DNA DOUBLE-STRAND BREAKS IN RELATION TO PERSISTENT ORGANIC POLLUTANTS IN A FASTING SEABIRD

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Abstract

Lipophilic persistent organic pollutants (POPs) are released from fat reserves during fasting, causing increased blood concentrations. Thus, POPs represent a potential anthropogenic stressor during fasting periods. We analysed the blood of female common eiders (*Somateria mollissima*) by using agarose gel electrophoresis and image data analysis to quantify the DNA-fraction, of total DNA, that migrated into the gel (DNA-FTM) as a relative measure of DNA double strand-breaks (DSBs) during the fasting incubation period in the high arctic. In 2008 and in 2009 blood samples were obtained for analysis of 9 POPs and DNA-FTM at day 5 of the incubation period, and then in the same individuals at day 20. This unique study design gave us the opportunity to analyse the same individuals throughout two points in time, with low and high stress burdens. During the incubation period the body mass (BM) decreased by 21-24%, whereas the POP levels increased by 148-639%. The DNA-FTM increased by 61-67% (being proportional to the increase in DSBs). At day 5, but not day 20, DNA-FTM was positively correlated with most analysed POPs. The increase in DNA-FTM was positively correlated with the decrease in BM (g) during incubation. Thus, we suggest that fasting stress (BM loss) decreases DNA integrity and that stress caused by fasting on BM loss appeared to override the additional stress caused by concurrent increase in levels of the analysed POPs in the eiders. Blood levels of POPs in the eiders in Svalbard were relatively low, and additive and/or synergistic genotoxic effects of fasting stress and POP exposure may occur in populations with higher POP levels.

Keywords—Arctic, Common eiders, Genotoxicity, Anthropogenic Contaminants

1. Introduction

Man-made chemicals, such as halogenated persistent organic pollutants (POPs) have been produced in large numbers and quantities due to demands for new materials and for pesticides (Tanabe et al., 1994). Persistent organic pollutants include industrial chemicals, such as polychlorinated biphenyls (PCBs), brominated flame retardants (BRFs) and perfluorinated compounds (PFCs), by-products from industrial processes like hexachlorobenzene (HCB) and dioxins, and pesticides, such as dichlorodiphenyltrichloroethane (DDT) (Letcher et al., 2010). Persistent organic pollutants are, albeit to various degrees, resistant to biodegradation and they can reach areas remote from their source by air- and water transport (Pacyna and Oehme, 1988; Barrie et al., 1992). Over the last decades it has become increasingly evident that POPs are of great concern in the Arctic environment (Bustnes, 2006; Jenssen, 2006; Erikstad et al., 2010).

Many animals have regular fasting periods during their life-cycle, where accumulated fat reserves are mobilized and metabolized. Most POPs are lipophilic and after uptake they are distributed to fatty tissue in organisms. During fasting periods lipophilic contaminants are released into the blood and redistributed to sensitive tissue such as the brain, liver and kidneys (Debier et al., 2003; 2006; Henriksen et al., 1996). Thus, POPs represent a challenge for species that rely on their lipid reserves for vital life-functions during fasting periods.

The genotoxicity of mixtures of POPs has generally been little studied (Marabini et al., 2011). There are, however, studies reporting possible genotoxic effects from exposure to persistent halogenated compounds and their metabolites (Butterworth et al., 1995; Marabini et al., 2011). Sirinivasan et al. (2001) showed that PCB metabolites can induce breakage in DNA strands *in vitro*. DNA double–strand breaks (DSBs) are among the most severe DNA lesion because they disrupt the continuity of the DNA template, which is essential for replication and transcription. If not repaired, DSBs may result in loss of chromosomes and/or

cell death, mutations, chromosomal rearrangements (Thacker, 1986; Jackson, 1999) and carcinogenesis (Jeggo, 1998; Kanaar et al., 1998; Pfeiffer, 1998).

Dubois et al. (1995) have shown that, when exposed *in vitro*, PCBs caused genotoxic effects on avian cells. Also, gel electrophoresis has been applied to study DSBs after genotoxic exposure in glaucous gulls (*Larus hyperboreus*) (Krøkje et al., 2006) and in fish blood cells (Theodorakis et al., 1994). By conducting the electrophoresis under neutral pH conditions, the detection of relative DSB frequency is possible because the duplex structure of DNA is not disrupted, and migration of DNA within the gel depends on duplex structures produced by DSBs. The amount of DNA migrating out of the sample well relative to the total amount of DNA loaded (the DNA-fraction of total DNA, that migrated, DNA-FTM), can be used to measure the increase in DSB frequency (Theodorakis et al., 1994; Wlodek et al., 1991). Also, a lower median molecular length (MML) of the DNA in the gel generally indicates more damaged DNA (Theodorakis et al., 1994).

There are very few studies on genotoxic effects of POPs in birds (Krøkje et al., 2006; Matson et al., 2004; Østby et al., 2005). Regardless of probable causes or functional significance, several genomic characteristics unique to avian species suggest that extrapolation of genetic toxicity data from a microbial or mammalian model to presumed avian genotoxic effect might not be appropriate. Reliable avian models for genetic toxicity are therefore needed (Krøkje et al., 2006).

In the present study female common eiders *(Somateria mollissima)*, breeding in Svalbard were investigated during the incubation period. Common eiders feed at a low trophic level (Dahl et al., 2003), and are thus exposed to relatively low concentrations of POPs (Bustnes et al., 2010) compared to other birds feeding at higher trophic levels (Letcher et al., 2010). However, the female common eiders fast for about four weeks during their incubation period, and may lose 30-45% of their initial body mass (BM) (Korschgen, 1977;

Parker and Holm, 1990). During fasting, POPs are redistributed into the blood of the females and blood levels increase 2-10 folds towards the end of their incubation period (Bustnes et al., 2010; 2012). At this time, their body reserves are depleted and the immune system of the female common eiders is severely suppressed (Hanssen et al., 2003; 2005). Although poorly studied (Holmstrup et al., 2010), there is some evidence of increased toxic effects from pollution when combined with the additional stress that starvation causes (Jørgensen et al., 1999; Kajiwara et al., 2008; Leung and Furness, 2001). Hence, there is a cause for concern that the increased circulatory levels of POPs that common eider females experience during their incubation fast may elicit toxic effects. The common eider is, therefore, a good model species for studying possible effects from POPs during breeding stress or other periods where animals undergo nutritional stress and must rely on stored body lipids.

Previously, altered vitamin levels (Murvoll et al., 2007) and increased CYP 450 1A activities (Murk et al., 1994; Rozemeijer et al., 1995) have been documented in common eider chicks after exposure to POPs. These observations indicate that the common eider is a sensitive species to PCB toxicity and may be at risk of developing adverse health effects in relatively highly contaminated areas (Murk et al., 1994).

In the present study, neutral gel electrophoresis was used to investigate the DNA integrity in blood of female common eiders. The DNA-fraction of total DNA, that migrated into the gel and median molecular length was analysed as possible genotoxic endpoints from environmental POP exposure and concurrent incubation fast. The objectives of the study were to examine whether POPs affected DNA integrity in the blood of female common eiders, and if the increase in blood levels of POPs and reduction in BM during the fasting incubation period, had any effect on the occurrence of DSBs in the blood of the female common eiders.

2. Materials and methods

2.1. Field sampling

Blood samples were obtained from incubating female common eiders in 2008 (N=8) and from new individuals in 2009 (N=15) at Storholmen, Kongsfjorden, in Svalbard (78°56'N, 12°13'E). Nests were searched at three-day intervals from the beginning of the breeding season. Every new nest was marked with a nest identification sticker and its GPS position was recorded. After three days, nests were re-visited and day zero of incubation was defined as the day when the last egg was laid. The females were caught on the nest at day 5 and 20 of the incubation using a fishing rod with a nylon snare at the end. Body mass was recorded using a spring balance (Pesola Medio-Line 42500, Ecotone-Poland, 2500 g), the clutch size was recorded and the wing length (mm), i.e. the distance from the carpal joint to the tip of the longest primary, was measured using a ruler with a stop. Blood (8-10 ml) was sampled from the jugular vein using a heparinised syringe. A sample of 500 µl blood was transferred to an Eppendorf tube (1.5 ml) for later DNA analyses. The Eppendorf tube was immediately frozen in a thermos containing a mixture of ice and salt (~5 table spoons of salt/l ice, ~ -20°C). The rest of the blood was transported to the laboratory in Ny-Ålesund within six hours, and four ml whole blood was frozen (-20°C) for later POP analyses. Frozen samples were transported to the laboratories at Norwegian Institute for Air Research (NILU), Tromsø, and Norwegian University of Science and Technology (NTNU), Trondheim, at the end of field season. The samples were kept at -80°C until analysis. The study complies with the Norwegian regulation on animal experimentation and permissions of field work were granted by the Governor of Svalbard.

2.2. Chemicals

Solvents, n-hexane: of pesticide grade (Merck, Darmstadt, Germany); florisil (0.150-0.250 mm, Merck, Darmstadt, Germany); crystalline reference materials were obtained from Promochem (Wesel, Germany); All ^{12}C and ^{13}C-labeled internal standards were purchased

from Cambridge isotope laboratories (Wolborn, MA, USA). Agarose (#A9539), DNA/electrophoretic grade (#A5093), boric acid (#B7901), ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA) (#E5134), proteinase K (#P2308), lauryl sulphate (SDS) (#L-3771), sodium chloride (S#3014) and trizma base (#T6066) were purchased from Sigma (USA), while low melt preparative grade agarose (#162-0019) and ethidium bromide (10 mg/ml, #161-0433) were purchased from BioRad. Loading dye (# R0611) was purchased from Fermentas. DNA standard Hind III digested λ DNA (Fermentas SMO-101, 0.5 µg/µl) and λ DNA (Fermentas SD0011, 0.3 µg/µl) were used as size markers.

2.3. POP analysis

The analysis of POPs was performed at NILU in Tromsø as described by Bustnes et al. (2008). Briefly, after blood samples were denatured with ethanol, deionised water saturated with ammonium sulphate was added, followed by a double extraction with 6 ml n-hexane. The extract was concentrated and run over a florisil (activated at 120 ˚C for 8 h) column. After volume reduction, a recovery standard (octachloronaphtalene) was added all samples prior to quantification. Native ^{12}C and ^{13}C-labelled equivalents were analysed, representing the groups of PCBs (28, 52, 99, 101, 118, 138, 153, 180, 183, 187 and 194), DDT, dichlorodiphenyldichloroethylene (DDE), hexachlorocyclohexanes (HCHs), HCB, chlordanes and nonachlores.

To ensure quality of the results, blank samples and reference material (standard reference material 1589b human serum from National Institute of Standards and Technology (NIST)) were analysed with every 10^{th} sample. No contamination of blank samples was observed except for HCB. The limit of detection (LOD) for HCB was calculated by using three times blank signal. Results from the analysed reference samples were within the given limits of accuracy (±20%). The limit of detection for PCBs, DDT and organochlorines (OCs) ranged from 0.7 to 178 pg/g ww depending on the specific compound. Compounds that were

below the LOD in 50% of the individuals at both incubation stages, either year were excluded from further statistical analysis. The specific compounds further analysed were HCB, *trans*-chlordane (*t*-chlordane), oxy-chlordane, *trans*-nonachlor (*t*-nonachlor), *p,p'*DDE (DDE), and the PCBs 118, 138, 153 and 180. At day 5 in 2008, only HCB, *t*-nonachlor and DDE were detectable in 50% of the individuals and, thus, included in statistical analyses. Concentrations below the LOD were set to 50% of the detection limit. When used in correlations with biological variables, POP levels are presented in pmol/g ww concentrations.

2.4. Detection of DNA double-strand breaks

Agarose plugs for electrophoresis were prepared according to the procedure described by Krøkje et al. (2006) and others (Wlodek et al., 1991; Theodorakis et al., 1994) with modifications. A small volume of whole blood (10 µl) was diluted in 500 µl of TE buffer (10 mM Tris base, 1 mM EDTA, pH 8) at 37°C, and then mixed with 500 µl of premelted 1% low-melting agarose at 37°C. From this mixture, 50 µl plugs were cast in plug moulds (BioRad, #170-3713). The plugs were set at 4°C for 30 min, before being placed into lysis buffer (100 mM NaCl, 10 mM Tris, 25 mM EDTA, 0.5% SDS, pH 7.6) with proteinase K newly added at 1mg/ml, and incubated at 55°C overnight. After incubation, the plugs were cooled at 4°C for 30 min and loaded into the wells of a 0.6% agarose electrophoresis gel in TBE running buffer (Sambrook and Maniatis, 1989). Premelted, 1% low-melting-point agarose (37°C) was used to seal the wells prior to electrophoresis.

DNA was prepared from lysed blood samples of 23 individuals in which samples were obtained at both day 5 and day 20 of incubation. DNA fragments released from the lysed blood cells embedded in low-melting-point agarose plugs were electrophoretically separated by size. Lambda-DNA Hind III fragments plus whole linearized Lambda DNA were used as molecular size markers. The electrophoresis was run at 2.1 V/cm for 18 hours in running buffer at room temperature, followed by staining of the gel in ethidium bromide

solution (~0.1 mg/l) for two hours. Gel image data was acquired using the BioRad Gel Doc 2000 system. Calculation of median molecular length (MML) of DNA fragments in the gel was performed, using densitometric data obtained from the gel image analysis. The relative amounts of DNA left in the well and the DNA that had migrated into the gel after electrophoresis were determined by the area under the respective DNA staining intensity curves. The fraction of the DNA released by electrophoresis into the gel of the total DNA loaded in the well was calculated (DNA in the gel/ (DNA in well + DNA in gel)*100, DNA-FTM) and used as an indication of DSB frequency. The DNA fragment size distribution and MML of the fraction that migrated into the gel, determined from gel image data, was quantified with standard curve obtained from the Lambda size markers separated on the same gel.

Samples from day 5 and day 20 of incubation, from the same individual, were run on the same gel. The mean results were obtained from multiple analyses (6-17) of each sample. The results were highly reproducible (% Coefficient of variation, ″% CV″ range = 1.2-19.3), thus, the precision of the measurement of the fraction of DNA that migrated into gel of each individual was high.

By sampling the same individuals at both day 5 and day 20 of incubation, each individual was investigated at a low and high stress burden, and with lower and higher blood levels of POPs. Thus, in this unique study design each wild living bird could be its own control.

2.5. Data analysis

Statistical analysis of the data were conducted using R 2.15.2 (R Core Team, 2013). Linear mixed effect models (*lme* function, *nmle* package), with individual identity as a random factor, were used to investigate the effect of incubation day and year, as well as their interaction, on blood levels (pg/g ww) of HCB, t-nonachlor, DDE, on BM (g), MML and DNA-FTM. General linear models (*lm* function, *stats* package) were used to assess the

effects of changes in blood POP levels (pmol/g ww) and body mass (g) on the change in DNA-FTM from day 5 to day 20 of incubation. Diagnostic plots in R were used to assess whether the data sufficiently met the assumption of the linear model, and when needed, variables were ln-transformed. The mixed effect linear model (*lme*) was used when the same individuals were measured twice (on day 5 and day 20) and the data were not independent. The full model included the main effects (e.g. incubation day and year) and their interaction. The final models were obtained with backward selection, and non-significant interactions and main effects were removed. Finally, principal component analysis (PCA) of the data was conducted using the software Simca-P+ version 12 (Umetrics AB, Umeå, Sweden). The PCA was used for exploratory analysis and to visualise how variables (including all the detected POPs at each incubation stage) were grouped and correlated at the two different incubation periods and between years. The data was scaled to unit variance (UV) and centred to make the variance within each variable equal before analysis. Pearson moment product correlation was used to analyse correlation between variables, with the exception of PCB 180 (pmol/g) at day 5 in 2009, which was not normally distributed (Shapiro-Wilk test). Thus, Spearman's rho was used to investigate possible correlations between PCB 180 and DNA-FTM. Mean values are presented with ±1 standard deviation (SD) of the mean. All tests were two-tailed, and the level of significance was set at $p<0.05$.

3. Results

3.1.POP levels, BM and DNA-FTM during the incubation period

Blood concentrations (pg/g ww) of HCB, *t*-nonachlor and DDE were affected by incubation day ($df=21$, $3.7<t<8.1$, $p<0.002$) and sampling year ($df=21$, $2.7<t<5.6$, $p<0.02$). Furthermore, there was an interaction between year and incubation day for HCB ($df=21$, $t=-3.6$, $p=0.002$), *t*-nonachlor ($df=21$, $t=-0.4.9$, $p<0.001$) and a trend for DDE ($df=21$, $t=-1.9$, $p=0.069$). Thus, the blood POP levels increased significantly from day 5 to day 20 of

250 incubation (Table 1), but the degree of change was stronger in 2008 compared to 2009. The
251 blood concentrations of all analysed POPs ($\sum_9$POPs) increased by 639% in 2008 and by
252 148% in 2009, during the incubation period (Table 1). Also, the BM of the females was
253 dependent on incubation day (df=21, t=-29.3, p<0.001) and the interaction between
254 incubation day and sampling year (df=21, t=2.1, p=0.046). Hence, in both 2008 and 2009 the
255 BM of the female common eiders was lower at day 20 compared to day 5 of incubation
256 (Table 2), but the decrease in BM from day 5 to day 20 was higher in 2008 (23% BM loss)
257 compared to in 2009 (21% BM loss). The BM loss (g) was 404 ±45g in 2008 and 367 ±36g in
258 2009.
259 Collectively, the 2008 and 2009 data showed that blood concentrations of the POPs did
260 not change notably until the birds had a BM less than 1600 g (Fig. 1). However, when the
261 birds starved to a BM below 1600 g, blood concentrations increased rapidly with decreasing
262 BM (Fig. 1).
263 The DNA-FTM was significantly higher at day 20 than at day 5 (df=22, t=4.1, p=0.001,
264 Table 2) and there was no interaction between incubation day and year (df=21, t=-1.5,
265 p=0.14). This increase in DNA-FTM corresponds to a 61.0% and 66.5% increase in DSB
266 frequencies from day 5 to day 20 of incubation in 2008 and 2009, respectively (Table 2).
267 Finally, the MML (Table 2) did not change from day 5 to day 20 of incubation (df=22, t=-0.4,
268 p=0.7) and there was no interaction between year and incubation day (df=21, t=1.1, p=0.3).
269 The mean cutch sizes of the females were 3.6 ±0.9 in 2008 and 3.0 ±0.8 in 2009, and
270 the mean wing lengths were 297.1 mm ±6.1 in 2008 and 297.5 mm ±3.1 in 2009 (Table 2).
271 *3.2. Associations between increased POP levels, decreased BM and the increase in DNA-*
272 *FTM*
273 The increase in blood concentrations of all POPs and the decrease in BM during the
274 15 days of incubation were included as independent variables with sampling year as a factor

in general linear models, with the fold increase in DNA-FTM as dependent variable. There were no correlations between the fold increase of any of the POP compounds and the fold increase in DNA-FTM from day 5 to day 20 of incubation ($-1.1<t_{1,21}<0.9$, $0.2<p<0.9$), and there were no interactions between the fold increase in POP compound and sampling year ($-0.8< t_{1,19}<0.3$, $0.4<p<0.9$). However, body mass loss (delta BM, g) during the incubation period was correlated with the fold increase in DNA-FTM ($t_{1,21}=2.11$, $p=0.047$) and there were no interactions between year and BM loss ($t_{1,19}=-0.26$, $p=0.8$).

3.3. Associations between POPs and DNA-FTM at day 5 of incubation

In 2008, only HCB, *t*-nonachlor and DDE were in concentrations above the LOD in 50% of the individuals and were, thus, included in the PCA. Principal component 1 and PC2 explained 55% and 16%, respectively, of the variation in 2008 (Fig. 2A). Most POPs, as well as DNA-FTM, were clustered at PC1= -0.94 − -0.74, whereas BM and clutch size were clustered at PC1= 0.60 − 0.78. This indicates positive relationships between blood levels of POPs and DNA-FTM. Indeed, correlation analysis confirmed that there was a positive correlation between DDE and DNA-FTM in 2008 ($n=8$, $r_p=0.78$, $p=0.023$). However, there were no statistical correlations between DNA-FTM and any of the other POP compounds ($n=8$, $0.12<r_p<0.67$, $p>0.07$). Furthermore, there were no statistical correlations between DNA-FTM and BM ($n=8$, $r_p=-0.6$, $p=0.11$), between POPs and BM ($n=8$, $-0.42<r_p<-0.62$, $p>0.1$), between BM and clutch size ($n=8$, $r_p=0.45$, $p=0.26$) or between POPs and clutch size ($n=8$, $-0.5<r_p<-0.2$, $p>0.17$). HCB and DDE intercorrelated positively ($n=8$, $r_p=0.82$, $p=0.012$).

In 2009 PC1 and PC2 explained 65% and 15%, respectively, of the variation (Fig. 2B). Most POPs and DNA-FTM were clustered at PC1= -0.96 − -0.73, and BM and clutch size were clustered at PC1= 0.41 − 0.51. As for the data in 2008, this indicates positive relationships between some POPs and DNA-FTM. Indeed, correlation analysis confirmed

that blood concentrations of all POPs (n=15, $0.51<r_p< 0.77$, $0.001<p<0.05$), with the exception of PCB 180 (n=15, $r_s=0.44$, p=0.10), correlated positively with the DNA-FTM at day 5 of incubation. The strongest relationship was seen between DNA-FTM and blood concentration of HCB (n=15, $r_p=0.8$, $p<0.001$). There were no statistical correlations between BM and DNA-FTM (n=15, $r_p=-0.36$, p=0.18), between BM and clutch size (n=15, $r_s=-0.56$, p=0.97), between BM and POPs (n=15, $-0.49<rp<0.04$, $p>0.06$) or between POPs and clutch size (n=15, $-0.26<r_s<-0.03$, $p>0.3$). All the POPs intercorrelated positively (n=15, $0.5<r_p<0.8$, $p<0.05$).

3.4. Associations between POPs and DNA-FTM at day 20 of incubation

In 2008, in the PCA of the data from day 20 of incubation, PC1 and PC2 explained 51% and 18%, respectively, of the variation (Fig. 2C). The POPs were clustered at PC1= -0.99 − -0.50, while BM and clutch size were clustered at PC1= 0.52 − 0.55. The DNA-fraction of total DNA, that migrated was situated at PC2= 0.65, slightly opposite of some of the contaminants, such as t-nonachlor and PCB 118 as well as BM and clutch size. PCB 180 correlated negatively with clutch size (n=8, $r_p=-0.71$, p=0.047). However, there were no statistical correlations between DNA-FTM and any of the other variables (n=8, $-0.47<r_p<-0.02$, $p>0.2$), between BM and POPs (n=8, $-0.66<r_p<-0.21$, $p>0.07$), between BM and clutch size (n=8, $r_p=0.44$, p=0.27) or between POPs and clutch size (n=8, $-0.5<rp<-0.05$, $p>0.2$). All the pesticides intercorrelated positively (n=8, $0.91<rp<0.99$, $p<0.01$).

In 2009, in the PCA of the data from day 20 of incubation, PC1 and PC2 explained 60% and 14%, respectively, of the variation (Fig. 2D). All the POPs were clustered at PC1= -0.96 − -0.76, opposite of BM (PC1= 0.78). The DNA-fraction of total DNA, that migrated was situated at PC2= 0.37, slightly opposite of some contaminants, such as HCB and t-chlordane, as well as clutch size (PC2= -0.4 − -0.75). There were no correlations between the DNA-FTM and the other variables (n=15, $-0.11<r_p<0.18$, $p>0.5$). Furthermore, the PCA

indicated a negative relationship between POPs and BM. Indeed, an inverse relationship between BM and POPs was statistically confirmed ($n=15$, $-0.85<r_p<-0.59$, $p<0.03$). Clutch size was not related to BM ($n=15$, $r_p=0.10$, $p=0.7$) or POPs ($n=15$, $-0.92<r_p<0.12$, $p>0.6$). All of the POPs intercorrelated positively ($n=15$, $0.60<r_p<0.98$, $p<0.02$), with the exception of HCB and DDE ($n=15$, $r_p=0.48$, $p=0.07$).

4. Discussion

In the present study we documented a significant increase in the DNA-fraction of total DNA, that migrated (>60%) from day 5 to day 20 of incubation. This indicates that the female common eiders in Svalbard have lower DNA integrity at the end of their incubation period. Although the sample size in 2008 was lower than preferred, each individual operated as its own control, excluding variation that is normally present when comparing different groups of individuals. Also, the pattern of decreasing DNA integrity during the incubation period was consistent both in the individuals sampled in 2008 and in the individuals sampled in 2009, strengthening the reliability of the observation.

Blood concentrations of several POPs (in 2008: DDE, in 2009: HCB, *t*-chlordane, oxy-chlordane, *t*-nonachlor, DDE, PCB 118, PCB 138, PCB 153) were positively associated with DNA-FTM in blood cells of the female common eiders early in the incubation (i.e. at day 5 of incubation). The strongest relationships were seen between the DNA-FTM and DDE in 2008, and the DNA-FTM and HCB in 2009. Since most POPs co-varied (Fig. 2A-2D), and might interact with each other, identification of single compounds that cause the genotoxic effect is not possible. Nevertheless, it should be mentioned that the blood levels of HCB (pmol/g) at day 5 in 2009 were high compared to the HCB levels at day 5 in 2008 (3 times higher) and to blood concentrations of the other POPs (2-222 times higher), with the exception of DDE (Table 1). From previous studies on mammalian cells, HCB is known to generate free radicals (Mrema et al., 2013). However, i*n vitro* tests have provided conflicting

results concerning the genotoxicity of HCB (Canonero et al., 1997; Salmon et al., 2002; Ennaceur et al., 2008). The results from the present study indicate that environmental exposure to HCB can potentially cause genotoxic effects.

Elevated genetic damage has previously been documented in blood of common eiders in the polluted Baltic Sea compared to a control area in the Beaufort Sea (Matson et al., 2004). The present study suggests that early in the incubation, POPs may affect the DNA integrity of female common eiders in Svalbard.

The blood levels of POPs were higher in the female common eiders at day 20 of incubation, compared to day 5 (Table 1), presumably due to redistribution of contaminants from fatty tissue to blood during fasting. This is consistent with previous findings by Bustnes et al. (2010; 2012). The combined data from 2008 and 2009 showed that at a body mass of 1600 g, blood concentrations of POPs increased rapidly (Fig. 1). This corresponds to a BM decrease of 9-11% from their initial BM. Thus, at least in eiders, a decrease in BM of ca. 10% appears to be a threshold for initiating the release of POP body lipid stores to the circulation. The identification of such a “critical lower BM” for redistribution of POPs from lipid storage to the circulation during fasting provides helpful information to risk assessments related to effects of POPs in fasting animals, and/or in animals that undergo nutritional constrains due to periodic starvation periods.

In the present study, no correlations between blood levels of POPs and DNA-FTM were found at day 20 of incubation, neither in 2008 nor in 2009. Furthermore, there were no correlations between the increase in blood concentrations of POPs and the increase in DNA-FTM during incubation. The increased DNA-FTM at day 20 of incubation might, thus, be caused by stress factors other than POPs. There are numerous factors, both endo- and exogenic, influencing DNA integrity. For instance heat energy produces thousands of abasic sites per cell per day, which are rapidly repaired by a number of supplementary mechanisms

(Preston and Hoffmann, 2008). DNA double-strand breaks can arise during replication of single strand breaks (Houtgraaf et al., 2006), and oxidative stress can directly cause DNA strand breaks (Preston and Hoffmann, 2008). Studies on birds have demonstrated induction of oxidative stress with high reproduction efforts (Wiersma et al., 2004) caused by increased formation of oxidative metabolites and free radicals (von Schantz et al., 1999). Limited availability of exogenous antioxidants, buffering toxic effects from ROS, may mediate reproduction costs (Wiersma et al., 2004), such as genotoxic effects from ROS. In a study with zebra finch (*Taeniopygia guttata*) Alonso-Alvarez et al. (2004) showed that increased breeding effort induced a cost of BM loss and decreased anti-oxidant defence. Thus, the susceptibility to oxidative stress increased. Hence, the decreased DNA integrity observed at day 20, compared to day 5 of incubation in the present study might be caused by reproductive stress, increasing oxidative damage and, possibly, reducing energy expenditure available for anti-oxidant defence. Supporting this particular theory in the present study was the positive relationship between BM loss and the increase in DNA-FTM during incubation. This indicates decreased DNA integrity with increased fasting stress (BM loss (g)). Therefore, the decreased DNA integrity was most likely due to stressful environmental conditions and there appeared to be no additional or combined effects due to the additional POP exposure on DNA integrity of the eiders. This indicated that the stress inflicted by the fasting on the BM overrides the additional stress caused by the concurrent increased levels of the analysed POPs. It is, however, still possible that POPs that were not analysed for may have played a role in the % increase in DSB frequencies identified in 2008 and 2009. It should also be noted that the POP levels in Svalbard are low compared to more industrialized regions. It is thus possible that combined genotoxic effects caused by fasting on BM *per se* and additional stress caused by increased levels of POPs due to fasting might occur in populations with higher POP levels.

The mechanisms behind genotoxic effects in wild living birds and the contribution from the multiple stress factors that potentially cause genotoxic effects during incubation fast warrants further study, preferably with more individuals due to individual variation with regard to the mechanisms involved in DNA integrity.

5. Conclusion

The present study showed that the % frequency of DNA double-strand breaks in the blood of female common eiders increases during their fasting incubation period. The increase in blood levels of the specific POPs analysed in the present study did not affect the DNA integrity during incubation. However, the BM decrease during incubation correlated positively with the % increase in DNA double-strand break frequencies, indicating that stress inflicted by fasting on BM reduces DNA integrity in wild living common eiders. Furthermore, the observation that the individuals with higher blood levels of POPs at day 5 of incubation had a higher DNA-FTM indicates that environmental exposure to POPs might affect DNA integrity, but that when the natural stress is high (day 20 of incubation) it overrides the potential effects from POPs. In particular, higher blood levels of HCB and DDE appeared to be associated with the DNA-FTM at day 5 of incubation.

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References

Alonso-Alvarez, C., Bertrand, S., Devevey, G., Prost, J., Faivre, B., Sorci, G., 2004. Increased susceptibility to oxidative stress as a proximate cost of reproduction. Ecology Letters. 7**,** 363-368.

Barrie, L. A., Gregor, D., Hargrave, B., Lake, R., Muir, D., Shearer, R., Tracey, B., Bidleman, T., 1992. Arctic contaminants: sources, occurrence and pathways. Science of The Total Environment. 122**,** 1-74.

Bustnes, J. O., 2006. Pinpointing potential causative agents in mixtures of persistent organic pollutants in observational field studies: a review of glaucous gull studies. J Toxicol Environ Health A. 69**,** 97-108.

Bustnes, J. O., Borga, K., Erikstad, K. E., Lorentsen, S. H., Herzke, D., 2008. Perfluorinated, brominated, and chlorinated contaminants in a population of lesser black-backed gulls (Larus fuscus). Environ Toxicol Chem. 27**,** 1383-92.

Bustnes, J. O., Moe, B., Hanssen, S. A., Herzke, D., Fenstad, A. A., Nordstad, T., Borga, K., Gabrielsen, G. W., 2012. Temporal dynamics of circulating persistent organic pollutants in a fasting seabird under different environmental conditions. Environ Sci Technol. 46**,** 10287-94.

Bustnes, J. O., Moe, B., Herzke, D., Hanssen, S. A., Nordstad, T., Sagerup, K., Gabrielsen, G. W., Borga, K., 2010. Strongly increasing blood concentrations of lipid-soluble organochlorines in high arctic common eiders during incubation fast. Chemosphere. 79**,** 320-5.

Butterworth, F. M., Pandey, P., McGowen, R. M., Ali-Sadat, S., Walia, S., 1995. Genotoxicity of polychlorinated biphenyls (PCBs): recombinogenesis by biotransformation products. Mutat Res. 342**,** 61-9.

Canonero, R., Campart, G. B., Mattioli, F., Robbiano, L., Martelli, A., 1997. Testing of p-dichlorobenzene and hexachlorobenzene for their ability to induce DNA damage and micronucleus formation in primary cultures of rat and human hepatocytes. Mutagenesis. 12**,** 35-9.

Dahl, T. M., Falk-Petersen, S., Gabrielsen, G. W., Sagent, J. R., Hop, H., Millar, R. M., 2003. Lipids and stable isotopes in common eider, black-legged kittiwake and northern fulmar : a trophic study from an Arctic fjord. Mar Ecol Prog Ser. 256**,** 257-269.

Debier, C., Chalon, C., Le Bœuf, B. J., de Tillesse, T., Larondelle, Y., Thomé, J.-P., 2006. Mobilization of PCBs from blubber to blood in northern elephant seals (Mirounga angustirostris) during the post-weaning fast. Aquatic Toxicology. 80**,** 149-157.

Debier, C., Pomeroy, P. P., Dupont, C., Joiris, C., Comblin, V., Boulengé, E. L., Larondelle, Y., Thomé, J.-P., 2003. Quantitative dynamics of PCB transfer from mother to pup during lactation in UK grey seals Halichoerus grypus. Mar Ecol Prog Ser. 247**,** 237-248.

Dubois, M., Pfohl-Leszkowicz, A., Groose, Y., Kremers, P., 1995. DNA adducts and P450 induction in human, rat and avian liver cells after exposure to polychlorobiphenyls. Mutation Research/Genetic Toxicology. 345**,** 181-190.

Ennaceur, S., Ridha, D., Marcos, R., 2008. Genotoxicity of the organochlorine pesticides 1,1-dichloro-2,2- bis(p-chlorophenyl)ethylene (DDE) and hexachlorobenzene (HCB) in cultured human lymphocytes. Chemosphere. 71**,** 1335-1339.

Erikstad, K. E., Moum, T., Bustnes, J. O., Reiertsen, T. K., 2010. High levels of organochlorines may affect hatching sex ratio and hatchling body mass in arctic glaucous gulls: Sex ratio and organochlorines in glaucous gulls. Functional Ecology.

Hanssen, S. A., Folstad, I., Erikstad, K. E., 2003. Reduced immunocompetence and cost of reproduction in common eiders. Oecologia. 136**,** 457-464.

Hanssen, S. A., Hasselquist, D., Folstad, I., Erikstad, K. E., 2005. Cost of reproduction in a long-lived bird: incubation effort reduces immune function and future reproduction. Proc R Soc Biol Sci Ser B. 272**,** 1039-1046.

Henriksen, E. O., Gabrielsen, G. W., Skaare, J. U., 1996. Levels and congener pattern of polychlorinated biphenyls in kittiwakes (Rissa tridactyla), in relation to mobilization of body-lipids associated with reproduction. Environmental Pollution. 92**,** 27-37.

Holmstrup, M., Bindesbøl, A.-M., Oostingh, G. J., Duschl, A., Scheil, V., Köhler, H.-R., Loureiro, S., Soares, A. M. V. M., Ferreira, A. L. G., Kienle, C., Gerhardt, A., Laskowski, R., Kramarz, P. E., Bayley, M., Svendsen, C., Spurgeon, D. J., 2010. Interactions between effects of environmental chemicals and natural stressors: A review. Science of The Total Environment. 408**,** 3746-3762.

Houtgraaf, J. H., Versmissen, J., van der Giessen, W. J., 2006. A concise review of DNA damage checkpoints and repair in mammalian cells. Cardiovascular Revascularization Medicine. 7**,** 165-172.

Jackson, S. P., 1999. Detection, repair and signalling of DNA double-strand breaks. Biochem Soc Trans. 27**,** 1-13.

Jeggo, P. A., 1998. Identification of Genes Involved in Repair of DNA Double-Strand Breaks in Mammalian Cells. Radiation Research. 150**,** S80-S91.

Jenssen, B. M., 2006. Endocrine-Disrupting Chemicals and Climate Change: A Worst-Case Combination for Arctic Marine Mammals and Seabirds? Environ Health Perspect. 114**,** 76-80.

Jørgensen, E. H., Bye, B. E., Jobling, M., 1999. Influence of nutritional status on biomarker responses to PCB in the Arctic charr (Salvelinus alpinus). Aquatic Toxicology. 44**,** 233-244.

Kajiwara, N., Watanabe, M., Wilson, S., Eybatov, T., Mitrofanov, I. V., Aubrey, D. G., Khuraskin, L. S., Miyazaki, N., Tanabe, S., 2008. Persistent organic pollutants (POPs) in Caspian seals of unusual mortality event during 2000 and 2001. Environmental Pollution. 152**,** 431-442.

Kanaar, R., Hoeijmakers, J. H. J., van Gent, D. C., 1998. Molecular mechanisms of DNA double-strand break repair. Trends in Cell Biology. 8**,** 483-489.

Korschgen, C. E., 1977. Breeding stress of female eiders in Maine. J Wildl Manag. 41**,** 360-373.

Krøkje, Å., Bingham, C., Husmo Tuven, R., Wing Gabrielsen, G., 2006. Chromosome Aberrations and DNA Strand Breaks in Glaucous Gull (Larus Hyperboreus) Chicks Fed Environmentally Contaminated Gull Eggs. Journal of Toxicology and Environmental Health, Part A. 69**,** 159-174.

Letcher, R. J., Bustnes, J. O., Dietz, R., Jenssen, B. M., Jørgensen, E. H., Sonne, C., Verreault, J., Vijayan, M. M., Gabrielsen, G. W., 2010. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. Science of The Total Environment. 408**,** 2995-3043.

Leung, K. M. Y., Furness, R. W., 2001. Survival, growth, metallothionein and glycogen levels of Nucella lapillus (L.) exposed to sub-chronic cadmium stress: the influence of nutritional state and prey type. Marine Environmental Research. 52**,** 173-194.

Marabini, L., Calò, R., Fucile, S., 2011. Genotoxic effects of polychlorinated biphenyls (PCB 153, 138, 101, 118) in a fish cell line (RTG-2). Toxicology in Vitro. 25**,** 1045-1052.

519 Matson, C. W., Franson, J. C., Hollmén, T., Kilpi, M., Hario, M., Flint, P. L., Bickham, J. W.,
520 2004. Evidence of chromosomal damage in common eiders (Somateria mollissima)
521 from the Baltic Sea. Marine Pollution Bulletin. 49**,** 1066-1071.
522 Mrema, E. J., Rubino, F. M., Brambilla, G., Moretto, A., Tsatsakis, A. M., Colosio, C., 2013.
523 Persistent organochlorinated pesticides and mechanisms of their toxicity. Toxicology.
524 307**,** 74-88.
525 Murk, A. J., Van den Berg, J. H. J., Fellinger, M., Rozemeijer, M. J. C., Swennen, C.,
526 Duiven, P., Boon, J. P., Brouwer, A., Koeman, J. H., 1994. Toxic and biochemical
527 effects of 3,3′,4,4′-tetrachlorobiphenyl (CB-77) and clophen A50 on eider duckling
528 (Somateria mollissima) in a semi-field experiment. Environmental Pollution. 86**,** 21-
529 30.
530 Murvoll, K. M., Skaare, J. U., Jensen, H., Jenssen, B. M., 2007. Associations between
531 persistent organic pollutants and vitamin status in Brünnich's guillemot and common
532 eider hatchlings. Science of The Total Environment. 381**,** 134-145.
533 Pacyna, J. M., Oehme, M., 1988. Long-range transport of some organic compounds to the
534 Norwegian Arctic. Atmospheric Environment (1967). 22**,** 243-257.
535 Parker, H., Holm, H., 1990. Patterns of nutrient and energy expenditure in female common
536 eiders nestingin the high Arctic. The Auk. 107**,** 660-668.
537 Pfeiffer, P., 1998. The mutagenic potential of DNA double-strand break repair. Toxicology
538 Letters. 96–97**,** 119-129.
539 Preston, J. R., Hoffmann, G. R., Gene Toxicology. In: C. D. Klaassen, (Ed.), Casarett and
540 Dull's Toxicology, The Basic Science of Poisons. McGraw Hill, NY, USA, 2008, pp.
541 381-413.
542 R Core Team, R: A Language and Environment for Statistical Computing. R Foundation for
543 Statistical Computing, 2013. http://www.R-project.org, 31.03.14.
544 Rozemeijer, M. J. C., Boon, J. P., Swennen, C., Brouwer, A., Murk, A. J., 1995. Dioxin type
545 and mixed type induction of the cytochrome P-450 system of common eider ducklings
546 (Somateria mollissima) by PCBs: with indications for biotransformation. Aquatic
547 Toxicology. 32**,** 93-113.
548 Salmon, M. L., Madanagopal, S. G., Blando, R., Berner, J., Williams, K., 2002. Effects of
549 hexachlorobenzene on embryonic mammalian cells. Toxicology in Vitro. 16**,** 539-
550 548.
551 Sambrook, E. F., Maniatis, T., 1989. Molecular cloning a Laboratory Manual. Cold Spring
552 Harbor Laboratory Press, Melbourne, Australia.
553 Srinivasan, A., Lehmler, H.-J., Robertson, L. W., Ludewig, G., 2001. Production of DNA
554 Strand Breaks in Vitro and Reactive Oxygen Species in Vitro and in HL-60 Cells by
555 PCB Metabolites. Toxicological Sciences. 60**,** 92-102.
556 Tanabe, S., Iwata, H., Tatsukawa, R., 1994. Global contamination by persistent
557 organochlorines and their ecotoxicological impact on marine mammals. Science of
558 The Total Environment. 154**,** 163-177.
559 Thacker, J., 1986. The nature of mutants induced by ionising radiation in cultured hamster
560 cells III. Molecular characterization of HPRT-deficient mutants induced by γ-rays or
561 α-particles showing that the majority have deletions of all or part of the hprt gene.
562 Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 160**,**
563 267-275.
564 Theodorakis, C. W., D'Surney, S. J., Shugart, L. R., 1994. Detection of Genotoxic Insult as
565 DNA strand breaks in fish blood cells by agarose gel electrophoresis. Environmental
566 Toxicology and Chemistry. 13**,** 1023-1031.

von Schantz, T., Bensch, S., Grahn, M., Hasselquist, D., Wittzell, H., 1999. Good genes, oxidative stress and condition-dependent sexual signals. Proc R Soc Biol Sci Ser B. 7**,** 1-12.

Wiersma, P., Selman, C., Speakman, J. R., Verhulst, S., 2004. Birds sacrifice oxidative protection for reproduction. Proc R Soc Biol Sci Ser B. 271**,** S360-S363.

Wlodek, D., Banáth, J., Olive, P. L., 1991. Comparison between Pulsed-field and Constant-field Gel Electrophoresis for Measurement of DNA Double-strand Breaks in Irradiated Chinese Hamster Ovary Cells. International Journal of Radiation Biology. 60**,** 779-790.

Østby, L., Wing Gabrielsen, G., Krøkje, Å., 2005. Cytochrome P4501A induction and DNA adduct formation in glaucous gulls (Larus hyperboreus), fed with environmentally contaminated gull eggs. Ecotoxicology and Environmental Safety. 62**,** 363-375.

Table 1. Blood concentrations (pg/g ww) of POPs in female common eiders given as mean, standard deviation (SD), median and range at day 5 and day 20 of incubation in 2008 and 2009.

		day 5 of incubation					day 20 of incubation				
year	**POP**	**mean[b]**	**SD**	**median**	**range**	**detected[a]**	**mean**	**SD**	**median**	**range**	**detected [a]**
2008	HCB	124.01 (0.44)	65.88	127.15	31.0-236.3	7/8	636.04 (2.23)	390.33	602.95	195.9-1301.2	8/8
	t-chlordane	2.49 (0.01)	4.71	0.40	0.4-13.8	2/8	35.98 (0.09)	34.57	28.20	0.4-104.7	7/8
	oxy-chlordane	37.78 (0.09)	17.51	25.20	25.2-61.8	3/8	179.24 (0.42)	117.86	161.90	25.20-382.60	7/8
	t-nonachlor	11.75 (0.03)	15.93	6.25	0.5-47.5	5/8	107.13 (0.24)	97.58	83.50	22.7-302.5	8/8
	DDE	72.11 (0.23)	50.70	55.75	31.0-168.80	4/8	686.82 (2.16)	576.23	596.50	31.0-1562.0	7/8
	PCB 118	48.00 (0.15)	0.00	48.00	48.0-48.0	0/8	248.99 (0.76)	144.11	306.35	48.0-393.0	6/8
	PCB 138	45.10 (0.12)	31.40	34.00	34.0-123.0	1/8	436.74 (1.21)	212.36	497.35	34.0-708.0	7/8
	PCB 153	47.32 (0.13)	28.55	32.00	32.0-99.0	2/8	631.51 (1.75)	291.60	619.85	32.0-1009.0	7/8
	PCB 180	36.47 (0.09)	0.00	36.47	36.5-36.5	0/8	179.10 (0.45)	125.21	173.85	36.5-372.1	6/8
	∑POPs	425.03 (1.52)	165.49	413.87	238.6-744.8	-	3141.54 (9.32)	1640.47	3009.60	425.7-5588.2	-
2009	HCB	383.17 (1.35)	180.56	327.40	190.3-879.5	15/15	676.23 (2.37)	211.95	761.40	319.5-988.9	15/15
	t-chlordane	9.79 (0.02)	8.34	7.00	3.6-35.9	15/15	21.39 (0.05)	15.52	19.60	5.0-60.5	15/15
	oxy-chlordane	79.52 (0.18)	43.64	59.30	38.4-195.2	15/15	149.60 (0.35)	91.14	107.40	33.3-360.9	15/15
	t-nonachlor	22.30 (0.05)	23.76	13.10	6.6-100.1	15/15	48.79 (0.11)	47.59	39.80	9.4-173.3	15/15
	DDE	389.41 (1.22)	425.31	251.60	23.0-1762.1	13/15	1179.09 (3.71)	2333.44	401.30	23.0-9440.4	14/15
	PCB 118	101.35 (0.31)	65.36	85.60	43.5-275.8	11/15	207.81 (0.64)	294.53	106.80	43.5-1184.7	10/15
	PCB 138	188.94 (0.52)	168.11	110.80	58.5-695.9	15/15	533.59 (1.48)	670.65	384.80	64.0-2847.1	15/15
	PCB 153	250.40 (0.69)	162.03	187.00	99.9-738.7	15/15	692.79 (1.92)	725.18	485.00	78.0-3080.2	15/15
	PCB 180	53.74 (0.14)	20.18	41.68	36.0-100.8	10/15	151.98 (0.38)	112.16	124.30	41.7-441.9	13/15
	∑POPs	1473.62 (5.77)	1034.95	1081.10	591.3-4710.0	-	3661.28 (11.02)	4293.10	2459.58	711.6-18486.1	-

[a] Total number of individuals with blood concentrations over the limit of detection (LOD), when under the LOD, concentrations were set to 50% of the detection limit.

[b] The mean blood concentration in pmol/g ww is listed in parentheses.

Table 2. The body mass (BM, g), median molecular length (MML) and DNA-fraction of total DNA, that migrated into the gel (DNA-FTM) in blood of female common eiders in Svalbard, given as mean ± standard deviation at day 5 and day 20 of incubation in 2008 (N=8) and in 2009 (N=15).

year		day 5 of incubation mean	day 20 of incubation mean
2008	BM	1752 ± 117	1348 ± 109
	MML	67.6 ± 7.8	66.2 ± 6.7
	DNA-FTM	37.4 ± 19.8	60.2 ± 19.7
2009	BM	1790 ± 115	1422 ± 105
	MML	55.2 ± 2.2	55.4 ± 3.2
	DNA-FTM	16.7 ± 9.4	27.8 ± 17.6

[a] MML calculated in kilobases.

[b] DNA-FTM the % of DNA migrating out of sample well out of the total DNA loaded.

Figure legends

Figure 1: Blood concentrations (pg/g ww) of HCB (A), *t*-chlordane (B), *oxy*-chlordane (C), *t*-nonachlor (D), PCB 118 (E), PCB 138 (F), PCB 153 (G) and PCB 180 (H) of female common eiders at day 5 (blank circles (2008) + filled circles (2009)) and day 20 (blank triangles (2008) + filled triangles (2009)) of incubation as a function of body mass (g).

Figure 2: Principal component analysis (PCA) plots with the blood concentrations (pmol/g ww) of PCBs, DDE, oxychlordane (oxy-chlor), *t*-chlordane (t-chlor), *t*-nonachlor (t-nona), body mass (g) (BM), wing length (wing), clutch size (clutch) and the DNA-fraction of total DNA, that migrated into the gel (DNA-FTM) in blood of female common eiders at Svalbard at both incubation stages, both years.