Norsk institutt for luftforskning
Norwegian Institute for Air Research

Assessment of additives used in plastic in seabirds

Dorte Herzke, Pawel Rostkowski, Mikael Harju, Anders R. Borgen, Signe Christensen-Dalsgaard

NILU report 1/2019

NILU report 1/2019	ISBN: 978-82-425-2962-6 ISSN: 2464-3327	TILGJENGELIGHET: A
DATO 19.12.2019	ANSVARLIG SIGNATUR Ole-Anders Braathen, viseadministrerende direktør (sign.)	ANTALL SIDER 16
TITTEL Assessment of additives used in plastic in seabirds		PROSJEKTLEDER Dorte Herzke
		NILU PROSJEKT NR. O-118106
FORFATTER(E) Dorte Herzke, Pawel Rostkowski, Mikael Harju, Anders R. Borgen, Signe Christensen-Dalsgaard		KVALITETSSIKRER Eldbjørg S. Heimstad
OPPDRAGSGIVER Miljødirektoratet		OPPDRAGSGIVERS REF. Sjur Andersen

REFERAT

NILU- Norsk Institutt for Luftforskning har på oppdrag fra Miljødirektoratet gjennomført en kjemisk analyse av additiver assosiert med plastpartikler i sjøfugl. Norsk Institutt for Naturforskning - NINA har vært ansvarlig for innsamling, transport og oppbevaring av prøver, samt forsendelsen av prøver fra NINA til NILU.
Norsk Institutt for Vannforskning – NIVA har vært ansvarlig for analysering av kjemikalier som UV filtrere og stabilisatorer. Målet med prosjektet har vært og dokumentere forekomst av plast additiver i leverprøver fra sjøfugl som en mulig indikator på eksponering av plast i hav og mikroplast.

TITLE

Analyses of additives from plastic in liver of seabirds

EMNEORD

Plastic pollution | Additives | Seabirds

ABSTRACT

Liver samples from 10 herring gulls (*Larus argentatus*) were investigated for a broad range of chemicals used as additives in plastic products. The aim of this study was to clarify if the ingestion of plastic by seabirds would cause additives to leach out and get taken up by the organism, posing a potential harm. After chemical trace analyses of the liver samples, considerable concentrations of S/MCCPs and dechloranes were detected. Of the other additive classes analysed for, only sporadic detections were observed. In general, the results from chemical analysis of additives used in plastic do not indicate a relationship between gastric contents (plastic occurrence in the stomach) and additive concentration in the liver, in respect to the chemical compounds investigated here.

PUBLISERINGSTYPE: Digitalt dokument (pdf) | FORSIDEBILDE: Kilde: NILU

1 Preface

NILU - The Norwegian Institute for Air Research has, on behalf of the Norwegian Environment Agency, conducted chemical analysis of additives associated with ingested plastic particles in seabirds. The Norwegian Institute for Nature Research – NINA was responsible for sampling, transport and storage of the samples, as well as shipping to NILU for analysis. The Norwegian Institute for Water Research – NIVA was responsible for the analyses of a number of chemicals used as UV-screens and –stabilisers. The aim of the project was to document the occurrence of plastic additives in liver samples from seabirds as a possible indication of exposure to marine plastic litter/ microplastic.

We thank all participating researchers and technical personnel, especially:

Signe Christensen-Dalsgaard; Magdalene Langset og Audun Rikardsen
NINA

Malcolm Reid
NIVA

Merete Miøen og Arntraut Götsch
NILU

Content

Summary

Liver samples from 10 herring gulls (*Larus argentatus*) were investigated for a broad range of chemicals used as additives in plastic products. The aim of this study was to clarify if the ingestion of plastic by seabirds would cause additives to leach out of ingested plastic particles and get taken up by the organism, posing a potential harm.

In 20 % of the investigated stomach contents, plastic was found (1 male and 1 female individual) with 1 and 2 particless for the female and male, respectively.

Herring gulls belong to the seabird species regurgitating undigested stomach contents (e.g. bone remnants, shells and plastic). The plastic content found in this study should therefore be considered as a "snapshot" of what the birds have recently eaten.

After chemical trace analyses of the liver samples, considerable concentrations of S/MCCPs and dechloranes were detected. Of the other additive classes analysed for, only sporadic detections were observed.

The considerable concentrations of S/MCCPs in herring gull liver are most probably caused by exposure via prey and bioaccumulation through the food chain. The absence of other additives in herring gull liver can be attributed to either fast degradation/metabolism of these chemicals in the environment since they are designed to be chemical reactive, and/or the small amount of plastic found ingested by herring gulls could be explained by their regurgitating habits.

In general, the results from chemical analysis of the additives used in plastic do not indicate a relationship between gastric contents (plastic occurrence in the stomach) and plastic additive concentration in the individual liver samples, in respect to the chemical compounds investigated here.

2 Background

Seabird liver is a well-documented matrix, known for its ability to take up environmental pollutants that birds are exposed to through their diet. Both polar and non-polar pollutants can be found in seabirds, and the hypothesis is that plastic additives will be taken up if birds are exposed to microplastics. Additives are used in plastic in relatively large amounts (up to 70% by weight) [1, 2]. and have the potential to be absorbed through the gastrointestinal system by organisms that ingest plastic through the food, such as Norwegian seabirds. The Northern fulmar is known to ingest large amounts of plastic, but the abundance of additives in their tissues is unknown [3, 4]. Seabirds can either be exposed to such additives through direct intake of plastic, through the food chain if the prey has been exposed to plastic or through direct intake of the additives via water and air.

In a previous study under the auspices of the Norwegian Polar Institute, NILU has measured additives such as OPFR and PFAS [5]. No significant relationship was found between the amount of plastic found in the stomach and the concentrations of PFAS and OPFR.

Other substances have also been previously analysed in marine organisms by NILU (dechloranes, chlorinated paraffins (S/ MCCP), TBBPA, BPA, etc.)

Dechloranes are used as flame retardants, especially in plastic products. The other substances selected for analysis represent antioxidants in products (UV-substances) or flame retardants and plasticizers (S/MCCP). Phthalates are a separate group of chemicals with softening properties that are widely used in plastic production.

3 Objective

The goal of this study was to investigate whether plastic additives can be found in tissues of seabirds and to find out more about:

1. The occurrence of plastic additives in seabird liver
2. Clarify whether these substances can be taken up in seabirds

4 Methods and materials

4.1 Sampling

The Norwegian Institute for Nature Research (NINA) was the supplier of liver and plastic samples included in the study. The herring gulls (*Larus argentatus*) used in the study were sampled in Skulsfjord in Troms, Northern Norway in January 2017 (Map 1). The birds were found dead and washed ashore on the beach. To investigate the cause of death, the birds were subsequently collected, frozen and sent to Trondheim for necropsy. The necropsy showed that the birds had died of acute drowning. The cause of death for these birds was most probably linked to professional fishing activities in the area.

Five adult birds of each sex was included in the study. During necropsy at NINA, the whole stomach and samples of liver tissue were collected from each individual. Tissue samples were put in aluminium foil, enveloped, and frozen to −18 °C for further analysis.

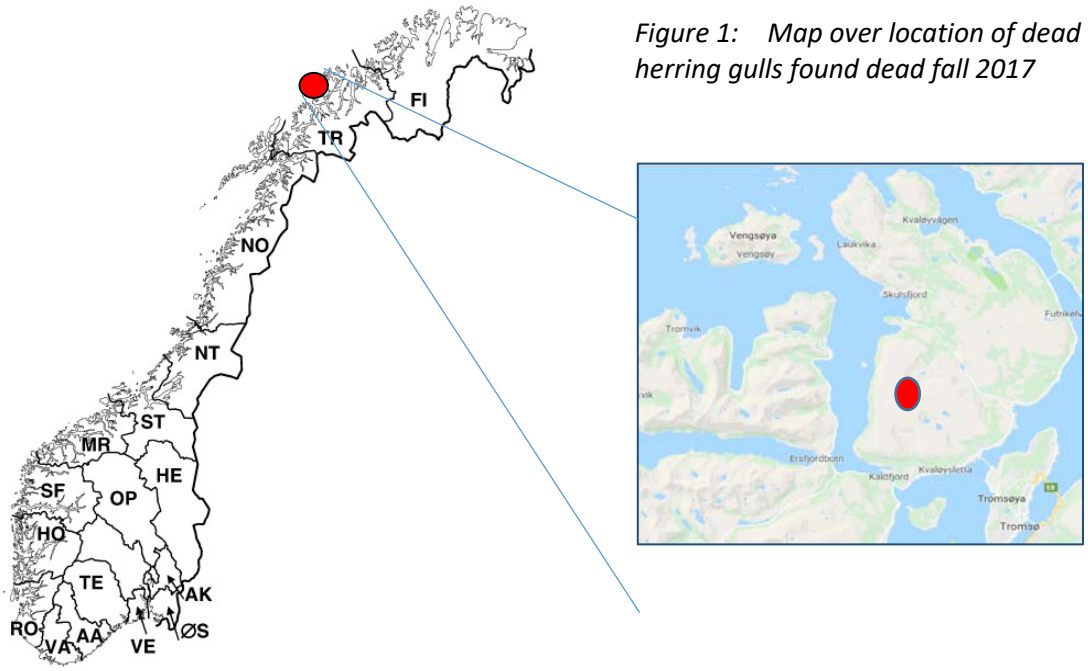

Figure 1: Map over location of dead herring gulls found dead fall 2017

Figure 2: Illustration of the situation of the found dead Herring gulls in the fall, 2017 (n > 100)

4.2 Chemical Analyses and analyses for ingested plastic

Plastic particles were extracted by NINA from the stomach samples following an internationally standardized procedure by rinsing the proventriculus and gizzard over a 1-mm sieve, drying their contents in a Petri dish at 40 °C, and sorting it into different categories (i.e. plastic, non-plastic waste and natural food items) [6].

Chemical substances selected for analysis are presented in Table 1.

Table 1: Overview over analysed compounds

Substance name	**CAS-number**
Priority 1	
Dechlorane plus (605)	13560-89-9
Dechlorane Plus Syn	135821-03-3
Dechlorane Plus Anti	135821-74-8
Dechlorane 601	13560-90-2
Dechlorane 602	31107-44-5
Dechlorane 603	13560-92-4
Dechlorane 604	34571-16-9
Dibromoaldrin	20389-65-5
Benzenesulfonic acid, 5-chloro-2-[(2-hydroxy-1-naphthalenyl)azo]-4-methyl-, barium salt (2:1)	5160-02-1
2-(4,6-Diphenyl-s-triazin-2-yl)-5-hexyloxyphenol	147315-50-2
1-[(2-chloro-4-nitrophenyl)diazenyl]-2-naphthol; D&C Red No. 36	2814-77-9
2-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol; Octrizole	3147-75-9
4,4'-diamino-1,1'-bianthracene-9,9',10,10'-tetrone; Pigment Red 177	4051-63-2
2,5-di-tert-pentylhydroquinone	79-74-3
Bisphenol A	80-05-7
m-Cresol, 4,4'-butylidenebis-6-tert-butyl-	85-60-9
4,4'-Thiobis(6-tert-butyl-m-cresol)	96-69-5
TBBPA	79-94-7
MCCP	85535-85-9
SCCP	85535-84-8
Priority 2	
Octocrylen*	6197-30-4
Benzophenone-3*	131-57-7
Ethylhexylmethoxycinnamate*	5466-77-3
UV-327 *	3864-99-
UV-328 *	25973-55-1
UV-320 *	3846-71-7
UV-326 *	3896-11-5
DEHP	117-81-7

DBP	84-74-2
BBP	85-68-7
DIBP	84-69-5
DCUP	80-43-3
Bis(tert-butylperoxyisopropyl)benzene	25155-25-3
Isodecyl diphenyl phosphate	29761-21-5
Phenol, isopropylated, phosphate (3:1)	68937-41-7
2,2-bis-(chloromethyl)-trimethylene bis(bis(2-chloroethyl)phosphate) (V6)	38051-10-4

*Analysed by Norwegian Institute of water research (NIVA)

For most compound groups, several extraction and processing methods were used:

OPFR and phthalates

For the OPFR and phthalates, we used a newly established in-house method, which included work in clean rooms to lower the contamination risk to a minimum. Blank samples were used at all stages of sample cleaning to check for possible contamination. Two gram of sample was homogenized with dry Na_2SO_4. Surrogate standard was added and samples was extracted by ultrasonication using acetone/n-hexane. Extract was evaporated to near dryness and cleanup of the samples was done using solid phase extraction using Supelclean EZ-POP NP, the sample was eluted using 15mL of acetonitrile and evaporated and transferred analytical glass and added PFR recovery standard and 0.2% formic acid in cleaned deionized water.

Chlorinated paraffins and dechloranes. Briefly, 1-2 grams of sample were mixed and homogenized with a 20-fold amount of dry Na_2SO_4. The homogenate was extracted using a mixture of acetone/cyclohexane (1/1 v/v). The organic extract was evaporated and treated 2-4 times with 3-4 mL of concentrated sulfuric acid to remove the lipids. Extracts were measured using GC/HRMS.

UV-compounds. Chrysene-d_{12} and benzophenone-d_{10} were used as internal standards. Liver was extracted with iso-hexane/isopropanol (50/50) by ultrasonication for 1 hour. Samples were centrifuged and the solvent decanted. This extraction was repeated and the extracts combined. The iso-hexane fraction was isolated by the addition of 0.5% NaCl and the evaporated to approximately 1 ml before solvent exchange to cyclohexane. Different clean-up methods were used for each matrix in response to differing interferences.

Phenolic- and other compounds. Samples were first digested with enzymes and then were extracted using ultrasonic assisted liquid extraction. Fat was removed by liquid-liquid extraction with hexane and remaining interferences were removed with two different SPE columns. All samples were analysed with the use the Agilent 1290 UHPLC coupled to Agilent 6550 HR-QTOF equipped with Agilent Dual Jet Stream electrospray source operating in a negative mode.

Since in this study, only partially established methods were present for some compounds, no reference material or laboratory intercalibrations were available. For these compounds, an

uncertainty for the analyses of at least 40-50% needs to be assumed. However, the certainty is sufficient for a rough first overview of the presence of these compounds.

5 Results

Studies of the stomach contents showed that only 2 out of 10 individuals had plastic pieces in the stomach (1 female and 1 male). The occurance varied between 1-2 pieces with a total weight of 0.0012 and 0.061 g for each individual, respectively. Results for the chemical analyses are summarised in Table 2.

Table 2: Average concentrations (min/max)
n = 10 liver samples from herring gull

Compound	Average ng/g ww	Min ng/g ww	Max ng/g ww
Dechloranes			
Dibromoaldrin	<0.18	<0.18	<0.18
Dechlorane 601	0.03	<0.011	0.29
Dechlorane 602	0.25	0.1	0.69
Dechlorane 603	0.01	< 0.01	0.09
Dechlorane 604	< 0.39	< 0.39	< 0.39
Dechlorane plus syn	0.15	0.09	0.34
Dechlorane plus anti	0.38	0.19	1.23
Phenols and bisphenols			
Bisphenol A	<0.6	<0.6	<0.6
m-Cresol, 4,4'-butylidenebis-6-tert-butyl-	<10	<10	<10
4,4'-Thiobis(6-tert-butyl-m-cresol)	<10	<10	<10
TBBPA	< 2	< 2	< 2
Chlorinated paraffins			
MCCP	87.8	<63	372
SCCP	210	< 156	698
UV filters and stabilisers			
Octocrylen	<2	<2	<2
Benzophenone-3	<0.14	<0.14	<0.14
Ethylhexylmethoxycinnamate	<0.3	<0.3	<0.3
UV-320	0.04	<0.04	0.15
UV-326	<0.3	<0.3	<0.3
UV-327	0.02	<0.03	0.07
UV-328	<0.08	<0.08	<0.08
Phthalates			
DEHP	< 370	< 370	< 370
DBP	< 6.0	< 6.0	17.5

BBP	< 11.5	< 11.5	< 11.5
DIBP	< 6.0	< 6.0	< 6.0
OPFRs			
Isodecyl diphenyl phosphate	< 0.1	< 0.1	< 0.1
Phenol, isopropylated, phosphate (3:1)	< 1.0	< 1.0	< 1.0
2,2-bis-(chloromethyl)-trimethylene bis(bis(2-chloroethyl) phosphate) (V6)	< 0.05	< 0.05	< 0.05
Other additives			
Benzenesulphonic acid, 5-chloro-2-[(2-hydroxy-1-naphthalenyl)azo]-4-methyl-, barium salt (2:1)	Detected in n = 3 <10	<10	<10
2-(4,6-Diphenyl-s-triazin-2-yl)-5-hexyloxyphenol	<10	<10	<10
1-[(2-chloro-4-nitrophenyl)diazenyl]-2-naphthol; D&C Red No. 36	<10	<10	<10
2-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol; Octrizole	<10	<10	<10
4,4'-diamino-1,1'-bianthracene-9,9',10,10'-tetrone; Pigment Red 177	<10	<10	<10
2,5-di-tert-pentylhydroquinone	<10	<10	<10

5.1 Chlorinated paraffins

Chlorinated paraffins dominated the analytical results with mean concentrations varying between 88 and 210 ng/g for MCCPs and SCCPs, respectively. In previous projects, we detected S/MCCPs in liver of Atlantic and polar cod with concentrations of 10.3 and 2.28 ng/g ww for SCCP and 0.9 and 1.5 ng/g ww for MCCP [7]. The Environmental quality standard (EQS) for SCCP and MCCP in biota is 6000 and 170 ng/g ww, respectively [8]. In the present study, 6 out of 10 liver samples exceeded the EQS for MCCP (see Figure 3). Similar elevated concentrations of S/MCCP were detected in liver from cod sampled in harbours along the Norwegian coast [9]; cod is an important prey to the herring gull and likely a source of the findings reported here.

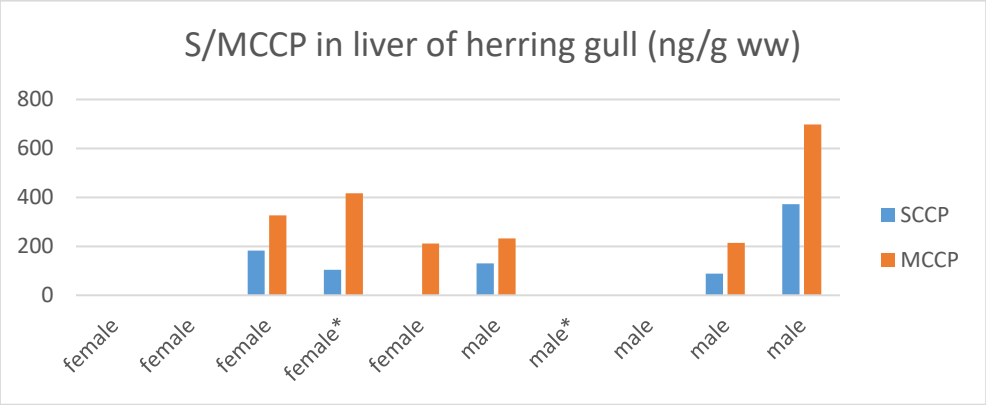

*Figure 3: Concentrations of chlorinated paraffins (S/MCCP) in seabird liver. The samples marked with * are the individuals with plastic findings in the stomach.*

As Figure 3 shows, there is no correlation between S/MCCP findings in gull liver and the occurrence of plastic in their stomach.

5.2 Dechloranes

Dechloranes were among the additives found with high detection rates, specifically dechlorane 602, dechlorane plus syn and dechlorane plus anti. The concentrations of these varied between 0.09 and 0.69 ng/g ww for the dechlorane 602; between 0.08 and 0.38 ng/g ww for plus syn and 0.19 and 1.23 ng/g ww for plus anti (Figure 4).

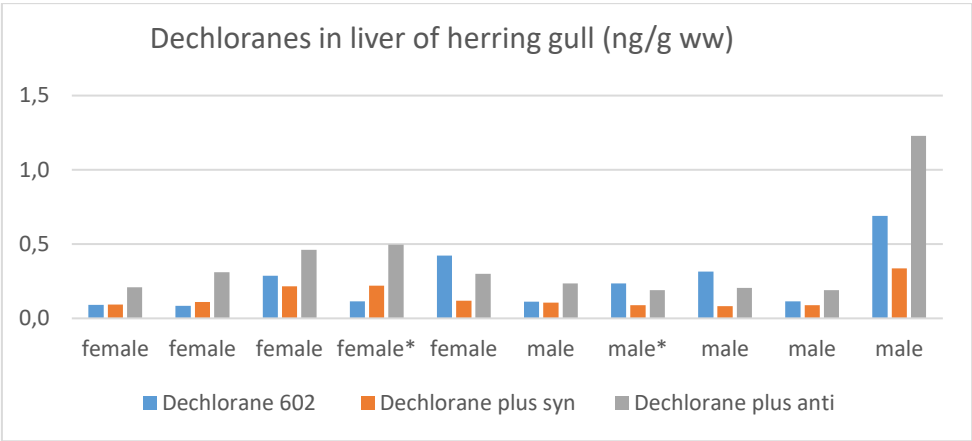

*Figure 4: Concentrations of detected dechloranes in seabirds liver. The samples marked with * are the individuals with plastic findings in the stomach.*

Also for dechloranes, no correlation between plastic occurrence and liver concentrations can be found.

5.3 UV compounds

Of the 10 UV filters and stabilizers measured, UV 320 was detected with the highest detection rate (40%) and a maximum concentration of 0.15 ng/g ww. Additionally, UV 327 was found in four individuals out of ten. The two gulls with plastic in the stomach showed only UV 327 in the female individual (0.07 ng/g ww) and no UV stabilizer was detected in the male.

5.4 Phthalates

Of the 4 phthalates measured, only DBD was detected, and also only in 1 sample (17.5 ng/g ww). No connection with intestinal plastic could be found. A probable cause of our findings is the fast degradation in the environment and metabolism in organisms of phthalates.

5.5 Bisphenols and other phenols

No phenols and bisphenols could be detected in gulls. This may be due to the rapid metabolism of these substances in gulls and their prey, and that they are generally easily biodegradable in nature. This is consistent with earlier findings reported for cod liver [9]. Also, to the fact that there was little plastic in the stomachs of the birds can be a reason for our findings.

5.6 OPFRs

No OPFR could be detected in the gulls. The very limited detection of these chemicals may be due to a rapid metabolism of these substances in gulls and their prey, that they are generally easily biodegradable, in addition to the fact that there was little plastic in the stomach of the birds.

5.7 Other additives

None of the other analysed substances could be detected in gulls. For substances with no available standard and/ or internal standard, the following grouping was used:

1: detected with standard available and High Resolution MS → certain identification but no quantification
2: no standard available; detected with High Resolution MS → uncertain identification and no quantification
3: standard available; not detected with High Resolution MS → no identification and no quantification
4: no standard available; not detected with High Resolution MS → no identification and no quantification

Of the chemicals within this group (see Table 2), all belonged to category 3 or 4, except 3 individuals. For them the benzenesulfonic acid, 5-chloro-2-[(2-hydroxy-1-naphthalenyl)azo]-4-methyl-, bariumsalt was found as category 2, resulting in a positive detection but without possible quantification. One of these instances of positive detection belong to one gull containing ingested plastic.

6 Discussion and Conclusion

The herring gulls are generalists and a highly opportunistic species. They can thus forage on everything from marine invertebrates, pelagic fish, terrestrial invertebrates as well as anthropogenic food sources (e.g. landfills, sewage outfall and household waste). In this study, considerable concentrations of S/MCCPs and dechloranes were detected in all 10 liver samples of herring gull (5 males and 5 females). UV 320 and 327 was sporadically found at low concentrations (< 0.15 ng/g ww). Two of the investigated individuals contained plastic in their stomachs.

Herring gulls belong to the seabird species regurgitating undigested stomach contents (e.g. bone remnants, shells and plastic). One can therefore probably not expect large amounts of plastic to accumulate in the stomachs of these birds, as compared to other species. The plastic content found in this study can therefore be assessed as a "snapshot" of what they have recently eaten.

Seif et al., categorised herring gulls from Canadian waters as species with only single reports of ingested plastic, similar to glaucous gulls and kittiwakes and opposite to species with high incidences of reported plastic ingested as Northern Fulmars and Common murre [10]. O'Hanlon reported herring gull as belonging to seabirds species with currently no reports of ingested plastic from Norwegian coasts [11].

The considerable concentrations of S/MCCPs in herring gull liver found in this study, are most probably caused by exposure via prey and bioaccumulation through the food chain. The absence of other additives in herring gull liver can be attributed to either fast degradation/metabolism of these chemicals in nature (they are designed to be chemical reactive) and/or the little plastic found ingested by herring gulls due to their regurgitating habits. Earlier studies showed no or only few detections of UV-filters, OPFRs and phenolic compounds in seabirds and the marine environment [12]. When reported in seabirds, egg samples were investigated rather than liver. For phthalates, earlier reporting shows some detection in herring gull eggs from Røst and Sklinna, varying between 6.9 and 23.7 ng/g ww while, opposite to our findings, no dechloranes were detected in the eggs [13]. Both dechlorane 602, dechlorane plus syn and dechlorane plus anti were detected in all liver samples analysed with concentrations up to 0.69, 0.34 and 1.23 ng/g ww, respectively.

In general, the results from chemical analysis of additives used in plastic do not indicate a relationship between gastric contents (plastic occurrence in the stomach) and additive concentration in the liver of herring gulls. However, only limited incidences of ingested plastic were found.

7 References

1. Hahladakis, J.N., et al., *An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling.* J Hazard Mater, 2018. **344**: p. 179-199.
2. Hermabessiere, L., et al., *Occurrence and effects of plastic additives on marine environments and organisms: A review.* Chemosphere, 2017. **182**: p. 781-793.
3. Trevail, A.M., et al., *Elevated levels of ingested plastic in a high Arctic seabird, the northern fulmar (Fulmarus glacialis).* Polar Biology, 2015. **38**(7): p. 975-981.
4. Herzke, D., et al., *Negligible Impact of Ingested Microplastics on Tissue Concentrations of Persistent Organic Pollutants in Northern Fulmars off Coastal Norway.* Environ Sci Technol, 2016. **50**(4): p. 1924-33.
5. Ask, A.A.-N., Tycho; Herzke, Dorte; Trevail, Alice; van Franeker, Jan Andries; Gabrielsen, Geir Wing, *Contaminants in northern fulmars (Fulmarus glacialis) exposed to plastic*, Nordic Council of Ministers. 2016.
6. van Franeker, J.A., et al., *Monitoring plastic ingestion by the northern fulmar Fulmarus glacialis in the North Sea.* Environmental Pollution, 2011. **159**(10): p. 2609-2615.

7 Herzke, D.; Kaasa, H.; Gravem, F.; Gregersen, H.; Jensen, J.G.B.; Hveding, Ø.P., et al. *Perfluorinated alkylated substances, brominated flame retardants and chlorinated paraffins in the Norwegian Environment* - Screening 2013. M-40-2013. 2013.

8 Miljødirektoratet. *Grenseverdier for klassifisering av vann, sediment og biota.* M-608 2016.

9 Green, N.; Schøyen, M.; Hjermann, d. Ø.; Øxnevad, S.; Ruus, R.; Lusher, A.; Beylich, B.; Lund,E.; Tveiten,L.; Håvardstun,J.; Jenssen, M. T. S.; Ribeiro, A. L.; Bæk, K. *Contaminants in coastal waters of Norway 2017*. M-1120 2018

10 Seif, S., Provencher, J. F., Avery-Gomm, S., Daoust, P. Y., Mallory, M. L., & Smith, P. A. *Plastic and non-plastic debris ingestion in three gull species feeding in an urban landfill environment.* Archives of environmental contamination and toxicology, 2018. **74**(3), p. 349-360.

11 O'Hanlon, N. J., James, N. A., Masden, E. A., & Bond, A. L. *Seabirds and marine plastic debris in the northeastern Atlantic: A synthesis and recommendations for monitoring and research*. Environmental Pollution, 2017. **231** p.1291-1301.

12 Lucia, M., Gabrielsen, G. W., Herzke, D., & Christensen, G. *Screening of UV chemicals, bisphenols and siloxanes in the Arctic.* 2016. Brief Report no. 039 Norwegian Polar Institute. M-598|2016.

13 Huber, S., Nygård, T., Warner, N. A., Remberger, M., Harju, M., Uggerud, H. T., ... & Hanssen, L. *Kartlegging av miljøgifter i sjøfuglegg fra Sklinna og Røst.* 2014. NILU OR 8/2014 (NILU Oppdragsrapport).

NILU

NILU - Norwegian Institute for Air Research is an independent foundation established in 1969. The purpose of NILU's research is to increase understanding of processes and effects associated with climate change, the composition of the atmosphere, air quality and environmental toxins. Based on the research, NILU delivers integrated services and products within analysis, monitoring and consulting. NILU is committed to informing and advising the community on climate change and pollution and its consequences.

NILU's values: Integrity - Expertise - Social benefit
NILU's vision: Research for a clean atmosphere

NILU – Norsk institutt for luftforskning
Postboks 100, 2027 KJELLER

E-post: nilu@nilu.no
http://www.nilu.no

ISBN: 978-82-425-2962-6
ISSN: 2464-3327

Norsk institutt for luftforskning
Norwegian Institute for Air Research