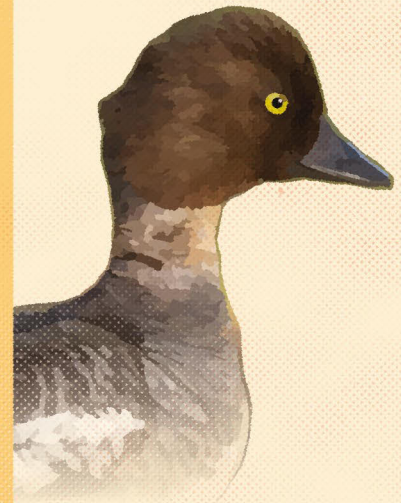
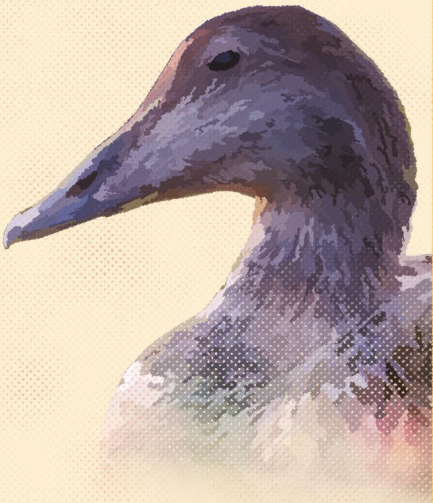




**TURUN
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CONTAMINANTS OF EMERGING CONCERN IN FINNISH WATERBIRDS

Amalie Vigdel Ask



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ABSTRACT

Chemical pollution of the environment is widespread with detrimental effects on wildlife. Currently, more than 350 000 different chemicals or mixtures thereof are registered on the global market and the chemical industry is expected to continue its growth. This necessitates an expansion of which chemicals wildlife toxicology studies target. In this thesis, I examine the occurrence and concentrations of 73 contaminants in waterbirds. In **Chapter I**, I investigate whether classes of common environmental pollutants, viz. bisphenols, benzophenones, phthalate metabolites, benzotriazoles, benzothiazoles, parabens, triclosan, and triclocarban are present in the plasma of breeding female common eiders (*Somateria mollissima*) from a colony in the Archipelago Sea, Finland. I detected 21 of the 58 contaminants targeted, with bisphenol A and benzophenone-3 being dominant in terms of concentrations and detection frequencies. I found evidence of late-breeding females having higher concentrations of some contaminants compared to early-breeding females. In **Chapter II**, I continued my investigation of the aforementioned contaminant families in the eider by collecting whole clutches and plasma samples from the mother. I found no evidence of the egg laying order affecting the concentrations of contaminants in the eggs. While 9 contaminants were detected in both eggs and plasma, 8 were detected in only eggs and 4 only in plasma. This, combined with overall higher detection frequencies of many of the contaminants in eggs compared to plasma, suggests that eggs may be a more suitable matrix for monitoring the targeted contaminants in eiders. Finally, in **Chapter III**, I examined the occurrence of neonicotinoids in plasma samples from female common goldeneyes (*Bucephala clangula*) breeding across Finland. Interestingly, I did not detect any of the seven targeted neonicotinoids—nor their transformation products—in any of the sampled goldeneyes. While the limits of detection (LOD) may have been too high to detect low levels of neonicotinoids in the females, the LODs were sufficiently low to assess whether neonicotinoid exposure poses a major risk to the goldeneyes. This thesis reveals that waterfowl, as exemplified by the eider, are exposed to a cocktail of contaminants, many of which are severely understudied and more scientific attention should be given to expanding our knowledge of the behaviour and fate of these contaminants in wildlife and in the environment.

KEYWORDS: personal care products, plasticizers, insecticides, industrial chemicals, ducks, wildlife toxicology

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Matemaattis-luonnontieteellinen tiedekunta

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Biologia

AMALIE V. ASK: Huolta aiheuttavat uudet haitta-aineet Suomen

vesilinnuissa

Väitöskirja, 131 s.

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TIIVISTELMÄ

Ympäristön kemikalisoituminen on laaja ongelma, joka vaikuttaa haitallisesti eliöihin. Tällä hetkellä markkinoilla on yli 350 000 rekisteröityä kemikaalia tai kemiallista seosta, ja kemianteollisuuden odotetaan edelleen kasvavan kansainvälisesti. Tämä tekee tarpeelliseksi laajentaa myös ympäristötoksikologiassa tutkittavien kemikaalien määrää. Tässä väitöskirjassa tutkin 73 haitta-aineen esiintyvyyttä ja pitoisuuksia vesilinnuissa. Luvussa I tutkin ympäristössä yleisesti esiintyvien haitallisten aineiden esiintymistä Saaristomerellä pesivien naarashaahkojen (*Somateria mollissima*) veri-plasmassa. Tutkittavat aineryhmät ja aineet olivat bisfenolit, bentsofenolit, ftalaattien metaboliitit, bentsotriatsolit, bentsotiatsolit, parabeenit, triklosaani ja triklokarbaani. Tutkitusta 58:sta haitta-aineesta haahkoissa esiintyi 21, joista bisfenoli-A:n ja bentsofenoli-3:n pitoisuudet ja esiintyvyys olivat suurimmat. Joidenkin tutkittujen aineiden pitoisuudet vaikuttivat olevan suurempia myöhään pesivissä haahkanaaraisissa verrattuna aikaisemmin pesinnän aloittaviin naaraisiin. Luvussa II jatkoin edellä mainittujen haitta-aineryhmien tutkimusta haahkoissa keräämällä kokonaisia pesällisiä munia sekä plasmanäytteet ne munineilta emoilta. En löytänyt viitteitä siitä, että munimisjärjestys vaikuttaisi munien haitta-ainepitoisuuksiin. Havaittiin yhdeksää tutkituista aineista sekä munissa että plasmassa, kahdeksaa ainetta vain munissa, ja neljää vain plasmassa. Koska useita tutkittuja aineita esiintyi useammin munissa kuin plasmassa, vaikuttaa että munat soveltuvat plasmaa paremmin tutkittujen haitta-aineiden seurantaan haahkoissa. Luvussa III tutkin neonikotinoidien esiintymistä eri puolilla Suomea pesivien naarastelkkien (*Bucephala clangula*) plasmassa. En havainnut yhtään tutkituista seitsemästä neonikotinoidista tai niiden trans-formaatiotuotteista telkissä. Vaikka aineiden toteamisrajat olivat mahdollisesti liian korkeita matalien neonikotinoidipitoisuuksien havaitsemiseen, toteamisrajat olivat tarpeeksi alhaiset neonikotinoidien telkille aiheuttaman terveysriskin arviointia varten. Väitöskirjani osoittaa, että vesilinnut, esimerkkinä haahka, altistuvat samanaikaisesti usealle haitta-aineelle, joista usean haittavaikutuksia ei ole tutkittu tarpeeksi. Tulevaisuudessa tarvitaankin lisää tutkimusta näiden haitta-aineiden käyttäytymisestä ja vaikutuksesta eliöissä ja ympäristössä.

ASIASANAT: hygieniatuotteet, muovinpehmentimet, hyönteismyrkyt, teollisuus-kemikaalit, sorsat, ekotoksikologia, ympäristötoksikologia

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Abbreviations

1H-BTH	1H-benzothiazole
1H-BTR	1H-benzotriazole
2-OH-BTH	2-hydroxy-benzothiazole
2-S-BTH	2-mercaptobenzothiazole
2-SCNMeS-BTH	2-thiocyanomethylthio-benzothiazole
BPA	Bisphenol A
BPS	Bisphenol S
BzP	Benzophenone
BzP-2	Benzophenone-2
BzP-3	Benzophenone-3, also oxybenzone
CEC	Contaminant of emerging concern
DDT	Dichlorodiphenyltrichloroethane
K _{ow}	Octanol/water partition coefficient
LOD	Limit of detection
MetP	Methyl paraben
mBP	Monobutyl phthalate
mEP	Monoethyl phthalate
mIBP	Monoisobutyl phthalate
mMP	Monomethyl phthalate
mHxP	Mono-n-hexyl phthalate
MTF	Maternal transfer factor
NTNU	Norwegian University of Science and Technology
PrP	Propyl paraben
T1	Sample at early incubation
T2	Sample at late incubation
TTR	Tolyltriazole
UV	Ultraviolet

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

- I** Ask, A. V., Jaspers, V. L. B., Zhang, J., Asimakopoulos, A. G., Frøyland, S. H., Jolkkonen, J., Prian, W. Z., Wilson, N. M., Sonne, C., Hansen, M., Öst, M., Koivisto, S., Eeva, T., Vakili F. S., and Arzel, C. Contaminants of emerging concern in an endangered population of common eiders (*Somateria mollissima*) in the Baltic Sea. *Environmental Pollution*, 2025; 365: 125409. <https://doi.org/10.1016/j.envpol.2024.125409>
- II** Ask, A. V., Zhang, J., Devineau, O., Asimakopoulos, A. G., Frøyland, S. H., Jolkkonen, J., Wilson, N. M., Noreikiene, K., Prian, W. Z., Hansen, M., Ruuskanen, S., Koivisto, S., Sonne, C., Jaspers, V. L. B., and Arzel, C. Contaminants of emerging concern in whole clutches and plasma of the common eider (*Somateria mollissima*): implications for monitoring programs. Unpublished manuscript.
- III** Ask, A. V., Gómez-Ramírez, P., Jaspers, V. L. B., Fenoll, J., Cava, J., Vakili, F. S., Lemesle, P., Eeva, T., Davranche, A., Koivisto, S., Hansen, M., and Arzel, C. Pilot study on neonicotinoids in Finnish waterbirds: no detectable concentrations in common goldeneye (*Bucephala clangula*) plasma. *Environmental Science and Pollution Research*, 2024; 31: 61950-61958. <https://doi.org/10.1007/s11356-024-35197-3>

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1 Introduction

Wildlife faces many threats such as habitat destruction, climate change, invasive species, and chemical pollution (Tilman et al., 2017). History has several examples of chemical pollution causing widespread population declines: of birds of prey and Baltic Sea seals by the insecticide dichlorodiphenyltrichloroethane (DDT) and other organochlorinated contaminants (Helle et al., 1976; Opdam et al., 1987; Porter and Wiemeyer, 1969; Ratcliffe, 1967), of marine invertebrates due to antifouling paint containing tributyltin (Bryan et al., 1986; Oehlmann et al., 1996), and of vulture populations on the Indian subcontinent from feeding on diclofenac-contaminated cattle (Green et al., 2004; Oaks et al., 2004; Prakash et al., 2012), to name a few. Given the estimated 350 000 chemicals and mixtures registered for use and the expected growth of the chemical industry (UNEP, 2019; Wang et al., 2020), environmental contamination continues to be a pressing issue.

Contaminants are chemically diverse with different physicochemical properties (Borrel et al., 2023; Samanipour et al., 2024), and while scientific and regulatory concerns predominantly focus on environmentally persistent chemicals, many chemicals are produced in high volumes with usage patterns that result in more or less continuous release into the environment. This gives these contaminants a *pseudo-persistence* (Mackay et al., 2014) and, yet, for many there is little knowledge regarding their occurrence and concentration in wildlife.

In this thesis I have focused on eight contaminant families for which there are knowledge gaps regarding occurrence in wildlife but which are frequently detected in the aquatic environment. Collectively, I refer to the chemicals I targeted as contaminants of emerging concern (CECs). There is no consensus on the definition of the term CEC (Nilsen et al., 2019; Sauv e and Desrosiers, 2014) and it is inherently a subjective term (Field et al., 2006). My use of the term is relational and collective: to distinguish the contaminants I targeted from the so-called legacy contaminants for which there is a large body of knowledge within wildlife toxicology as well as to have an encompassing term for my targeted contaminants.

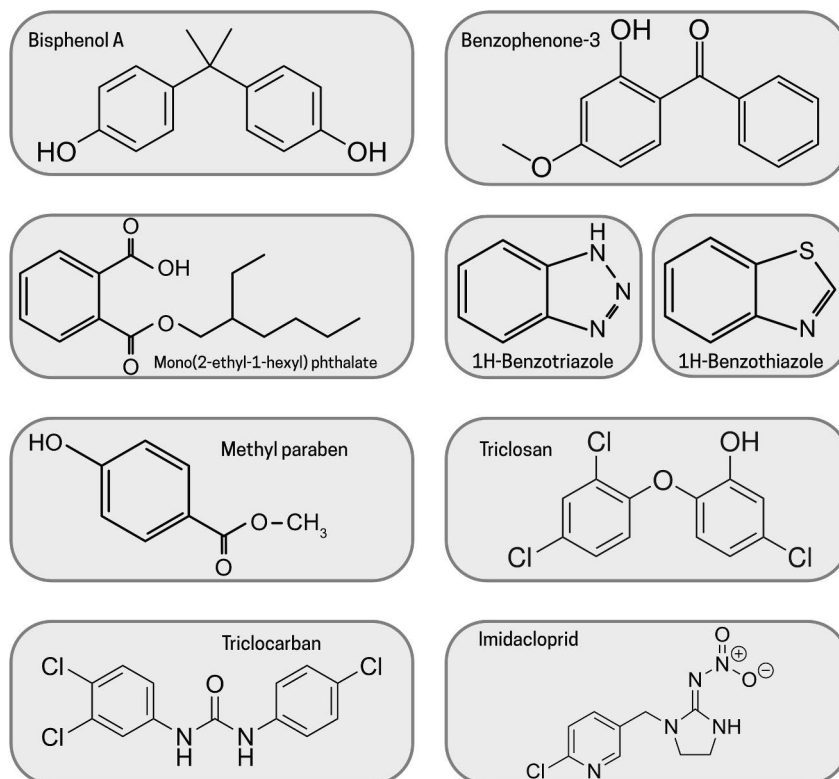


Figure 1. Chemical structures of representative compounds from each of the families of contaminants of emerging concern I targeted in this thesis.

1.1 Contaminants of Emerging Concern

1.1.1 Bisphenols

Bisphenol A (BPA; Figure 1) is a high production volume chemical used in the manufacturing of epoxy resins, polycarbonate plastics, and thermal paper (Chen et al., 2016; Geens et al., 2012; Pan et al., 2024). Scientific and public concerns over BPA's endocrine activity led to its usage being restricted (European Union, 2011) and BPA analogues are being used as substitutes. BPA and its analogues are collectively referred to as bisphenols. There are 16 known BPA analogues used in industry (Chen et al., 2016).

Bisphenols enter the environment through multiple routes with some important sources being industrial and municipal wastewater, urban rainfall runoff, and leachate from landfills (Huang et al., 2021, 2020; Xue and Kannan, 2019; Yamamoto et al., 2001). Bisphenols are ubiquitous in the aquatic environment where they may present a risk to wildlife (reviewed by Chen et al., 2016; Im and Löffler, 2016; Liu et al., 2021). Indeed, bisphenols have been detected in invertebrates (Staniszewska et al., 2014; Wang et al., 2017; Zhao et al., 2021), fish (Wang et al., 2017; Zhao et al., 2021), and birds (Elliott et al., 2019; Gewurtz et al., 2021; González-Rubio et al., 2020; Heimstad et al., 2018; Lucia et al., 2016; Nehring et al., 2017; Ruus et al., 2018; Staniszewska et al., 2014).

There is a lack of studies on the effects of bisphenols in wildlife. Still, BPA's endocrine disrupting properties and effects on reproduction are well-documented (e.g. Oehlmann et al., 2009; Santoro et al., 2019), and the analogues replacing BPA are also associated with a range of effects on, for example, the endocrine system (Liu et al., 2021; Rochester and Bolden, 2015), reproduction (Shi et al., 2015; Siracusa et al., 2018), maternal behaviour (Catanese and Vandenberg, 2017), and development (Berg et al., 2001; Kinch et al., 2015; Moreman et al., 2017).

1.1.2 Benzophenones

Benzophenone (BzP) and its derivatives are organic chemicals which absorb ultraviolet (UV) radiation and are commonly used as UV filters in sunscreens (Li and Kannan, 2022; Narla and Lim, 2020). Benzophenone-3 (BzP-3; Figure 1) is the main BzP-type UV filter and it is also used as a photostabilizer in products such as plastics, inks, and textiles (ECHA, 2022; Li and Kannan, 2022; J. Xue et al., 2017).

Wastewater treatment plant effluents and direct release through recreational activities are the major sources of benzophenones to the aquatic environment (Cuderman and Heath, 2007; Kim et al., 2017; Mao et al., 2019). Accordingly, benzophenones have been detected in a variety of free-ranging biota such as aquatic invertebrates (Emnet et al., 2020; Langford et al., 2015; Sang and Leung, 2016), fish (Balmer et al., 2005; Gago-Ferrero et al., 2015; Langford et al., 2015; Molins-Delgado et al., 2018; Sang and Leung, 2016), corals (Mitchellmore et al., 2019; Tsui et al., 2017), mammals (Heimstad et al., 2018), and birds (González-Rubio et al., 2020; Molins-Delgado et al., 2017; Oró-Nolla et al., 2021).

In vitro and experimental studies, primarily on fish, have revealed that benzophenones have sex and thyroid hormone disrupting properties (Kunz and Fent, 2006; Lee et al., 2018; Molina-Molina et al., 2008; Schlumpf et al., 2004; Schmutzler et al., 2007). Other experimental studies on fish report links between BzP-3 and BzP-2 and effects on reproduction, gonadal development, and the phenotypic sex ratio

(Coronado et al., 2008; Fent et al., 2008; Kim et al., 2014; Kinnberg et al., 2015; Weisbrod et al., 2007).

1.1.3 Phthalate Metabolites

Phthalates, or phthalic acid esters, are the most commonly used plasticizers globally (European Plasticisers, 2021) but are also found in personal care products, cosmetics, and household cleaning products (Cacho et al., 2015; Hubinger and Havery, 2006; Schettler, 2006).

Phthalates are widespread in the environment (Net et al., 2015); indeed, they are so ubiquitous that contamination of samples by sampling and laboratory equipment is an issue (Ikonomou et al., 2012). Thus, the metabolites formed after phthalates are absorbed by an organism are often targeted in the chemical analyses instead of the parent phthalates (Asimakopoulos et al., 2016). Phthalates and phthalate metabolites have been found in a range of wildlife species, including invertebrates (Adeogun et al., 2015; Blair et al., 2009; Hu et al., 2016), fish (Adeogun et al., 2015; Blair et al., 2009; Fossi et al., 2014; Fourgous et al., 2016; Hu et al., 2016; Martins et al., 2016; Molbert et al., 2021; Ros et al., 2016, 2015), reptiles (Brock et al., 2016), marine mammals (Baini et al., 2017; Fossi et al., 2014; Hart et al., 2018; Rian et al., 2020; Routti et al., 2021; Xie et al., 2023), and birds (Allen et al., 2021; Elliott et al., 2019; Hardesty et al., 2015; Huber et al., 2015; Mackintosh et al., 2004; Padula et al., 2020; Sühling et al., 2022).

Phthalates and phthalate metabolites have endocrine disrupting properties, embryo toxicity, and affect development and reproduction (Mankidy et al., 2013; Oehlmann et al., 2009; Sohn et al., 2016; Xu et al., 2022). Many studies on effects of phthalates have been done using *in vitro* techniques or with laboratory animals, but there are some studies finding associations between phthalates and phthalate metabolites and biological effects in wildlife, such as oxidative stress in bird eggs (Allen et al., 2021), altered expression of immune system related genes in a salmonid (Martins et al., 2016), and lipid metabolism in a cetacean (Xie et al., 2023).

1.1.4 Benzotriazoles

1H-benzotriazole (1H-BTR; Figure 1) and its derivatives are corrosion inhibitors and high production volume chemicals found in aircraft deicing fluids, dishwasher detergents, textiles, and hydraulic fluids (Janna et al., 2011; Liu et al., 2017; Pillard et al., 2001). Benzotriazole derivatives containing a phenyl group are used as UV stabilizers and are outside the scope of this thesis.

Wastewater treatment plants are one of the main sources of benzotriazoles to the environment (Giger et al., 2006; Shi et al., 2019) and benzotriazoles have been found

in groundwater, rivers, and seawater (Giger et al., 2006; Loos et al., 2010; Nödler et al., 2014). Indeed, 1H-BTR was one of the compounds found in highest concentrations in a monitoring study of polar organic pollutants in the Baltic Sea (Fisch et al., 2021).

Despite benzotriazoles being widespread in the environment and having been detected in human urine (Asimakopoulos et al., 2013), there are very few studies on their occurrence in wildlife: Gkotsis et al. (2023) found tolyltriazole (TTR) in one Eurasian curlew (*Numenius arquata*) egg while Schlabach et al. (2019) did not detect any benzotriazoles in herring gull (*Larus argentatus*) eggs. Apart from these investigations, to the best of my knowledge, these contaminants have only been detected in marine invertebrates and freshwater fish in China (Jia et al., 2019; Yao et al., 2018a, 2018b, 2016).

While benzotriazoles have low acute toxicity to aquatic organisms (Seeland et al., 2012), experimental studies focusing on sublethal and chronic effects in invertebrates and fish have reported associations between benzotriazole exposure and effects on reproduction (Seeland et al., 2012), the endocrine system (Fent et al., 2014; Giraud et al., 2017; Liang et al., 2014; Tangtian et al., 2012), embryo development (Kadar et al., 2010), hepatotoxicity (Liang et al., 2017, 2014), and the brain proteome indicating potential neurotoxicity (Liang et al., 2016).

1.1.5 Benzothiazoles

1H-benzothiazole (1H-BTH; Figure 1) and its derivatives are high production volume chemicals with many industrial applications including vulcanizing accelerators in rubber production, corrosion inhibitors, and fungicides in the tanning and timber industries (Brownlee et al., 1992; Milanova et al., 2001; Reddy and Quinn, 1997; Reemtsma et al., 1995).

Industrial and municipal wastewater as well as road surface runoff are the main sources of benzothiazoles to the environment (Asheim et al., 2019; Kloepfer et al., 2005; Ni et al., 2008; Zhang et al., 2023). Benzothiazoles have been detected in rivers, marine sediment, and seawater (Fries et al., 2011; Hidalgo-Serrano et al., 2019; Zhao et al., 2023).

As with benzotriazoles, despite benzothiazoles being ubiquitous chemicals and detected in humans (Asimakopoulos et al., 2013; Wang et al., 2015) there is a lack of knowledge regarding occurrence and concentrations in free-ranging biota. Jia et al. (2019) found benzothiazoles in marine invertebrates from China and other studies have targeted benzothiazoles in seafood purchased from markets, where 1H-BTH and four of its derivatives were detected (Castro et al., 2023a, 2023b, 2023c; Chen et al., 2020; Trabalón et al., 2017). Further, a non-target analysis of common

guillemot (*Uria aalge*) eggs from the Baltic Sea tentatively identified 2-mercaptobenzothiazole (2-S-BTH) in the eggs (Rebryk et al., 2022).

As mentioned above, some benzothiazoles are produced specifically for their toxicity (e.g. the fungicide 2-thiocyanomethylthio-benzothiazole [2-SCNMeS-BTH]) and many of the benzothiazoles possess biological activity. Indeed, *in vitro*, animal experimental, and human studies have linked benzothiazoles to effects on development (Evans et al., 2000; Korhonen et al., 1983), thyroid disruption (Hornung et al., 2015; Tietge et al., 2013), cytotoxicity (Evans et al., 2000; Zeng et al., 2016a), oxidative stress and DNA damage (Zeng et al., 2016b), and cancer (Sorahan, 2009).

1.1.6 Parabens

Parabens—esters of 4-hydroxybenzoic acid—possess antimicrobial activity and are used as preservatives in personal care products, pharmaceuticals, and food (Wei et al., 2021). Methyl paraben (MetP; Figure 1) and propyl paraben (PrP) are the most frequently used parabens (Soni et al., 2005; Wei et al., 2021).

While some parabens are synthesized by bacteria (Peng et al., 2006; Quévrain et al., 2009), their anthropogenic production likely overshadows the natural synthesis and wastewater has been identified as a major source of parabens to the environment (Haman et al., 2015). Parabens have been detected in water, sediment, and soil (Feng et al., 2019; Núñez et al., 2008). As a result of their widespread presence in the environment, wildlife is exposed to them as seen in aquatic invertebrates (Emnet et al., 2020; García-Fernández et al., 2022), fish (Peng et al., 2018; Xue and Kannan, 2016; X. Xue et al., 2017; Yao et al., 2016), mammals (Xue et al., 2015; Xue and Kannan, 2016), and birds (Elliott et al., 2019; Xue and Kannan, 2016).

Parabens interfere with the endocrine system (Nowak et al., 2018), for example, they show estrogenic and anti-androgenic activity *in vitro* and exposure to PrP was associated with decreased sperm production and testosterone in rats (Byford et al., 2002; Chen et al., 2007; Oishi, 2002).

1.1.7 Triclosan and Triclocarban

Triclosan and triclocarban (Figure 1) are also antimicrobial chemicals and have been extensively used in personal care products and household products (Adolfsson-Erici et al., 2002; Chalew and Halden, 2009). They are found in the environment (Kobusińska et al., 2018; Xie et al., 2022) and given that triclosan and triclocarban have been produced since 1957 and 1964, respectively (Halden, 2014), there are surprisingly few studies on their occurrence in wildlife. Still, they have been detected in marine invertebrates (García-Fernández et al., 2022; Lu et al., 2019), fish (Das

Sarkar et al., 2020), mammals (Fair et al., 2009), and birds (Morrow et al., 2015; Sherburne et al., 2016).

Triclosan and triclocarban are endocrine disrupting chemicals and *in vitro* and experimental studies on invertebrates, amphibians, and fish have linked them to effects on reproduction and development (Geiß et al., 2016; Gomes et al., 2021; Halden et al., 2017; Veldhoen et al., 2006). Exposure to triclosan *in ovo* was associated with liver oxidative stress, increased mortality, and altered liver transcriptome in bird embryos (Guo et al., 2018; Possenti et al., 2019).

1.1.8 Neonicotinoids

Neonicotinoids are synthetic insecticides which have been used extensively in plant protection products in agriculture, but they are also used in veterinary medicine products and biocides (Jeschke et al., 2011; KemiDigi, 2024a; Perkins et al., 2021). Neonicotinoids are moderately to highly water soluble (Bonmatin et al., 2015) and this, combined with their moderate persistence (Jones et al., 2014), leads to neonicotinoids being widely transported in the environment and they have been found in aquatic environments across the world (Casillas et al., 2022; Mehtonen et al., 2023; Morrissey et al., 2015).

Given the neonicotinoids' high usage in agriculture, much of the scientific literature has focused on their exposure to pollinators and on farmland-associated non-target organisms such as birds. However, as they are mobile in the environment, neonicotinoids have also been detected in wildlife not typically associated with agriculture (e.g. Anderson et al., 2023; Crayton et al., 2020; Distefano et al., 2022).

Neonicotinoids have high toxicity towards insects, targeting the nicotinic acetylcholine receptors, with lower acute toxicity towards fish, mammals, and birds (Tomizawa and Casida, 2005). Nevertheless, neonicotinoid exposure has been linked to adverse effects in vertebrates on reproduction, development, and behaviour (Eng et al., 2019; Gao et al., 2016; Gibbons et al., 2015; Humann-Guillemot et al., 2019; Lopez-Antia et al., 2015; Wang et al., 2016).

1.2 Avian Ecotoxicology

Birds are often used as sentinels of environmental pollution (e.g. Fox, 1993; González-Rubio et al., 2021). A well-known example is the elucidation of the chemical pollution of the North American Great Lakes through meticulous and long-term study of different bird species (e.g. Grasman et al., 1998; Hebert et al., 1999). While the contaminants behind the historic contamination of the Great Lakes are now well-studied and are under regulatory obligations (Stockholm Convention, 2025), for most chemicals—including the CECs introduced above—there are gaps

in our knowledge on their potential occurrence in the environment, exposure risks to birds and wildlife, and possible toxic effects. Given that the CECs are frequently detected in the aquatic environment, I studied these contaminants in two waterbird species, the common eider (*Somateria mollissima*) and the common goldeneye (*Bucephala clangula*).

1.2.1 Common Eider

The common eider (hereafter referred to as eider) is a ground-nesting sea duck (Figure 2A). The female typically lays 4-5 eggs (Waldeck et al., 2004). She begins the approximately 26-day long incubation after laying 2-4 eggs (Hanssen et al., 2002). Only the female incubates the eggs and she fasts during most of the incubation, leading to a mass loss of up to 45% (Criscuolo et al., 2002; Garbus et al., 2018). The eider primarily feeds on blue mussels (*Mytilus edulis*) but is also known to prey on amphipods and other marine invertebrates (Kristjánsson et al., 2013; Öst and Kilpi, 1998). In wintertime the Finnish eider population migrates to the southern Baltic Sea and the North Sea (Laursen et al., 2019). The Baltic Sea eider population is declining and in Europe the eider is listed as endangered (BirdLife International, 2021; Ekroos et al., 2012).

The Baltic Sea is one of the most polluted seas globally (HELCOM, 2023; Kanwischer et al., 2022) and I therefore study the potential occurrence of CECs in eiders breeding in the Finnish part of the Baltic Sea. To the best of my knowledge, the only reports of any of the targeted CECs in eiders are of BPA (Evenset et al., 2009) which was not detected and BzP-3 (Ruus et al., 2023; Schlabach et al., 2018) which was detected in eggs but not in whole blood. Lastly, while not directly targeted in my thesis, parent phthalates have been detected in eider livers and eggs (Evenset et al., 2009; Huber et al., 2015).

1.2.2 Common Goldeneye

The common goldeneye (hereafter referred to as goldeneye) is a cavity-nesting diving duck (Figure 2B). It breeds in tree cavities and nest boxes near lakes, rivers, and the sea shore. The clutch size is usually 7-12 eggs (Milonoff et al., 1998). As with the eider, only the female goldeneyes incubate the eggs. However, unlike the eider, goldeneye females forage daily during their incubation and their diet comprises mostly aquatic invertebrates (Eadie and Keast, 1982; Zicus and Hennes, 1993). Goldeneyes spend the winters out at sea—mainly in the Baltic Sea and North Sea for the Finnish goldeneyes (Scott and Rose, 1996). The European goldeneye population was listed as Least Concern in 2021 but the population trend is decreasing (BirdLife International, 2021).

Neonicotinoids are in use in Finland (KemiDigi, 2024a, 2024b, 2024c) and have been detected in Finnish surface waters (SYKE, 2024). Given the goldeneye's distribution across Finland and close association with surface water, it is a good species with which to assess potential occurrence of neonicotinoids in waterbirds. As far as I know, there are no published studies on neonicotinoids in goldeneyes nor in waterfowl in general, but a recent study found neonicotinoids in seabird species (Distefano et al., 2022).

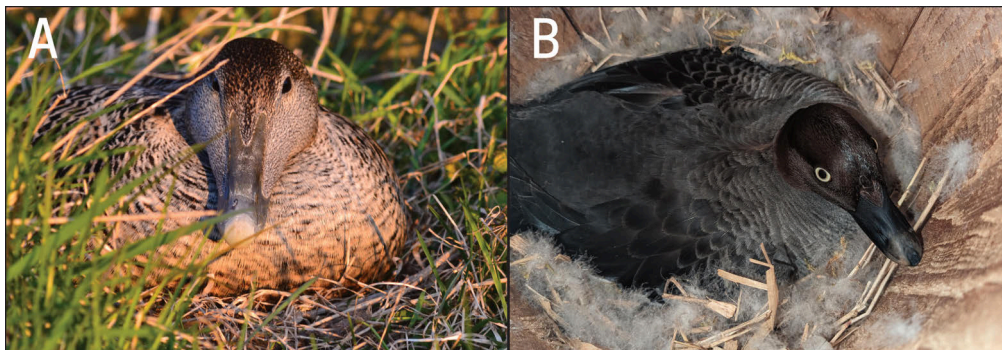


Figure 2. A) Female common eider (*Somateria mollissima*) incubating her brood. Photo by Juho Jolkkonen. B) Female common goldeneye (*Bucephala clangula*) incubating her brood, utilizing a nest box. Photo by Farshad Vakili.

1.3 Aims of the Thesis

The overarching aim of this thesis is to contribute to filling in the knowledge gap regarding the exposure of wildlife to the CECs introduced above. More specifically, in **Chapter I**, I examine whether seven families of contaminants (bisphenols, benzophenones, phthalate metabolites, benzotriazoles, benzothiazoles, parabens, and triclosan and triclocarban) can be found in the plasma of incubating female common eiders before delving deeper to investigate if early and late breeders and/or the incubation period affect the concentrations and profiles. Then, in **Chapter II**, I expand on the work of **Chapter I** by examining i) if these seven contaminant families are detectable in eider eggs and, if so, at which concentrations, ii) whether the egg laying order affects the concentration of the contaminants, and iii) the suitability of eggs and plasma as a matrix for biomonitoring these contaminants. Finally, in **Chapter III**, I investigate—for the first time—whether a waterfowl species, the common goldeneye, is exposed to neonicotinoids.

2 Materials and Methods

2.1 Target Analytes

In total, I targeted 73 chemicals in my thesis—58 in **Chapters I and II** and 15 in **Chapter III**—which are listed in Table 1.

Table 1. The abbreviations (Abbr.), full chemical names, and CAS registry numbers for the contaminants I targeted in my thesis.

CHAPTER	ABBR.	CHEMICAL NAME	CAS RN
BISPHENOLS			
	BPA	Bisphenol A; 2,2-bis(4-hydroxyphenyl) propane	80-05-7
	BPB	Bisphenol B; 2,2-bis(4-hydroxyphenyl) butane	77-40-7
	BPF	Bisphenol F; 4,4'-dihydroxydiphenylmethane	620-92-8
	BPM	Bisphenol M; 1,3-bis(2-(4-hydroxyphenyl)-2-propyl)benzene	13595-25-0
I & II	BPP	Bisphenol P; 4,4'-(1,4-phenylenediisopropylidene)bisphenol	2167-51-3
	BPS	Bisphenol S; 4,4'-sulfonyldiphenol	80-09-1
	BPZ	Bisphenol Z; 4,4'-cyclohexylidenebisphenol	843-55-0
	BPAF	Bisphenol AF; 4,4'-(hexafluoroisopropylidene)diphenol	1478-61-1
	BPAP	Bisphenol AP; 4,4'-(1-phenylethylidene)bisphenol	1571-75-1
BENZOPHENONES			
	4-OH-BzP	4-hydroxybenzophenone	1137-42-4
	BzP-1	Benzophenone-1	131-56-6
I & II	BzP-2	Benzophenone-2	131-55-5
	BzP-3	Benzophenone-3	131-57-7
	BzP-8	Benzophenone-8	131-53-3

PHTHALATE METABOLITES

	mMP	Monomethyl phthalate	4376-18-5
	mEP	Monoethyl phthalate	2306-33-4
	mBP	Mono-n-butyl phthalate	131-70-4
	mIBP	Monoisobutyl phthalate	30833-53-5
	mPeP	Mono-n-pentyl phthalate	24539-56-8
	mIPeP	Monoisopentyl phthalate	17866-76-1
	mHxP	Mono-n-hexyl phthalate	24539-57-9
	mCHP	Monocyclohexyl phthalate	7517-36-4
I & II	mHpP	Mono-n-heptyl phthalate	24539-58-0
	mOP	Mono-n-octyl phthalate	5393-19-1
	mNP	Mono-n-nonyl phthalate	24539-59-1
	mDP	Mono-n-decyl phthalate	24539-60-4
	mBzP	Monobenzyl phthalate	2528-16-7
	mEHP	Mono(2-ethyl-1-hexyl) phthalate	4376-20-9
	mEOHP	Mono(2-ethyl-5-oxohexyl) phthalate	40321-98-0
	mEHHP	Mono(2-ethyl-5-hydroxyhexyl) phthalate	40321-99-1
	PA	Phthalic acid	88-99-3

BENZOTRIAZOLES

	1H-BTR	1H-benzotriazole	95-14-7
	TTR	Tolyltriazole; 4-methyl-1H-benzotriazole	29878-31-7
	5-ABTR	5-amino-1H-benzotriazole	3325-11-9
I & II	1-OH-BTR	1-hydroxy-benzotriazole	2592-95-2
	XTR	Xyliltriazole	4184-79-6
	5-Cl-BTR	5-chloro-1H-benzotriazole	94-97-3
	BTR-COOH	Benzotriazole-5-carboxyl acid	23814-12-2

BENZOTHIAZOLES

	1H-BTH	Benzothiazole	95-16-9
	2-Me-BTH	2-methylbenzothiazole	120-75-2
	2-M-BTH	2-morpholin-4-yl-benzothiazole	4225-26-7
	2-ABTH	2-aminobenzothiazole	136-95-8
I & II	2-OH-BTH	2-hydroxy-benzothiazole	934-34-9
	2-S-BTH	2-mercaptobenzothiazole	149-30-4
	2-Cl-BTH	2-chlorobenzothiazole	615-20-3
	2-Me-S-BTH	2-methylthio-benzothiazole	615-22-5
	2-SCNMeS-BTH	2-thiocyanomethylthio-benzothiazole	21564-17-0

PARABENS			
	4-HB	4-hydroxybenzoic acid	99-96-7
	MetP	Methyl paraben	99-76-3
	3,4-DHB	3,4-dihydroxybenzoic acid	99-50-3
	EtP	Ethyl paraben	120-47-8
I & II	PrP	Propyl paraben	94-13-3
	OH-EtP	Ethyl protocatechuate	3943-89-3
	BuP	Butyl paraben	94-26-8
	BezP	Benzyl paraben	94-18-8
	HeP	Heptyl paraben	1085-12-7
TRICLOSAN & TRICLOCARBAN			
I & II	TCS	Triclosan	3380-34-5
	TCC	Triclocarban	101-20-2
NEONICOTINOIDS			
		Imidacloprid	138261-41-3
		Thiamethoxam	153719-23-4
		Thiacloprid	111988-49-9
		Acetamiprid	160430-64-8
		Clothianidin	210880-92-5
		Dinotefuran	165252-70-0
		Nitenpyram	150824-47-8
III		6-chloronicotinic acid	5326-23-8
		Hydroxy-imidacloprid	380912-09-4
		Imidacloprid-urea	120868-66-8
		Imidacloprid-olefin	115086-54-9
		Thiamethoxam-urea	902493-06-5
		Thiacloprid-amide	676228-91-4
		Acetamiprid-acetate	*
		Acetamiprid-desmethyl	190604-92-3

* No CAS registry number available.

2.2 Chapters I and II

2.2.1 Fieldwork

In 2021 we sampled blood from breeding female eiders at the Bengtskär colony, outer Archipelago Sea, Finland (Figure 3, red circle). We selected this colony as it has a high number of breeding females, unlike nearby eider colonies which have

declining breeding numbers (Hermansson et al., 2023), yet the Bengtskär colony has a high level of hatching failure (unpublished data) which prompted further investigation.

We received approval to handle and sample the eiders by the Animal Experiment Board at the State Provincial Office of Southern Finland (ESAVI/9500/2021) and the Finnish Wildlife Agency (2021-5-000-18962-8).

2.2.1.1 Chapter I

For each female in **Chapter I**, we sampled blood at the start (T1) and end (T2) of her incubation period, as past research on eiders shows that the concentrations of some contaminants are affected by the fasting during incubation (Bustnes et al., 2012; McPartland et al., 2020). Furthermore, in order to investigate if early- and late-breeding females showed different concentrations of contaminants, we sampled females in April-May and May-June.

We caught the female on the nest using a hand net. For all the blood samples, a maximum of 6 mL blood was collected from the brachial vein and transferred to a vacutainer containing heparin. The blood was stored cold and dark until centrifugation. The plasma was transferred to Eppendorf tubes and frozen at -20 °C while on Bengtskär and transferred to a -80 °C freezer upon returning to the University of Turku. We also collected blank samples while in the field (consisting of molecular-grade water), which were subjected to the same materials and procedures as the blood samples. I shipped the plasma and blank samples on dry ice to the Norwegian University of Science and Technology (NTNU) for sample preparation and instrumental analyses.

2.2.1.2 Chapter II

For **Chapter II**, we searched the colony for all nests containing 1-2 eggs and gave these eggs unique IDs using a permanent marker. We continued visiting these nests every day, labelling each new egg with a unique ID. Once the female had ceased egg-laying and towards the end of the first week of incubation, we captured her and took a blood sample according to the description above. On 19 May 2021, we collected all the eggs from the 10 nests with largest clutch sizes ($n = 44$ eggs), wrapped them in bubble-wrap, and froze them at -20 °C while on Bengtskär and transferred them to a -80 °C freezer upon returning to the University of Turku. The plasma samples were sent on dry ice to NTNU for chemical analyses and the eggs were sent on dry ice to Aarhus University, Denmark for homogenization.

2.2.2 Egg Homogenization and Sample Preparation

For the egg samples, while the eggs were still frozen, I used a Dremel tool to cut the eggs open at the equator. I placed the egg contents in a beaker and removed some embryonic material for maternity analysis. After the eggs thawed, I homogenized them using an immersion blender. I made four “homogenization” blanks consisting of ultra-pure water which were subjected to the same materials and procedure as the eggs. The egg homogenates and blanks were shipped on dry ice to NTNU for chemical analyses.

To extract bisphenols, benzophenones, benzotriazoles, benzothiazoles, parabens, triclosan, and triclocarban I weighed 100 mg plasma or egg homogenate into Eppendorf tubes before adding 10 μL of internal standard mix to each tube. I then added 300 μL 1% formic acid in methanol, vortexed and ultrasonicated the tubes. Afterwards, I centrifuged the tubes and transferred the supernatant to hybridSPE cartridges which had been conditioned with 1 mL 1% formic acid in methanol. Finally, I transferred 150 μL of the eluate to glass vials for instrumental analysis.

For the phthalate metabolites, I extracted the plasma and egg homogenate samples according to the method described by Asimakopoulos et al. (2016) and Rian et al. (2020). Briefly, I weighed 100 mg plasma or egg homogenate into Eppendorf tubes before adding 10 μL of internal standard mix and 50 μL 1M ammonium acetate (containing β -glucuronidase). After an overnight incubation at 37 °C and 220 rpm, I added 450 μL 10% formic acid in acetonitrile to the tubes, vortexed and centrifuged them, and transferred the supernatant to hybridSPE cartridges which had been conditioned with 1 mL 10% formic acid in acetonitrile. The eluates were collected in 15 mL polypropylene tubes and I used a gentle stream of nitrogen to remove the solvent, before reconstituting the samples with 500 μL acetonitrile:ultra-pure water. After a final centrifugation step, I transferred the supernatant to a glass vial for instrumental analysis.

2.2.3 Instrumental Analyses

The extracts were analysed using ultra-performance liquid chromatography (UPLC; Waters, Milford, U.S.) coupled to a triple quadrupole mass analyser (Xevo TQ-S, Waters, Milford, U.S.) at the mass spectrometry core facility at NTNU, Trondheim.

2.2.4 Maternity Analysis

In waterfowl species like the eider, conspecific brood parasitism is prevalent, meaning that eider females lay eggs in other females' nests (Waldeck et al., 2004). Thus, to control for this in **Chapter II**, we performed a maternity analysis, based on

microsatellite genotyping, using tissues from the eggs and the blood pellet formed after centrifuging the female eider's blood.

2.2.5 Data Analyses

2.2.5.1 Chapter I

I performed the data analyses using Python (v. 3.9.12, Python Software Foundation) and R (v. 4.1.1, R Core Team, 2024). To investigate whether contaminants differed between early and late incubation and/or between early- and late-breeding females, only contaminants which were above the limit of detection (LOD) in $\geq 60\%$ of the samples in at least one group were included. Observations below LOD were replaced by $1/2\text{LOD}$. Depending on whether or not the assumptions of normality and homoscedasticity were met, the concentration differences between early and late incubation were tested with either the paired t-test or the Wilcoxon signed-rank test, respectively. Similarly, concentration differences between early and late breeders were tested with either the independent t-test or the Mann-Whitney U test. I adjusted the p -values using the Benjamini-Hochberg false discovery rate and I interpreted the outcomes of the statistical tests using the language of evidence, where little or no evidence corresponds to p -values above 0.1, weak evidence to p -values between 0.05-0.1, moderate evidence to p -values between 0.01-0.05, and strong evidence to p -values below 0.01 (Muff et al., 2022).

2.2.5.2 Chapter II

I performed the data analyses using Python (v. 3.9.12, Python Software Foundation) and R (v. 4.1.1, R Core Team, 2024). Observations below the LOD were replaced with $1/2\text{LOD}$. To examine whether the laying order had an effect on the contaminant concentrations, I used an R script developed by Prof. Olivier Devineau (University of Inland Norway). Shortly, the contaminant concentrations were compared with a hierarchical Gamma model with a log link function, fitted in a Bayesian framework. Marginal predictions were obtained from the best-fitting model to examine potential differences in concentrations across the laying order.

I calculated maternal transfer factors (MTFs) to assess maternal transfer efficiencies for compounds detected in $\geq 60\%$ of samples for either egg or plasma. The MTFs were calculated by applying a \log_{10} transformation to the fraction of mean concentration in eggs to mean concentration in plasma. Log ratios > 0 suggest higher maternal transfer efficiency. Lastly, I visually compared detection frequencies in eggs and plasma through a heatmap.

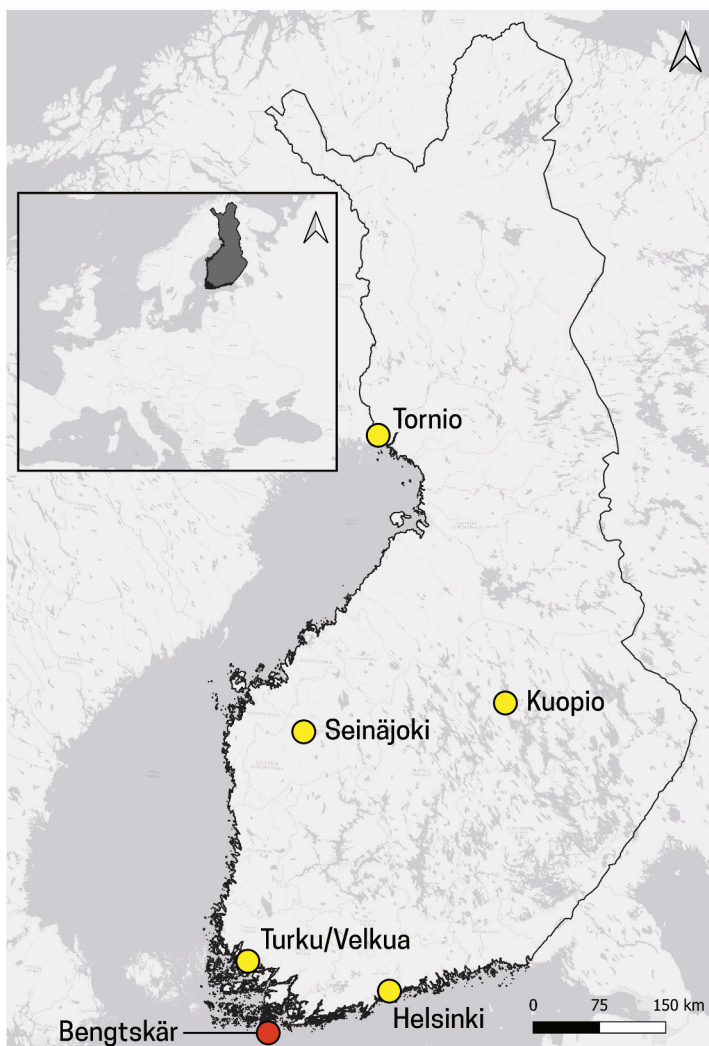


Figure 3. The sampling locations of eiders (in red – Chapters I and II) and goldeneyes (in yellow – Chapter III). The map inset shows Finland’s position within Europe.

2.3 Chapter III

2.3.1 Fieldwork

During the goldeneye breeding season of 2022, we collected blood samples from incubating female goldeneyes from five different regions in Finland: Tornio ($n = 10$), Seinäjoki ($n = 10$), Kuopio ($n = 15$), Helsinki ($n = 10$), and Velkua/Turku ($n = 6$)

(Figure 3, yellow circles). We collaborated with local bird ringers who deploy goldeneye nest boxes. The goldeneye was caught while in the nest box, then we collected a maximum of 4 mL blood from the brachial vein. The blood was transferred to a vacutainer, kept cold and dark until centrifugation later the same day, then the plasma was frozen at -20 °C. Approval for the study was given by the Animal Experiment Board at the State Provincial Office of Southern Finland (ESAVI/9500/2021) and the Finnish Wildlife Agency (2021-5-000-18962-8).

At each of the five regions, we also took field blanks consisting of molecular-grade water. These were subjected to the same steps as the blood samples. Finally, the plasma samples and field blanks were sent to the University of Valencia, Spain on dry ice for sample preparation.

2.3.2 Sample Preparation

I used the method described by Martínez et al. (2022) to extract the plasma and blank samples for the targeted neonicotinoids (Table 1). Shortly, I pipetted 100 µL of plasma into an Eppendorf tube, added 1 mL acetonitrile:water (1:1 v/v), vortexed and placed the tubes into an ultrasound bath. Afterwards, I added 100 mg NaCl to each tube, vortexed them for 5 min, and then centrifuged the tubes before collecting the supernatant in a syringe and filtered it through a 0.2 µm filter into an autosampler vial with insert. The extracts were stored at -80 °C before instrumental analysis.

2.3.3 Instrumental Analysis

The samples were sent to the Instituto Murciano de Investigación y Desarrollo Agrario y Medioambiental in Murcia, Spain for analysis using high pressure liquid chromatography-mass spectrometry.

2.3.4 Data Analysis

None of the target analytes were detected and, accordingly, I did not perform any data analysis.

3 Results and Discussion

3.1 Contaminants in Plasma

In the eider females, I detected 21 out of the 58 targeted contaminants in at least one plasma sample (**Chapters I and II**). Some of these contaminants have never before been reported in a free-ranging bird species, namely the benzothiazoles 2-SCNMeS-BTH and 2-hydroxy-benzothiazole (2-OH-BTH), highlighting the importance of expanding the range of contaminants targeted in wildlife toxicology studies.

In both **Chapters I and II**, when considering all observations, including those below LOD, the contaminants detected at the highest concentrations in the eider plasma were BPA and BzP-3 (median concentration ranges: 7.76-10.8 and 1.43-3.75 ng/g ww, respectively) (Table 2). Similarly, BPA and BzP-3 were detected at the highest frequencies in both **Chapter I and II** (61-78% and 61-90%, respectively). Of the 58 targeted contaminants in these Chapters, BPA was the contaminant released on the Finnish market in the greatest number of products and at the highest volume in 2021 (see Table S10 in Ask et al., 2025). Hence, the prevalence of BPA in the eider is reflected in its production statistics. Interestingly, compared to plasma concentrations of BPA in avian species from North America (Elliott et al., 2019; Gewurtz et al., 2021), the BPA concentration is an order of magnitude higher in the eiders of this thesis. This likely reflects the ongoing use of BPA in Finland as well as the contaminated status of the Baltic Sea. With regards to BzP-3, it was used in 20 products and 0.454 tonnes were placed on the Finnish market in 2021 (Table S10 in Ask et al., 2025). To the best of my knowledge, only one other study has investigated BzP-3 in blood of eiders and it was not detected in any of the three pooled whole blood samples collected from the Oslofjord, Norway (Ruus et al., 2023). Similarly, BzP-3 has not been detected in herring gull (*Larus argentatus*) whole blood samples collected as part of a multi-year monitoring program of the Oslofjord (Grung et al., 2021; Ruus et al., 2023, 2020, 2019, 2018). This may indicate that the Baltic Sea is more heavily contaminated by BzP-3 as I detected the compound in the eiders from Finland. Furthermore, studies of wild birds have found that BzP-3 partitions to tissues (González-Rubio et al., 2020; Oró-Nolla et al., 2021), so another reason could be related to the eiders' fast which may remobilize stored

BzP-3 into the circulation, but this does not explain the differences with the eiders from the Oslofjord.

With respect to contaminants being remobilized during the eiders' fast, one of the aims in **Chapter I** was to investigate whether the concentrations of the targeted contaminants changed between early and late incubation, as has been observed for several persistent organic pollutants and trace elements in eiders (Bustnes et al., 2012; McPartland et al., 2020). Interestingly, I found no strong evidence that the concentrations changed during the incubation period (Figure 4). The studies of eider females which found that contaminant concentrations increased from early to late incubation targeted recalcitrant compounds with considerably longer plasma half-lives compared to the contaminants I targeted. Hence, a possible explanation for not observing strong differences between early and late incubation in **Chapter I** is that as the females lose weight throughout their fast, remobilized contaminants are metabolized and therefore not detected at higher concentrations.

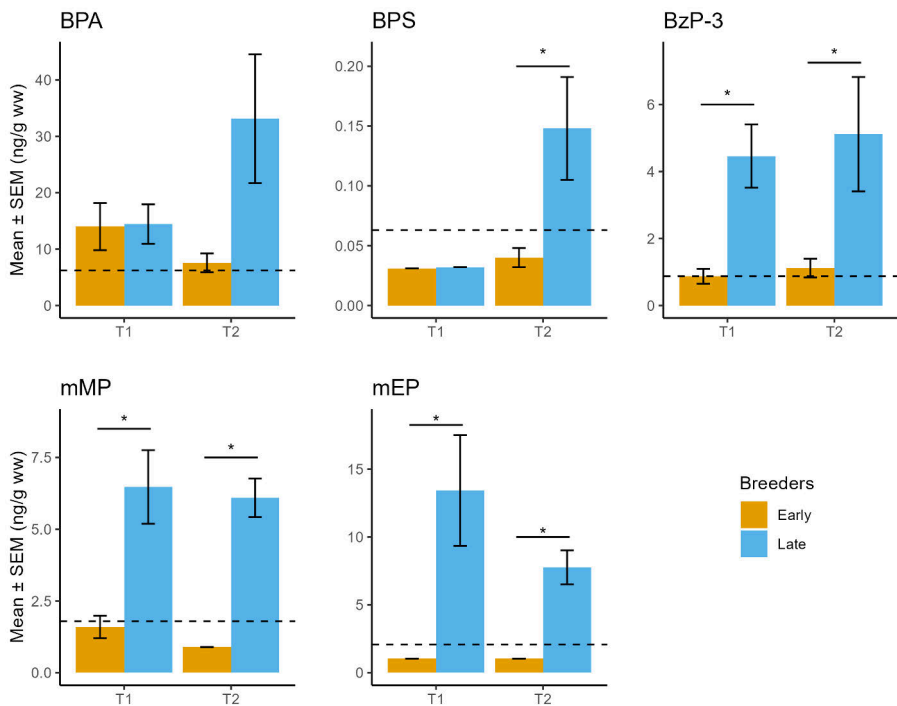


Figure 4. Mean ± standard error of the mean (SEM) concentrations of bisphenol A (BPA), bisphenol S (BPS), benzophenone-3 (BzP-3), monomethyl phthalate (mMP), and monoethyl phthalate (mEP) in plasma of female common eiders, Finland, 2021. Early- and late-breeding females were sampled twice: at the beginning and end of their incubation (T1 and T2, respectively). Sample sizes: early breeders $n = 10$ and late breeders $n = 8$ (except for mMP and mEP where $n = 4$ for the late breeders). The dashed lines indicate the compound-specific limits of detection. The asterisks show when there was moderate or strong evidence of a difference in concentrations.

Another aim in **Chapter I** was to examine whether concentrations differed between early and late breeders. As can be seen in Figure 4 there was moderate to strong evidence that late breeders had higher concentrations of BzP-3, monomethyl phthalate (mMP), and monoethyl phthalate (mEP) in both their first (T1) and second (T2) plasma samples. For the T2 sample only, I found moderate evidence of bisphenol S (BPS) being higher in the late breeders compared to early breeders, but it should be noted that the mean difference was only 0.11 ng/g ww and it is unlikely that this small difference of BPS is biologically meaningful.

At present, it is not clear what might be driving the differences in circulating contaminants between early and late breeders. Early-breeding eiders rely more on nutrients from the wintering areas for financing reproduction compared to late breeders (Jaatinen et al., 2016; Sénéchal et al., 2011) and based on the higher human population density surrounding the watersheds draining into their wintering areas (Bollmann et al., 2019), one might expect higher environmental contamination in the eiders' winter grounds. Yet, in my thesis, it was the late breeders who had higher concentrations of the tested contaminants. Furthermore, past research has found that eiders in poorer condition tend to breed later (Descamps et al., 2011; Jaatinen and Öst, 2016), which may influence circulating contaminants, however, in our study the body condition of early and late breeders was comparable. Thus, more research is needed to elucidate the contaminant exposure and toxicokinetics in the eiders.

In **Chapter III** I investigated the occurrence of neonicotinoids in goldeneyes from five different regions of Finland. Surprisingly, none of the neonicotinoids were above the LOD in any of the plasma samples. Given that neonicotinoids are used in Finland, are mobile and persistent in the environment, and have been detected in honey buzzards (*Pernis apivorus*) in Finland and seabirds elsewhere (Byholm et al., 2018; Distefano et al., 2022; European Food Safety Authority, 2021; KemiDigi, 2024b, 2024a, 2022), I expected to detect neonicotinoids in at least some samples. In particular, I expected to find imidacloprid, thiamethoxam, and acetamiprid in at least the samples from the Helsinki and Seinäjoki regions, due to these neonicotinoids' use cases, the land uses in those regions and/or human population numbers. Indeed, Byholm et al. (2018) detected imidacloprid, as well as thiacloprid, in whole blood from honey buzzards sampled close to where we sampled goldeneyes in Seinäjoki. However, Byholm et al. (2018) sampled the honey buzzards in 2013 which was just before the use of neonicotinoids in the European Union was restricted (Reg. (EU) No 485/2013). Furthermore, honey buzzards feed on larvae of insects which likely were directly exposed to plant protection products containing neonicotinoids. In contrast, the goldeneyes would be exposed through water and/or aquatic invertebrates and dilution of the neonicotinoids in the environment combined with the restrictions discussed above, is probably why I did not detect any in the goldeneyes. Previous research has reported low concentrations of neonicotinoids in

bird plasma and whole blood (Byholm et al., 2018; Hao et al., 2018) and it might be that neonicotinoids were present in the plasma but at too low concentrations to be detected. Nevertheless, the LODs in **Chapter III** were sufficiently sensitive for assessing whether neonicotinoid exposure in the sampled goldeneyes was high and cause for further investigation.

Table 2. Plasma concentrations (ng/g ww) of contaminants of emerging concern in female common eiders (*Somateria mollissima*), Archipelago Sea, Finland, 2021. Detection rate (DR) shows the number of observations above the limit of detection (LOD) out of the total number of plasma samples analyzed for that contaminant. For Chapter I, the females were sampled twice: during early (T1) and late (T2) incubation.

Compound	LOD	Chapter I - T1			Chapter I - T2			Chapter II		
		DR	Median	Max	DR	Median	Max	DR	Median	Max
BPA	6.21	14/18	10.8	46.6	11/18	10.2	87.8	7/10	7.76	110.1
BPS	0.06	0/18			6/18	<0.06	0.36	3/10	<0.06	0.30
BPAF	0.02	2/18	<0.02	0.09	5/18	<0.02	0.06	2/10	<0.02	0.10
BzP-1	0.66	2/18	<0.66	1.24	3/18	<0.66	2.30	0/10		
BzP-3	0.87	11/18	1.81	9.58	12/18	1.43	14.8	9/10	3.75	22.4
mMP	1.79	7/14	<1.79	8.85	4/14	<1.79	7.33	4/8	<1.79	7.91
mEP	2.08	4/14	<2.08	25.2	4/14	<2.08	10.5	4/8	2.34	23.0
mBP	3.61	1/18	<3.61	6.42	0/18			0/10		
mIBP	1.81	1/18	<1.81	1.89	0/18			1/10	<1.81	2.10
mHxP	0.45	0/18			1/18	<0.45	0.73	0/10		
mDP	1.33	3/18	<1.33	6.60	2/18	<1.33	5.35	1/10	<1.33	8.97
mEHP	10.1	4/18	<10.1	52.2	5/18	<10.1	25.0	2/10	<10.1	19.8
TTR	0.61	0/18			1/18	<0.61	0.85	1/10	<0.61	0.86
2-OH-BTH	0.60	1/18	<0.60	1.57	0/18			2/10	<0.60	2.44
2-S-BTH	0.29	1/18	<0.29	0.58	2/18	<0.29	5.96	1/10	<0.29	0.34
2-SCNMeS-BTH	0.05	1/18	<0.05	0.10	0/18			0/10		
MetP	0.21	0/18			2/18	<0.21	0.85	0/10		
EtP	0.03	3/18	<0.03	0.07	1/18	<0.03	0.03	0/10		
PrP	0.01	3/18	<0.01	0.05	3/18	<0.01	0.09	1/10	<0.01	0.06
BuP	0.01	3/18	<0.01	0.02	0/18			0/10		
BezP	0.06	0/18			2/18	<0.06	0.13	0/10		

3.2 Contaminants in Eggs

Of the 44 eggs we collected in **Chapter II**, seven were parasitic, thus “eggs” henceforth refers to the 37 non-parasitic eggs unless specifically stated otherwise. Seventeen contaminants were detected in at least one egg. The three dominant contaminants in terms of concentration and detection frequencies were BzP-1, BPA, and BzP-3 with mean concentrations \pm standard error of the mean (SEM) of 6.46 ± 1.46 , 3.70 ± 0.58 , and 3.56 ± 0.71 ng/g ww in the first-laid eggs and detection frequencies of 100, 95, and 73%, respectively, out of the 37 eggs.

While several other studies on BPA in seabird eggs did not find detectable concentrations (Gewurtz et al., 2021; Ruus et al., 2018; Schlabach et al., 2018), the concentrations in the eider eggs are comparable to the concentrations reported in black-legged kittiwake (*Rissa tridactyla*) eggs (Lucia et al., 2016). Furthermore, the concentration of BzP-3 in the eider eggs in my thesis are similar to concentrations found in eider eggs collected from Svalbard (Schlabach et al., 2018).

For the other contaminants, concentrations and/or detection frequencies were generally low. Nevertheless, of particular note is that I detected the benzotriazole tolyltriazole (TTR) in 23 out of the 44 eggs which is comparatively high as the two other studies targeting TTR in bird eggs report detection frequencies of 4 and 0% (Gkotsis et al., 2023; Schlabach et al., 2019). The higher detection frequency in the eider eggs may be related to the polluted state of the Baltic Sea and/or species differences in absorption, distribution, metabolism, and excretion (including maternal transfer) of TTR.

An important aim in **Chapter II** was to examine whether the laying order of the eggs had an effect on the contaminant concentrations in the eggs. Such an effect has previously been found in other avian species for other contaminants and could have an impact on the results in monitoring programs which often use one randomly collected egg per clutch (Ackerman et al., 2016; Bianchini et al., 2022; Lasters et al., 2019; Van den Steen et al., 2009). Nine contaminants were detected above the LOD for $\geq 60\%$ of the observations per laying order position and were thus included in the model: namely BPA, BzP-1, BzP-3, mMP, monobutyl phthalate (mBP), monoisobutyl phthalate (mIBP), mono-n-hexyl phthalate (mHxP), TTR, and MetP. All the intervals of the marginal predicted concentrations across the egg laying order overlap for those contaminants, thus there is no evidence of the laying order affecting the concentrations (Figure 5). This result is in accordance with studies on mercury and persistent organic pollutants in terrestrial bird species (Brasso et al., 2010; Reynolds et al., 2004; Van den Steen et al., 2006).

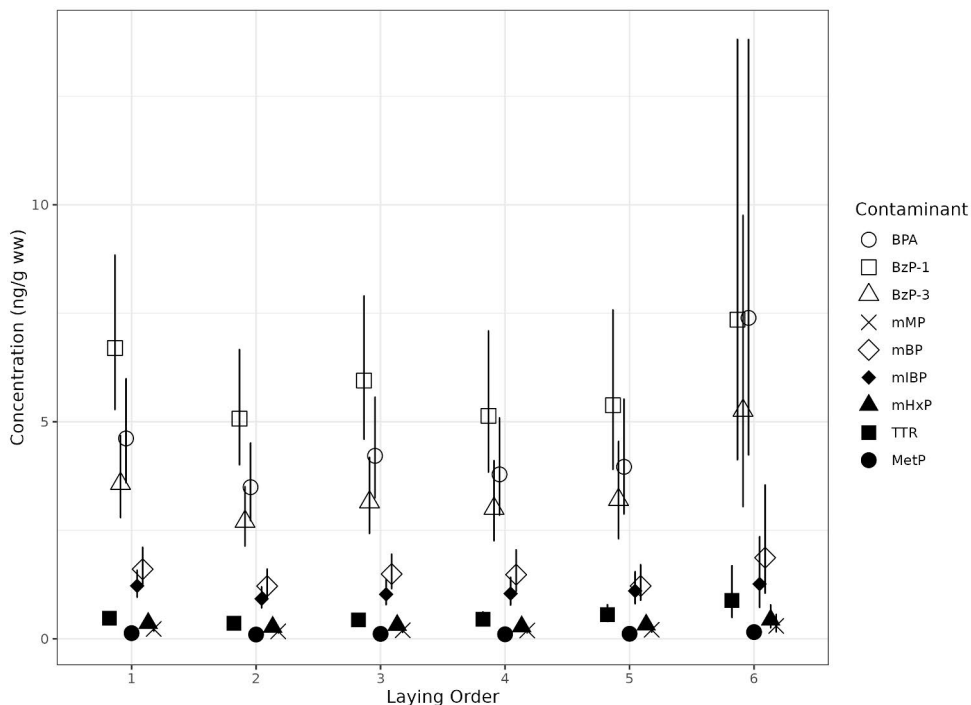


Figure 5. The marginal predicted concentrations (ng/g wet weight) of contaminants across the egg laying order in ten clutches of common eider (*Somateria mollissima*). The data is displayed as medians with 95% credible intervals.

3.3 Comparing Eggs and Plasma

Contaminants are not transferred with equal efficiencies from mother to egg and in **Chapter II** I calculated maternal transfer factors (MTFs) to assess the transfer efficiencies for my targeted contaminants. I calculated the MTFs for contaminants detected in $\geq 60\%$ of observations in either eggs or plasma. BzP-1 had MTFs > 1.0 for all laying order positions, whereas the opposite was seen for BzP-3 and BPA (Figure 6). An MTF greater than zero indicates that the compound is found preferentially in eggs. Thus, BzP-1 seems to be preferentially transferred from the mother to the egg, whereas BzP-3 and BPA were found at higher concentrations in the plasma. mMP had an MTF of -0.93, but this could only be calculated for the 5th laying order position which consisted of only four eggs. The MTFs for the remaining contaminants were closer to zero.

As far as I know, there is only one other study examining any of the targeted contaminants in paired samples of mother and eggs in a wild bird. Ruus et al. (2018) detected BPA in one blood sample from herring gull, but not in any of the eggs. Given the low detection frequency in the herring gull blood samples, it is difficult to

draw any conclusions from this. With regards to BzP-1 and -3, it is interesting that I found MTFs in opposite directions. In addition to being manufactured, BzP-1 is also the major metabolite of BzP-3. A possible explanation for the different MTFs is the lower octanol/water partition coefficient ($\log K_{OW}$) of BzP-1 compared to BzP-3—past research on maternal transfer has found that contaminants with lower $\log K_{OW}$ are transferred more easily to the egg (Li et al., 2021; Verreault et al., 2006).

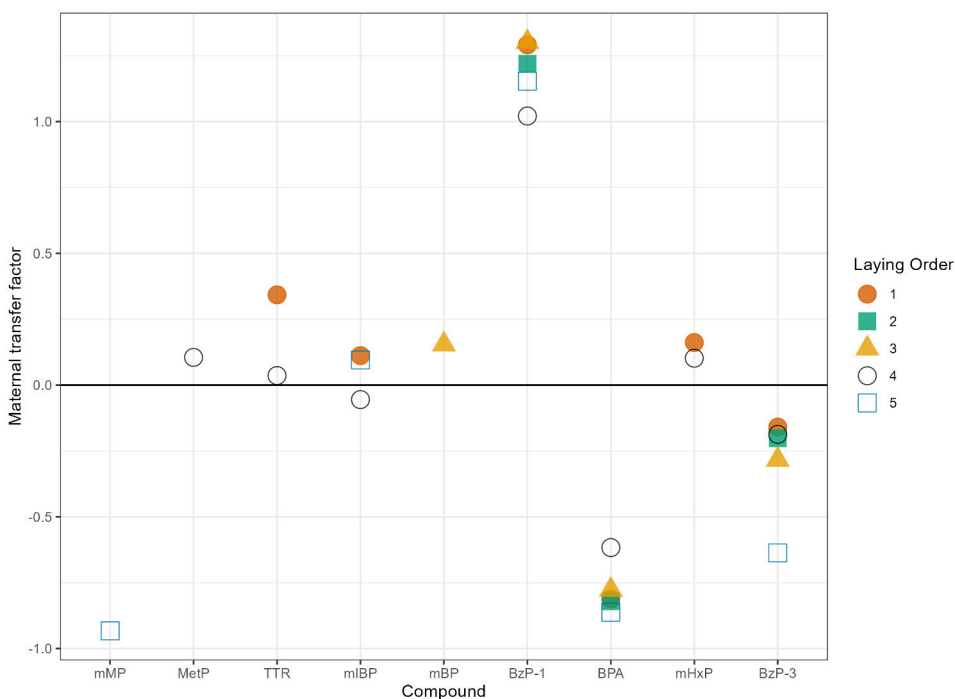


Figure 6. Maternal transfer factors (\log_{10} transformation of the ratio of mean concentration in eggs to mean concentration in plasma) of contaminants of emerging concern in common eider (*Somateria mollissima*), Finland, 2021. The compounds are listed with increasing $\log K_{OW}$ values from left to right.

MTFs are based on the concentrations in eggs and plasma, but it is also informative to consider the detection frequencies in the matrices. In **Chapter II** I found that nine compounds were detected in at least one sample in both matrices, whereas eight compounds were only found in eggs and four compounds were only found in plasma. Further, as can be seen in Figure 7, eggs had overall higher detection frequencies compared to plasma samples.

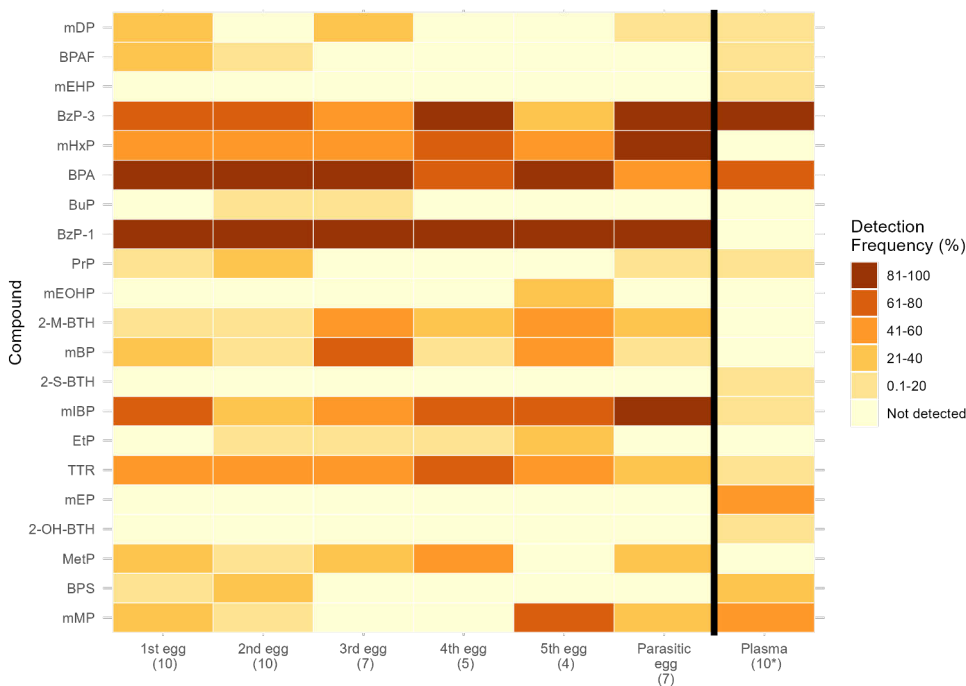


Figure 7. Detection frequencies of contaminants of emerging concern in eggs and plasma of common eider (*Somateria mollissima*), Finland, 2021. The sample sizes of the respective groups are given in parenthesis. The 6th laying order position was excluded as it consists of only one egg. The compounds are listed in ascending order of their log K_{ow} values. *For mMP and mEP in plasma, the sample size is $n = 8$, instead of 10.

3.4 Limitations and Possibilities for Further Studies

In my thesis, plasma was only investigated from breeding females, so the situation for juveniles, non-breeding females, and males is unknown. In the future it would be very interesting to develop a practical and safe method for capturing males to investigate the occurrence and concentrations of the contaminants in them.

For **Chapter I**, simultaneous collection and analyses of the target contaminants in water and the eiders’ prey species would have allowed for a deeper exploration of the eiders’ exposure. Given the considerable financial cost of chemical analysis, we decided to focus only on the eider for this exploratory study and now that I have established that they are exposed to some of the targeted contaminants, follow-up studies should be conducted to elucidate exposure pathways.

My primary aim in **Chapter II** was to investigate whether the egg laying order affected contaminant concentrations in the eggs and the study was designed around that. However, I was also interested in evaluating the utility of plasma and eggs for biomonitoring of my targeted contaminants. Monitoring programs frequently collect

abandoned and unhatched eggs as collecting actively incubated eggs causes a greater disturbance and may—for critically endangered species—have impacts on the population. As far as I can tell, there are no studies comparing my contaminants of interest in actively incubated eggs with abandoned and unhatched eggs. Even so, the concentrations in abandoned and unhatched eggs will likely change due to microbiological degradation as well as the eggs' exposure to temperature fluctuations and sunlight. Thus, it may be that the tentative conclusion of **Chapter II** (i.e. eggs appear to be more suited than plasma for monitoring the targeted contaminants) does not hold when considering unhatched eggs instead. As such, I recommend that a follow-up study on unhatched eggs should be conducted.

A limitation in **Chapter III** is that I did not collect and analyze water and aquatic invertebrates. Thus, while I can say that neonicotinoids are of minor concern to Finnish goldeneyes, I do not know whether this is because neonicotinoid occurrence in the studied regions is low in general or if neonicotinoids in goldeneyes are low due to their prey species being severely affected by the neonicotinoids and consequently may die before a goldeneye ingests it.

A recurring constraint in wildlife toxicological studies is low sample sizes. Especially in **Chapter II** the number of whole clutches we collected was small. Nevertheless, our ethics permit limited us to collecting ten clutches. Our final sample size was further limited by the fact that of the nests we followed and marked (all nests containing one or two eggs upon our first inspection of the colony), many were either abandoned or the females ceased laying after two to three eggs. We selected the nests with the greatest number of eggs to maximize the sample size.

Finally, I did not incorporate biological endpoints in my thesis, consequently, even though some of the contaminants were detected in eider plasma and eggs, I do not know whether this exposure might have had a biological effect. Now that I have established that eider females are exposed to these contaminants, an important next step is to incorporate biological study parameters such as hormones, behavioural changes, and/or population dynamics.

4 Conclusions

The overarching aim of my thesis was to contribute to bridging the knowledge gap of CECs in wildlife by exploring their occurrence in eiders and goldeneyes. Accordingly, I have shown that eiders in Finland are exposed to a cocktail of contaminants of emerging concern—many of which are severely understudied in wildlife—which are also transferred to their eggs. Whilst I did not find any evidence of a difference in contaminant concentrations from early to late incubation, I did find evidence of possible differential exposure of early- and late-breeding eider females for some compounds. I did not find any evidence of an effect of laying order on contaminant concentrations in the eggs, indicating that monitoring programs can collect a randomly selected egg in the clutch. Related to this, my results further indicate that in a choice between utilizing plasma or eggs as the study matrix, eggs appear to have higher detection frequencies of contaminants and appear to be a more appropriate matrix than plasma if the aim is monitoring the occurrence of the targeted contaminants in eiders.

In **Chapter III**, I have provided the first report of an investigation of neonicotinoids in a waterfowl species. For the Finnish goldeneye population, neonicotinoids appear to be of little concern. Nevertheless, as studies have found neonicotinoids in surface water in Finland, I recommend future studies to examine the potential occurrence of neonicotinoids in aquatic macroinvertebrates as well as in avian species foraging on aquatic macroinvertebrates such as white-throated dippers (*Cinclus cinclus*) and on emergent aquatic insects such as barn swallows (*Hirundo rustica*).

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