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DNA Damage in Arctic Seabirds: Baseline, Sensitivity to a Genotoxic Stressor, and Association with Organohalogen Contaminants

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Abstract: Environmental contaminants are found throughout Arctic marine ecosystems, and their presence in seabirds has been associated with toxicological responses. However, there are few studies of genotoxicity in Arctic avian wildlife. The purpose of the present study was to quantify deoxyribonucleic acid (DNA) damage in lymphocytes of selected seabird species and to examine whether accumulation of organohalogen contaminants (ΣOHCs) affects DNA damage. Blood was sampled from common eider (*Somateria mollissima*), black guillemot (*Cepphus grylle*), black-legged kittiwake (*Rissa tridactyla*), glaucous gull (*Larus hyperboreus*), arctic skua (*Stercorarius parasiticus*), and great skua (*Stercorarius skua*) in Kongsfjorden, Svalbard (Norway). Contaminant concentrations found in the 6 species differed, presumably because of foraging ecology and biomagnification. Despite large differences in contaminant concentrations, ranging from ΣOHCs 3.3 ng/g wet weight in the common eider to ΣOHCs 895 ng/g wet weight in the great skua, there was no strong difference among the species in baseline DNA damage or sensitivity to a genotoxic stressor (hydrogen peroxide). Baseline levels of DNA damage were low, with median values ranging from 1.7% in the common eider to 8.6% in the great skua. There were no associations between DNA damage and contaminants in the investigated species, suggesting that contaminant concentrations in Kongsfjorden are too low to evoke genotoxic effects, or possibly that lymphocytes are resistant to strand breakage. Clearly, genotoxicity is a topic for future studies of Arctic seabirds. *Environ Toxicol Chem* 2018;37:1084–1091. © 2017 SETAC

Keywords: Arctic; Seabirds; Genotoxicity; Comet Assay; Persistent organic pollutants; Perfluoroalkyl substances

INTRODUCTION

Arctic breeding seabirds may be affected by climate change, habitat degradation, predation, changes in their marine food sources, and environmental contaminants [1]. Seabirds are ecologically relevant study organisms in ecotoxicological studies and monitoring because they are considered to be good indicators of environmental health and human-induced environmental changes [2]. Because of their trophic level in the marine food web, many seabird species accumulate high concentrations of persistent contaminants [3]. Organohalogen contaminants (OHCs) encompass a wide range of anthropogenic compounds, such as chlorinated, fluorinated, and brominated industrial compounds and byproducts, pesticides, and consumer products. Although structurally and functionally diverse, many OHCs share the common characteristics of being persistent, accumulative in the environment and biota, and toxic [1]. Despite international regulations, OHCs are still considered a threat to wildlife, particularly apex predators in the Arctic such as the polar bear (*Ursus maritimus*) and the glaucous gull (*Larus hyperboreus*) [1].

Several OHCs are known to affect the endocrine and immune systems, enzymes, vitamin homeostasis, bioenergetics, and reproduction of seabirds [1]. However, relatively few studies have addressed the genotoxic effects of contaminants on

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birds [4,5]. Deoxyribonucleic acid (DNA) strand breakage is a promising biomarker of contaminant exposure and effect because DNA integrity and maintenance is vital for cellular and tissue function [6]. Several contaminants are known genotoxicants because they can affect genome integrity by adduct formation, production of reactive oxygen species, strand breaks, and impairment of DNA repair mechanisms [7–9]. Double-strand breaks represent a severe type of DNA lesion, because the continuity of the DNA molecule is potentially disrupted. Double-strand breaks may, if not repaired, lead to cell death, chromosomal aberrations, and mutations [10], and may result in adverse health effects (such as cancer), accelerated aging, reproductive impairment, and developmental malformations [11]. Several cellular mechanisms can protect the DNA against damaging agents, including the antioxidant defence system and DNA repair systems [12]. Defence capacity is also related to various individual factors such as energy expenditure, health status, and amount of antioxidants obtained from the diet [13,14].

Because studies on genotoxicity in avian models are scarce, little is known about baseline levels of DNA damage, sensitivity to genotoxicants, the ability to repair induced damage, and the relationship between DNA damage and contaminants. These knowledge gaps are addressed in the present study on 6 Arctic breeding seabird species. The selection of different seabird species provided a broad basis for comparison among trophic level, dietary habitats, elimination abilities, and contaminant accumulation. For example, the common eider (*Somateria mollissima*) feeds low in the trophic web on benthic invertebrates; the diving alcid black guillemot (*Cepphus grylle*) and the surface-feeding gull black-legged kittiwake (*Rissa tridactyla*) are both considered midtrophic species feeding on fish and crustaceans; the kleptoparasitic arctic skua (*Stercorarius parasiticus*) is specialized in stealing food from other seabirds, and may also forage on eggs and chicks; the top-level predator great skua (*Stercorarius skua*) is the largest of the skuas, feeding on fish and other seabirds; and the glaucous gull (*Larus hyperboreus*) is also a top predator and a generalist feeding on eggs, chicks and adults of other birds, but also crustaceans, fish, and carrion [15]. Because of their diets, high trophic level species accumulate greater levels of persistent contaminants compared with species feeding at lower trophic levels [3]. The present study was conducted in Ny Ålesund (78.9235 °N, 11.9099 °E), a small research settlement by Kongsfjorden located west on the Spitsbergen Island, Svalbard (Norway). Although long-range transport is considered the main source of contaminants in this area, some areas may reflect significant local sources of pollution, including sites affected by historical mining activities, fuel storage, the local airport, and the sewage systems [16].

The purpose of the present study was to clarify whether accumulated anthropogenic organohalogens in 6 seabird species sampled from natural populations were related to levels of DNA strand breaks in lymphocytes. The comet assay was adapted for use on avian lymphocytes, and used to ascertain baseline levels of DNA strand breaks, sensitivity of the cells to hydrogen peroxide (H_2O_2), and ability to repair induced DNA damage [17]. Blood concentrations of 51 different OHCs were determined, including polychlorinated biphenyls (PCBs), chlorinated pesticides, perfluoroalkyl substances (PFAS), and polybrominated diphenyl ethers (PBDEs). It was hypothesized that contaminant concentrations would affect baseline levels of DNA damage and H_2O_2 sensitivity. The high trophic level species (the glaucous gull and great skua) were expected to have greater levels of both contaminants and DNA damage compared with the low and midtrophic level species such as the common eider, black guillemot, black-legged kittiwake, and arctic skua.

MATERIALS AND METHODS

Species and field procedures

Fieldwork was conducted from 5 June to 7 July 2015 during the incubation and hatching period. Glaucous gulls ($n = 15$), Arctic skuas ($n = 15$), and great skuas ($n = 6$) were caught on the nest using a remote triggered nest trap or a hand-held net gun. Black guillemots ($n = 10$) were caught using a snare placed on rocks in the colony. Black-legged kittiwakes ($n = 20$) and common eiders ($n = 15$) were caught on the nest using a rod with a nylon snare. Birds were weighed to the nearest 5 g (10 g for the glaucous gull), using Pesola spring balances. Skull length (head + bill) was measured with a sliding caliper (±0.5 mm) and wing length with a ruler (±1 mm). Two to 10 mL of blood (depending on the species) was taken from the brachial vein or the jugular (common eider) using a syringe flushed with heparin (Heparin Leo® 5000 IE/mL; Pharma AS) with a 25- or 23-gauge one-time use cannula. Only female common eiders incubate the eggs and can be caught on the nest, and thus only females are included in the present study. Sex determination was done morphologically by size for the glaucous gull and kittiwake [18,19] and by molecular methods for the black guillemot and the skuas with the DNeasy kit (Qiagen) using the primers 2550F and 2718R. In the field, samples were kept in the dark and on ice, and within 5 to 15 h, blood samples were brought to the laboratory in Ny-Ålesund for further processing. Fresh blood (300 μL) was kept for comet assay analyses, and the remaining blood sample was separated by centrifugation and stored at –20 °C for contaminant analyses.

Comet assay

The comet assay method for detecting DNA strand breaks at the single-cell level was first described by Ostling and Johanson [20]. It has been applied to cells from mussels and fish, humans, and other mammals, and has been used for in vitro clinical studies and biomonitoring [21,22]. In the present study, the comet assay was performed as described by Gutzkow et al. [17], with minor adjustments. A DNA damage percentage of approximately 10% tail intensity is considered acceptable for baseline measurements and indicates that cells are in good condition for scoring [23].

Lymphocytes were isolated from freshly sampled blood (300 μL) on a Percoll® gradient. The isolated lymphocytes were diluted in phosphate-buffered saline ethylenediamine tetraacetic acid (PBS EDTA) to ensure optimal cell density for scoring.

Cells were mixed with agarose gel and cast onto 3 Gelbond® films. The DNA damage experiments had 3 different treatments, as follows: 1) To investigate baseline DNA strand breaks in the different species, films were placed directly in lysis buffer after the gels had solidified; 2) to investigate sensitivity to a genotoxic stressor, H_2O_2, films were placed in a 10 μM hydrogen peroxide solution (in PBS EDTA) for 15 min before being placed in lysis buffer; and 3) to investigate the cells' ability to repair induced DNA strand breaks, films were incubated for 1 h in PBS EDTA buffer after exposure to H_2O_2. All films were left overnight in lysis buffer at 4 °C. For unwinding and relaxation of supercoiled DNA, films were placed in electrophoresis buffer at alkaline conditions (pH 13–14). Electrophoresis was run for 25 min at 4 °C. Films were treated with neutralizing buffer, dehydrated with 96% ethanol, and stored dry and without direct light exposure. The DNA was stained with SYBR® Gold, and scoring of the cells was done using the Comet Assay IV software (Perspective Instruments, version 4.2). Cells around the edge of the gel, overlapping cells, cells with irregular shape, and cells close to foreign objects were not scored. For each treatment, 4 gels were scored/individual, with 50 cells/gel randomly selected. A single estimate of % DNA damage per individual per treatment consisted of the median of 50 cells/gel and the mean of 4 gels.

Contaminant analyses

Analysis of organochlorines and PBDEs was conducted as described in Bustnes et al. [24]. Analysis of PFASs was conducted as described in detail in Hanssen et al. [25]. In short, internal standards were added to the samples (1–2 g of whole blood or plasma; Table 1) prior to extraction. For the chlorinated and brominated compounds, n-hexane was used for extraction, adsorption chromatography was used for cleanup of the extracts, and an Agilent 7890 gas chromatograph with a triple quadropole mass spectrometer was used for instrumental analysis. Lipid content was determined gravimetrically. For fluorinated compounds, methanol was used for extraction, cleanup was conducted using activated carbon (EnviCarb), and an ultra–high-pressure liquid chromatography triple quadropole mass spectrometer was used for instrumental analysis. The internal standards (25 pg/μL C^{13} persistent organic pollutant mix in iso-octane and 0.1 ng/μL C^{13} PFAS mix in methanol) were used to produce a standard curve from which the concentrations of the different OHCs were calculated. Reference material (from the AMAP Ring Test, organized by the Institut National de Santé Publique du Quebec, Canada) was included together with a solvent blank sample in all analytical batches. For the chlorinated and brominated contaminants, the recovery of spiked samples was on average 99%, ranging between 77 and 137% for the organochlorine pesticides (OCPs), 63 and 96% for the DDTs, and 67 and 122% for the PCBs. For PFAS, the achieved accuracy ranged between 84 and 105%. In case of contamination of the blanks, the limit of detection (LOD) was set to 3 times the blank signal. Otherwise, the LOD was set to 3 times the instrumental noise. A total of 51 compounds were analyzed, of which 41 compounds were included in the statistical analyses. Tencompounds were not detected in any of the samples, including perfluoroheptanoic acid (PFHpA), perfluorobutane sulfonate (PFBS), branched perfluorooctane sulfonate (br PFOS), perfluorododecanoic sulfonate (PFDcS), α-hexachlorocyclohexane (α-HCH), γ-HCH, heptachlor, *o,p′*-dichlorodiphenyl trichloroethane (*o,p′*-DDT), *o,p′*-dichlorodiphenyldichlorethane (*o,p′*-DDD), and *o,p′*-dichlorodiphenyldichloroethylene (*o,p′*-DDE). See the Supplemental Data for further details on which compounds were omitted for each species. Among all species, the LOD for the PCBs ranged from 5 to 235 pg/g wet weight; DDTs ranged from 2 to 328 pg/g wet weight; non-DDT pesticides ranged from 0.3 to 93 pg/g wet weight; PBDEs ranged from 2 to 141 pg/g wet weight; and PFAS ranged from 6 to 246 pg/g wet weight.

Data treatment

All statistical analyses were performed using R [26], and the significance level was set at $p = 0.05$. Wet weight data (ng/g wet wt) of the contaminants were used in the analyses. Contaminant concentrations were log-transformed prior to data analyses to ensure normality and homogeneity of variance. Normal distribution was assessed using the Shapiro–Wilks test, and homogeneity of variance was assessed using the Fligner–Killen test.

For each species, compounds that were below the LOD in more than 30% of the individuals were omitted from the data analyses. Compounds below the LOD in less than 30% of the individuals were assigned random values between LODX0.5 and LOD, and were included in the data analyses. A cutoff at 30% was chosen to include as much information on contaminant concentrations as possible without having a dataset with too many randomly assigned low datapoint values. A total of 46 random values were substituted, representing 1.2% of the total dataset.

Scale mass index (SMI) was used as body condition indices, calculating "the mass each individual would have at a fixed body size" [27]. Body condition indices were calculated for each sex for black guillemot, glaucous gull, and Arctic skua to account for differences in size between the sexes. The SMI for each species is presented in the Supplemental Data (Table S1).

To account for potential effects of storage time in the field on DNA integrity, linear regression models were run with storage time and baseline DNA damage (Supplemental Data, Figure S1). Linear models were used to investigate differences in biometric data and DNA damage among species, as well as associations between DNA damage and body condition. In addition, *t* tests were conducted to investigate differences between sexes for biometric data and DNA damage. Linear mixed effect models were used to investigate the relationships among the 3 DNA damage treatments (% tail intensity). Treatment, species, and body condition (SMI) were used as fixed effects, and each individual's unique identity was used as a random effect. The model to best predict the response (% tail intensity) was chosen based on Akaike Information Criterion (AIC), corrected for low sample size (AICc). Paired *t* tests were conducted to investigate DNA damage between treatments within each species.

TABLE 1: Lipid content (%), concentrations of organohalogen contaminants (OHCs; ng/g wet wt), and DNA damage (% tail intensity) in blood from 6 different Arctic breeding seabirds sampled in Kongsfjorden, Svalbard, June-July, 2015[a]

	Common eider[b]	Black guillemot[b]		Black-legged kittiwake[c]	Glacous gull[b]		Arctic skua[d]		Great skua[b]
	$n = 15$[e]	$n = 10$		$n = 20$[e]	$n = 15$		$n = 15$		$n = 6$[f]
	Females ($n = 15$)	Females ($n = 6$)	Males ($n = 4$)	Females ($n = 20$)	Females ($n = 11$)	Males ($n = 4$)	Females ($n = 7$)	Males ($n = 8$)	
Lipid (%)	0.7 ± 0.3	1.3 ± 0.5	1.0 ± 0.5	0.13 ± 0.1	0.80 ± 0.1	0.9 ± 0.2	0.3 ± 0.1	0.2 ± 0.1	0.8 ± 0.4
	0.01–1.0	0.7–1.9	0.5–1.5	0.01–0.3	0.5–0.9	0.8–1.2	0.1–0.3	0.2–0.3	1.3 – 2.5
ΣPCBs	0.9 ± 0.4	14.0 ± 5.9	24.0 ± 14.0	16.0 ± 6.5	97.0 ± 63.0	311 ± 192	10.0 ± 5.8	15.0 ± 12.0	575 ± 315
	0.4–1.9	8.2–24.8	12.0–44.0	7.4–31.0	41.0–230	149–536	5.5–21.0	3.8–40.0	255–1040
ΣPesticides	1.2 ± 0.7	5.7 ± 3.2	8.2 ± 4.3	6.0 ± 2.2	42.0 ± 23.0	129 ± 74.0	9.0 ± 7.2	9.0 ± 4.0	268 ± 103
	0.6–2.6	2.8–11.7	4.3–14.0	2.5–11.0	11.5–82.0	57.0–197	3.2- 24.0	4.1–16.0	163–424
ΣPBDEs	n.d.	n.d.	n.d.	0.60 ± 0.40	2.4 ± 1.0	4.20 ± 2.2	0.7 ± 0.4	0.6 ± 0.3	14.5 ± 5.3
				0.20–1.60	1.1–3.9	1.5–6.9	0.1–1.3	0.2–1.2	8.8–24.0
ΣPFASs	1.3 ± 2.1	6.6 ± 4.5	15.0 ± 16.0	11.0 ± 3.5	27.0 ± 36.0	23.0 ± 10.0	8.7 ± 8.7	19.0 ± 4.7	44.8 ± 16.9
	0.04–7.7	2.3–14.0	4.2–39.0	4.2–33.0	3.4–127	10.0–32.0	3.4–28.0	14.0–26.0	27.3– 67.9
Σtotal OHCs	3.3 ± 2.4	26.0 ± 13.0	47.0 ± 33.0	33.0 ± 9.5	170 ± 84.0	467 ± 258	29.0 ± 15.0	43.0 ± 19.0	888 ± 422
	0.9–10.0	16.0–51.0	24.0–96.0	19.0–56.0	87.0–316	237–756	14.0–50.0	23.0–80.0	470–1511
DNA baseline	1.7 ± 1.5	0.4 ± 0.2	0.40 ± 0.20	1.7 ± 2.3	1.1 ± 1.0	2.8 ± 3.6	3.2 ± 3.7	4.6 ± 3.7	8.6 ± 5.5
	0.3–4.9	0.2–0.9	0.20–0.60	0.10–10.5	0.2–3.6	0.5–8.0	0.3–7.7	0.5–8.1	3.6–15.0
DNA peroxide	39.0 ± 25.5	35.0 ± 16.0	17.0 ± 3.60	47.0 ± 17.5	40.0 ± 7.0	n.a.	42.0 ± 36.0	52.0 ± 28.0	27.0 ± 14.0
	9.4–77.0	19.0–63.0	15.0–23.0	10.0–71.0	30.0–51.0		1.6–71.0	1.9–69.0	14.5–46.0
DNA repair	44.0 ± 17.0	37.0 ± 30.0	36.5 ± 16.0	65.0 ± 16.5	49.0 ± 19.0	n.a.	40.0 ± 35.0	48.0 ± 31.0	34.0 ± 32
	19.0–70.0	8.1–88.0	12.0–65.0	28.0–90.0	21.0–81.0	n.a.	2.3–75.0	1.2–79.0	8.9–79.0

[a]Data are mean ± standard deviation, min–max.
[b]All organohalogen contaminants measured in plasma.
[c]Perfluorinated contaminants measured in whole blood; chlorinated and brominated contaminants measured in plasma.
[d]All organohalogen contaminants measured in whole blood.
[e]Only females sampled.
[f]Only one female sampled, and sexes are therefore pooled.
PCB = polychlorinated biphenyl; PBDE = polybrominated diphenyl ether; n.d. = not detected; n.a. = not available.

Multivariate analyses were performed to explore the variation in contaminant concentrations (principal component analysis [PCA]), and to relate response variables to a set of explanatory variables (redundancy analysis [RDA]) between species, and also within each species (Supplemental Data, Figure S2). Species, sex, and body condition were used as explanatory variables, and contaminant concentrations were used as response variables. The significance of the explanatory variables was assessed using forward permutation tests. Because amount of lipid (%) in the blood varied among the species, different matrices were used for extraction of contaminants (Table 1), and because the contaminants have different affinities to lipids, lipid was used as a covariate in the analyses of contaminant concentrations among species to remove the variation in contaminant concentrations explained by the variation in lipid content [28].

To assess the relationship between contaminants and DNA damage within each species, regression analyses were run with baseline, peroxide (adjusted for baseline), and repair treatments as response variables and the principal components explaining most of the variation in contaminant concentrations (PC1 and PC2) as explanatory variables. Regression analyses were also run with single contaminants and contaminant groups (as inferred from relative grouping of the response variables [contaminants] in the PCA plot) as explanatory variables.

RESULTS AND DISCUSSION

The present study provides a first screening of DNA strand break levels, quantified with the comet assay, in relation to contaminant concentrations in 6 seabird species with different dietary ecology and physiology.

DNA damage

Baseline DNA damage levels were expected to differ among the 6 seabird species, reflecting varying contaminant exposure and accumulation. However, no such variation was found, and mean baseline DNA strand break levels, reported as % tail intensity, was below 4% for all species except for the great skua (i.e., baseline levels significantly greater; linear model, 8.6%, $p = 0.002$; Figure 1). The effect of storage time (ranging between 5 and 15 h) was considered negligible. Although a significantly positive relationship between baseline DNA damage and storage time was found for the common eider (Supplemental Data, Figure S1). the variation in DNA damage was very low (ranging from 1 to 5% tail intensity) and thus not considered important for the overall results and interpretations. No association between baseline DNA damage and body mass, body condition, lipid %, or sex was found (linear model, $p > 0.1$ for all combinations). For all species, DNA damage increased after exposure to H_2O_2 (Figure 1). No differences in sensitivity to H_2O_2 were found among species or between sexes, and no association with body condition or lipid % was found (linear

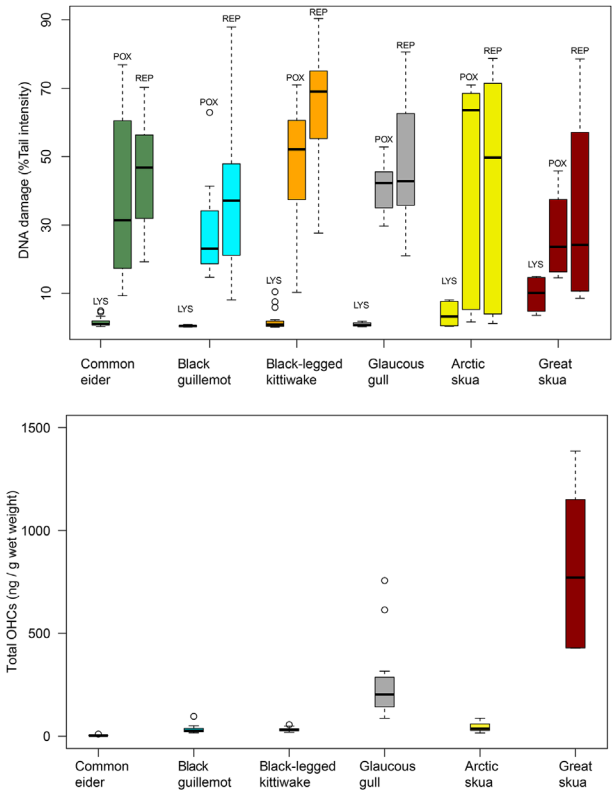

FIGURE 1: (**A**) DNA strand breaks (% tail intensity) of all DNA damage treatments: baseline (LYS, untreated cells placed directly in lysis buffer after gels had fastened on the films), peroxide (POX, 15-min incubation of 10 μM hydrogen peroxide solution before being placed in lysis buffer), and repair (REP, 1-h incubation in phosphate-buffered saline ethylenediamine tetraacetic acid following peroxide treatment) for the common eider ($n = 15$), black guillemot ($n = 10$), black-legged kittiwake ($n = 20$), glaucous gull ($n = 15$), Arctic skua ($n = 15$), and great skua ($n = 6$). (**B**) Total organohalogen contaminant concentrations (ng/g wet wt) for each species. The boxes cover the first and third quantile, and the median is indicated with a horizontal line. Error bars extend to the outermost measurements of the data, and the outliers are represented as individual points.

model, $p > 0.05$ for all combinations), which indicates that the genotoxic mode of action for H_2O_2 is not modulated by this type of biological variance. These results indicate that H_2O_2 is a suitable agent to generate genotoxic stress when assessing DNA damage in avian lymphocytes with the comet assay.

There was no indication of recovery from induced DNA damage in any of the species after 1 h following exposure to H_2O_2. Damage to DNA did not decrease after the repair treatment (Figure 1). Contrary to expectation, an increase in DNA damage after the repair treatment was found in kittiwakes (paired t test, $p < 0.05$). Repair activity to DNA involves the temporary removal of nucleotides or bases before DNA strands are rejoined [29], which may be interpreted as DNA damage. When one is conducting the comet assay, it is not possible to distinguish between DNA strand breaks because of genotoxicants or because of repair mechanisms or apoptosis [30,31]. In cultured mammalian cells, repair of DNA strand breaks occurs quickly [31]. However, repair activity and repair rates in different types of cells ex vivo and how this can manifest as an increase in DNA strand breaks is not well understood. In echinoderm immune cells, H_2O_2 -induced DNA strand breaks continued to increase up to 6 h after acute exposure, and complete recovery was not evident until 24 h after exposure [32]. In freshly isolated human lymphocytes, an additional increase in DNA strand breaks was observed after exposure to H_2O_2 and also in the unexposed control cells [13]. The additional increase was suggested to be the result of the cells' poor adaptations to atmospheric oxygen compared with cultured cells, resulting in a temporal delay in the repair process [13]. Thus, the continued increase in DNA strand breaks in kittiwakes in the repair treatment might be because of DNA strand break intermediates as a result of ongoing repair activity or might be a reaction of the lymphocytes to atmospheric oxygen. These results warrant further research on the rate of DNA repair activity in avian cells ex vivo and species specificity.

Contaminant concentrations

The 6 seabird species were expected to differ in contaminant concentrations and thus to reflect their different ecological niches and phylogenetic background, which affect biotransformation and other elimination processes [33]. In the unconstrained multivariate plot (PCA), there was a clear concentration gradient along the first axis (Figure 2). Mean ΣOHC concentrations increased in common eider, kittiwake, black guillemot, Arctic skua, glaucous gull, and great skua (Table 1). The bird occupying the lowest trophic level, the benthic feeding common eider, had the lowest concentrations of OHCs (ΣOHCs 3.3 ng/g wet wt), and the top predator glaucous gull and great skua had the highest concentrations (ΣOHCs 255 and 895 ng/g wet wt, respectively; Table 1). This was in agreement with expectations and was consistent with previous findings [34–36]. There was no difference in total contaminant concentrations among the black guillemot, kittiwake, and Arctic skua, which indicates that they are feeding on similar trophic levels. In 5 of the species investigated, ΣPCB concentrations exceeded those of the other compounds, whereas for the common eider, concentrations of ΣOCPs were higher than the other measured compounds (Table 1).

The constrained multivariate analyses (RDA) of contaminant concentrations accounted for 83% of the total variation in contaminant concentrations. Species was the most important explanatory factor, explaining 53% of that variation. Because of significant differences in contaminant concentrations between the species, multivariate analyses were also run on each species separately (Supplemental Data, Figure S2). Body condition (SMI) was negatively associated with contaminant concentrations in all species except for the common eider. This negative association was in agreement with previous findings [18] indicating that remobilization of stored body reserves results in an increase of organic contaminants into the bloodstream. Differences in contaminant concentrations between the sexes were observed for the glaucous gull, black guillemot, and Arctic skua, although not all differences were statistically significant. For the glaucous gull, sex accounted for 19% of the variation in contaminant concentrations (RDA, permutation test, $p = 0.037$). There were

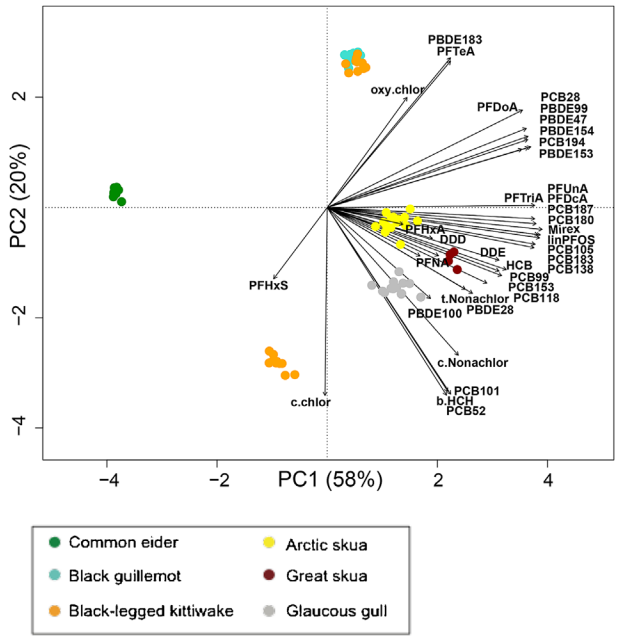

FIGURE 2: Principle component analysis (PCA) biplot showing the relative positioning of all species based on logarithmically transformed contaminant concentrations (ng/g wet wt), with lipid as covariate. Different colors indicate the different species. Contaminants are projected onto the plot as arrows. Direction and length of arrows indicate increasing values and amount of variation, respectively. The cosine of the angle between each arrow indicates degree of correlation: the smaller the angle the higher the correlation. Arrows pointing in the same direction are positively correlated with each other, arrows pointing in opposite directions are negatively correlated, and arrows perpendicular to each other are uncorrelated. The % of the total variance explained by each principal component (PC1 and PC2) is given on each axis. PFHxS = perfluorohexane sulfonate; PBDE = polybrominated diphenyl ether; PFTeA = perfluorotetradecanoic acid; PFDoA = perfluoro dodecanoic acid; PCB = polychlorinated biphenyl; PFUnA = perfluorounde canoic acid; PFDcA = perfluorodecanoic acid; linPFOS = linear perfluoroo ctane sulfonate; PFTriA = perfluorotridecanoic acid; PFHxA = perfluorohe xanoic acid; DDD = dichloro-diphenyl-dichloroethane; DDE = dichloro-diphenyl-dichloroethylene; PFNA = perfluorononanoic acid; HCB = hexachl orobenzene; HCH = hexachlorocyclohexane.

tendencies to higher contaminant concentrations in male black guillemots and male Arctic skuas compared with females, but because of the relatively small sample size, this apparent difference could not be shown with statistical significance. Only one individual out of 6 great skuas sampled was female, and thus no further analyses of differences between sexes were conducted. Difference in contaminant concentrations between the sexes may be because of female egg production and the transfer of contaminants from hen to egg, or because of foraging ecology: males may have alternative diets or a higher food intake during the incubation period [36,37].

Association between DNA damage and contaminants

It was hypothesized that high concentrations of contaminants in the blood would result in adverse effects such as elevated baseline DNA damage, reduced ability of the DNA to tolerate a genotoxic stressor, and reduced ability to recover from induced DNA damage. In contrast, chronic exposure to genotoxicants could potentially also induce defence capacity and thereby reduce DNA damage [31,38]. Increasing DNA strand breaks (measured with the comet assay) with increasing blood concentrations of contaminants was shown for white storks (*Ciconia ciconia*) [39], barn swallows (*Hirundo rustica*) [40], and broiler chickens (*Gallus gallus*) [41]. In contrast, reduced DNA damage with increasing contaminant levels was observed in Svalbard polar bears [42] and royal terns from North Carolina (USA) [38]. However, in the present study, no positive or negative associations could be confirmed with statistical significance between contaminant occurrence and DNA damage. Although the small sample size for the great skua ($n = 6$) clearly reduced the power of the statistical analyses and results must thus be interpreted with care, contaminant concentrations and baseline DNA strand breaks were significantly greater compared with the other species. This finding warrants further research on genotoxic effects of contaminants in the same concentration range as in the great skua.

Previous studies have shown associations between DNA damage and contaminant exposure when periods of low and high stress are compared, such as early and late in the incubation period [4], or when individuals from high exposure areas are compared with those from to low exposure areas [39,43]. Fenstad et al. [4] showed increased DNA double strand breaks in whole blood of incubating and fasting common eiders in Kongsfjorden, but argued that loss of body mass was a more important stress factor and thus a more important inducer of DNA damage than the simultaneous increase of contaminants. Furthermore, a positive relationship between DNA double strand breaks and levels of mercury, PCB-118, and DDE was observed in common eiders from a highly polluted area in the Baltic Sea, but not in common eiders from the Kongsfjorden population [43].

The lack of associations between contaminants and DNA damage in the present study might be because of the relatively low contaminant concentrations found in all species. Kongsfjorden is considered a relatively clean environment with few local sources of contaminants [34]. However, certain areas are still influenced by historical mining activities, which could increase exposure to some organic contaminants, for example, polycyclic aromatic hydrocarbons and other organic compounds [16]. Nonetheless, seabirds breeding in Kongsfjorden have lower contaminant concentrations compared with populations breeding in other areas. For example, Bjørnøya (Norway) has been described as a "hot spot" area for contaminant exposure and accumulation [44]. Reported concentrations in great skuas breeding in Bjørnøya are up to ∑OHCs 7900 ng/g wet weight [45], which is more than 5 times higher than in the most contaminated individual from the present study, and reported plasma concentrations of PCBs in male glaucous gulls breeding in Bjørnøya are more than twice as high as those measured in the present study [46]. The Baltic Sea is also considered highly polluted compared with the Barents Sea and Kongsfjorden, and common eiders breeding in the Baltic have been reported to have concentrations of contaminants that are up to 26 times higher than those in Kongsfjorden

populations [34]. Other factors such as diet specializations, climate, food availability, and health and reproduction status may also affect contaminant exposure and sensitivity to adverse effects [33,45], and may represent factors that have not been included in the present study. Another explanation for the lack of association between contaminants and DNA damage may be lymphocyte sensitivity. Lymphocytes separated from blood as well as cells from disaggregated tissue are the most commonly used cells in comet assay studies [23]. However, based on the low variation in baseline DNA damage observed among the species in the present study, it is pertinent to ask whether lymphocytes are sufficiently responsive to be affected by contaminants. In animals that have nucleated erythrocytes, such as birds and fish, erythrocytes may be a more sensitive cell type for investigating genotoxicity [47]. However, because nucleated erythrocytes are targets of naturally occurring reactive oxygen species, adjustments would have to be made to take into account the characteristics of these cells. During adjustments of the method for the purpose of the present study, analyses of whole avian blood produced high background levels of DNA damage (~80% tail intensity), in contrast to lymphocytes that produced low background levels (~10%). This difference is most likely because of avian erythrocytes, which contain highly condensed chromatin that is alkaline sensitive [40]. During unwinding and electrophoresis at high pH (13–14), alkali labile sites are expressed as strand breaks that do not represent actual DNA damage [48]. These findings warrant further testing on the suitability of avian erythrocytes for use in the comet assay, and the effect of pH during unwinding and electrophoresis of these cells. The sensitivity of the comet assay, as exemplified above, makes comparisons between different laboratories and different species or matrices challenging. Conditions must be optimized to fit the study questions, and protocols must be standardized to obtain low baseline values in relevant cell types [49].

The purpose of the present study was to compare DNA damage in 6 seabird species with different levels of accumulated contaminants that might affect baseline DNA damage, sensitivity to H_2O_2, and ability to repair induced DNA damage. No effects of contaminant concentrations on DNA damage were observed, except for the great skua where baseline DNA strand break levels as well as total contaminant concentrations were higher compared with the other species. The observed results may be because of the relatively low contaminant levels in Kongsfjorden compared with regions closer to sources of contaminant production and release, both Arctic and temperate regions. Alternatively, the characteristics of lymphocytes may afford tolerance to contaminant concentrations. The present study contributes to the relatively unknown research area of genotoxicity in Arctic seabirds, and provides a basis for further developing and refining the comet assay as a tool for assessing DNA damage in avian blood.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4035.

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Data availability—Data, associated metadata, and calculation tools are available from the corresponding author (katrine.borga@ibv.uio.no).

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