzofurans, dibenzodioxins, retene, and presumably, polychlorinated naphthalenes (PCNs). PCN concentrations in fish are in the ppt to ppb range and somewhat lower in herring gull eggs (Kannan et al., 2000, 2001). The 380/430 ηm wavelength combination detects 3, 4, and 5-ring PAHs, which originate from pyrolytic sources including coke production. In addition to B[*a*]P, pyrene and fluoranthene are major contributors at this wavelength pair (Krahn et al., 1987).

Increasing exposure to a genotoxin should decrease MML. Sum PCBs, mono-ortho PCB congeners 118 and 105, TEQs, DDE, NAP-EQs and B[*a*]P-EQs were rejected as variables in stepwise linear regression to explain MML in 29 individuals. On a colony basis, we found significant inverse statistical associations between median erythrocyte MML and hepatic concentrations of PCDDs, bile concentrations of NPH-EQs, kidney concentrations of Hg and Pb, and the trophic level of the diet. Regression models suggested that the combination of PCDDs, Se, SOSIP, and TEQs (R^2 = 0.999) or NPH-EQs alone (R^2 = 0.30) could predict adult median MML. Regression models suggested that median MML could be explained by Hg in prefledglings (R^2 = 0.40), and PCBs (R^2 = 0.79) when adults and prefledglings were combined. Although the biological significance of these agents in Great Lakes herring gulls is unclear, they have been found to induce genotoxic effects in other species. For example, Wahba et al. (1988) showed that 2,3,7,8-TCDD is capable of inducing hepatic DNA single strand breaks in rats. Although PCDDs in bile would be detected by the 290/335 nm wavelength pair and contribute to NAP-EQs, these two parameters are not significantly correlated in our data set. Yang et al. (2003) found a significant association between the occurrence of barbell abnormalities in brown bullheads collected from Lake Erie tributaries and NPH-like metabolites in their bile. Exposure to Pb has been shown to result in DNA damage in both marine and freshwater bivalves (Avery et al., 1996; Black et al., 1996). *In vitro* exposure of human hepatocytes to the parent PCB mixture Aroclor 1254 significantly increased DNA adduct formation (Borlak et al., 2003). Using the unscheduled DNA synthesis assay to measure excision repair in a brown bullhead cell line, Ali et al. (1993) demonstrated that PAH- and organochlorine-containing fractions of sediment extracts from the Detroit River system were equipotent, but exhibited a significant synergism (3–7×) when the two fractions were combined.

There is evidence of DNA damage and Ah receptor activation

DNA strand length

The high degree of within-colony dispersion and among-colony variability in median MML we observed in DNA isolated from erythrocytes of both adults and prefledglings is suggestive of widespread DNA damage in herring gulls in the Great Lakes in the early 1990s. These results were similar to DNA strand breakage seen in other species exposed to complex mixtures of genotoxins. The MMLs measured for erythrocytes isolated from our herring gulls are in the same range as those measured by the same technique in bluegill sunfish (*Leopomis macrochirus*) exposed to sediments containing high levels of PAHs, PCBs and heavy metals (10.9 ± 10.7 kb) and in unexposed fish (31.9 ± 7.8 kb) (Theodorakis et al., 1994). Extensive inter-animal heterogenetity (dispersion) in DNA migration was also observed among

golden mice (*Ochrotomys nuttalli*) live trapped from both a control and hazardous waste site in North Carolina and damage was increased in animals from the hazardous waste site (Tice, 1995). At pH = 12, the agarose gel electrophoresis technique detects single- and double-strand breaks. It does not separate DNA fragments greater than 50 kb, thus the MMLs may appear to be much lower than they actually are.

In both adults and prefledglings, DNA damage was greatest in gulls from Saginaw Bay (SAG), a site known to be badly contaminated with PCBs, TCDD, and a variety of other contaminants. However, we did not detect SOS activity in breast muscle extracts from this site and concentrations of FACs in bile were intermediate. In prefledglings, the other sites with severely damaged DNA were the two colonies in Lake Ontario (HAM, SBI) and the colony in the St. Lawrence River (STR) where Hg, TCDD, and DDE exposure were greater. Using the Ames microbial assay, Metcalfe et al. (1990) found some extracts of sediments from Hamilton Harbour, but not relatively uncontaminated Georgian Bay, were mutagenic with and without S9, and induced hepatocellular carcinomas in rainbow trout when injected into sac fry.

In interpreting our results, it should be kept in mind that all measures of DNA damage reflect the net result of two opposing processes: damage and repair. DNA damage detected by alkaline electrophoretic techniques can arise through multiple mechanisms, including DNA:DNA crosslinking, single strand breaks, alkali labile sites, sites of incomplete excision repair, and fragmentation associated with cell death (Tice, 1995). The very low MMLs we measured in some individuals may represent predominantly single strand breaks. Single strand breaks may represent DNA damage and/or temporary single strand gaps resulting from excision repair of adducts, may be formed *in vitro* under alkaline conditions (pH > 13) at alkali labile sites (adducts, abasic sites, or oxidized bases), or may be a result of damage to the deoxyribose molecules caused by reactive oxygen species. Reactive oxygen species produced by normal cellular respiration or by physical and chemical environmental stressors are normally detoxified by antioxidant enzymes and scavenger molecules. DNA strand breaks, the most common DNA alteration, are produced when the antioxidant capacity of the cell is overwhelmed, but are usually quickly repaired. Thus, strand breaks are observed in exposed individuals only when the rate of formation of strand breaks exceeds the rate of repair.

Since mature erythrocytes are 100% heterochromatin, the single strand gaps present at the time the cell matures are "fixed" because repair mechanisms are no longer functional. The DNA repair systems of the prefledglings may be less developed than those of adults. It is therefore likely that erythrocytes accumulate damage from oxyradicals and reactive metabolites. Jones and Johanson (1972) suggest the relatively short, 4–6 week life-span of the avian erythrocyte may be related to the high energy metabolism and body temperature of birds. The lower median MMLs and higher SOSIPs in gull prefledglings at KNT and SBI is likely a reflection of their higher metabolic rate, greater food consumption, and therefore greater daily genotoxic exposure, and potentially, the immaturity of their repair mechanisms.

We did not find a biologically significant association between DNA strand length and PAH exposure, measured as FACs in the bile of individual herring gulls. However, there was a significant association with NAP-EQs on a colony basis. Our results differ from those of Custer et al. (2000) who found increased variability (CV) in DNA content of lesser scaup (*Aythya affinis*) collected from the heavily polluted Indiana Harbour Canal on the south shore of Lake Michigan during the winter of 1993–1994, relative to game farm-raised scaup (increased intercellular coefficient of variation in DNA content is a measure of chromosome damage). They found a significant correlation between the CV of erythrocyte DNA and total PAH concentrations in carcasses ($R^2 = 0.45$, $p = 0.01$) and Hg ($R^2 = 0.22$, $p = 0.10$) but not with TPCBs, PCB TEQs, or PCDD + PCDF TEQs.

Other investigators have suggested birds living in the Great Lakes are exposed to genotoxic agents. Custer et al. (1994) found increased CV in DNA content in spleen cells of nestling black-crowned night herons (*Nycticorax nycticorax*) from a colony near KID in lower Green Bay. Yauk et al. (2000) found a significantly increased incidence of germline mutations in minisatelite DNA isolated from erythrocytes of herring gull

families from Great Lakes colonies located near large steel mills operating coking facilities. Outside the Great Lakes, Maness and Emslie (2001) assessed damage in DNA from the erythrocytes of adult and prefledgling royal terns (*Sterna maxima*) from five colonies along the coast of North Carolina in 1999 using the alkaline single cell gel electrophoresis (Comet) assay. Both adults and juveniles from the two colonies in Core Sound had greater DNA damage (measured as "tail" moment) than the other sites. At all sites, adults had significantly greater damage than prefledglings. Wickliffe and Bickham (1998) found alterations in the CV of erythrocyte DNA of brown pelicans (*Pelecanus occidentalis*) collected at the same coastal North Carolina sites in 1995. Pastor et al. (2001) using the Comet assay, found a greater than 10× increase in DNA damage in the DNA isolated from periferal blood lymphocytes of nestling white storks (*Ciconia ciconia*) from Donana National Park (Spain) one year after the April 1998 spillage of large quantities of toxic acid waste rich in heavy metals from the processing of pyrite ore.

Discrepancies between findings for DNA strand length, incidence of mutations in minisatelite DNA isolated from erythrocytes, chromosome aberration-inducing potency of egg extracts of gulls, and genome size in scaup from Great Lakes locations may simply be due to the degree of DNA alteration measured by the biomarker employed. Strand breakage is regarded as an early secondary modification of DNA that occurs after the primary interaction of the chemical with the DNA molecule (adduct formation), whereas cytogenetic effects such as chromosome aberrations, sister chromatid exchanges, altered DNA content, and mutations represent fixed, irreversible lesions which occur later in the genotoxicity sequence (Shugart et al., 1992; Shugart, 2000).

The associations between EROD activity levels and concentrations of NPH-like metabolites in individual adult herring gulls are consistent with the relationship with total PAH concentrations in carcasses of lesser scaup (*Aythya affinis*) from Lake Michigan in the winter of 1993–1994 (Custer et al., 2000). EROD activity was significantly correlated with PCDDs, PCBs, and TEQs in herring gulls from this study (on a colony basis) but was not significantly correlated with PCBs, or PCDD/PCDF TEQs in individual lesser scaup. There was a significant correlation with Pb in both species which was negative in scaup and positive in adult gulls. There was a strong association between EROD activity and CYP1A protein concentration in a subset of adult gulls from our collection (Kennedy et al., 2003).

Under captive conditions, the relationship between exposure to PAH-rich petroleum hydrocarbons and induction of the cytochrome P450-[A] system has been documented in a variety of non-domesticated avian species: Atlantic puffins (*Fratercula artica*) (Peakall et al., 1987), mallard ducks (*Anas platyrhynchos*) (Gorsline and Holmes, 1981), and European starlings (*Sturnus vulgaris*) (Trust et al., 1994). Peakall et al. (1989) found single doses of Prudoe Bay crude oil caused induction of EROD activity in herring gulls and concluded that this hepatic mixed function oxidase activity was a sensitive, but short-lived (< 72 h), biological indicator of recent and possibly continuous exposure to oil and PAHs. Liver microsomes of wild mallards and common mergansers (*Mergus merganser americanus*) from contaminated areas metabolized B[*a*]P at a much greater rate and converted a greater proportion of the administered dose to dihydrodiols than those from non-polluted areas (Honey et al., 2000). The hepatic enzymatic activity for metabolizing naphthalenes increased almost fourfold following dietary exposure to crude oil (Gorsline and Holmes, 1981).

It is clear from this extensive screening survey that Great Lakes herring gulls continue to be exposed to genotoxins and Ah-receptor activating agents in biologically significant concentrations. These agents appear to be persistent bioaccumulative compounds and/or their metabolites. The specific patterns of response appear to be dependent on age, trophic level of the diet, winter movements, and local deposition patterns of constituents of complex mixtures of contaminants. Different constituents in complex mixtures may produce complex interactive responses that vary from site to site. NAP-like compounds, EROD activity, tissue accumulation of SOS-inducing agents, and DNA damage were generally greatest in colonies located in industrialized urban environments and designated AOCs. Similarly, Stein et al. (1995) found juvenile Chinook salmon (*Onchorhynchus tshawytscha*) sampled from urban

estuaries in Puget Sound, WA, had mean concentrations of FACs, hepatic cytochrome P4501A, and levels of hepatic DNA adducts that were significantly higher than in those from non-urban estuaries or hatcheries. Unlike salmon, the exposure of gulls to B[*a*]P-like compounds was greatest in colonies in remote, non-urbanized Lake Superior, reflecting the importance of atmospheric deposition. To better understand the significance and nature of these chemical exposures, follow-up investigations of herring gulls should (1) focus on the condition of gametic DNA, (2) identify the SOS-inducing agents in their tissues using a combination of chemical fractionation and the SOS bioassay, and (3) identify and quantify the various FACs present in their bile.

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