

Plastic ingestion in Procellariiformes: Assessing *in vitro* release of plastic- associated chemicals during gastric digestion and interactions with estrogen receptors

Thèse présentée par **Liesbeth Van Hassel** en vue de
l'obtention du grade de docteur en sciences agronomiques et
ingénierie biologique.

*Thesis presented by Liesbeth Van Hassel for the degree of Doctor of
Agronomic Sciences and Biological Engineering.*

Promotrice: Prof. Cathy Debier
Co-promotrice: Prof. Myra Finkelstein

Président du jury: Prof. Michel Ghislain

Membres du jury: Prof. Krishna Das
Prof. Isabelle Donnay
Prof. Jean-François Rees
Dr. Christopher Tubbs

Acknowledgments

Je n'aurais jamais été capable d'arriver au bout de cette incroyable aventure sans le soutien de nombreuses personnes. I have been surrounded by wonderful people throughout these four years (or should I say six?), who never stopped believing in me. I would like to thank all of them.

Merci, thank you, to both of my advisors for believing in me since the first day we met in 2019, during my internship at UC Santa Cruz. Cathy, je suis tellement reconnaissante d'avoir pu construire ce projet avec toi. J'ai toujours admiré ta rigueur mélangée à ton côté tellement humain. Tu m'as appris à développer mon esprit critique, à toujours penser plus loin. Au-delà de ça, tu as été bien plus qu'une promotrice ; merci pour ta présence et ton soutien dans les moments plus difficiles, et pour tous ces moments de rires, parfois brefs dans les couloirs du labo, ou plus longs, lorsque je vous apprenais à faire la tortue dans les eaux bleues de Santa Cruz avec Tanguy.

Myra, I am so grateful I had the opportunity to start working with you as a trainee in 2019. You made me believe in myself like I never did, and you opened the door to a research project that I had never been more passionate about. You helped me so much to improve my communication and ability to synthesize information. Thank you for always pushing me. Thank you also for constantly valuing my work.

Merci aussi à mes deux mémorants, Arthur et Maxime, pour leur contribution. Mon travail n'aurait pas été le même sans les nombreux mois que vous avez passés dans les labos du CRA-W à scanner et décoder le spectre d'innombrables petits morceaux de plastique. Bravo, vous avez été super !

I would like to warmly thank the BNTE team for their wonderful energy and constant support during this journey. Every time I passed the lab door, a feeling of joy and security came over me, even during the harder times. I will always cherish the numerous talks and laughs we had in room A.253, the cafeteria, the toxico room, the corridors, and even outside of the lab. Merci à Eric, Cécile et Ineke pour leur disponibilité et leur soutien, sans oublier Aurélien qui nous a rejoints et a apporté avec lui une énergie pétillante. Thank you also to the wonderful team of doctoral students: Alexandra, Antoine, Julien, Marine, Maud, Oksana, Perrine, Kaan, Sarah, and Zhuo, as well as former doctoral students Aline, Apolline, Caroline, Hélène, Emeline, and Laura. My thanks also go to Jean-François, Melissa, and Yvan for their support. I wish I could have brought you all with me during my time in Santa Cruz.

Un merci tout particulier à Laura, qui n'aura jamais cessé d'être à mes côtés depuis notre rencontre au BNTE en 2019, quelques mois avant mon premier départ pour Santa Cruz. Merci pour ton soutien, ma Laulau ; tu n'as jamais cessé de croire en moi. Merci pour tous ces rires, qui nous accompagnaient d'autant plus dans les moments difficiles, comme lors des journées intenses à Liège... Cheers à la magnifique et unique amitié qui est née de cette aventure.

I was also fortunate to meet wonderful people during my time at UC Santa Cruz. Thank you, Myra, for always making me feel welcome in your lab. I'm also grateful to Don for his support and valuable ideas. A big thank you to Tom, Nick, Shanna, Ellie, Mika, Kaitlyn, and Megan for their positive energy and encouragement.

I would also like to thank the reproduction team at the San Diego Zoo Wildlife Alliance for their warm welcome and for granting me access to their well-equipped facility. Thank you, Chris, for agreeing to collaborate on this project; without your support, I could not have realized the dream of starting

this thesis. I'm also grateful for your constant availability and kindness. Rachel, I would never have been able to run 8 plates (sometimes 9!) per assay without your perfect training—thank you for making me the queen of the luciferase assay! I would also like to express my heartfelt thanks to Candace for welcoming me into her home during my numerous stays in San Diego. Thank you, Elena, for your ever-radiant, loving energy; you always put a smile on my face whenever I crossed your path in the building. Thank you also to Megan and Lindsey for your support and positivity. Wine tastings, the Halloween party, and the beach/surf days are memories I will not forget.

J'aimerais également remercier du fond de mon cœur ma famille et mes amis pour avoir cru en moi, m'avoir écouté, conseillé, et pour m'avoir fait rire et relâcher la pression dans les moments parfois difficiles. Un merci tout particulier à mes parents et à mon frère, qui n'ont cessé d'être fiers de moi et qui m'ont toujours ouvert leurs bras pour partager avec eux mes défis et mes doutes. Merci aussi à Alicia, qui, malgré la distance, m'a souvent donné l'impression d'être à ses côtés durant la fin de la rédaction, nous permettant de nous soutenir mutuellement. Une mention spéciale à mes amies Liévine, Nathalie, et Mathilde pour leurs encouragements constants et tout l'amour qu'elles m'ont donné et envoyé, même à 9 000 km de distance. Merci aussi à Athina, qui m'a toujours rappelé combien je pouvais être fière de mon parcours et de ce que j'accomplissais chaque jour.

I am also grateful for the wonderful friends in Santa Cruz who surrounded me with constant encouragement, believed in me, made space for me to share difficult times, and brought so much joy and happiness into my life. Thank you, Pato, for being my number one supporter, for cheering me on for every small thing I accomplished, for always showing interest when I shared my passion with you, and for your love during difficult times. Kadisha and Mike, thank you for always being there to make me smile. Thank you also to Jeanne, Andrew, Steve and their friends for their support and love.

Enfin, je remercie les membres de mon jury, Christopher Tubbs, Isabelle Donnay, Jean-François Rees, et Krishna Das, ainsi que le président de mon jury, Michel Ghislain, pour le temps consacré à la lecture de cette thèse ainsi que pour les échanges enrichissants qui ont eu lieu durant mon parcours doctoral et la défense privée. Merci pour vos précieux conseils et retours positifs.

Summary

Every year, millions of tons of plastic enter the ocean, threatening marine wildlife. Seabirds, especially Procellariiformes are particularly prone to plastic ingestion due to their infrequent regurgitation and unique gastric physiology. While the physical damage of plastic ingestion is well documented, there is limited knowledge about the toxicological risks from plastic-associated chemicals. This thesis addresses this gap by studying the digestive fate and endocrine-disrupting potential of ingested plastics in Procellariiformes.

An *in vitro* Procellariiform gastric model was developed to simulate digestion and examine the leaching dynamics of chemicals (i.e., additives and sorbed POPs) from plastic. The model revealed that both lipid and aqueous components enhance chemical leaching, with dynamics influenced by the chemical properties of plastic-associated molecules, diet, and polymer type. Results indicate that plastic additives likely pose a higher risk than sorbed POPs due to their prolonged leaching (i.e., for weeks to months) at high concentrations.

The potential hormonal disruption caused by the leaching of chemicals from ingested plastic was studied *in vitro* by using northern fulmar (*Fulmarus glacialis*) estrogen receptors (ERs). Fulmar and human ER response were compared in the presence of known chemicals and showed species-specific sensitivity differences. Plastics recovered from fulmar stomachs were also incubated for 14 days in solvents mimicking stomach fluids. Over half of the fulmars exhibited ER activation and/or inhibition after one day of leaching, with more than a third showing a subsequent ER response after 5 and/or 14 days. These findings suggest that plastic ingestion may contribute to hormonal disruption in Procellariiformes. This work provides

valuable insight into the toxicological risks of plastic ingestion in Procellariiformes.

Liesbeth Van Hassel was born in Namur on October 21st, 1994. She graduated as a biologist in 2019.

List of abbreviations

4-OP	4-tert-octylphenol
ABS	Acrylonitrile butadiene styrene
AhR	Aryl-hydrocarbon receptor
AR	Androgen receptor
BBP	Benzyl butyl phthalate
BDEs	Brominated diphenyl ethers
BFRs	Brominated flame retardants
B-gal	β -galactosidase
BHA	Butylated hydroxyanisole
BPA	Bisphenol A
BPS	Bisphenol S
BPZ	Bisphenol Z
Cr ₂ O ₃	Chromium trioxide
CYPs	Cytochromes P450
DBP	Di-n-butyl phthalate
DEP	Diethyl phthalate
DEHP	Di(2-ethylhexyl) phthalate
DEHT	Bis(2-ethylhexyl) terephthalate
DDT	Dichlorodiphenyltrichloroethane
DIBP	Diisobutyl phthalate
DMP	Dimethyl phthalate

DMSO	Dimethyl sulfoxide
E1	Estrone
E2	17 β -estradiol
E3	Estriol
E4	Estetrol
EC50	Half maximal effective concentration
EDCs	Endocrine disruptor chemicals
ER	Estrogen receptor
ER α	Estrogen receptor alpha
ER β	Estrogen receptor beta
ERR	Estrogen-related receptor
ESR	Estrogen receptor sequence
GC-MS	Gas chromatography-mass spectrometry
GPCR	G-protein coupled receptor
HBCDD	Hexabromocyclododecane
HCB	Hexachlorobenzene
HDPE	High density polyethylene
HEK293	Human embryonic kidney 293 cells
HRE	Hormone responsive element
HSP90	Heat shock protein 90
K _{ow}	Octanol-water partition coefficient
LC-MS	Liquid chromatography-mass spectrometry

LDPE	Low density polyethylene
LOD	Limit of detection
LOQ	Limit of quantification
MHRs	Membrane hormone receptors
MIR	Mid-infrared spectroscopy
NBR	Nitrile butadiene rubber
NHRs	Nuclear hormone receptors
NIR	Near-infrared spectroscopy
NIRM	Near-infrared microscopy
NP	Nonylphenol
OPAs	Organophosphate antioxidants
OPEs	Organophosphate esters
PAHs	Polycyclic aromatic hydrocarbons
PBDE-209	Decabromodiphenyl ether
PBDEs	Polybrominated diphenyl ethers
PBT	Polybutylene terephthalate
PC	Polycarbonate
PCBs	Polychlorinated biphenyls
PCB-28	2,4,4'-trichloro-1,1'-biphenyl
PCB-52	2,2',5,5'-tetrachlorobiphenyl
PCB-80	3,3',5,5'- tetrachlorobiphenyl
PCB-138	2,2',3,4,4',5'-hexachlorobiphenyl

PE	Polyethylene
PET	Polyethylene terephthalate
PFRs	Phosphorous flame retardants
PGM	Procellariiform gastric model
POPs	Persistent organic pollutants
PP	Polypropylene
PPAR γ	Peroxisome proliferator activated receptor γ
PR	Progesterone receptor
PS	Polystyrene
PU	Polyurethane
PUFAs	Polyunsaturated fatty acids
PVC	Polyvinyl chloride
RAR	Retinoic acid receptor
RT	Room temperature
RXR	Retinoid X receptor
TAGs	Triacylglycerols
TBBPA	Tetrabromobisphenol A
TCEP	Tris(2-chloroethyl) phosphate
TCPP	Tris(chloropropyl) phosphate
TDBPP	Tris(2,3-dibromopropyl) phosphate
TDCP	Tris(1,3-dichloro-2-propyl) phosphate
T _g	Glass transition temperature

TiCl ₃	Titanium trichloride
ToF-SIMS	Time-of-Flight Secondary Ion Mass Spectrometry
TPP	Triphenyl phosphate
TR	Thyroid receptor
WEs	Wax esters

Table of Contents

Chapter 1. Literature survey	19
1.1. Plastic	22
1.1.1. Definition and production	22
1.1.2. Types of polymers.....	23
1.1.3. Plastic additives	26
1.2. Plastic pollution.....	33
1.2.1. From terrestrial to marine plastic debris	33
1.2.2. Plastic interaction with chemicals.....	36
1.3. Impact of plastic pollution on marine wildlife	46
1.3.1. Physical impact: entanglement and ingestion	46
1.3.2. Chemical impact of plastic ingestion.....	47
1.3.3. Risks associated with plastic ingestion in seabirds with a focus on Procellariiformes.....	56
References.....	63
Chapter 2. Aims of the thesis and scientific strategy	87
Chapter 3. Influence of the type of food, gastric secretions and polymer type on the leaching of additives and sorbed POPs from plastic in a Procellariiform gastric model	91
Abstract.....	94
3.1. Introduction	95
3.2. Materials and methods	98
3.2.1. Chemicals and materials	98
3.2.2. Plastic sample preparation.....	99
3.2.3. Leaching experiments	104
3.2.4. Quality controls.....	105
3.2.5. Chemical analysis	106
3.2.6. Statistical analysis.....	109
3.3. Results and discussion	110
3.3.1. PCB sorption onto HDPE and PVC	110
3.3.2. Influence of food and gastric secretions on chemical leaching	111

3.3.3. Influence of the type of polymer on chemical leaching	118
3.4. Conclusion	122
Supplementary material	124
Acknowledgments.....	127
References.....	128

Chapter 4. Dynamics of leaching of POPs and additives from plastic in a Procellariiform gastric model: implications for long-term exposure..... 139

Abstract.....	141
4.1. Introduction	142
4.2. Materials and methods	143
4.2.1. Leaching experiments	143
4.2.2. Statistical analysis.....	145
4.3. Results and discussion	146
4.3.1. PGM compared to individual gastric components	146
4.3.2. Influence of contact time.....	152
4.3.3. Model limitations	156
4.3.4. Implications of plastic ingestion on seabird's health	156
Supplementary material	159
Acknowledgments.....	160
References.....	161

Chapter 5. Hormonal disruption from plastic ingestion in northern fulmar: activation and inhibition of estrogen receptors..... 167

Abstract.....	170
5.1. Introduction	172
5.2. Materials and methods	174
5.2.1. Chemicals and materials	174
5.2.2. Human and fulmar ER cloning	175
5.2.3. Plastic-associated chemicals for human and fulmar ER comparison	176
5.2.4. Plastic collection from northern fulmar stomachs.....	176
5.2.5. Plastic leaching	177
5.2.6. Cell culture and receptor activation/inhibition assay	180
5.2.7. Plastic identification.....	181

5.2.8. Qualitative assessment of plastic leachates with mass spectrometry	182
5.2.9. Statistical analysis	183
5.3. Results.....	184
5.3.1. Fulmar ER exhibits similar response to human ER, with chemical and receptor type-specific sensitivity differences.....	184
5.3.2. Plastic leachates activated and inhibited fulmar ERs	187
5.4. Discussion	192
5.4.1. Fulmar ERs exhibit similar response to human ER, with chemical and receptor type-specific sensitivity differences.....	193
5.4.2. Plastic leachates activate and inhibit fulmar ERs	194
5.4.3. Biological significance	195
5.5. Conclusion	197
Supplementary material	198
Acknowledgments.....	231
References.....	232

Chapter 6. General discussion and perspectives 245

6.1. General discussion	247
6.1.1. Leaching dynamic of plastic-associated chemicals in a Procellariiform stomach.....	247
6.1.2. Health risks associated with exposure to plastic associated-chemicals	255
6.1.3. Conservation implications of plastic ingestion	260
6.1.2. Plastic pollution: current situation and future directions	261
6.2. Conclusion	263
6.3. Perspectives	266
References.....	270

Chapter 1

Literature survey

Table of Contents

1.1.	Plastic.....	22
1.1.1.	Definition and production.....	22
1.1.2.	Types of polymers.....	23
1.1.3.	Plastic additives	26
1.1.3.1.	Plasticizers	26
1.1.3.2.	Flame retardants.....	28
1.1.3.3.	Antioxidants.....	31
1.2.	Plastic pollution	33
1.2.1.	From terrestrial to marine plastic debris	33
1.2.2.	Plastic interaction with chemicals.....	36
1.2.2.1.	Migration of plastic additives and monomers.....	37
1.2.2.2.	Sorption and desorption of marine contaminants	41
1.3.	Impact of plastic pollution on marine wildlife.....	46
1.3.1.	Physical impact: entanglement and ingestion	46
1.3.2.	Chemical impact of plastic ingestion	47
1.3.2.1.	Microplastics.....	48
1.3.2.2.	Leaching of plastic-associated chemicals	48
1.3.2.3.	Endocrine disruption.....	51
1.3.3.	Risks associated with plastic ingestion in seabirds with a focus on Procellariiformes.....	56
1.3.3.1.	Plastic ingestion	56

1.3.3.2.	Gastric models to study the leaching of chemicals from plastics	59
1.3.3.3.	Risks associated with plastic ingestion	61
References.....		63

1.1. Plastic

1.1.1. Definition and production

Plastic is commonly defined as a synthetic or semi-synthetic material made of organic polymers primarily consisting of carbon, hydrogen, and oxygen (Pilapitiya and Ratnayake, 2024). The first synthetic plastic “Bakelite” was considered to have been invented in 1907 (Klun *et al.*, 2022). However, significant progress in plastic production began in the 1950s with the introduction of various types of plastics, including polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), nylon and others (Pilapitiya and Ratnayake, 2024). Due to the numerous advantages of plastics, including versatility, lightweight, durability and cost-effectiveness, plastic production has grown exponentially, reaching an estimated 390.7 million tons in 2021 compared to 2 million tons in 1950 (Plastics Europe, 2022).

The process of plastic manufacturing is illustrated on Figure 1.1. Single molecules known as “monomers” are synthesized through the “cracking” of naphtha, a byproduct derived from crude oil. Monomers are then combined together through a process called “polymerization”, to form the repeating units of long-chain polymers (Mills, Jenkins and Kukureka, 2020). Catalysts such as titanium trichloride (TiCl_3) and chromium trioxide (Cr_2O_3) play crucial roles in initiating and propagating the polymerization reaction by activating monomers to form reactive species and by stabilizing reactive intermediates (Mills, Jenkins and Kukureka, 2020). Chemical additives are subsequently added to modify the properties, long-term use and performance of the polymer. The resulting material is shaped into industrial pellets, subsequently melted and molded to produce a wide range of final plastic products (Mills, Jenkins and Kukureka, 2020). Residual catalysts and monomers that did not react during the polymerization,

Chapter 1 – Introduction

together with plastic additives, which are often not chemically bound to the polymer chains are raising health concern due to their tendency to migrate out of the final plastic product (Hahladakis *et al.*, 2018).

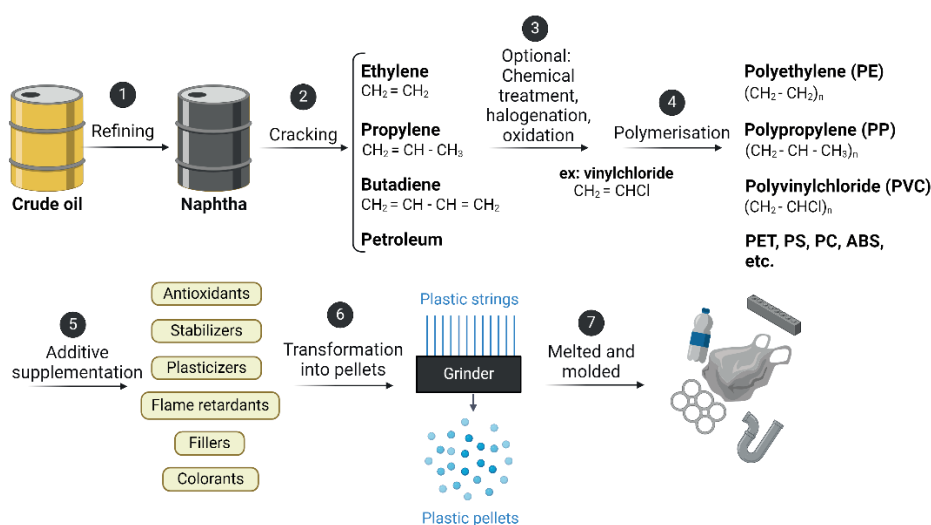


Figure 1.1. Process of plastic fabrication. PET, polyethylene terephthalate; PS, polystyrene; PC, polycarbonate; ABS, Acrylonitrile butadiene styrene [adapted from Mills, Jenkins and Kukureka (2020)]. Created with BioRender.com.

1.1.2. Types of polymers

Polymers can be classified according to different characteristics like their origin, structure, physico-chemical properties and application. The number of different polymers is high due to ongoing research and development. The top three-most produced polymers are PE (24% total production), PP (19.3% total production) and PVC (12.9% total production) (Plastics Europe, 2022).

Chapter 1 – Introduction

PE is made out of monomers of ethylene (Figure 1.2) and comes in different forms depending on its density. Low-density polyethylene (LDPE) is more flexible (density 0.91–0.94 g/cm³) and often used for film application (e.g. plastic bag) while high-density polyethylene (HDPE) is more rigid (density 0.95–0.97 g/cm³) and commonly used for containers and solid packaging (Shah *et al.*, 2008; Mills, Jenkins and Kukureka, 2020). PP is made out of monomers of propylene (Figure 1.2) and is a versatile, more flexible polymer (density 0.89–0.92 g/cm³) used in a wide range of applications, such as plastic packaging, textiles, furniture, and medical materials (Shah *et al.*, 2008; Mills, Jenkins and Kukureka, 2020). PVC is composed of vinyl chloride monomers, characterized by the presence of a chlorine atom bound to the second carbon of the monomer (Figure 1.2) (Shah *et al.*, 2008). This polymer is typically rigid (density 1.35 g/cm³) and commonly used in construction, pipes, and electronics. However, PVC is also utilized for the production of flexible plastics, such as shower curtains, through the addition of plasticizers (Mills, Jenkins and Kukureka, 2020) (Shah *et al.*, 2008).

Other well-known polymers include polystyrene (PS), polyurethane (PU) and polyethylene terephthalate (PET) representing 5.3%, 5.5% and 6.3% of the global production of plastic (Plastics Europe, 2022). PS is composed of monomers of styrene containing a benzene ring (Figure 1.2) and is primarily used in the fabrication of packaging and insulation materials (Shah *et al.*, 2008). PU common use includes foams for furniture, mattresses and insulation while PET is commonly used in the production of bottles, fibers, films, and other packaging materials (Shah *et al.*, 2008). The remaining fossil-based plastics includes other thermoplastics [e.g. polybutylene terephthalate (PBT)] and thermosets [e.g. polyester, silicone and epoxy] each of them representing 7.1% of plastic

Chapter 1 – Introduction

production. Circular plastics (recycled and bio-based) account for 9.8% of world plastic production (Plastics Europe, 2022).

Overall, packaging stands as the predominant application for plastic, constituting 44% of global plastic production, followed by building and construction, which represents 18%. The remaining 38% finds application in various sectors, including automotive, electrical and electronics, household, leisure and sports, agriculture, and other materials (Plastics Europe, 2022).

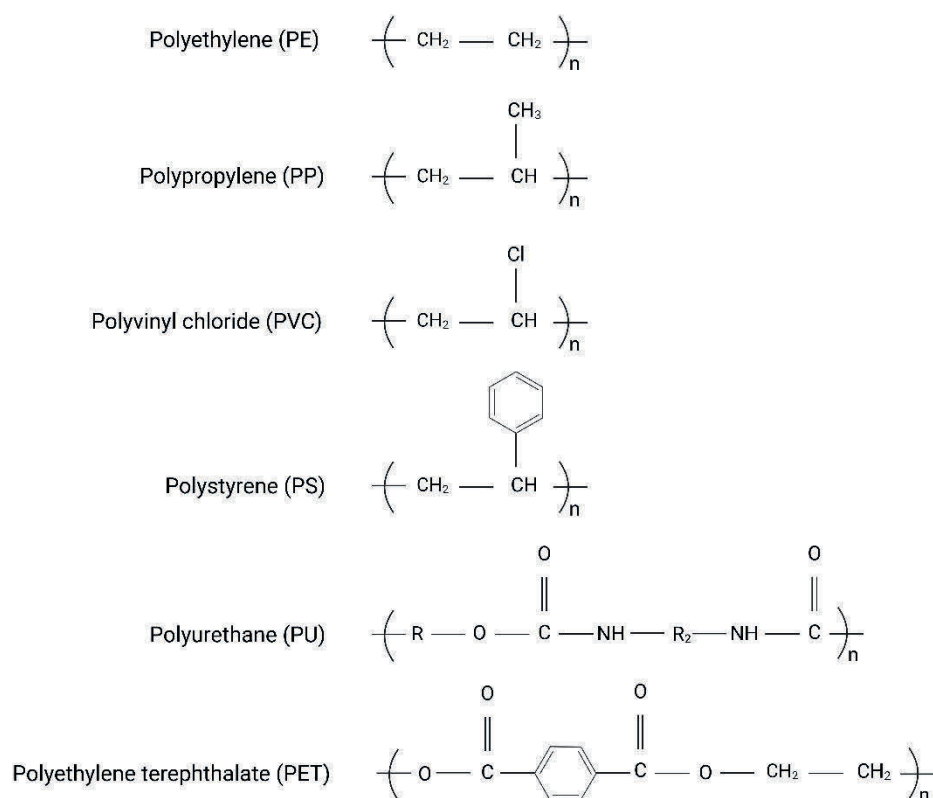


Figure 1.2. Molecular structure of the most commonly found polymers [adapted from Shah *et al.* (2008)].

1.1.3. Plastic additives

During plastic manufacture, chemicals called “additives” are added to the polymer matrix to modify the plastic properties and improve its resistance to environmental stresses (Hahladakis *et al.*, 2018). Plastic additives are added at concentrations ranging from 0.1 to 70% of the plastic weight, depending on the type of polymer and the intended application of the product (Hahladakis *et al.*, 2018). The most common plastic additives used in plastic industry are plasticizers (e.g., phthalates), flame retardants (e.g., polybrominated diphenyl ethers), antioxidants (e.g., alkylphenol), UV- and heat stabilizers (e.g. benzophenones), fillers (e.g., calcium carbonate) and colorants (e.g., azocolorants) (Hahladakis *et al.*, 2018).

It is estimated that one single piece of plastic contains between 5 to 30 different additives (Chun Z. Yang *et al.*, 2011). Plastic additives pose a significant concern for both human health and the environment due to their predominantly non-covalent binding to the plastic matrix, facilitating their easy release into the external environment, including food, water, and soil (Hahladakis *et al.*, 2018; Pinto da Costa *et al.*, 2023). This is particularly problematic as many plastic additives are recognized for their endocrine-disrupting effects (Pinto da Costa *et al.*, 2023).

1.1.3.1. Plasticizers

Plasticizers are low molecular weight molecules added to plastic to enhance its flexibility and workability by increasing the free volume and reduce intermolecular forces between the polymer chains (Czogala, Pankalla and Turczyn, 2021). This is achieved through the branched structure of plasticizers, which creates additional space within the polymer matrix (Figure 1.3).

Chapter 1 – Introduction

Additionally, their polar functional groups such as hydroxyl (-OH) and carbonyl (C=O) also interact with the polar segments of the polymer chains through dipole-dipole interaction or hydrogen bonding, disrupting the cohesive forces between polymer chains, further reducing the overall strength of intermolecular attractions (Czogala, Pankalla and Turczyn, 2021).

The most common plasticizers used in industry are phthalates, which accounted for 55% of world consumption plasticizers in 2022 (Li *et al.*, 2024). Examples include di(2-ethylhexyl) phthalate (DEHP), diethyl phthalate (DEP) and di-n-butyl phthalate (DBP). Plasticizers are added at concentrations ranging from 10 to 70% of the plastic weight and are commonly used in PVC products such as cables or medical material. They can also be found in other polymers such as PE and PP in plastic films, sheets and fibers and PS in packaging materials (Luo *et al.*, 2022).

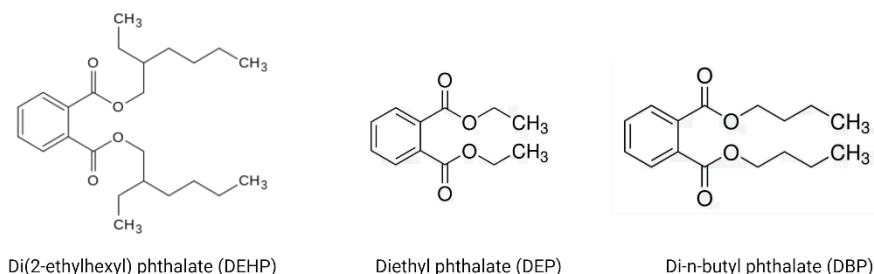


Figure 1.3. Molecular structure of di(2-ethylhexyl) phthalate (DEHP), diethyl phthalate (DEP) and di-n-butyl phthalate (DBP).

Due to concerns about the potential adverse health effects of phthalates, particularly on reproductive and developmental health (Sedha *et al.*, 2021), regulations have been established in many countries such as the European Union (EU) and the United States (US) to limit their use in certain products. For

Chapter 1 – Introduction

example, in the EU, the use of DBP, benzyl butyl phthalate (BBP), DEHP, diallyl phthalates, dimethyl isophthalate, and bis(2-ethylhexyl) terephthalate (DEHT) has been restricted in contact food materials by implementing maximum permitted amounts of the substances to be released into food (ranging from 0.05 mg/kg to 60 mg/kg) (Ong, Samsudin and Soto-Valdez, 2022). Additionally, all phthalates classified as toxic to reproduction have generally been prohibited in toys (ECHA, 2022). More recently, in 2020, reprotoxic phthalates have been restricted in children swimming aids, flooring, coated fabrics and paper, recreational gear, mattresses, footwear, office supplies, consumer clothing or related accessories, as well as in other textiles that come into contact with the skin in the EU (maximum concentration of 0.1% of plastic material) (ECHA, 2022). However, there are still many phthalates for which more data is needed to confirm their potential hazard. Moreover, old plastic items may still contain some of the phthalates that are no longer allowed to be used, posing risks for the environment and consumers in the context of waste or plastic recycling/reuse (ECHA, 2022).

1.1.3.2. Flame retardants

Flame retardants (FRs) are incorporated into plastic to reduce their flammability and improve their resistance to combustion, which is essential to enhance the fire safety of various products (Cheng *et al.*, 2020). Brominated flame retardants (BFRs) are the most widely used FRs due to their low cost, wide application and effectiveness (Marturano, Cerruti and Ambroggi, 2017). During combustion, BFRs release brominated radicals which react with hydrogen and hydroxyl radicals formed from the polymer combustion. This mechanism inhibits the formation of free radical, preventing the spread of flames (Marturano, Cerruti and Ambroggi, 2017).

Chapter 1 – Introduction

The most common BFRs are polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCDD) and tetrabromobisphenol A (TBBPA) (Figure 1.4), often found in electronic devices, construction materials, textiles, automotive components, and furniture (Cheng *et al.*, 2020). BFRs are usually added at concentrations ranging from 5 to 30% of the plastic weight (Hahladakis *et al.*, 2018). However, due to concerns about their toxicity, persistence and bioaccumulation, some regions have implemented restrictions on some of these compounds.

In the EU, the use of PBDEs has been restricted to a maximum concentration of 0.05% plastic weight for products placed on the market since 2021. Before that, the concentration was limited to 0.1% since 2006 for decabromodiphenyl ether (PBDE-209) and since 2011 for other PBDEs (Sharkey *et al.*, 2020). In the EU, HBCDD concentration is limited to 0.01% in products placed on the market since 2019. The US has no restriction on BFRs at the federal level, although 13 states have implemented a maximum concentration of 0.1% PBDEs and HBCDD in certain commercial goods such as children's products and furniture (Sharkey *et al.*, 2020). TBBPA and its derivative are restricted for use in electronic displays at a limit concentration of 0.1% per weight in EU (ECHA, 2021).

Chapter 1 – Introduction

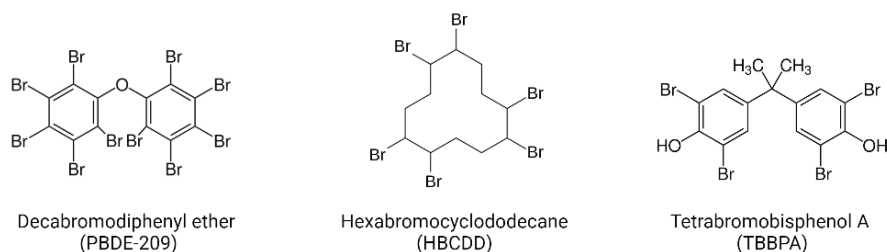


Figure 1.4. Molecular structure of decabromodiphenyl ether (PBDEs), hexabromocyclododecane (HBCDD) and tetrabromobisphenol A (TBBPA).

In response, alternative flame retardants, such as phosphorus flame retardants (PFRs), have been considered (Pilapitiya and Ratnayake, 2024). PFRs react with radicals in the same way that BFRs do during the burning process. Additionally, they reduce flammability by releasing phosphoric acid, which reacts with the material and create a protective layer – called “char layer” – at the surface of the plastic (Marturano, Cerruti and Ambroggi, 2017). PFRs are generally added into polyamides, polyesters, PE, PP and PS, especially in electrical and electronic insulation components (Cheng *et al.*, 2020).

Some PFRs such as tris(2-chloroethyl) phosphate (TCEP), tris(2,3-dibromopropyl) phosphate (TDBPP) and triphenyl phosphate (TPP) have also been identified as toxic substances, especially for the reproductive system, leading to similar restriction than BFRs (limit of 0.1% of plastic weight) (ECHA, 2023). Alternative PFRs such as tris(chloropropyl) phosphate (TCPP) and tris(1,3-dichloro-2-propyl) phosphate (TDCP) are currently used as replacement at non restricted concentrations (Figure 1.5) (ECHA, 2023).

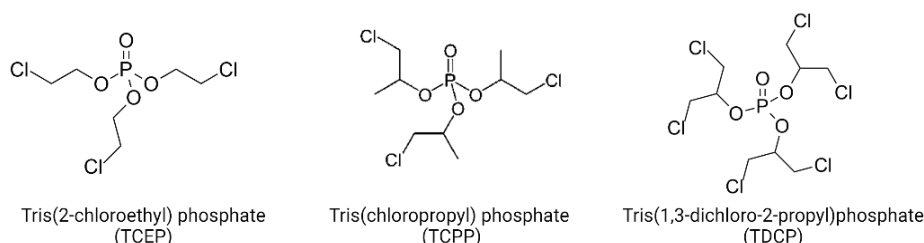


Figure 1.5. Molecular structure of tris(2-chloroethyl) phosphate (TCEP), tris(chloropropyl) phosphate (TCPP) and tris(1,3-dichloro-2-propyl) phosphate (TDCP).

1.1.3.3. Antioxidants

Antioxidants play an important role in extending the lifespan of plastic. They are commonly used to inhibit or slow down the oxidative degradation of the material in the presence of heat and light (especially UVs) (Luo *et al.*, 2022). When plastics are exposed to oxygen, heat or UV-radiation, they can undergo oxidative degradation, leading to the formation of free radicals. These free radicals can then propagate chain reactions, causing the plastic to degrade further, resulting in discoloration, embrittlement, and other forms of deterioration (Marturano, Cerruti and Ambroggi, 2017). Antioxidants function by trapping reactive free radicals, generating inactive free radicals, or by decomposing hydroperoxide into inert secondary products (Figure 1.6) (Marturano, Cerruti and Ambroggi, 2017).

Antioxidants can be divided between two groups: phenols and amines (Luo *et al.*, 2022). Phenols include monophenols, biphenols, tri-phenols, polyphenols, hydroquinone, alkylphenol (e.g. nonylphenol and octylphenol) and thiobisphenols, etc. Amines include naphthylamine, diphenylamine, p-phenylenediamine and quinoline derivatives, etc (Regan and Fogarty, 2005; Luo

Chapter 1 – Introduction

et al., 2022). Butylated hydroxyanisole (BHA) is an example of phenolic antioxidant widely used in plastics and has been detected in various environments such as surface water, landfill leachates and house dust (Luo *et al.*, 2022).

Antioxidants can be found in a many different types of polymers such as PE, PP, PVC, PS, PC, PET, ABS, etc. and are used at smaller concentrations than plasticizers and flame retardants, ranging between 0.05 – 3% of the plastic weight (Luo *et al.*, 2022). Due to their classification as toxic substances, certain antioxidants have been restricted. For instance, BHA should not exceed 6 mg/dm² in food contact coatings, and nonylphenol is restricted to a maximal concentration of 0.1% by weight (ECHA, 2010).

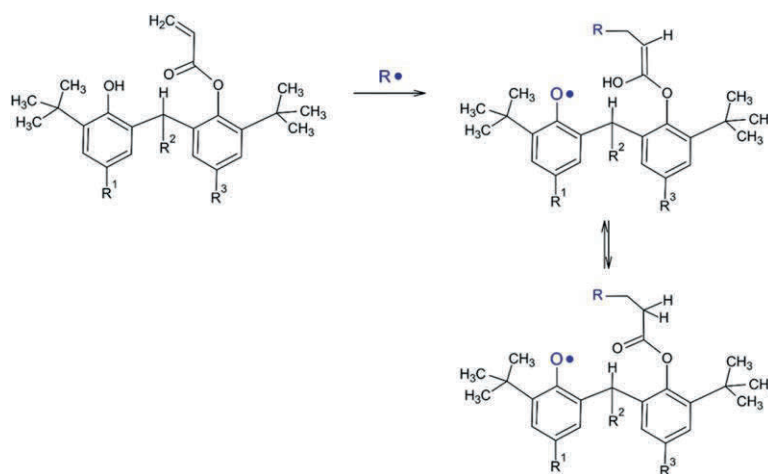


Figure 1.6. Molecular reaction of acrylated bisphenol (antioxidant) with a free radical (Marturano, Cerruti and Ambrogi, 2017).

1.2. Plastic pollution

1.2.1. From terrestrial to marine plastic debris

In 2018, the annual generation of plastic waste was estimated to be more than 300 million tons compared to 0.35 million tons in 1950, with only ~ 9% being recycled, and a remaining 80% accumulating in the environment (Kibria *et al.*, 2023). Plastic inherent durability and resistance to degradation (degradation rate ranges from 100 to 1000 years) is a key characteristic that contributes to its persistence in the environment (Pilapitiya and Ratnayake, 2024). Plastic lifespan also highly influences environmental pollution. It is estimated that two-third of global plastic has a lifespan shorter than two months (Pilapitiya and Ratnayake, 2024). Ultimately, plastics designed for single-use purposes, such as packaging materials, significantly account for plastic waste generation compared to longer-lasting plastic products like building, automotive, household and electronic material (Pilapitiya and Ratnayake, 2024). For example, in 2015, 42% of plastic produced was used for packaging and 19% was used for construction material. The same year, 54% of plastic waste were generated from packaging while 5% were generated from construction (Geyer, Jambeck and Law, 2017).

Due to littering and inadequate waste disposal, plastic waste rapidly spread out on land which can then be transported by winds, stormwaters and rivers to water bodies such as the ocean (Pilapitiya and Ratnayake, 2024). It is estimated that 8 million tons of plastic waste end up in the ocean every year, accounting for 80% of all marine pollution and with an estimated 80% originating from land (Pilapitiya and Ratnayake, 2024). Rivers are identified as the major source of marine plastic, transporting large amounts of debris from inland areas to the

Chapter 1 – Introduction

ocean. According to a study lead by The Ocean Cleanup in 2021, 1000 rivers account for approximately 80% of riverine plastic emission into the ocean, with the largest contributing countries being the Philippines, India, Malaysia and China (Meijer *et al.*, 2021). Fishing industry also accounts for a significant portion of ocean plastics, leaving behind abandoned or lost fishing gear (called “ghost gear”) such as nets and traps (Lebreton *et al.*, 2022). Accidental leaks can also significantly contribute to marine plastic pollution, as seen in the case of the MV X-Press accident in 2021, when a container ship caught fire and sank, releasing tons of plastic pellets into the Indian Ocean (Perera *et al.*, 2022).

Negatively buoyant plastic (e.g. PVC, PC, ABS) will generally remain closer to their sources (mostly lands) while positively buoyant plastic (e.g. PE, PP, PS, PU) will be transported across oceans to remote areas, influenced by various processes including current, wind and waves (Lebreton *et al.*, 2022). The accumulation of biofilm and organisms at the surface of buoyant plastic debris can increase their density, causing them to sink below the water surface (Gaylarde *et al.*, 2023). The transport of plastic across vast distances will contribute to their accumulation in specific oceanic regions such as gyres, where they will slowly degrade into smaller particles under the effect of sun, waves and marine life (Lebreton *et al.*, 2022). The great Pacific Garbage Patch is the largest of the five offshore plastic accumulation area in the world’s ocean, covering an estimated surface of 1.6 million square kilometers (three times the size of France) (Lebreton *et al.*, 2018).

Plastic wastes can be found in different sizes in both terrestrial and marine environments, ranging from macroplastics (> 25 mm), to mesoplastics (5 – 25 mm) and microplastics (< 5 mm) (Pilapitiya and Ratnayake, 2024). The term nanoplastics can also be used for particles ranging from 1 to 1000 nm (Pilapitiya

Chapter 1 – Introduction

and Ratnayake, 2024). The most commonly found macroplastics in the environment include single use plastics such as plastic bottles, bags, caps, straws, utensils, food wrappers, packaging and fishing gear (Morales-Caselles *et al.*, 2021). Macroplastics represent most of the emitted mass of plastic waste in the ocean, with the largest contributing nations being China, India, Indonesia, Africa, Middle East and South America (Boucher and Friot, 2017). However, in term of numerical concentration (km^{-2}), microplastics represent an important portion of marine plastic. In the Great Pacific Garbage patch, the number of microplastics per square kilometers is estimated to 100 thousand to 1 million compared to less than 1 thousand macroplastics per km^2 (Lebreton *et al.*, 2018).

Microplastics can be classified as “primary” or “secondary” depending on their source. Primary microplastics are intentionally produced at their micrometric size and can be used as a raw material (industrial plastic pellets) for the manufacture of larger plastic products or added to personal care products to provide scrubbing or abrasive effects, (Thacharodi *et al.*, 2024). Over the past 5 years, the use of plastic microbeads in personal care products such as exfoliating scrubs, toothpaste, and shower gels has been restricted in several countries including the US, Canada, UK and a few European countries (Anagnosti *et al.*, 2021; Singh and Mishra, 2023). Microfibers formed from the abrasion of synthetic textiles and rubber dust originating from tire erosion during driving are also classified as primary microplastics, which account for two thirds of the world’s primary microplastic discharges into the ocean (ETRMA, 2011; FAO/ICAC, 2013). Secondary microplastics result from the fragmentation of larger plastics, due to mechanical weathering and chemical breakdown (Thacharodi *et al.*, 2024).

Chapter 1 – Introduction

In Europe and the US, where waste management systems are more advanced, the mass of primary microplastics equals or exceeds that of macroplastics entering the ocean annually. In contrast, in other countries, the major contribution originates from mismanaged waste (i.e., macroplastics) (Boucher and Friot, 2017). While it is estimated that 1.5 million tons of primary microplastics enter the ocean every year (in addition to the 8 million tons of macroplastics), estimating the contribution of secondary microplastics is challenging due to the difficulty in assessing the amount of macroplastics that fragment into microplastics annually (Boucher and Friot, 2017).

1.2.2. Plastic interaction with chemicals

Throughout their entire lifecycle, plastics are exposed to a wide range of chemicals that interact with the polymer matrix in various ways, including chemical additives and environmental contaminants. Different types of interactions are encountered and described in the literature, depending on the chemical, the polymer and the external environment.

- 1) *Migration/leaching* refers to the movement of chemicals within the plastic material or between the plastic and the external environment (Omeroğlu, Özdal and Bulut, 2017). For instance, many plastic additives, which are not covalently bound to the polymer, can migrate from the plastic into the surrounding environment, including food, water, soil, and other mediums (Hahladakis *et al.*, 2018). Plastic monomers may also migrate from the matrix to the external environment (Hahladakis *et al.*, 2018). Migration of chemicals out of the plastic is also known as leaching (Li *et al.*, 2024).

Chapter 1 – Introduction

- 2) *Sorption* is the process of transferring a chemical from a fluid phase, such as air or water, to a solid phase like plastic (Pinto da Costa *et al.*, 2023). Throughout its lifecycle, plastic is exposed to environmental contaminants that may sorb onto it due to their significant affinity with the material. Two processes of sorption can occur in the environment: (1) absorption, where the substance penetrates into the internal structure of the material and (2) adsorption, where the contaminant adhere to the surface of the plastic (Rodrigues *et al.*, 2019).
- 3) *Desorption* is the process by which a substance is released or removed from the material on which it was previously sorbed (Pinto da Costa *et al.*, 2023). For instance, chemical contaminants that sorb onto plastic, particularly in aquatic environments, may be released from the plastic when conditions in the surrounding environment change.

1.2.2.1. Migration of plastic additives and monomers

During plastic manufacture, additives are introduced into the polymer matrix to modify its properties and performance. The majority of these additives do not form covalent bonds with the polymer, making them prone to migration within the plastic material or into the surrounding environment.

The leaching of phthalates such as DEHP has been documented in various contexts, including in agricultural soils from plastic films (Xu *et al.*, 2024), in blood from medical material (Luo *et al.*, 2014), in water from children toys (Jang *et al.*, 2023), in seawater from marine plastic debris (Dimassi *et al.*, 2023) and in house dust from household products (Lee *et al.*, 2021) to name a few. Flame retardants primarily used in electronics and electronical devices are suspected of leaching out in small proportions during plastic use, potentially releasing significant amounts later in their lifecycle, when exposed to abiotic

Chapter 1 – Introduction

factors such as sludge from dumpsite and weathering (physical abrasion, sunlight irradiation and oxidation) (Ilyas *et al.*, 2013; Cheng *et al.*, 2020). The migration of organophosphate antioxidants (OPAs) and organophosphate esters (OPEs), popular as flame retardants and plasticizers has also been observed from single-use food packaging into food matrix simulants (e.g. isooctane) (Bi, Meng and Su, 2023; Zhou *et al.*, 2023). Recent studies suggests that OPEs in food packaging could originate from the oxidation of OPAs (Liu and Mabury, 2019; Bi, Meng and Su, 2023). It is estimated that one single piece of plastic contains between 5 to 30 different additives (Chun Z. Yang *et al.*, 2011), yet the exact chemical composition is complex and often confidential, hindering accurate risk assessments regarding the release of additives from plastic material (Ong, Samsudin and Soto-Valdez, 2022).

Catalysts and solvents used during the manufacture process of plastics are also products added intentionally that can potentially migrate out of the plastic into the environment (Ong, Samsudin and Soto-Valdez, 2022). Other non-bounded chemicals found in plastic material are residual monomers that did not polymerize during the manufacturing process. Abiotic processes (e.g. heat, light, acids) can also favor the formation of monomers and oligomers via plastic degradation (e.g. thermal- and photo-oxidation and hydrolysis), which may subsequently leach in the external environment (Chamas *et al.*, 2020). One famous example is bisphenol A (BPA), a monomer of PC primarily used in the production of hard plastics and epoxy resins, also used as an antioxidant in PE, PP and PVC (Hermabessiere *et al.*, 2017; Czarny-Krzyżmińska, Krawczyk and Szczukocki, 2023). BPA monomers can originate from non-polymerization during plastic manufacture, and from hydrolysis of PC polymer chains at the polymer surface in contact with aqueous substances (Mercea, 2009).

Chapter 1 – Introduction

BPA migration from plastic material has been documented in various studies, involving leaching from (i) marine microplastics into seawater (Dimassi *et al.*, 2023), (ii) canned soft drink and plastic-bottles water (Kumar *et al.*, 2023; Mârşolea *et al.*, 2023) and (iii) industrial plastics into artificial gut fluid (Coffin *et al.*, 2019). Notably, in 2010, the leaching of BPA from baby bottles raised concerns due to BPA's classification as a potent endocrine disruptor (Siddique *et al.*, 2021). BPA's harmful effects on reproduction and development prompted restrictions in Europe, Canada, the United States, and China for products intended for children. However, BPA is still detected in many environments such as indoor dust (Zhang *et al.*, 2020), seawater (Xie *et al.*, 2022) and drinking water (Kumar *et al.*, 2023) as well as in biological samples such as human urine (Zhang *et al.*, 2020) and fish (Barboza *et al.*, 2020).

Due to the toxicity of BPA, substitutes such as bisphenol S, F, E, B, AF have been developed (Czarny-Krzywińska, Krawczyk and Szczukocki, 2023). However, studies already indicate endocrine-disrupting properties associated with these new BPA analogues (Qiu *et al.*, 2019; Lee *et al.*, 2022; Metruccio *et al.*, 2024). The release of other monomers such as styrene from PS food containers has also been observed, primarily due to hydrolysis and oxidation of the plastic under acidic conditions and elevated temperature, raising concerns for human health and the environment (Wang *et al.*, 2023).

The migration of intentionally added (e.g. additives, catalysts, solvents) and non-intentionally added [e.g. monomers and break down products (oligomers)] substances in the polymer matrix is governed by Fick's law, which postulates that a flux of a given compound occurs from regions of high concentrations to regions of low concentrations and that the degree of migration is proportional to the concentration gradient (Pinto da Costa *et al.*, 2023).

Chapter 1 – Introduction

Migration is also size-dependent, with small molecules such as monomers migrating faster (Pinto da Costa *et al.*, 2023). The characteristics of the polymer as well as the external media will also influence the migration rate.

The log K_{ow} , or octanol-water partition coefficient, is used to assess the differential solubility of environmental contaminants in two solvents (octanol and water) and their potential for bioaccumulation (Walters *et al.*, 2011). A large proportion of additives have an elevated log K_{ow} , resulting in their leaching being facilitated by a hydrophobic/lipophilic environment. For example, PBDEs exhibit log K_{ow} from 5.08 for mono-BDE to 8.70 for deca-BDE. Some phthalates, such as BBP (log K_{ow} = 5.20) or di-n-octyl phthalate (log K_{ow} = 6.07) also have hydrophobic properties, enhancing their leaching in lipidic environment such as oils (Tanaka *et al.*, 2015; Sun *et al.*, 2019) and lipid-rich food (Yang *et al.*, 2019; Baranenko *et al.*, 2022). In contrast, other additives such as bisphenol analogous [log K_{ow} 1.2 (BPS) – 5.6 (BPZ) (Rifa and Lavado, 2024)] or some phthalates such as dimethyl phthalate (DMP) [Log K_{ow} = 1.60 (Bajagain *et al.*, 2023)] have shown significant leaching in aqueous environments such as water or saliva (Nilsson *et al.*, 2016; Mârşolea *et al.*, 2023).

Abiotic factors such as weathering (Luo *et al.*, 2022), heat (Dimassi *et al.*, 2023), salinity (Suhrrhoff and Scholz-Böttcher, 2016), or acidity (Coffin *et al.*, 2019) will also favor the leaching of additives and monomers from plastic. Once released from the plastic matrix, these components can potentially cross biological membranes and pose a risk to human and environmental health, due to their toxicity (Pilapitiya and Ratnayake, 2024).

Chapter 1 – Introduction

1.2.2.2. Sorption and desorption of marine contaminants

In addition to being contaminated with plastic, the ocean also contains a wide range of chemicals, including persistent organic pollutants, heavy metals and antibiotics which can interact with plastic in the marine environment (Rafa *et al.*, 2024).

Persistent organic pollutants (POPs) are extensively studied in the context of marine contamination due to their recognized detrimental impact on both the environment and human health, making them a focal point in research exploring their interaction with oceanic plastic (Chen *et al.*, 2024). The Stockholm Convention defines persistent organic pollutants (POPs) as “organic chemical substances that persist in the environment, bioaccumulate through the food web, and pose a risk of causing adverse effects to human health and the environment”. The most concerning and well-known POPs were predominantly used after World War II (Li *et al.*, 2022). Today, those legacy POPs can still be found in terrestrial and marine environment due to their resistance to degradation (Rafa *et al.*, 2024).

POPs can be classified into two main categories: intentionally produced chemicals, which include pesticides (e.g. DDTs and its metabolites) and industrial chemicals, and unintentionally produced chemicals, such as polycyclic aromatic hydrocarbons (PAHs), dioxins, and furans. Industrial chemical category encompasses a wide range of chemicals used in industrial processes and applications such as polychlorobiphenyls (PCBs) mainly used as dielectric fluids in transformers and capacitors and brominated diphenyl ethers (BDEs) used as flame retardants in a variety of products, including electronics, furniture and textiles. PAHs are formed during the incomplete combustion of fossil fuels, wood, and other organic materials while dioxins and furans are often formed as

Chapter 1 – Introduction

by-products of combustion processes, such as waste incineration and industrial activities (Ashraf, 2017). POPs can be transported to the ocean through rivers, atmospheric currents – mainly by interacting with suspended and airborne particles – and can also be directly released through marine activities and accidental spills (Rodrigues *et al.*, 2019).

One main characteristic of POPs is their high hydrophobicity characterized by a log K_{ow} usually > 5 , favoring their interaction with non-polar phases in water environments such as organic matter and sediments (Rafa *et al.*, 2024). Plastics are also hydrophobic, which therefore promotes their interaction with POPs in the ocean through a mechanism called “sorption” (Rafa *et al.*, 2024). Studies have shown that POPs were found to be twice as concentrated on plastics compared to sediments and organic matter and ten times more concentrated than in saltwater (Mato *et al.*, 2001). PCBs, PAHs and DDTs (and their metabolites) are the POPs most commonly found sorbed at the surface/within marine microplastics, with concentrations detected as high as 14,000 ng/g plastic (Bouhroum *et al.*, 2019; Rodrigues *et al.*, 2019; Rafa *et al.*, 2024).

Chemicals can be sorbed onto plastics in two different ways: absorption and adsorption. During absorption, the molecules are taken up by the material’s internal structure rather than adhering to its surface. This process involves relatively weak Van-der-Waals forces (Rodrigues *et al.*, 2019). Adsorption occurs at the interface between the sorbent and media. This phenomenon involves a boarder array of forces from Van-der-Waals to ionic or covalent bonds (Rodrigues *et al.*, 2019). The sorption process is influenced by many parameters such as the concentration of chemical in the environment, the polymer properties, the size of plastic and environmental conditions such as weathering and acidity

Chapter 1 – Introduction

(Rafa *et al.*, 2024). Both absorption and adsorption can happen simultaneously which can make it difficult to distinguish one process from the other (Tourinho *et al.*, 2019).

One major characteristic influencing the sorption process is the crystallinity of the polymer, which is the degree to which polymer chains are aligned with one another. A polymer can contain both crystallin and amorphous regions which distribution depends on the polymer composition (i.e. type of monomer) and structure (i.e. chain length and branching) (Rafa *et al.*, 2024) (Figure 1.3). Crystallin areas are characterized by tightly-packed polymer chains arranged in a highly ordered and repetitive manner, while amorphous areas are defined by a more random and disordered arrangement with more free volume, allowing a better diffusion of the chemicals (Rodrigues *et al.*, 2019) (Figure 1.3). For example, HDPE is characterized by a higher crystallinity than LDPE due to LDPE high branching degree preventing for the polymer chains to be arranged with each other (Li *et al.*, 2019). POPs will tend to sorb to the amorphous region of the polymer due to its higher free volume, providing a higher mobility for the pollutant to diffuse (Rodrigues *et al.*, 2019). As a results, high crystallin polymers such as PS will have a lower sorption capacity than PE or PP which possess more amorphous regions (Rodrigues *et al.*, 2019).

An amorphous region can be characterized as glassy, when the chains are more condensed and cross-linked (e.g. PS and PVC), or rubbery, when there is a higher diffusivity, permeability and free volume between chains (e.g. PE and PP) (Figure 1.7) (Rodrigues *et al.*, 2019). This state depends on the glass transition temperature (T_g) of the polymer, namely the temperature at which the polymer will transition from a glassy rigid state to a rubbery state (Mills, Jenkins and Kukureka, 2020). For instance, PE and PP are defined as rubbery polymers

Chapter 1 – Introduction

because of their low T_g ($< 0^\circ\text{C}$) while PS and PVC, exhibiting higher T_g values ($> 80^\circ\text{C}$) are defined as glassy polymers (Joseph P, 2021). Hüffer et al. (2016) found that absorption was the primary mode of sorption for rubbery polymers due to the higher free volume within the amorphous region, facilitating greater chemical diffusibility. In contrast, adsorption is considered as the dominant mode of sorption for glassy polymers which are more compact and only allow interaction at their surface (Hüffer and Hofmann, 2016). In PS, adsorption is also influenced by the monomeric composition of the polymer allows it to form $\Pi - \Pi$ interactions with the sorbate (Hüffer and Hofmann, 2016)

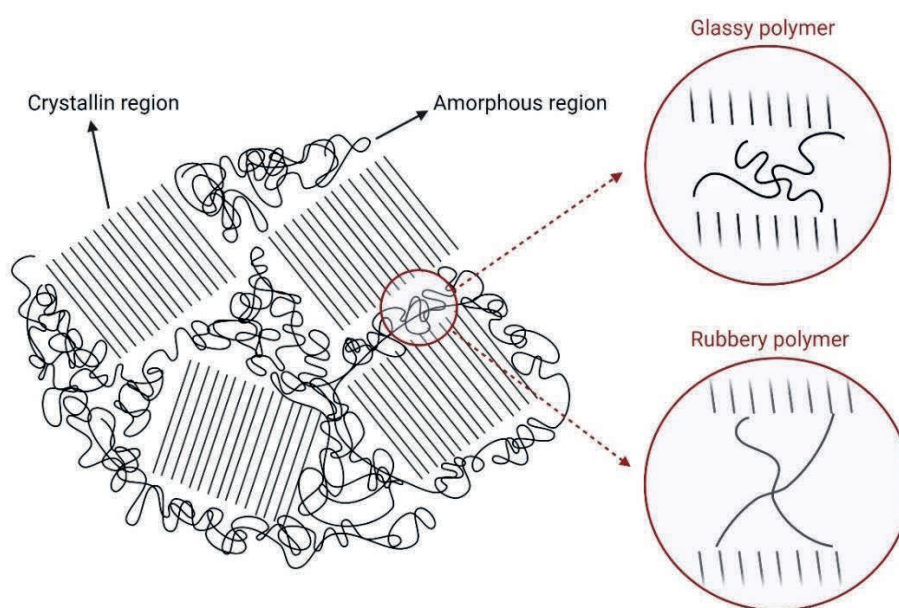


Figure 1.7. Illustration of a polymer crystallin and amorphous region [adapted from Li et al. (2019)]. Created with BioRender.com.

The size of the plastic will also highly influence its ability to concentrate large amounts of contaminants at its surface. Microplastics are known for their

Chapter 1 – Introduction

high adsorption capacity because of their higher surface/volume ratio (Cui *et al.*, 2023; Rafa *et al.*, 2024). Weathering and environmental conditions also influence the sorption process, for example, the formation of cracks within the plastic matrix will increase the surface area, leading to a higher sorption capacity (Rodrigues *et al.*, 2019; Rafa *et al.*, 2024).

The tendency of POPs to experience sorption or desorption from plastic will depend on the environment. In theory, chemicals will tend to distribute from a more concentrated environment to one with lower concentration (Rodrigues *et al.*, 2019). However, this distribution will also be highly influenced by the chemical properties and the environmental conditions, as migration will also be dictated by the chemical's affinity for the surrounding medium or matrix. POPs sorption at the surface of plastic will mainly occur in water, as their affinity for plastic is higher than for water (Rochman *et al.*, 2013; Lee, Shim and Kwon, 2014; Abihssira-García *et al.*, 2022). However, in a lipidic environment, their behavior will invert and a desorption process will likely be observed. For example, Nor *et al.* (2024) found that PCBs, initially sorbed onto PE's surface, were desorbed in a lipid-rich digestive solution. The process was reversed, following digestive breakdown of lipids (Mohamed Nor *et al.*, 2023).

1.3. Impact of plastic pollution on marine wildlife

1.3.1. Physical impact: entanglement and ingestion

Ocean plastic debris has a profound impact on marine wildlife, influencing a spectrum of organisms from plankton, corals, and crustaceans to more sizable inhabitants like turtles, seabirds, seals, and whales. Whether existing in the microscopic realm as microplastics or in larger forms known as mesoplastics, marine debris wields a versatile range of physical effects on oceanic life, encompassing entanglement and ingestion. Entanglement, primarily caused by lost fishing nets and lines, has been documented across 354 marine species and plastic ingestion has been recorded in 701 marine species (Kühn and van Franeker, 2020). The first documentation of plastic ingestion dates back to 1962 with a leach's storm Petrel (Rothstein, 1973). Today, plastic ingestion has been documented to affect 363 species of fish, 180 species of seabirds, 82 species of invertebrate, 69 species of marine mammals (e.g. polar bears, seals, whales, etc.) and 7 species of turtles (Kühn and van Franeker, 2020).

Animals ingest plastic through three main pathways: (1) mistaking plastics for prey, (2) direct ingestion while feeding and (3) ingestion of contaminated prey (Ryan, 2019). Sea turtles are the most commonly known species to mistaken plastics for prey (Ryan, 2019). Plastic debris commonly found in sea turtle digestive tracts are flexible and transparent plastics, presumably because they look alike jellyfish and other gelatinous zooplankton (Moon *et al.*, 2022; Noh *et al.*, 2024).

Chapter 1 – Introduction

The accidental ingestion of plastic together with seawater during prey consumption can occur at all levels of the food chain. Filter feeders, especially those at the base of the food chain like plankton and small crustaceans are particularly susceptible to the intake of microplastics while filtering water for their food (Setälä, Fleming-Lehtinen and Lehtiniemi, 2014). Larger marine organisms such as seabirds and marine mammals are also susceptible to unintentional ingestion of plastic while feeding. For example, most seabird will experience plastic ingestion while trying to catch prey at the surface of water, as most marine plastic debris float at the surface of the ocean (Ryan, 2019; Arat, 2024). In some case, plastic will also be directly associated with prey species. For example, *Phoebastria* albatrosses often eat masses of flying fish eggs that attach to floating debris (Fry, Fefer and Sileo, 1987). Large pieces of plastics such as those found in seabirds, sea turtles and marine mammals are usually the result of direct ingestion.

The ingestion of plastic can, in some cases, lead to obstruction or perforation of the digestive tract, particularly when dealing with larger plastic items (Ryan, 2019). It can also lead to a reduction of the appetite which may eventually lead to death (Pierce *et al.*, 2004; Roman *et al.*, 2021). Plastic ingested by organism on the bottom of the food chain can also be transferred to higher trophic level of individuals, such as via the ingestion of contaminated plankton, mollusks and fish (Miller, Hamann and Kroon, 2020).

1.3.2. Chemical impact of plastic ingestion

In addition to physical damage (e.g., digestive tract perforation and obstruction), plastic ingestion can result in physiological exposure to microplastics and toxic plastic-associated chemicals.

1.3.2.1. Microplastics

Following ingestion, microplastics pass through the digestive tract and are transferred into the intestine where they can either be excreted (Schwabl *et al.*, 2019) or, if small enough, be absorbed by the intestinal barrier and circulate in the organism where they can translocate into other tissues and organs (Huang *et al.*, 2021). Microplastics have been detected in almost every part of the human body including gastrointestinal tract, blood, liver, lungs, kidneys, lymphatic system, reproductive system, placenta and spleen (Li *et al.*, 2023). They have also been detected in the tissue of marine organisms such as mollusks (Expósito *et al.*, 2022), fish (Hossain *et al.*, 2023) and seabirds (Charlton-Howard *et al.*, 2023).

Microplastic can exert toxicity on the organism through its physical presence (i.e., plastic itself) or by acting as a carrier for toxic chemicals (e.g., additives) (Li *et al.*, 2023). At a physical level, microplastics can be internalized by the cell through processes such as endocytosis and passive diffusion (Liu *et al.*, 2021) which can lead to membrane damage (Wang *et al.*, 2022), oxidative stress (Kadac-czapska and Knez, 2024) and immune responses such as inflammation (Yang *et al.*, 2022; Xu *et al.*, 2023).

1.3.2.2. Leaching of plastic-associated chemicals

Once ingested, plastics are subjected to a variety of conditions in the digestive tract, including higher temperatures, acids, enzymes and food, which can impact plastic integrity, leading to their fragmentation into microplastics, while facilitating the release of plastic-associated compounds (monomers, additives, sorbed POPs). In humans, the migration of additives such as phthalates and bisphenol A from plastic toys has been demonstrated at the initial stage of

Chapter 1 – Introduction

the digestive process, in the presence of saliva (Noguerol-Cal *et al.*, 2011; Souza *et al.*, 2022). The desorption of POPs from microplastics has been demonstrated to be enhanced under gut conditions (bile salts with proteins and seawater) (Bakir, Rowland and Thompson, 2014; Mohamed Nor and Koelmans, 2019). Coffin *et al.* also demonstrated the ability of acidic pepsin condition to enhance the leaching of BPA and DEHP from commercial plastics (Coffin *et al.*, 2019). Marine oil [i.e., oil extracted from marine animals (e.g., salmon oil, stomach oil collected from seabirds)] has also shown to facilitate the leaching of hydrophobic chemicals such as PBDE-209 from industrially prepared plastic (Tanaka *et al.*, 2015; Kühn *et al.*, 2020).

The presence of contaminants such as POPs in the environment adds complexity to establishing a direct link between plastic-associated chemicals in the tissue of marine organisms and plastic ingestion. Studies suggest that the intake of POPs through plastic ingestion is negligible compared to the amount of POPs marine organisms are exposed to via prey consumption (Herzke *et al.*, 2016). On the other hand, the potential risk posed by additives is noteworthy, considering their significantly higher concentrations within the plastic bulk compared to POPs adsorbed to the surface (Barrick *et al.*, 2021). Jang *et al.* (2024) found a difference in concentrations between plastic additives (phthalates, UV stabilizers, antioxidants and BPA) and conventional sorbed POPs [PCBs, DDTs, hexachlorobenzene (HCB), etc.] in fishing gear with differences of up to 100,000 times greater for additives than for POPs in the analyzed samples. PBDEs and non-brominated flame retardants were also quantified at high concentrations in a few samples (Jang *et al.*, 2024), their presence possibly resulting both from their incorporation as an additive within the matrix as well as from environmental adsorption. It is important to keep in

Chapter 1 – Introduction

mind that part of the additives may also be released from plastic debris in seawater, exposing individuals to smaller amount than what was initially present, although these concentrations may still remain higher than those of POPs (Jang *et al.*, 2024).

Some chemicals, like phthalates and bisphenols, are mostly found in plastic as additives and/or residual monomers, distinguishing them from POPs, such as PCBs, DDTs, or PAHs, that sorb on plastic within the environment (Bridson *et al.*, 2021). Because phthalates are classified as non-persistent, meaning that they do not bioaccumulate in marine organisms and biomagnify up the food chain, their presence in the body indicates recent exposure to plastics (seawater or direct plastic ingestion). Sambolino *et. al* (2023) observed a positive correlation between the level of diisobutyl phthalate (DIBP) measured in the tissue of pelagic squid and fish and the abundance of microplastics found in their digestive tract (Sambolino *et al.*, 2023). High concentrations of PBDE-209 and UV-stabilizers have also been detected in seabird species having high levels of plastics in their stomach (Yamashita *et al.*, 2021). Furthermore, an *in vivo* feeding experiment with seabird chicks showed the accumulation of additives (UV-stabilizers and PBDE-209) from plastic pellets in their adipose tissue and muscles, providing evidence of additive transfer from plastic to seabird tissues (Tanaka *et al.*, 2020). Tanaka *et al.* (2013) also found a correlation between the presence of PBDE-209 in the tissue of northern fulmar (*Fulmarus glacialis*) and plastic ingestion due to the absence of this PBDE congener in the bird's prey (Tanaka *et al.*, 2013). Another study suggests that the presence of PBDE-209 in fulmar tissues is caused by plastic ingestion, as it was only detected in individuals that had ingested plastics (Neumann *et al.*, 2021).

Chapter 1 – Introduction

While the plastic-associated chemicals represent only a fraction of the total exposure of marine organisms to various substances, it does not preclude a potential impact on the organism. Additionally, the cumulative effect of these chemicals, added to existing exposure from other sources, may result in increased overall exposure, potentially leading to distinct effects (Barrick *et al.*, 2021; Pinto da Costa *et al.*, 2023).

1.3.2.3. Endocrine disruption

Most plastic-associated chemicals are known as endocrine disruptors (EDCs). The European Chemical Agency (ECHA) defines EDCs as chemicals that may interfere with the hormonal system and thereby produce harmful effects in both humans and wildlife. The endocrine system is based on the secretion of hormones by endocrine glands and organs, such as the pituitary gland, thyroid gland, adrenal glands, pancreas, ovaries, and testes (Greathouse and Walker, 2010). These hormones are then transported through the bloodstream to target organs, where they induce responses by binding to specific hormone receptors (Greathouse and Walker, 2010). For example, the secretion of 17 β -estradiol by ovaries is responsible for the development of secondary sexual characteristics (Alva-Gallegos, Carazo and Mladěnka, 2023).

EDCs can disrupt the hormonal system by interfering with ligand-binding (activate or inhibit the hormone receptor), hormone biosynthesis/degradation, hormone transport and receptor synthesis (Combarnous and Diep Nguyen, 2019). Additionally, EDCs can affect hormone regulation, altering the feedback mechanisms that control hormone levels and maintain homeostasis. One of the most-well known mechanisms by which EDCs act is via binding to hormone receptors. Hormone receptors can be located on the plasma membrane, where they primarily bind hydrophilic ligands that cannot

Chapter 1 – Introduction

cross the plasma membrane (e.g., catecholamines, glycoproteins, insulin), or they can be located inside the cell (cytoplasm or nucleus), where they bind hydrophobic ligands that can diffuse through the plasma membrane (e.g., estrogen, androgen, thyroid hormones) (Greathouse and Walker, 2010).

Hydrophobic EDCs readily cross the cell membrane and commonly bind to nuclear hormone receptors (NHRs), a family of ligand-regulated transcription factors activated by steroid hormones and other lipid-soluble ligands (Sever and Glass, 2013). NHRs can be classified into four types depending on their mode of action and ligand binding. Type I receptors, also called “steroid receptors” [e.g. estrogen receptor (ER), androgen receptor (AR) and progesterone receptor (PR)] are located in the cytoplasm under their non-activated form, often anchored with a chaperone protein such as heat shock protein 90 (HSP90) (Figure 1.8). After ligand binding, they form a homodimer and translocate into the nucleus where they bind to a hormone-response element (HRE) within the promoter of a target gene, controlling different pathways such as cell proliferation, development, metabolism, and reproduction (Greathouse and Walker, 2010; Sever and Glass, 2013; Weikum, Liu and Ortlund, 2018) (Figure 1.4).

Type II receptors, also called “non-steroid receptors” bind to non-steroidal ligands [e.g. thyroid receptor (TR), peroxisome proliferator activated receptor γ (PPAR γ), retinoic acid receptor (RAR) and retinoid X receptor (RXR)]. Non-steroid receptors are primarily located in the nucleus of the cell regardless of the presence of a ligand (Figure 1.8) (Weikum, Liu and Ortlund, 2018). Under their non-activated form, they bound to specific DNA-response elements, usually as heterodimers, and interact with corepressor proteins. After ligand-binding, co-repressors are exchanged with coactivator complexes promoting the transcription of target genes (Weikum, Liu and Ortlund, 2018).

Chapter 1 – Introduction

Type II NHRs can also be found in the cytoplasm of certain cell types where they play a role in the cross-talk with other transduction pathways. For example, RXR can also interact with signaling pathways such as PI3K/Akt which plays a role in suppressing cancer cell growth (Zhang *et al.*, 2018). Type III receptors, also called “orphan receptors” do not have a well-defined endogenous ligand [e.g., estrogen-related receptors (ERR)]. They have the same mechanism of action as type I receptors, except that the HRE is organized differently. Type IV receptors bind as monomers to hormone-response elements (Sever and Glass, 2013).

EDCs can also disrupt the hormonal system by binding to membrane hormone receptors (MHRs). Unlike NHRs, which directly interact with DNA to modulate gene expression, MHRs typically trigger short-term, non-genomic responses within cells (Combarnous and Nguyen, 2022). For example, the membrane estrogen receptor GPR30 is a known target for EDCs such as BPA, whose binding has been shown to trigger spermatocyte apoptosis in male rats (Wang *et al.*, 2017).

EDCs can function as agonists, mimicking the activity of endogenous hormones to activate receptors, or as antagonists, inhibiting receptor activity (Combarnous and Diep Nguyen, 2019). Agonists can induce a partial response of the receptors compared to the maximal biological response induced by endogenous ligands (Autrup *et al.*, 2020).

Chapter 1 – Introduction

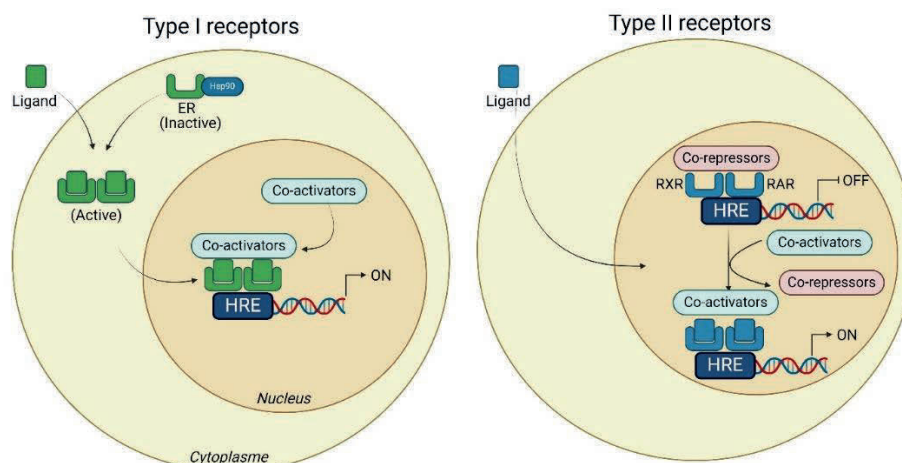


Figure 1.8. Mechanism of action of type I and type II nuclear hormone receptors. ER, estrogen receptor; HRE, hormone responsive elements; RXR, retinoid X receptor; RAR, retinoid acid receptor (adapted from Weikum et al. 2018 and Sever and Glass, 2013). Created with Biorender.com

One of the major class of EDCs are xenoestrogens, which are defined as substances able to bind to estrogen receptors and activate or prevent the expression of estrogen-responsive genes (Alva-Gallegos, Carazo and Mladěnka, 2023). There exist two subtypes of nuclear estrogen receptors (ER): ER α , located in the adipocytes, uterus, pancreatic β -cells, prostate and ER β , found mainly in the colon, prostate epithelium, granulosa cells in the ovaries, bone marrow, adipocytes, and some brain regions (Alva-Gallegos, Carazo and Mladěnka, 2023). The principal ligands of ERs are endogenous estrogen hormones including estradiol (E2) – produced throughout the individual’s life at varying concentrations depending on the sex and age – estrone (E1) and estriol (E3) – produced during menopause and fetal life (Alva-Gallegos, Carazo and Mladěnka, 2023).

Chapter 1 – Introduction

In mammals, E2 regulates the menstrual cycle, promotes the growth of the uterine lining, supports the maturation of eggs in the ovaries and promotes mammary development. In males, E2 plays an important role in spermatogenesis and other sexual functions (Amenyogbe *et al.*, 2020). In birds, E2 is primarily responsible for the development of the reproductive tract in females, and plays a role in the sexual behavior of both male and females (e.g., activation of copulation) (Balthazart, Arnold and Adkins-Regan, 2017). Additionally, E2 affects various processes in different parts of the body, including the liver, adipocytes, muscles, bone marrow, and brain (Alva-Gallegos, Carazo and Mladěnka, 2023).

In vivo exposure to xenoestrogens has shown to negatively impact both male and female reproductive system and development at many levels. BPA is one of the most well-known plastic-associated xenoestrogens, acting as an agonist of ERs (Paris *et al.*, 2002; Rochester and Bolden, 2015; Molina-Molina *et al.*, 2019; Alva-Gallegos, Carazo and Mladěnka, 2023). Human exposure to BPA has been associated with testicular dysgenesis syndrome, decreased anogenital distance in boys whose mothers were exposed to BPA during pregnancy, decreased sperm concentration, increased risk of miscarriage, offspring gonadal abnormalities and cancers (Marlatt *et al.*, 2022). BPA was also shown to disrupt gonadal development, spermatogenesis, testis maturation in fish (Leet *et al.*, 2015).

Phthalates also have the ability to bind ERs and activate or inhibit them, resulting in adverse outcomes such as reduction of testosterone and sperm production, fertility impairment, perturbation in estradiol levels and gonad malformation to name a few (Amir *et al.*, 2021). Other EDCs able to bind nuclear estrogen receptors include PCBs, DDTs and its metabolites, PBDEs, TBBPA

Chapter 1 – Introduction

and HBCDD (Plíšková *et al.*, 2005; Hamers *et al.*, 2006; Kojima *et al.*, 2009; Sunday *et al.*, 2022), which have also been associated with toxic effects on male and female reproductive system such as reproductive diseases, lower pregnancy rate, miscarriage and sperm abnormalities (Meeker and Hauser, 2010; Arowolo *et al.*, 2022; Wu *et al.*, 2024).

1.3.3. Risks associated with plastic ingestion in seabirds with a focus on Procellariiformes

1.3.3.1. Plastic ingestion

Seabirds are among the most studied marine species and among the most affected by plastic pollution. The number of seabird species documented with plastic in their stomach keeps increasing, with 180 out of 409 species (44%) documented in 2019 compared to 164 species (40%) in 2015 (Kühn and van Franeker, 2020). If we relate these figures to the number of seabird species studied, 80% seabird species have been found with plastic in their stomach, with more than a quarter of all individuals affected (12,065 individuals, 27.7%) (Kühn and van Franeker, 2020).

Procellariiform seabirds, which include petrels, albatrosses, and shearwaters, represent the largest taxon among seabirds, comprising 144 species. This taxa is among the most threatened groups of seabirds, with 25% of species listed as endangered and 19.6% as vulnerable on the International Union for Conservation of Nature red list of threatened species (*IUCN Red List of Threatened Species*, 2023). In addition to climate change, invasive species and fisheries bycatch, plastic ingestion is increasingly recognized as a significant threat to Procellariiformes. Indeed, this group displays the highest frequency of plastic occurrence, with 88% of the species and 41.5% of all individuals analyzed

Chapter 1 – Introduction

documented with plastic in their stomachs (Kühn and van Franeker, 2020). Examples of the most affected species are the Laysan albatross (*Phoebastria immutabilis*), northern fulmar (*Fulmarus glacialis*) and short-tailed shearwater (*Ardenna tenuirostris*) with 93%, 82% and 74% of the individuals studied having ingested plastic (Kühn and van Franeker, 2020).

The elevated prevalence of plastic ingestion is attributed, in part, to the foraging strategy of these birds, which predominantly feed at the ocean's surface. The majority of marine plastic debris is buoyant, increasing the risk of accidental ingestion during feeding or when mistaking plastic for prey (Ryan, 2019). In addition, unlike gulls which regurgitate indigestible items within a day, Procellariiformes only regurgitate to feed their chicks or as a defense mechanism (Ryan, 2019). Because of their gastric morphology (i.e. constriction between the two stomach chambers), only the content of the first gastric chamber (proventriculus) can be regurgitated, resulting in a higher propensity to accumulate plastics in their stomachs that are too large to be transferred into the rest of the digestive tract (Roman, Paterson, *et al.*, 2019) (Figure 1.9).

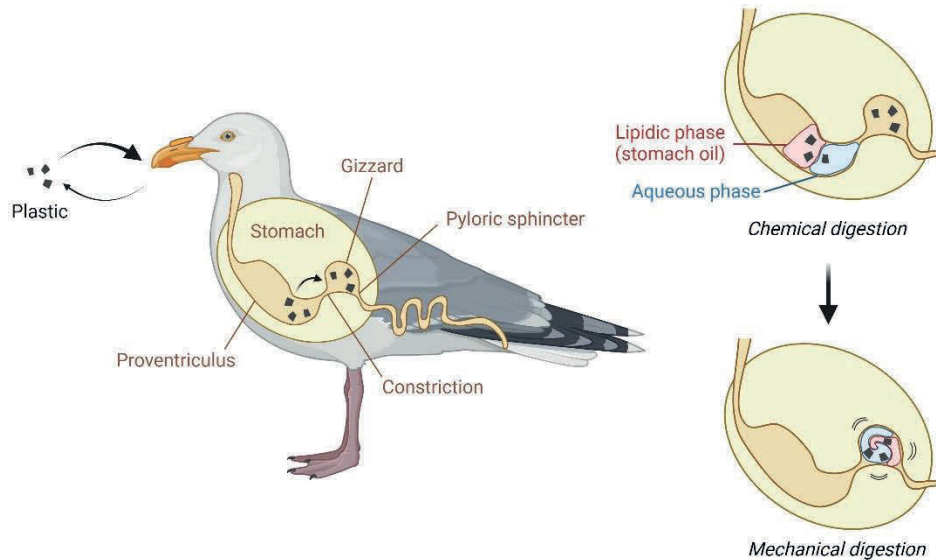


Figure 1.9. Procellariiform stomach morphology and digestion process (adapted from Tanaka et al. 2018). Created with Biorender.com.

The average number of plastic pieces measured within the stomach of Procellariiformes is 9.88 (Kühn and van Franeker, 2020). Based on the size of plastic debris found in the stomach of different Procellariiform species, Roman et al. (2019) estimate that plastic debris exceeding 2 mm in size are too large to exit the gizzard into the intestine and will likely be retained in the bird (Roman, Paterson, *et al.*, 2019). It is estimated that plastic pieces can remain in a Procellariiform stomach for weeks to months or even years, depending on the size of the ingested debris (van Franeker *et al.*, 2011; Nania and Shugart, 2021).

1.3.3.2. Gastric models to study the leaching of chemicals from plastics

Once retained in the stomach of a Procellariiform, plastic will be continuously exposed to chemical and mechanical stresses. A seabird stomach is divided in two chambers: the proventriculus, where the chemical digestion takes place and the gizzard, where the mechanical digestion occurs (Figure 1.9). In the proventriculus, enzymes and acid are secreted for the digestion of prey. Unlike other birds, Procellariiformes do not secrete lipase in their stomach, resulting in the formation of two phases in the first chamber: an upper lipidic phase called “stomach oil”, and a lower aqueous phase (Figure 1.9). Stomach oil can be transferred to chicks as a source of energy or regurgitated on predators as a defense mechanism (rare) (Warham, 1977). After chemical digestion, the content of the proventriculus is emptied into the gizzard, with the aqueous phase being transferred first, followed by the lipidic phase (Figure 1.9) (Tanaka, Yamashita and Takada, 2018).

In vitro models have been developed to understand the influence of gastric fluids on the leaching of chemicals from plastic trapped in a Procellariiform stomach. Industrial salmon oil and stomach oil collected from streaked shearwaters (*Calonectris leucomelas*) have shown to promote the leaching of PBDE-209 present as an additive from HDPE (Tanaka *et al.*, 2015). Kuhn *et al.* (2020) also observed the leaching of several plastic additives such as DEHP (plasticizer), bumetrizole (UV-stabilizer), dibutyl phenol (antioxidant) and TCEP (flame retardant) from a marine plastic debris leached in northern fulmar stomach oil (Kühn *et al.*, 2020). Gastric secretions (i.e., acidic pepsin solution) enhanced the leaching of BPA and DEHP from shopping bags, strings and polystyrene foam (Coffin *et al.*, 2019). Simulated gut fluids (bile salts sometimes

Chapter 1 – Introduction

in the presence of albumin) have also induced the desorption of POPs previously sorbed at the surface of LDPE, PVC and ABS (Bakir, Rowland and Thompson, 2014; Guo *et al.*, 2019; Mohamed Nor and Koelmans, 2019).

The digestive models used in the prior studies strongly suggest that plastics leach/desorb additives and contaminants into a Procellariiform stomach following ingestion. However, *in vitro* gastric models have not assessed both the aqueous and oil components of digestion to evaluate the potential leaching of plastic-associated chemicals in a Procellariiform stomach. Gastric components (lipids, acids, enzymes and proteins) may act together in different ways depending on the properties of the plastic-associated chemical. Moreover, the leaching of sorbed POPs together with plastic additives has never been tested, which is crucial to understand the potential difference between the risks associated with the leaching of each type of chemical, since additives are present at significantly higher concentration than POPs and suspected to be able to leach in the environment for years (Barrick *et al.*, 2021). Finally, it is crucial to incorporate a temporal factor to understand the leaching behavior of plastic-associated chemicals over time, as plastics can remain trapped in the bird's stomach for extended periods. Daily monitoring of chemical leaching over several days has not yet been fully explored, and is essential to accurately assess the long-term exposure risks. Accordingly, a more complete gastric model could be developed by combining gastric components together, testing the simultaneous leaching of sorbed POPs and plastic additives, and including a temporal factor.

Chapter 1 – Introduction

1.3.3.3. Risks associated with plastic ingestion

Once released in digestive fluids, plastic-associated chemicals can be absorbed by the intestinal barrier and circulate in the organism. Although Tanaka *et al.* (2019) identified specific additives such as PBDE-209, HBCDD, and UV-stabilizers in plastic retrieved from the stomachs of northern fulmars, the overall impact of the complete chemical mixture potentially released in a Procellariiform digestive tract is poorly understood (Tanaka *et al.*, 2019). Three studies on quails revealed symptoms of endocrine disruption following plastic ingestion including an increased propensity for developing cysts in the epididymis, reduced estrogen levels in females and effects on lipid metabolism (Roman, Lowenstine, *et al.*, 2019; de Souza *et al.*, 2022; McCann Smith *et al.*, 2024). Moreover, chemicals such as phthalates have been detected in egg yolk, indicating maternal transfer of plastic-associated chemicals to offspring, which could alter embryonic development (Herzke *et al.*, 2005; Ottinger *et al.*, 2011; Allen *et al.*, 2021). These findings underline the importance of understanding the potential endocrine-disrupting effects associated with plastic ingestion, with a focus on the leached chemicals potentially responsible for these effects.

Since many chemicals associated with plastic have estrogenic activity, analyzing the estrogenic effects of plastic leachates is a logical and established method for investigating endocrine disruption caused by plastic exposure (Chun Z Yang *et al.*, 2011; Coffin *et al.*, 2019; Michishita *et al.*, 2023). Recently, a study found that leachates of microplastics from the stomach of common murre (*Uria aalge*) induced the activation of human estrogen receptors (Michishita *et al.*, 2023). However, the temporal release of chemicals and their ability to activate seabird ERs instead of human has never been considered. Since interspecific variations of ER sequences exist, likely resulting in differential

Chapter 1 – Introduction

responses to endocrine disrupting chemical exposure (Tubbs *et al.*, 2012), it would be essential to consider Procellariiform ERs to realistically assess the estrogenic risks of plastic ingestion in seabirds. This highlights the need for more comprehensive investigations into the ability of plastic-associated chemicals released from plastic ingested by Procellariiformes to disrupt their endocrine system through estrogen receptor binding.

References

- Abihssira-García, I. S. *et al.* (2022) ‘Distinct polymer-dependent sorption of persistent pollutants associated with Atlantic salmon farming to microplastics’, *Marine Pollution Bulletin*, 180(January). doi: 10.1016/j.marpolbul.2022.113794.
- Allen, S. F. *et al.* (2021) ‘Phthalate diversity in eggs and associations with oxidative stress in the European herring gull (*Larus argentatus*)’, *Marine Pollution Bulletin*, 169(May), p. 112564. doi: 10.1016/j.marpolbul.2021.112564.
- Alva-Gallegos, R., Carazo, A. and Mladěnka, P. (2023) ‘Toxicity overview of endocrine disrupting chemicals interacting in vitro with the oestrogen receptor’, *Environmental Toxicology and Pharmacology*, 99(February). doi: 10.1016/j.etap.2023.104089.
- Amenyogbe, E. *et al.* (2020) ‘A Review on Sex Steroid Hormone Estrogen Receptors in Mammals and Fish’, *International Journal of Endocrinology*, 2020. doi: 10.1155/2020/5386193.
- Amir, S. *et al.* (2021) ‘Endocrine disruptors acting on estrogen and androgen pathways cause reproductive disorders through multiple mechanisms: A review’, *International Journal of Environmental Research and Public Health*, 18(4), pp. 1–20. doi: 10.3390/ijerph18041464.
- Anagnosti, L. *et al.* (2021) ‘Worldwide actions against plastic pollution from microbeads and microplastics in cosmetics focusing on European policies. Has the issue been handled effectively?’, *Marine Pollution Bulletin*, 162(January),

Chapter 1 – Introduction

pp. 1–6. doi: 10.1016/j.marpolbul.2020.111883.

Arat, S. A. (2024) ‘An Overview of Microplastic in Marine Waters: Sources, Abundance, Characteristics and Negative Effects on Various Marine Organisms’, *Desalination and Water Treatment*, p. 100138. doi: 10.1016/j.dwt.2024.100138.

Arowolo, O. *et al.* (2022) ‘Mechanisms of Male Reproductive Toxicity of Polybrominated Diphenyl Ethers’, *International Journal of Molecular Sciences*, 23(22). doi: 10.3390/ijms232214229.

Ashraf, M. A. (2017) ‘Persistent organic pollutants (POPs): a global issue, a global challenge’, *Environmental Science and Pollution Research*, 24(5), pp. 4223–4227. doi: 10.1007/s11356-015-5225-9.

Autrup, H. *et al.* (2020) ‘Human exposure to synthetic endocrine disrupting chemicals (S-EDCs) is generally negligible as compared to natural compounds with higher or comparable endocrine activity: how to evaluate the risk of the S-EDCs?’, *Archives of Toxicology*, 94(7), pp. 2549–2557. doi: 10.1007/s00204-020-02800-8.

Bajagain, R. *et al.* (2023) ‘Determination of partition coefficients of phthalic acid esters between polydimethylsiloxane and water and its field application to surface waters’, *Journal of Hazardous Materials*, 448(February), p. 130933. doi: 10.1016/j.jhazmat.2023.130933.

Bakir, A., Rowland, S. J. and Thompson, R. C. (2014) ‘Enhanced desorption of persistent organic pollutants from microplastics under simulated physiological conditions’, *Environmental Pollution*, 185, pp. 16–23. doi: 10.1016/j.envpol.2013.10.007.

Chapter 1 – Introduction

Balthazart, J., Arnold, A. P. and Adkins-Regan, E. (2017) ‘Sexual Differentiation of Brain and Behavior in Birds’, in *Hormones, Brain and Behavior*. Third Edit. Elsevier, pp. 185–224. doi: 10.1016/b978-0-12-803592-4.00101-2.

Baranenko, D. *et al.* (2022) ‘Translocation of Phthalates From Food Packaging Materials Into Minced Beef’, *Frontiers in Nutrition*, 8(January), pp. 1–8. doi: 10.3389/fnut.2021.813553.

Barboza, L. G. A. *et al.* (2020) ‘Bisphenol A and its analogs in muscle and liver of fish from the North East Atlantic Ocean in relation to microplastic contamination. Exposure and risk to human consumers’, *Journal of Hazardous Materials*, 393(December 2019), p. 122419. doi: 10.1016/j.jhazmat.2020.122419.

Barrick, A. *et al.* (2021) ‘Plastic additives: Challenges in ecotox hazard assessment’, *PeerJ*, 9, pp. 1–26. doi: 10.7717/peerj.11300.

Bi, R., Meng, W. and Su, G. (2023) ‘Organophosphate esters (OPEs) in plastic food packaging: non-target recognition, and migration behavior assessment’, *Environment International*, 177(May), p. 108010. doi: 10.1016/j.envint.2023.108010.

Boucher, J. and Friot, D. (2017) *Primary Microplastics in the Oceans: A Global Evaluation of Sources*. Gland, Switzerland: IUCN. doi: dx.doi.org/10.2305/IUCN.CH.2017.01.en Julien.

Bouhroum, R. *et al.* (2019) ‘Concentrations and fingerprints of PAHs and PCBs adsorbed onto marine plastic debris from the Indonesian Cilacap coast and the North Atlantic gyre’, *Regional Studies in Marine Science*, 29, p. 100611. doi:

Chapter 1 – Introduction

10.1016/j.rsma.2019.100611.

Bridson, J. H. *et al.* (2021) ‘Leaching and extraction of additives from plastic pollution to inform environmental risk: A multidisciplinary review of analytical approaches’, *Journal of Hazardous Materials*, 414(February), p. 125571. doi: 10.1016/j.jhazmat.2021.125571.

Chamas, A. *et al.* (2020) ‘Degradation Rates of Plastics in the Environment’. doi: 10.1021/acssuschemeng.9b06635.

Charlton-Howard, H. S. *et al.* (2023) “‘Plasticosis’: Characterising macro- and microplastic-associated fibrosis in seabird tissues’, *Journal of Hazardous Materials*, 450(January), p. 131090. doi: 10.1016/j.jhazmat.2023.131090.

Chen, H. *et al.* (2024) ‘Monitoring, fate and transport, and risk assessment of organic pollutants in the environment: CREST publications during 2019–2023’, *Critical Reviews in Environmental Science and Technology*, 54(1), pp. 1–12. doi: 10.1080/10643389.2023.2273603.

Cheng, H. *et al.* (2020) ‘Release kinetics as a key linkage between the occurrence of flame retardants in microplastics and their risk to the environment and ecosystem: A critical review’, *Water Research*, 185, p. 116253. doi: 10.1016/j.watres.2020.116253.

Coffin, S. *et al.* (2019) ‘Fish and Seabird Gut Conditions Enhance Desorption of Estrogenic Chemicals from Commonly-Ingested Plastic Items’, *Environmental Science and Technology*, 53(8), pp. 4588–4599. doi: 10.1021/acs.est.8b07140.

Combarrous, Y. and Diep Nguyen, T. M. (2019) ‘Comparative overview of the mechanisms of action of hormones and endocrine disruptor compounds’, *Toxics*,

Chapter 1 – Introduction

7(1), pp. 1–11. doi: 10.3390/toxics7010005.

Combarnous, Y. and Nguyen, T. M. D. (2022) ‘Membrane Hormone Receptors and Their Signaling Pathways as Targets for Endocrine Disruptors’, *Journal of Xenobiotics*, 12(2), pp. 64–73. doi: 10.3390/jox12020007.

Cui, W. *et al.* (2023) ‘Sorption of representative organic contaminants on microplastics: Effects of chemical physicochemical properties, particle size, and biofilm presence’, *Ecotoxicology and Environmental Safety*, 251(September 2022), p. 114533. doi: 10.1016/j.ecoenv.2023.114533.

Czarny-Krzymińska, K., Krawczyk, B. and Szczukocki, D. (2023) ‘Bisphenol A and its substitutes in the aquatic environment: Occurrence and toxicity assessment’, *Chemosphere*, 315(July 2022). doi: 10.1016/j.chemosphere.2023.137763.

Czogała, J., Pankalla, E. and Turczyn, R. (2021) ‘Recent Attempts in the Design of Efficient PVC Plasticizers with Reduced Migration’, *Materials*, 14(844).

Dimassi, S. N. *et al.* (2023) ‘Effect of temperature and sunlight on the leachability potential of BPA and phthalates from plastic litter under marine conditions’, *Science of the Total Environment*, 894(May), p. 164954. doi: 10.1016/j.scitotenv.2023.164954.

ECHA (2021) *Assessment of regulatory needs. Group Name: Tetrabromobisphenol A (TBBPA) and its derivatives.*

ECHA (2022) *ANNEX XV REPORT AN ASSESSMENT OF WHETHER THE USE OF TEN PHTHALATES IN ARTICLES SHOULD BE RESTRICTED IN ACCORDANCE WITH ARTICLE 69(2) OF REACH.*

Chapter 1 – Introduction

ECHA (2023) *Regulatory strategy for flame retardants*. doi: 10.2823/854233.

ETRMA (2011) 'European Tyre & Rubber Industry. Statistics. Edition 2011. no3.'

Expósito, N. *et al.* (2022) 'Levels of microplastics and their characteristics in molluscs from North-West Mediterranean Sea: Human intake', *Marine Pollution Bulletin*, 181(May). doi: 10.1016/j.marpolbul.2022.113843.

FAO/ICAC (2013) 'Summary of the World Apparel Fiber Consumption Survey - Update 2013.'

van Franeker, J. A. *et al.* (2011) 'Monitoring plastic ingestion by the northern fulmar *Fulmarus glacialis* in the North Sea', *Environmental Pollution*, 159. doi: 10.1016/j.envpol.2011.06.008.

Fry, M. D., Fefer, S. I. and Sileo, L. (1987) 'Ingestion of Plastic Debris by Laysan Albatrosses and Wedge-tailed Shearwaters in the Hawaiian Islands', *Marine Pollution Bulletin*, 18(61), pp. 339–343.

Gaylarde, C. C. *et al.* (2023) 'The Importance of Biofilms on Microplastic Particles in Their Sinking Behavior and the Transfer of Invasive Organisms between Ecosystems', *Micro*, 3(1), pp. 320–337. doi: 10.3390/micro3010022.

Geyer, R., Jambeck, J. R. and Law, K. L. (2017) 'Production, use, and fate of all plastics ever made', *Science Advances*, 3(7), pp. 25–29.

Greathouse, K. L. and Walker, C. L. (2010) 'Mechanisms of endocrine disruption', in *Environmental Impacts on Reproductive Health and Fertility*. Cambridge University Press, pp. 72–91. doi: 10.1017/CBO9780511674686.007.

Chapter 1 – Introduction

Guo, H. *et al.* (2019) ‘The leaching of additive-derived flame retardants (FRs) from plastics in avian digestive fluids: The significant risk of highly lipophilic FRs’, *Journal of Environmental Sciences (China)*, 85, pp. 200–207. doi: 10.1016/j.jes.2019.06.013.

Hahladakis, J. N. *et al.* (2018) ‘An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling’, *Journal of Hazardous Materials*, 344, pp. 179–199. doi: 10.1016/j.jhazmat.2017.10.014.

Hamers, T. *et al.* (2006) ‘In vitro profiling of the endocrine-disrupting potency of brominated flame retardants’, *Toxicological Sciences*, 92(1), pp. 157–173. doi: 10.1093/toxsci/kfj187.

Hermabessiere, L. *et al.* (2017) ‘Occurrence and effects of plastic additives on marine environments and organisms: A review’, *Chemosphere*, 182, pp. 781–793. doi: 10.1016/j.chemosphere.2017.05.096.

Herzke, D. *et al.* (2005) ‘Brominated flame retardants and other organobromines in Norwegian predatory bird eggs’, *Chemosphere*, 61(3), pp. 441–449. doi: 10.1016/j.chemosphere.2005.01.066.

Herzke, D. *et al.* (2016) ‘Negligible Impact of Ingested Microplastics on Tissue Concentrations of Persistent Organic Pollutants in Northern Fulmars off Coastal Norway’, *Environmental Science and Technology*, 50(4), pp. 1924–1933. doi: 10.1021/acs.est.5b04663.

Hossain, M. B. *et al.* (2023) ‘Microplastics in Different Tissues of a Commonly Consumed Fish, *Scomberomorus guttatus*, from a Large Subtropical Estuary: Accumulation, Characterization, and Contamination Assessment’, *Biology*,

Chapter 1 – Introduction

12(11). doi: 10.3390/biology12111422.

Huang, Z. *et al.* (2021) ‘Microplastic: A potential threat to human and animal health by interfering with the intestinal barrier function and changing the intestinal microenvironment’, *Science of the Total Environment*, 785, p. 147365. doi: 10.1016/j.scitotenv.2021.147365.

Hüffer, T. and Hofmann, T. (2016) ‘Sorption of non-polar organic compounds by micro-sized plastic particles in aqueous solution’, *Environmental Pollution*, 214, pp. 194–201. doi: 10.1016/j.envpol.2016.04.018.

Ilyas, M. *et al.* (2013) ‘Characterization of polychlorinated biphenyls and brominated flame retardants in sludge, sediment and fish from municipal dumpsite at Surabaya, Indonesia’, *Chemosphere*, 93(8), pp. 1500–1510. doi: 10.1016/j.chemosphere.2013.07.048.

IUCN Red List of Threatened Species (2023) *International Union for Conservation of Nature*. Available at: <https://www.iucnredlist.org/>.

Jang, M. *et al.* (2023) ‘Method to analyze phthalate esters from soft toys dissolving into water mimicking infant playing’, *Chemosphere*, 330(October 2022), p. 138695. doi: 10.1016/j.chemosphere.2023.138695.

Jang, M. *et al.* (2024) ‘Hazardous chemical additives within marine plastic debris and fishing gear: Occurrence and implications’, *Journal of Cleaner Production*, 442(January), p. 141115. doi: 10.1016/j.jclepro.2024.141115.

Joseph P, G. (2021) ‘3 - Microstructures of Polymers’, in *Automotive Plastics and Composites*, pp. 27–37.

Kadac-czapska, K. and Knez, E. (2024) ‘Microplastics and Oxidative Stress —

Chapter 1 – Introduction

Current Problems and Prospects’, *Antioxidant*, 13(579).

Kibria, M. G. *et al.* (2023) *Plastic Waste: Challenges and Opportunities to Mitigate Pollution and Effective Management*, *International Journal of Environmental Research*. Springer International Publishing. doi: 10.1007/s41742-023-00507-z.

Klun, B. *et al.* (2022) ‘The first plastic produced , but the latest studied in microplastics research : The assessment of leaching , ecotoxicity and bioadhesion of’, *Environmental Pollution*, 307(January). doi: 10.1016/j.envpol.2022.119454.

Kojima, H. *et al.* (2009) ‘Nuclear hormone receptor activity of polybrominated diphenyl ethers and their hydroxylated and methoxylated metabolites in transactivation assays using Chinese hamster ovary cells’, *Environmental Health Perspectives*, 117(8), pp. 1210–1218. doi: 10.1289/ehp.0900753.

Kühn, S. *et al.* (2020) ‘Transfer of Additive Chemicals From Marine Plastic Debris to the Stomach Oil of Northern Fulmars’, *Frontiers in Environmental Science*, 8. doi: 10.3389/fenvs.2020.00138.

Kühn, S. and van Franeker, J. A. (2020) ‘Quantitative overview of marine debris ingested by marine megafauna’, *Marine Pollution Bulletin*, 151(110858). doi: 10.1016/j.marpolbul.2019.110858.

Kumar, A. *et al.* (2023) ‘Bisphenol A in canned soft drinks, plastic-bottled water, and household water tank from Punjab, India’, *Journal of Hazardous Materials Advances*, 9(July 2022), p. 100205. doi: 10.1016/j.hazadv.2022.100205.

Lebreton, L. *et al.* (2018) ‘Evidence that the Great Pacific Garbage Patch is

Chapter 1 – Introduction

rapidly accumulating plastic’, *Scientific Reports*, 8(1), pp. 1–15. doi: 10.1038/s41598-018-22939-w.

Lebreton, L. *et al.* (2022) ‘Industrialised fishing nations largely contribute to floating plastic pollution in the North Pacific subtropical gyre’, *Scientific Reports*, 12(1), pp. 1–11. doi: 10.1038/s41598-022-16529-0.

Lee, Byoung cheun *et al.* (2021) ‘Occurrence of bisphenols and phthalates in indoor dust collected from Korean homes’, *Journal of Industrial and Engineering Chemistry*, 99, pp. 68–73. doi: 10.1016/j.jiec.2021.03.051.

Lee, H., Shim, W. J. and Kwon, J. H. (2014) ‘Sorption capacity of plastic debris for hydrophobic organic chemicals’, *Science of the Total Environment*, 470–471, pp. 1545–1552. doi: 10.1016/j.scitotenv.2013.08.023.

Lee, S. J. *et al.* (2022) ‘Reproductive and developmental toxicity screening of bisphenol F by oral gavage in rats’, *Regulatory Toxicology and Pharmacology*, 136(September), p. 105286. doi: 10.1016/j.yrtph.2022.105286.

Leet, J. K. *et al.* (2015) ‘Environmental hormones and their impacts on sex differentiation in fathead minnows’, *Aquatic Toxicology*, 158, pp. 98–107. doi: 10.1016/j.aquatox.2014.10.022.

Li, D. *et al.* (2019) ‘Effect of Crystallinity of Polyethylene with Different Densities on Breakdown Strength and Conductance Property’, *Materials*, 12(1746).

Li, L. *et al.* (2022) ‘What do we know about the production and release of persistent organic pollutants in the global environment?†’, *Environmental Science: Advances*, 2(1), pp. 55–68. doi: 10.1039/d2va00145d.

Chapter 1 – Introduction

Li, Y. *et al.* (2023) ‘Potential Health Impact of Microplastics: A Review of Environmental Distribution, Human Exposure, and Toxic Effects’, *Environment & Health*, 1(4), pp. 249–257. doi: 10.1021/envhealth.3c00052.

Li, Y. *et al.* (2024) ‘Leaching of chemicals from microplastics: A review of chemical types, leaching mechanisms and influencing factors’, *Science of the Total Environment*, 906(October 2023), p. 167666. doi: 10.1016/j.scitotenv.2023.167666.

Liu, L. *et al.* (2021) ‘Cellular internalization and release of polystyrene microplastics and nanoplastics’, *Science of the Total Environment*, 779, p. 146523. doi: 10.1016/j.scitotenv.2021.146523.

Liu, R. and Mabury, S. A. (2019) ‘Organophosphite Antioxidants in Indoor Dust Represent an Indirect Source of Organophosphate Esters’, *Environmental Science & Technology*, 53, p. 1805–1811. doi: 10.1021/acs.est.8b05545.

Luo, H. *et al.* (2014) ‘Evaluation of the Di(2-ethylhexyl)phthalate released from polyvinyl chloride medical devices that contact blood Hongyu.Pdf’, *SpringerPlus*, 3(58), pp. 1–8.

Luo, H. *et al.* (2022) ‘Effects of aging on environmental behavior of plastic additives: Migration, leaching, and ecotoxicity’, *Science of the Total Environment*, 849(July), p. 157951. doi: 10.1016/j.scitotenv.2022.157951.

Marlatt, V. L. *et al.* (2022) ‘Impacts of endocrine disrupting chemicals on reproduction in wildlife and humans’, *Environmental Research*, 208. doi: 10.1016/j.envres.2021.112584.

Mârşolea, A. C. *et al.* (2023) ‘Migration and leaching behaviour of Bisphenol A

Chapter 1 – Introduction

from polyethylene terephthalate water bottles under different storage conditions', *International Journal of Food Science and Technology*, 58(10), pp. 5609–5615. doi: 10.1111/ijfs.16583.

Marturano, V., Cerruti, P. and Ambrogi, V. (2017) 'Polymer additives', *Physical Sciences Reviews*, (20160130), pp. 1–20. doi: 10.1515/psr-2016-0130.

Mato, Y. *et al.* (2001) 'Plastic Resin Pellets as a Transport Medium for Toxic Chemicals in the Marine Environment', *Environmental Science and Technology*, 35(2), pp. 318–324.

McCann Smith, E. *et al.* (2024) 'Exposure to microplastics affects fatty acid composition in the Japanese quail depending on sex and particle size', *Science of the Total Environment*, 912(November 2023), p. 169019. doi: 10.1016/j.scitotenv.2023.169019.

Meeker, J. D. and Hauser, R. (2010) 'Exposure to polychlorinated biphenyls (PCBs) and male reproduction', *Systems Biology in Reproductive Medicine*, 56(2), pp. 122–131. doi: 10.3109/19396360903443658.

Meijer, L. J. J. *et al.* (2021) 'More than 1000 rivers account for 80% of global riverine plastic emissions into the ocean', *Science Advances*, 7(18), pp. 1–13. doi: 10.1126/sciadv.aaz5803.

Mercea, P. (2009) 'Physicochemical Processes Involved in Migration of Bisphenol A from Polycarbonate', *Journal of Applied Polymer Science*, 112, pp. 579–593. doi: 10.1002/app.

Metruccio, F. *et al.* (2024) 'Teratogenic and neuro-behavioural toxic effects of bisphenol A (BPA) and B (BPB) on *Xenopus laevis* development', *Reproductive*

Chapter 1 – Introduction

Toxicology, 123(November 2023), p. 108496. doi: 10.1016/j.reprotox.2023.108496.

Michishita, S. *et al.* (2023) ‘Microplastic in northern anchovies (*Engraulis mordax*) and common murrelets (*Uria lomvia*) from the Monterey Bay, California USA - Insights into prevalence, composition, and estrogenic activity’, *Environmental Pollution*, 316(P2), p. 120548. doi: 10.1016/j.envpol.2022.120548.

Miller, M. E., Hamann, M. and Kroon, F. J. (2020) ‘Bioaccumulation and biomagnification of microplastics in marine organisms: A review and meta-analysis of current data’, *PLoS ONE*, 15(10 October), pp. 1–25. doi: 10.1371/journal.pone.0240792.

Mills, N., Jenkins, M. and Kukureka, S. (2020) ‘Molecular structures and polymer manufacture’, in *Plastics*. Fourth Edi, pp. 13–31. doi: 10.1016/b978-0-08-102499-7.00002-3.

Mohamed Nor, N. H. *et al.* (2023) ‘How Digestive Processes Can Affect the Bioavailability of PCBs Associated with Microplastics: A Modeling Study Supported by Empirical Data’, *Environmental Science and Technology*, 57(31), pp. 11452–11464. doi: 10.1021/acs.est.3c02129.

Mohamed Nor, N. H. and Koelmans, A. A. (2019) ‘Transfer of PCBs from Microplastics under Simulated Gut Fluid Conditions Is Biphasic and Reversible’, *Environmental Science and Technology*. doi: 10.1021/acs.est.8b05143.

Molina-Molina, J. M. *et al.* (2019) ‘Determination of bisphenol A and bisphenol S concentrations and assessment of estrogen- and anti-androgen-like activities in

Chapter 1 – Introduction

thermal paper receipts from Brazil, France, and Spain’, *Environmental Research*, 170, pp. 406–415. doi: 10.1016/j.envres.2018.12.046.

Moon, Y. *et al.* (2022) ‘What type of plastic do sea turtles in Korean waters mainly ingest? Quantity, shape, color, size, polymer composition, and original usage’, *Environmental Pollution*, 298(January), p. 118849. doi: 10.1016/j.envpol.2022.118849.

Morales-Caselles, C. *et al.* (2021) ‘An inshore–offshore sorting system revealed from global classification of ocean litter’, *Nature Sustainability*, 4(6), pp. 484–493. doi: 10.1038/s41893-021-00720-8.

Nania, T. G. and Shugart, G. W. (2021) ‘Are plastic particles reduced in size in seabirds’ stomachs?’, *Marine Pollution Bulletin*, 172(June), p. 112843. doi: 10.1016/j.marpolbul.2021.112843.

Neumann, S. *et al.* (2021) ‘Ingested plastics in northern fulmars (*Fulmarus glacialis*): A pathway for polybrominated diphenyl ether (PBDE) exposure?’, *Science of the Total Environment*, 778, p. 146313. doi: 10.1016/j.scitotenv.2021.146313.

Nilsson, N. H. *et al.* (2016) ‘Determination of Migration Rates for Certain Phthalates: Survey of chemical substances in consumer products’, *The Danish Environmental Protection Agency*, (149), pp. 1–67. Available at: <https://www2.mst.dk/Udgiv/publications/2016/08/978-87-93529-01-4.pdf>.

Noguerol-Cal, R. *et al.* (2011) ‘Effect of several variables in the polymer toys additive migration to saliva’, *Talanta*, 85(4), pp. 2080–2088. doi: 10.1016/j.talanta.2011.07.035.

Chapter 1 – Introduction

Noh, H. J. *et al.* (2024) ‘Experimental study on color and texture as cues for plastic debris ingestion by captive sea turtles’, *Marine Pollution Bulletin*, 200(June 2023), p. 116055. doi: 10.1016/j.marpolbul.2024.116055.

Omeroğlu, P. Y., Özdal, T. and Bulut, R. (2017) ‘Chemical Migration from Plastic Types of Food Contact Materials’, *Eurasian Journal of Food Science and Technology*, 1(1), pp. 22–32.

Ong, H. T., Samsudin, H. and Soto-Valdez, H. (2022) ‘Migration of endocrine-disrupting chemicals into food from plastic packaging materials: an overview of chemical risk assessment, techniques to monitor migration, and international regulations’, *Critical Reviews in Food Science and Nutrition*, 62(4), pp. 957–979. doi: 10.1080/10408398.2020.1830747.

Ottinger, M. A. *et al.* (2011) *Endocrine Disruption of Reproduction in Birds, Hormones and Reproduction of Vertebrates - Volume 4*. Elsevier. doi: 10.1016/B978-0-12-374929-1.10009-5.

Paris, F. *et al.* (2002) ‘Phenylphenols, biphenols, bisphenol-A and 4-tert-octylphenol exhibit α and β estrogen activities and antiandrogen activity in reporter cell lines’, *Molecular and Cellular Endocrinology*, 193(1–2), pp. 43–49. doi: 10.1016/S0303-7207(02)00094-1.

Perera, U. L. H. P. *et al.* (2022) ‘Maritime pollution in the Indian Ocean after the MV X-Press Pearl accident’, *Marine Pollution Bulletin*, 185(PA), p. 114301. doi: 10.1016/j.marpolbul.2022.114301.

Pierce, K. E. *et al.* (2004) ‘Obstruction and starvation associated with plastic ingestion in a Northern Gannet *Morus bassanus* and a Greater Shearwater *Puffinus gravis*’, *Marine Ornithology*, 32(2), pp. 187–189.

Chapter 1 – Introduction

Pilapitiya, P. G. C. N. T. and Ratnayake, A. S. (2024) ‘The world of plastic waste: A review’, *Cleaner Materials*, 11(August 2023), p. 100220. doi: 10.1016/j.clema.2024.100220.

Pinto da Costa, J. *et al.* (2023) ‘Plastic additives and microplastics as emerging contaminants: Mechanisms and analytical assessment’, *TrAC - Trends in Analytical Chemistry*, 158. doi: 10.1016/j.trac.2022.116898.

Plastics Europe (2022) *Plastics – the Facts 2022*.

Plíšková, M. *et al.* (2005) ‘Impact of polychlorinated biphenyls contamination on estrogenic activity in human male serum’, *Environmental Health Perspectives*, 113(10), pp. 1277–1284. doi: 10.1289/ehp.7745.

Qiu, W. *et al.* (2019) ‘The occurrence, potential toxicity, and toxicity mechanism of bisphenol S, a substitute of bisphenol A: A critical review of recent progress’, *Ecotoxicology and Environmental Safety*, 173(November 2018), pp. 192–202. doi: 10.1016/j.ecoenv.2019.01.114.

Rafa, N. *et al.* (2024) ‘Microplastics as carriers of toxic pollutants: Source, transport, and toxicological effects’, *Environmental Pollution*, 343(September 2023), p. 123190. doi: 10.1016/j.envpol.2023.123190.

Regan, F. and Fogarty, B. (2005) ‘Endocrine disrupting chemicals’, *Encyclopedia of Analytical Science (Second Edition)*. doi: 10.4161/spmg.1.3.18019.

Rifa, R. A. and Lavado, R. (2024) ‘Unveiling the next generation of bisphenol analogs and their impact on human health using in vitro methods’, *Emerging Contaminants*, 10(2). doi: 10.1016/j.emcon.2023.100296.

Chapter 1 – Introduction

Rochester, J. R. and Bolden, A. L. (2015) ‘Bisphenol S and F: A systematic review and comparison of the hormonal activity of bisphenol a substitutes’, *Environmental Health Perspectives*, 123(7), pp. 643–650. doi: 10.1289/ehp.1408989.

Rochman, C. M. *et al.* (2013) ‘Long-term field measurement of sorption of organic contaminants to five types of plastic pellets: Implications for plastic marine debris’, *Environmental Science and Technology*, 47(3), pp. 1646–1654. doi: 10.1021/es303700s.

Rodrigues, J. P. *et al.* (2019) ‘Significance of interactions between microplastics and POPs in the marine environment: A critical overview’, *TrAC - Trends in Analytical Chemistry*, 111, pp. 252–260. doi: 10.1016/j.trac.2018.11.038.

Roman, L., Lowenstine, L., *et al.* (2019) ‘Is plastic ingestion in birds as toxic as we think? Insights from a plastic feeding experiment’, *Science of the Total Environment*, 665, pp. 660–667. doi: 10.1016/j.scitotenv.2019.02.184.

Roman, L., Paterson, H., *et al.* (2019) ‘Size of marine debris items ingested and retained by petrels’, *Marine Pollution Bulletin*, 142, pp. 569–575. doi: 10.1016/j.marpolbul.2019.04.021.

Roman, L. *et al.* (2021) ‘Plastic ingestion is an underestimated cause of death for southern hemisphere albatrosses’, *Conservation Letters*, 14(3), pp. 1–12. doi: 10.1111/conl.12785.

Rothstein, S. I. (1973) ‘Plastic Particle Pollution of the Surface of the Atlantic Ocean: Evidence from a Seabird’, *The Condor*, 75(3), pp. 344–345. doi: 10.2307/1366176.

Chapter 1 – Introduction

Ryan, P. G. (2019) ‘Ingestion of Plastics by Marine Organisms’, *Handbook of Environmental Chemistry*, 78, pp. 235–266. doi: 10.1007/698_2016_21.

Sambolino, A. *et al.* (2023) ‘Microplastic ingestion and plastic additive detection in pelagic squid and fish: Implications for bioindicators and plastic tracers in open oceanic food webs’, *Science of the Total Environment*, 894(May), p. 164952. doi: 10.1016/j.scitotenv.2023.164952.

Schwabl, P. *et al.* (2019) ‘Detection of various microplastics in human stool: A prospective case series’, *Annals of Internal Medicine*, 171(7), pp. 453–457. doi: 10.7326/M19-0618.

Sedha, S. *et al.* (2021) ‘Reproductive toxic potential of phthalate compounds – State of art review’, *Pharmacological Research*, 167(December 2020), p. 105536. doi: 10.1016/j.phrs.2021.105536.

Setälä, O., Fleming-Lehtinen, V. and Lehtiniemi, M. (2014) ‘Ingestion and transfer of microplastics in the planktonic food web’, *Environmental Pollution*, 185, pp. 77–83. doi: 10.1016/j.envpol.2013.10.013.

Sever, R. and Glass, C. K. (2013) ‘Signaling by nuclear receptors’, *Cold Spring Harbor Perspectives in Biology*, 5(3), pp. 1–4. doi: 10.1101/cshperspect.a016709.

Shah, A. A. *et al.* (2008) ‘Biological degradation of plastics: A comprehensive review’, *Biotechnology Advances*, 26(3), pp. 246–265. doi: 10.1016/j.biotechadv.2007.12.005.

Sharkey, M. *et al.* (2020) ‘Phasing-out of legacy brominated flame retardants: The UNEP Stockholm Convention and other legislative action worldwide’,

Chapter 1 – Introduction

Environment International, 144(August), p. 106041. doi: 10.1016/j.envint.2020.106041.

Siddique, S. *et al.* (2021) ‘Investigation of the migration of bisphenols from baby bottles and sippy cups’, *Current Research in Food Science*, 4, pp. 619–626. doi: 10.1016/j.crfs.2021.08.006.

Singh, A. and Mishra, B. K. (2023) ‘Microbeads in personal care products: An overlooked environmental concern’, *Journal of Cleaner Production*, 427(November), pp. 1–7. doi: 10.1016/j.jclepro.2023.139082.

Souza, J. M. O. *et al.* (2022) ‘Levels of phthalates and bisphenol in toys from Brazilian markets: Migration rate into children’s saliva and daily exposure’, *Science of the Total Environment*, 828. doi: 10.1016/j.scitotenv.2022.154486.

de Souza, S. S. *et al.* (2022) ‘Toxicity induced via ingestion of naturally-aged polystyrene microplastics by a small-sized terrestrial bird and its potential role as vectors for the dispersion of these pollutants’, *Journal of Hazardous Materials*, 434(March). doi: 10.1016/j.jhazmat.2022.128814.

Suhrhoff, T. J. and Scholz-Böttcher, B. M. (2016) ‘Qualitative impact of salinity, UV radiation and turbulence on leaching of organic plastic additives from four common plastics - A lab experiment’, *Marine Pollution Bulletin*, 102(1), pp. 84–94. doi: 10.1016/j.marpolbul.2015.11.054.

Sun, B. *et al.* (2019) ‘Releases of brominated flame retardants (BFRs) from microplastics in aqueous medium: Kinetics and molecular-size dependence of diffusion’, *Water Research*, 151, pp. 215–225. doi: 10.1016/j.watres.2018.12.017.

Chapter 1 – Introduction

Sunday, O. E. *et al.* (2022) ‘Review of the environmental occurrence, analytical techniques, degradation and toxicity of TBBPA and its derivatives’, *Environmental research*, 206(July 2021), p. 112594. doi: 10.1016/j.envres.2021.112594.

Tanaka, K. *et al.* (2013) ‘Accumulation of plastic-derived chemicals in tissues of seabirds ingesting marine plastics’, *Marine Pollution Bulletin*, 69(1–2), pp. 219–222. doi: 10.1016/j.marpolbul.2012.12.010.

Tanaka, K. *et al.* (2015) ‘Facilitated Leaching of Additive-Derived PBDEs from Plastic by Seabirds’ Stomach Oil and Accumulation in Tissues’, *Environmental Science and Technology*, 49(19), pp. 11799–11807. doi: 10.1021/acs.est.5b01376.

Tanaka, K. *et al.* (2019) ‘Piece-by-piece analysis of additives and manufacturing byproducts in plastics ingested by seabirds: Implication for risk of exposure to seabirds’, *Marine Pollution Bulletin*, 145(May), pp. 36–41. doi: 10.1016/j.marpolbul.2019.05.028.

Tanaka, K. *et al.* (2020) ‘In Vivo Accumulation of Plastic-Derived Chemicals into Seabird Tissues’, *Current Biology*, 30(4), pp. 723–728.e3. doi: 10.1016/j.cub.2019.12.037.

Tanaka, K., Yamashita, R. and Takada, H. (2018) ‘Transfer of Hazardous Chemicals from Ingested Plastics to Higher-Trophic-Level Organisms’, in *Handbook of Environmental Chemistry*, pp. 267–280. doi: 10.1007/978-94-007-698-2_255.

Thacharodi, A. *et al.* (2024) ‘Mitigating microplastic pollution: A critical review on the effects, remediation, and utilization strategies of microplastics’, *Journal*

Chapter 1 – Introduction

of *Environmental Management*, 351(January), p. 119988. doi: 10.1016/j.jenvman.2023.119988.

Tourinho, P. S. *et al.* (2019) ‘Partitioning of chemical contaminants to microplastics : Sorption mechanisms , environmental distribution and effects on toxicity and’, 252, pp. 1246–1256. doi: 10.1016/j.envpol.2019.06.030.

Tubbs, C. *et al.* (2012) ‘Activation of southern white rhinoceros (*Ceratotherium simum simum*) estrogen receptors by phytoestrogens: Potential role in the reproductive failure of captive-born females?’, *Endocrinology*, 153(3), pp. 1444–1452. doi: 10.1210/en.2011-1962.

Walters, D. M. *et al.* (2011) ‘Trophic magnification of PCBs and its relationship to the octanol-water partition coefficient’, *Environmental Science and Technology*, 45(9), pp. 3917–3924. doi: 10.1021/es103158s.

Wang, C. *et al.* (2017) ‘Low concentration of BPA induces mice spermatocytes apoptosis via GPR30’, *Oncotarget*, 8(30), pp. 49005–49015. doi: 10.18632/oncotarget.16923.

Wang, J. *et al.* (2023) ‘Quantitative analysis of polystyrene microplastic and styrene monomer released from plastic food containers’, *Heliyon*, 9(5), p. e15787. doi: 10.1016/j.heliyon.2023.e15787.

Wang, W. *et al.* (2022) ‘Effects of polyethylene microplastics on cell membranes: A combined study of experiments and molecular dynamics simulations’, *Journal of Hazardous Materials*, 429(December 2021), p. 128323. doi: 10.1016/j.jhazmat.2022.128323.

Warham, J. (1977) ‘The incidence, functions and ecological significance of

Chapter 1 – Introduction

petrel stomach oils’, *Proceedings of the New Zealand Ecological Society*, 24, pp. 84–93.

Weikum, E. R., Liu, X. and Ortlund, E. A. (2018) ‘The nuclear receptor superfamily: A structural perspective’, *Protein Science*, 27(11), pp. 1876–1892. doi: 10.1002/pro.3496.

Wu, C. *et al.* (2024) ‘Advances in polychlorinated biphenyls-induced female reproductive toxicity’, *Science of the Total Environment*, 918(January), p. 170543. doi: 10.1016/j.scitotenv.2024.170543.

Xie, J. *et al.* (2022) ‘Occurrence and partitioning of bisphenol analogues, triclocarban, and triclosan in seawater and sediment from East China Sea’, *Chemosphere*, 287(P2), p. 132218. doi: 10.1016/j.chemosphere.2021.132218.

Xu, T. *et al.* (2023) ‘Microplastics induced inflammation and apoptosis via ferroptosis and the NF- κ B pathway in carp’, *Aquatic Toxicology*, 262(August), p. 106659. doi: 10.1016/j.aquatox.2023.106659.

Xu, Y. *et al.* (2024) ‘Exploring additives beyond phthalates: Release from plastic mulching films, biodegradation and occurrence in agricultural soils’, *Science of the Total Environment*, 918(January), p. 170763. doi: 10.1016/j.scitotenv.2024.170763.

Yamashita, R. *et al.* (2021) ‘Plastic additives and legacy persistent organic pollutants in the preen gland oil of seabirds sampled across the globe’, *Environmental Monitoring and Contaminants Research*, 1(0), pp. 97–112. doi: 10.5985/emcr.20210009.

Yang, Chun Z *et al.* (2011) ‘Most Plastic Products Release Estrogenic

Chapter 1 – Introduction

Chemicals : A Potential Health Problem that Can Be Solved’, *Environmental Health Perspectives*, 119(7), pp. 989–996. doi: 10.1289/ehp.1003220.

Yang, Chun Z. *et al.* (2011) ‘Most plastic products release estrogenic chemicals: A potential health problem that can be solved’, *Environmental Health Perspectives*, 119(7), pp. 989–996. doi: 10.1289/ehp.1003220.

Yang, J. *et al.* (2019) ‘Migration of phthalates from plastic packages to convenience foods and its cumulative health risk assessments’, *Food Additives and Contaminants: Part B Surveillance*, 12(3), pp. 151–158. doi: 10.1080/19393210.2019.1574909.

Yang, W. *et al.* (2022) ‘Impacts of microplastics on immunity’, *Frontiers in Toxicology*, 4(September), pp. 1–14. doi: 10.3389/ftox.2022.956885.

Zhang, H. *et al.* (2020) ‘Occurrence of bisphenol A and its alternatives in paired urine and indoor dust from Chinese university students: Implications for human exposure’, *Chemosphere*, 247, p. 125987. doi: 10.1016/j.chemosphere.2020.125987.

Zhang, R. *et al.* (2018) ‘RXR α provokes tumor suppression through p53/p21/p16 and PI3K-AKT signaling pathways during stem cell differentiation and in cancer cells article’, *Cell Death and Disease*, 9(5), pp. 1–13. doi: 10.1038/s41419-018-0610-1.

Zhou, R. *et al.* (2023) ‘Occurrence and migration of organophosphite and organophosphate esters into food simulants from single-use food packaging in China’, *Environmental Pollution*, 330(2), p. 121782. doi: 10.1016/j.envpol.2023.121782.

Chapter 2

Aims of the thesis and scientific strategy

Plastic pollution has emerged as a significant environmental threat, particularly affecting marine ecosystems (Kühn and van Franeker, 2020). Procellariiform seabirds, such as petrels, albatrosses, and shearwaters, are especially vulnerable due to their feeding habits and digestive physiology, leading to the ingestion and prolonged retention of plastics in their stomachs (Ryan, 2015, 2019). While the physical harm caused by plastic ingestion has been documented, the toxicological implications of plastic-associated chemical exposure on seabirds remain poorly understood. This thesis aims to fill this knowledge gap by addressing two primary objectives:

1. Understanding the leaching behavior of plastic-associated chemicals during gastric digestion in Procellariiformes
2. Investigating the potential hormonal disruption caused by the leaching of chemicals from plastic ingested by Procellariiformes.

To achieve these objectives, two different *in vitro* approaches were employed, encompassing the development of a Procellariiform gastric model and the novel use of a Procellariiform estrogen receptor bioassay.

In **Chapter 3**, we aimed to understand how the type of food, gastric secretions and polymer type influence the leaching of additives and sorbed

Chapter 2 – Aims and scientific strategy

persistent organic pollutants (POPs) from plastic within a Procellariiform stomach environment. High-density polyethylene (HDPE) and polyvinyl chloride (PVC) plastics were manufactured with two different additives: bisphenol S (BPS) or decabromodiphenyl ether (PBDE-209). Two PCBs [2,4,4'-trichloro-1,1'-biphenyl (PCB-28) and 2,2',3,4,4',5'-hexachlorobiphenyl (PCB-138)] were sorbed at the surface of plastic added with PBDE-209. Plastics were exposed to two different types of marine oil (i.e. oil extracted from marine animals) (salmon or calanus oil) and different aqueous solutions such as acidic water, acidic pepsin solution and acidic pepsin-protein solution for 20 hours. The amount of chemicals leached was determined using gas and liquid chromatography-mass spectrometry (GC-MS and LC-MS).

In **Chapter 4**, we aimed to understand the temporal leaching behavior of additives and sorbed POPs in Procellariiform stomach. We developed an *in vitro* Procellariiform gastric model (PGM) consisting of a mixture of marine oil (salmon or calanus oil) and acidic pepsin-protein solution. Plastics (HDPE and PVC) added with BPS or PBDE-209 supplemented with PCB-28 and -138 were incubated in the PGM for 100 hours. The amount of chemicals leached (additives and sorbed POPs) was quantified every 20 hours using GC-MS and LC-MS.

In **Chapter 5**, we aimed to investigate whether chemicals leached from ingested plastics could cause potential hormonal disruption. To achieve this objective, the response of northern fulmar (*Fulmarus glacialis*) estrogen receptors (ERs) to plastic associated chemicals was studied using an *in vitro* luciferase reporter gene assay. This chapter was divided in two sub-objectives:

Chapter 2 – Aims and scientific strategy

- The first sub-objective was to compare the activation and inhibition responses of human and northern fulmar ERs to plastic-associated chemicals. Fulmar and human ER α and ER β sequences were cloned into an expression vector which was transfected into human embryonic kidney (HEK293) cells to assess ER response to plastic-associated chemicals. Potential ER activation and inhibition was studied in the presence of six additives (bisphenol A, BPS, 4-octylphenol, benzyl butyl phthalate, PBDE-209, tetrabromobisphenol A) and two POPs commonly found sorbed at the surface of marine plastic (PCB-52 and PCB-138).
- The second sub-objective was to evaluate the estrogenic activity of chemicals leached from plastics ingested by northern fulmars using fulmar ERs. Plastic recovered from the stomach of 27 northern fulmars were leached in a mixture of hexane and methanol for 14 days. Leachates were collected daily and tested for estrogenic activity (ER activation and inhibition). The polymer composition of plastic was assessed with infrared spectroscopy to investigate whether the type of plastic influenced the response of ERs. Mass spectrometry was also run on several leachates to investigate for the presence of distinct compounds in leachate samples.

Chapter 2 – Aims and scientific strategy

References

Kühn, S. and van Franeker, J. A. (2020) ‘Quantitative overview of marine debris ingested by marine megafauna’, *Marine Pollution Bulletin*, 151(110858). doi: 10.1016/j.marpolbul.2019.110858.

Ryan, P. G. (2015) ‘How quickly do albatrosses and petrels digest plastic particles?’, *Environmental Pollution*, 207, pp. 438–440. doi: 10.1016/j.envpol.2015.08.005.

Ryan, P. G. (2019) ‘Ingestion of Plastics by Marine Organisms’, *Handbook of Environmental Chemistry*, 78, pp. 235–266. doi: 10.1007/698_2016_21.

Chapter 3

Influence of the type of food, gastric secretions and polymer type on the leaching of additives and sorbed POPs from plastic in a Procellariiform gastric model

Adapted from Van Hassel, L., Scholl, G., Eppe, G., Poleunisc, C., Dupont-Gillain, C., Finkelstein, M. and Debier, C. *Dynamics of leaching of POPs and additives from plastic in a Procellariiform gastric model : Diet- and polymer-dependent effects and implications for long-term exposure*. PLoS One 19, 1-19 (2024)

Table of Contents

Abstract.....	94
3.1. Introduction.....	95
3.2. Materials and methods	98
3.2.1. Chemicals and materials	98
3.2.2. Plastic sample preparation	99
3.2.2.1. Plastic manufacture.....	99
3.2.2.2. Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) analyses	102
3.2.2.3. Sorption of POPs in salt water	102
3.2.3. Leaching experiments	104
3.2.3.1. Procellariiform gastric conditions.....	104
3.2.3.2. Influence of lipids, acidity, enzymes and proteins on chemical leaching	105
3.2.4. Quality controls.....	105
3.2.5. Chemical analyses.....	106
3.2.6. Statistical analyses	109
3.3. Results and discussion	110
3.3.1. PCB sorption onto HDPE and PVC.....	110
3.3.2. Influence of food and gastric secretions on chemical leaching.....	111
3.3.2.1. Oil versus aqueous solution	111

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

3.3.2.2.	Calanus versus salmon oil.....	115
3.3.2.3.	Acidity, enzymes and proteins.....	116
3.3.3.	Influence of the type of polymer on chemical leaching.....	118
3.4.	Conclusion	122
	Supplementary material	124
	Acknowledgements.....	127
	References.....	128

Abstract

Procellariiform seabirds are known to have high rates of plastic ingestion. We investigated the influence of lipid type and gastric secretions on the bioaccessibility of plastic-associated chemicals [plastic additives and sorbed persistent organic pollutants (POPs)] leached in an *in vitro* Procellariiform gastric model. High-density polyethylene (HDPE) and polyvinyl chloride (PVC), commonly ingested by Procellariiformes, were manufactured with one additive [decabrominated diphenyl ether (PBDE-209) or bisphenol S (BPS)]. HDPE and PVC added with PBDE-209 were additionally incubated in salt water with 2,4,4'-trichloro-1,1'-biphenyl (PCB-28) and 2,2',3,4,4',5'-hexachlorobiphenyl (PCB-138) to simulate sorption of POPs on plastic in the marine environment. Our results indicate that the type of plastic (i.e. polymer), presence of food (i.e., lipids and proteins), gastric secretions (i.e., acid, pepsin) and chemical properties of plastic-associated compounds influence their leaching in a Procellariiform gastric model.

3.1. Introduction

Plastic ingestion is widespread among seabirds with 80% of studied species affected (Kühn and van Franeker, 2020). Procellariiformes (petrels, albatrosses and shearwaters) rarely regurgitate indigestible items (Ryan, 2019) and their digestive morphology prevents ingested plastics larger than 2 mm (Roman, Paterson, *et al.*, 2019) to pass through their digestive tract (Furness, 1985), which results in a larger proportion of species documented with plastic trapped in their stomach compared to other taxa (Kühn and van Franeker, 2020).

Plastic ingestion can harm Procellariiformes by mechanical damage and obstruction (Fry, Fefer and Sileo, 1987; Pierce *et al.*, 2004; Jacobsen, Massey and Gulland, 2010) but also by exposure to plastic-associated chemicals (Delaeter *et al.*, 2022). Broadly, plastic-associated chemicals can be categorized into two groups: those in the plastic matrix, including additives and those sorbed on the plastic surface, including persistent organic pollutants (POPs). Additives are chemicals mixed with polymers during the manufacture of plastic to modify their properties, performances and long-term use (e.g., flame retardants, plasticizers, antioxidants, UV stabilizers, lubricants, dyes and fillers) (Pfaendner, 2013). Most additives are not covalently bound to the polymeric structure of plastic and leach readily into the surrounding environment (Gunaalan, Fabbri and Capolupo, 2020). POPs can sorb onto plastic in aquatic environments; plastic has been shown to concentrate POPs, such as polychlorinated biphenyls (PCBs) (Perugini, 2009), by factors up to 10^7 compared to seawater (Koelmans *et al.*, 2016). While numerous studies have documented the prevalence of plastic ingestion in Procellariiformes

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

(Kühn and van Franeker, 2020; Omeyer *et al.*, 2023), the exposure risk to plastic-associated chemicals remains poorly understood (Pinto da Costa *et al.*, 2023).

One potential risk of exposure to plastic-associated chemicals is endocrine disruption (Aneck-Hahn *et al.*, 2018; Campos *et al.*, 2019; Park *et al.*, 2019), which is of concern to affect a seabird's hormonal and reproductive system (Mathieu-Denoncourt *et al.*, 2015; Roman, Lowenstine, *et al.*, 2019). A key data gap in understanding exposure risk is the bioaccessibility of plastic-associated chemicals in seabird's digestive tract (Pinto da Costa *et al.*, 2023). The few studies that have investigated the leaching of chemicals from plastics ingested by seabirds have only examined the effect of isolated gastric solutions on the leaching of additives [i.e. oil (Tanaka *et al.*, 2015; Kühn, Andy M Booth, *et al.*, 2020; Sun *et al.*, 2021) or acidic pepsin solution (Coffin *et al.*, 2019; Guo *et al.*, 2019)] and the leaching of POPs from plastic has only been studied under gut fluid conditions [i.e. bile solutions (Bakir, Rowland and Thompson, 2014; Mohamed Nor and Koelmans, 2019a)]. Notably, these investigations have never considered the co-existence of both additives and sorbed POPs. Moreover, Procellariiform stomach oil is mainly composed of triacylglycerols (TAGs) and wax esters (WEs) at proportions that vary according to diet (Wang *et al.*, 2007; Connan *et al.*, 2014; Akanbi and Barrow, 2016). Yet, the influence of different types of lipids on the leaching of plastic-associated chemicals has never been studied.

In this study, we evaluated the influence of gastric fluids (acidity and enzymes) and food (lipids and proteins) on the leaching of plastic-associated chemicals (additives and sorbed POPs). We used two plastic polymers known

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

to be commonly ingested by Procellariiformes: high-density polyethylene (HDPE), characterized by a high permeability and free volume, and polyvinyl chloride (PVC), more compact with less free volume (Menéndez-Pedriz and Jaumot, 2020). Each polymer was prepared with one additive: either decabrominated diphenyl ether (PBDE-209) or bisphenol-S (BPS), known for their reproductive toxicity (Howdeshell *et al.*, 2008; Li *et al.*, 2012, 2021; Murata and Kang, 2018; Santoro *et al.*, 2019; Adegoke, Rahman and Pang, 2020) and distinct chemical properties. The influence of food composition on the chemical leaching was assessed with two marine oils (salmon and calanus), commonly found in Procellariiform stomach oil (Wang *et al.*, 2007; Curie-paris *et al.*, 2014; Akanbi and Barrow, 2016). Our study highlights the pivotal role of food and gastric secretions in modulating the leaching of plastic-associated chemicals, with a dynamic depending on the type of polymer and the chemical properties of the associated compound.

3.2. Materials and methods

3.2.1. Chemicals and materials

High density polyethylene (HDPE) pellets (Hostalen GC7260LS) were purchased from Lyondellbasell (Brussels, Belgium) and polyvinylchloride (PVC) pellets (ER 027/W141/AC) were purchased from Bevnac (Dijon, France). Analytical-grade polybrominated diphenyl ether-209 (PBDE-209), polychlorinated biphenyl -28 and -138 (PCB-28 and PCB-138) and bisphenol-S (PBS) and bisphenol S-d₈ (BPS-d₈) were purchased from LGC Standards (Molsheim, France). Beaphar® Salmon oil was purchased from Aveve (Wavre, Belgium). Calanus® Oil was obtained from Zooca™ (Tromsø, Norway). NaCl and Pepsin A from porcine gastric mucosa were purchased from Sigma-Aldrich (Overijse, Belgium). Bovine serum albumin (V fraction) was purchased from Carl Roth® (Karlsruhe, Germany). Analytical-grade hydrochloric acid 37%, hexane and methanol were purchased from VWR™ (United Kingdom).

Glass bottles (500 mL) were used to incubate plastic samples in saltwater spiked with PCBs. Glass Erlenmeyers (50 mL) sealed with foil-lined rubber caps and parafilm were used to incubate plastic in gastric solutions, hexane and water. Round glass stirring bars used to mix the digestive solutions were purchased from Sigma-Aldrich (Overijse, Belgium). Samples were collected with cotton-plugged Pasteur pipets and stored in glass vials with polypropylene caps lined with aluminum foil.

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

3.2.2. Plastic sample preparation

3.2.2.1. Plastic manufacture

Four plastic plates (110 mm x 110 mm x 1 mm) were manufactured by Certech R&D Partner in Chemistry (Seneffe, Belgium) (Figure 3.1). One HDPE and one PVC plate were supplemented with PBDE-209 as an additive at a concentration of 1 % (w/w). PBDE-209 is a highly hydrophobic flame retardant [$\log K_{ow} = 9.97$ (Bao, You and Zeng, 2011)] which has been detected in ingested plastics and Procellariiform tissues (Tanaka *et al.*, 2013, 2015, 2019). The two other HDPE and PVC plates were supplemented with BPS as an additive at the same concentration (1 %, w/w). BPS is a weakly hydrophobic antioxidant [$\log K_{ow} = 1.65$ (Vasiljevic and Harner, 2021)] used as an analogous of the famous bisphenol-A (BPA), which has been detected in marine plastics (Staniszewska, Graca and Nehring, 2016). The concentration was chosen according to the typical concentration of flame retardants and anti-oxidants used in polymers (Hahladakis *et al.*, 2018; Gunaalan, Fabbri and Capolupo, 2020).

The addition of additives to the polymers was conducted in a manner similar to industrial processes. HDPE or PVC pellets were melted at 190°C and 170°C respectively for 1 minute in a monitor mixer containing two screws to ensure homogenization when rotating. One percent of additive (PBDE-209 or BPS; powder form) was added and mixed with the rest for 2 more minutes. Following that, the material was placed into a 1 mm thick frame, between 2 stainless steel plates coated with non-stick paper impregnated with Teflon and put into a fusing press at 190°C or 170°C for 5 minutes. After being cooled down to 40°C at 15°C/minute, the frame – steel plate – Teflon paper – plastic

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

system was removed from the press and the plastic plate was recovered. Plastic plates were cut in 5 mm x 5 mm x 1 mm square pieces (surface area of 70 mm²), according to the estimated size of plastic items trapped in the gizzard of Procellariiformes (2 – 10 mm) (Roman, Paterson, *et al.*, 2019) (Figure 3.1).

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

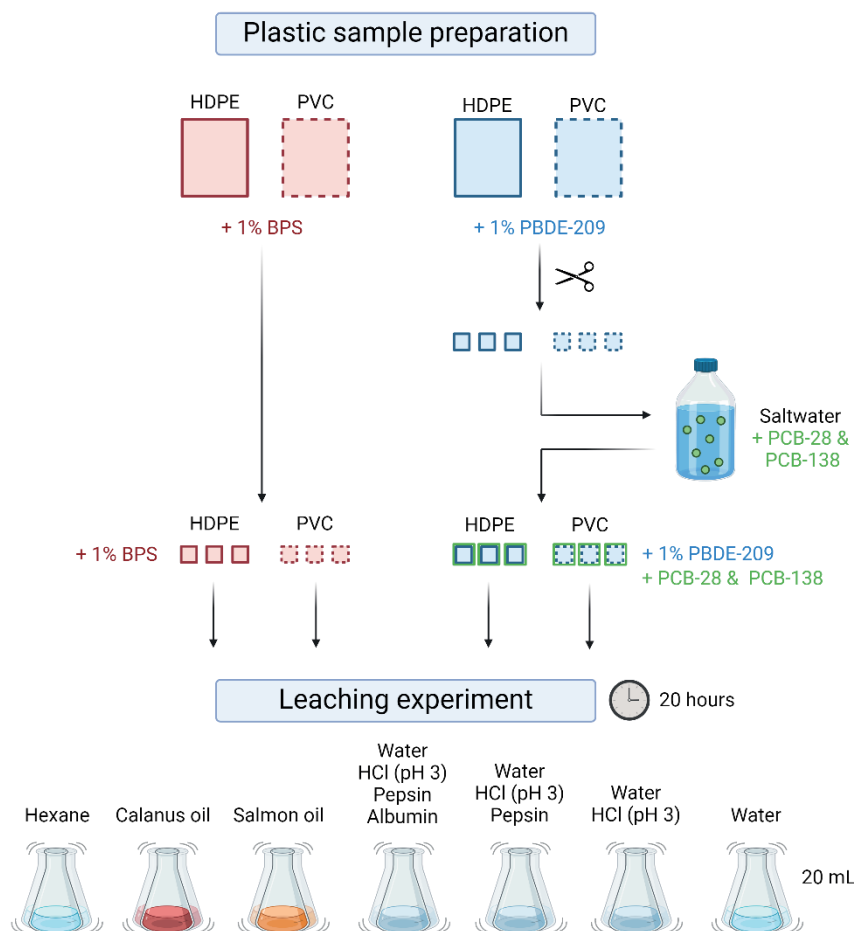


Figure 3.1. Experimental design. Two HDPE and PVC plastic plates were supplemented with decabrominated diphenyl ether (PBDE-209) or bisphenol S (BPS) at a concentration of 1 % (w/w). Plastic plates were cut in 5 mm x 5 mm x 1 mm square pieces. A total of 300 pieces of each polymer with 1% PBDE-209 were incubated in 100 ml of salt water under agitation (150 rpm; RT; in the dark) for three weeks with polychlorinated biphenyl -28 and -138 (PCB-28 and PCB-138). To study the influence of lipids, enzymes, proteins and acidity, ten plastic pieces (HDPE or

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

PVC) containing either 1% BPS or 1 % PBDE-209 + PCB-28 and -138 were exposed to either (i) salmon oil (ii) calanus oil, (iii) an acidic albumin-pepsin solution (iv) an acidic pepsin solution or (v) acidic water for 20 hours at 38°C. Hexane and milli-Q water were used as controls. Created with BioRender.com.

3.2.2.2. Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) analyses

Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) analyses were performed on HDPE and PVC pieces supplemented with BPS and PBDE-209 to characterize the distribution of additives within the polymer matrices. ToF-SIMS analyses were performed using a TOF.SIMS⁵ instrument (IONTOF GmbH, Münster, Germany), equipped with a Bi Nanoprobe-LMIG (liquid metal ion gun) and an Ar-GCIB (gas cluster ion beam) primary ion sources (beams oriented at 45° to the surface normal). Depth profiling experiments were conducted using the dual-beam mode. The 30 keV Bi₅⁺ pulsed ion beam (cycle time of 200 μs) with a raster of analysis of 200 x 200 μm² was used for spectral acquisition. The measured analysis current was ~0.4 pA. The 10 keV Ar₃₀₀₀⁺ with a raster set to 450 x 450 μm² was used for sputtering. The measured sputtering current was 4 nA. Charge compensation was achieved with a 20 eV electron flood gun.

3.2.2.3. Sorption of POPs in salt water

A total of 300 pieces of each polymer with 1% PBDE-209 were incubated in 100 mL of salt water (35 g NaCl/L) under agitation [150 rpm; room temperature (RT); in the dark] for three weeks with polychlorinated biphenyl -28 and -138 (PCB-28, a trichlorinated biphenyl and PCB-138, an

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

hexachlorinated biphenyl), two POPs known to be sorbed on plastic in the ocean (Antunes *et al.*, 2013; Bouhroum *et al.*, 2019; Mohamed Nor and Koelmans, 2019b) (Figure 3.1). The three-week incubation period was chosen according to Nor *et al.* (2019), who reported that longer incubation time assures a deeper sorption of the chemicals by the polymer, which can be considered as more environmentally relevant than the shorter equilibrium time (24 – 48h) used in prior studies (Mohamed Nor and Koelmans, 2019b). Salt water was spiked with 129.4 µg/L PCB-28 and 114.3 µg/L PCB-138 resulting in a maximum amount of 43.1 ng PCB-28 and 38.1 ng PCB-138 per plastic piece to approximate an environmentally relevant sorption of PCBs on plastic surface (Antunes *et al.*, 2013; Choi, Cho and Luthy, 2013; Koelmans *et al.*, 2016; Bouhroum *et al.*, 2019; Syberg *et al.*, 2020). Accordingly, the concentration of sorbed POPs was about ~10 thousand times smaller than the concentration of additives present within the plastic matrix – which is representative of what happens in the environment (Barrick *et al.*, 2021). After incubation, plastic pieces were removed with a sieve, briefly rinsed with milli-Q water, dried under a chemical-fume hood overnight, and stored in glass vials in the dark at RT. Sorption experiments with PCBs were not performed on plastics containing BPS as an additive as a three-week soaking in salt water may trigger the leaching of BPS from the plastic bulk [$\log K_{ow}$ 1.65 for BPS vs 9.97 for PBDE-209 (Bao, You and Zeng, 2011; Vasiljevic and Harner, 2021)].

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

3.2.3. Leaching experiments

3.2.3.1. Procellariiform gastric conditions

A seabird's stomach is divided in two chambers, the proventriculus and gizzard. During digestion, two phases are formed in a Procellariiform stomach: a lower aqueous phase and an upper lipidic phase. Plastic is majorly trapped in the gizzard chamber where it is exposed to both chemical and mechanical digestion, increasing the potential leaching of chemicals (Tanaka, Yamashita and Takada, 2018). In this study, the aqueous phase was simulated with 20 ml of an acidic pepsin-albumin solution made of milli-Q water, pepsin (10 g/L) and albumin (10 g/L) adjusted to a pH of 3 by adding first 37% and then 1 M HCl dropwise (Coffin *et al.*, 2019). The albumin concentration used was derived from the average mass of prey found in a Procellariiform stomach (Olsen *et al.*, 2002), along with an estimated associated gastric fluid volume of 120 ml (Warham, 1977; Connan *et al.*, 2007), assuming a protein concentration of 16% (Hilton, Furness and Houston, 2000). The lipidic phase was simulated with 20 ml of marine oil. Procellariiform stomach oil consists mainly of triacylglycerols (TAGs) and wax esters (WEs) (70 – 99% total lipids) at ratios varying according to the diet (Wang *et al.*, 2007; Connan *et al.*, 2014; Akanbi and Barrow, 2016). Accordingly, we investigated the effect of two marine oils: a salmon TAG-rich oil and a calanus WE-rich oil. Volumes were chosen according to the volume of stomach oil and gastric juice in Procellariiformes (Connan *et al.*, 2007; Coffin *et al.*, 2019; Kühn, Andy M. Booth, *et al.*, 2020).

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

3.2.3.2. Influence of lipids, acidity, enzymes and proteins on chemical leaching

Gastric components were tested individually to investigate the effect of food (lipids and proteins), digestive enzymes (pepsin) and acidity on the leaching of chemicals from plastic. Ten plastic pieces of HDPE (mass = ± 0.23 g) or ten plastic pieces of PVC (mass = ± 0.39 g) containing either 1% BPS or 1 % PBDE-209 + PCBs-28 and -138 (sorbed) were exposed to (i) salmon oil, (ii) calanus oil, (iii) an acidic aqueous solution containing both pepsin and proteins [milli-Q water, pepsin (10 g/L) and albumin (10 g/L), pH 3], (iv) an acidic aqueous solution containing only pepsin [milli-Q water with pepsin (10 g/L; pH 3)] or (v) acidic water (pH 3) for 20 hours at 38°C (800 rpm; 10 pieces/20 mL of solution) (Figure 3.1), based on the average time of gastric digestion in Procellariiformes (Roby, Brink and Place, 1989; Jackson, 1992; Hilton, Furness and Houston, 2000). The solid-to-liquid ratio was based on the average number of plastic pieces and the digestive fluid volume in the stomach of Procellariiformes (Connan *et al.*, 2007; Provencher *et al.*, 2017). After incubation, samples were stored in pre-cleaned glass vials at -20°C in the dark prior to analyses.

3.2.4. Quality controls

In order to ensure the reliability and accuracy of our experimental results, rigorous quality control measures were implemented throughout the study. Glassware used in POPs sorption and leaching experiments was pre-washed three times with analytical-grade hexane and analytical-grade methanol. Glassware intended for chemical analysis was baked in a muffle furnace at 450 °C for > 4 h prior to use. Additionally, plastic-free control

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

solutions were processed and analyzed in parallel to detect any potential contamination. All solutions contained no detectable chemicals, with the exception of marine oils, which contained a minimal amount of PCBs ($< 2.5 \mu\text{g/g}$) that was subsequently subtracted from the total concentrations measured. Hexane and water were used as positive controls as they are known to promote the leaching of hydrophobic chemicals (PBDEs and PCBs; hexane) (Tanaka *et al.*, 2015, 2019; Gripon *et al.*, 2021) and more water-soluble chemicals (bisphenol analogous) (Banaderakhshan *et al.*, 2022) respectively.

3.2.5. Chemical analyses

PBDE-209, PCB-28 and -138. Aqueous samples were liquid-liquid extracted according to the following procedure: 1 mL of n-hexane was added to 1 mL of sample and 10 μL of 200 $\text{pg}/\mu\text{L}$ ^{13}C -labelled PCB solution (PCB-28, -52, -101, -138, -153, -180) as well as 10 μL of 2,500 $\text{pg}/\mu\text{L}$ ^{13}C -labelled PBDE solution (PBDE-28, -47, -77, -99, -100, -138, -153, -154, -183, -209) were added for quantification and used for method recovery (range of accepted recoveries were 60-120%). The solution was vortexed for 30 seconds and settled for 5 minutes before recovering the upper n-hexane phase. Two additional liquid-liquid extractions were performed following the same procedure. For the oils, 10 mL n-hexane were added to 100 μL of sample and 10 μL of 200 $\text{pg}/\mu\text{L}$ ^{13}C -labelled PCB solution as well as 10 μL of 2,500 $\text{pg}/\mu\text{L}$ ^{13}C -labelled PBDE solution were added as internal standards. The resulting solutions (3 mL of hexane for aqueous samples and 10 mL of hexane for lipidic samples) were purified by solid-liquid adsorption chromatography. According to the Beltest I014 method, silica gel column composed of 0.5 g of

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

anhydrous Na₂SO₄, 1 g of Al₂O₃ containing 11% of water, 6 g of acidified silica gel (60/40 silica/H₂SO₄) and, on the top, 0.5 g of anhydrous Na₂SO₄ were conditioned with 40 ml of n-hexane. Solutions were then loaded onto the columns and rinsed three times with 5 ml of hexane. The column was further eluted with 20 ml of n-hexane ('Beltest I014-Rev3 Determination of polychlorinated biphenyls in animal feed, animal fat, egg and egg products, milk products and other foodstuffs. Beltest, Belgium', 2000). After evaporation under a gentle dried-air stream to approximately 90 µL, 10 µL of a 200 pg/µL ¹³C-labelled PCB-80 solution was added as internal standard for instrument calibration. Final concentration data were normalized to their recovery standard.

Analyses were performed on an Autospec Ultima mass spectrometer (Waters, Manchester,UK) coupled to an Agilent 6890 gas chromatograph (Palo Alto, CA) equipped with a splitless and a Programmable Temperature Vaporisation (PTV) injection port. For PCBs, 2 µL of samples were injected at 275°C in splitless mode on an HT8 (25m x 0.22mm x 0.25µm ; SGE) at a 0.8 mL/min He (6.0 purity ; Air Liquide) constant flow with the following temperature ramp: 140°C for 2 min, heated to 220°C at 15°C/min and held for 7.5 min, followed by an increase to 250°C at 6°C/min, to 265°C at 2°C/min and finally to 310°C at 28°C/min kept for 0.7 min. LOD and LOQ were 0.27 and 0.82 ng/ml for PCB-28 and 0.03 and 0.10 ng/mL for PCB-138. For BDE-209, 5 µL of samples were injected at 80°C in PTV mode on an RTX-1614 (15m x 0.25mm x 0.10µm; Restek) at a 1.5 mL/min He constant flow with the following temperature program: 120°C held for 2 min followed by a 15°C/min increase to 205°C and a 6°C/min to 315°C kept for 1 min. LOD was 0.003 ng/mL LOQ was 0.01ng/mL. Both analyses were performed in Selected Ion

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

Monitoring (SIM) at 10,000 mass resolution with Electronic Ionization at 35 eV. Two ions (quantifier and qualifier) were recorded for each analyte and their isotopic ion ratio was checked for interferences. The quantitative aspect was achieved through the isotope dilution technique using ^{13}C labelled analogous of each analyte.

BPS. Five μL of bisphenol S- d_8 at 100 $\mu\text{g/mL}$ water was added to 500 μL of aqueous sample as an internal standard. The solution was vortexed and centrifuged and 100 μL were transferred into a UPLC vial for injection. For hexane and oil samples, 5 μL of 100 $\mu\text{g/mL}$ bisphenol S- d_8 were added to 1 mL of sample as an internal standard. Five mL of hexane was added to the sample and mixed thoroughly. A volume of 2 mL of methanol was then added to the solution, shaken thoroughly, and centrifuged at 10,000 rpm for 2 minutes. The lower phase was filtered using a syringe filtration (0.2 μm filter) into a UPLC vial for injection.

An Agilent 6050B series single quadrupole LC/MS-ESI system equipped with an Agilent 1290 infinity UHPLC system was used for analysis. The chromatographic separation was carried out on an Agilent column RRHD Eclipse plus C18 (100 mm X 2.1 mm, 1.8 μm) with an Agilent Eclipse C18 (5mm x 2.1mm, 1.8 μm) guard column. All R^2 values of standard linear regression curves of measured BPS were > 0.99 . For BPS, LOD was 0.033 $\mu\text{g/mL}$ and LOQ was 0.125 $\mu\text{g/mL}$. For BPS- d_8 , LOD was 0.036 $\mu\text{g/mL}$ and LOQ was 0.133 $\mu\text{g/mL}$. Samples were analyzed in negative ionization mode. Under single ion monitoring (SIM), a m/z of 250 was used for BPS analysis $[\text{MM}+\text{H}]^+$ and a m/z of 258 for BPS- d_8 . The mobile phase consisted of milli-Q water: acetonitrile (95:5) with 0.1% of formic acid (A) and acetonitrile:

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

milli-Q water (95:5) with 0.1% of formic acid (B) and the flow rate was 0.3 mL/min. The gradient elution program was performed as follows: 0.0 – 1.0 min, 95% A – 5% B; 1.0 min – 7.0 min, 0% A – 100%; 7.0 – 9.0 min, 0% A – 100% B; 9.0 – 10.0 min, 95% A – 5% B; 10.0 – 12.0 min, 95% A – 5% B (total run time 12 minutes). The injection volume of the sample solution was 5 μ L. The temperature of the column was controlled at 40°C. Capillary voltage was set to 3.1 kV, nozzle voltage was set to 400 V (negative) and 1000 V (positive). Sheath gas temperature was 125°C, drying gas temperature was 250°C and drying gas flow was 9 L/min. The fragmentor was set to 90 eV.

3.2.6. Statistical analyses

The percentage of additive leached in each solution was calculated as the ratio of the mean additive concentration measured in the digestive medium (n=3) and the total concentration of additive added to the polymer (10,000 μ g/g). The percentage of PCBs leached in each medium was calculated as the ratio of the mean PCB concentrations (PCB-28 or PCB-138) measured in the digestive medium (n=3) and the mean PCB concentrations measured in hexane (positive control; n=3).

Unequal variance bilateral t-tests were conducted to compare the percentage of chemicals leached between HDPE and PVC within each treatment. To assess differences in chemical leaching between treatments, a Welch analysis of variance (ANOVA) was used. Subsequently, individual pairwise comparisons between treatments were performed using t-tests with a Bonferroni correction to account for multiple comparisons. These analyses elucidated how different treatments affected chemical leaching from the

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

plastic materials. All statistical analyses were conducted using JMP Pro 16 (JMP Software), and significance levels were set at $p < 0.05$.

3.3. Results and discussion

3.3.1. PCB sorption onto HDPE and PVC

To confirm our estimated amount of plastic-sorbed chemicals, plastic pieces were incubated for 20 hours in hexane. A maximum amount of 43.1 ng PCB-28 and 38.1 ng PCB-138 per plastic piece was expected to be measured if all the PCBs had homogeneously sorbed at the surface of plastic. We found 42.3 ± 1.4 ng PCB-28/piece of HDPE, 35.6 ± 3.3 ng PCB-138/piece of HDPE, 42.1 ± 8.1 ng PCB-28/piece of PVC and 35.8 ± 5.6 ng PCB-138/piece of PVC leached from the plastic in hexane (Figure 3.2). Negligible, non-quantifiable amounts of PCBs were detected in salt water after the 3-week incubation concluding that all PCBs were sorbed onto plastics after 3 weeks, without distinction between the type of polymer (Figure 3.2). Importantly, no trace of PBDE-209 were measured in salt water after 3 weeks, suggesting that no loss of PBDE-209 from plastics occurred during the PCB sorption experiment. The volume occupied by 42.2 ng of PCB-28 sorbed on a plastic particle represents $3.01 \cdot 10^{-5} \text{ mm}^3$ (density of PCB-28 = 1.4 mg/mm^3) and the one occupied by 35.7 ng of PCB-138 sorbed on a plastic particle represents $2.23 \cdot 10^{-5} \text{ mm}^3$ (density of PCB-138 = 1.6 mg/mm^3). Since one plastic particle represents a surface area of 70 mm^2 if the surface is considered smooth, it means that PCBs were most likely sorbed as a single layer onto plastic particles.

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

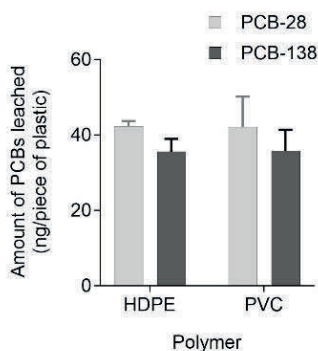


Figure 3.2. Amount of PCBs leached from HDPE and PVC in hexane after 20h. HDPE and PVC pieces with 1 % PBDE-209 + PCB-28 and -138 were leached to hexane for 20h at 38°C (800 rpm). Due to the difference in density between HDPE (0.96 g/cm³) and PVC (1.38 g/cm³), concentrations of PCBs released (ng per g of plastic) were normalized and expressed as the amount (ng) released per one unit of a piece of plastic. No differences were detected between the amount of PCB-28 (p-val = 0.96) and PCB-138 (p-val = 0.97) released from HDPE compared to PVC. Error bars represent standard deviation, n = 3.

3.3.2. Influence of food and gastric secretions on chemical leaching

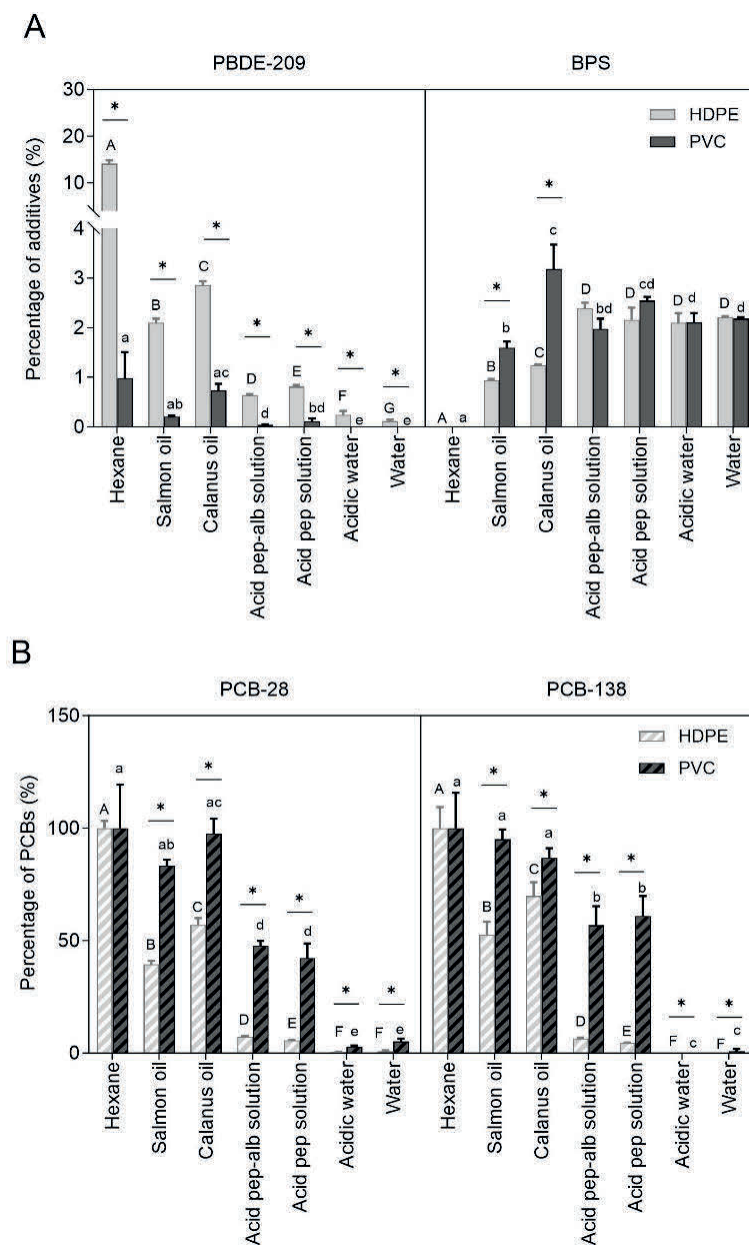
3.3.2.1. Oil versus aqueous solution

PBDE-209, PCB-28 and -138. For both polymer types, PBDE-209 and PCBs were leached at significantly higher proportions in oil than in aqueous solutions (acidic albumin-pepsin solution, acidic pepsin solution, acidic water and water) (Figure 3.3). For HDPE compared to the acidic albumin-pepsin solution, the leaching of PBDE-209 was 3.3-fold higher in

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

salmon oil and 4.5-fold higher in calanus oil (Figure 3.3A), the leaching of PCB-28 was 5.3-fold higher in salmon oil and 7.6-fold higher in calanus oil (Figure 3.3B), and the leaching of PCB-138 was 8.8-fold higher in salmon oil and 10.7-fold higher in calanus oil (Figure 3.3B). For PVC compared to the acidic albumin-pepsin solution, the leaching of PBDE-209 was 5.2-fold higher in salmon oil and 18.4-fold higher in calanus oil (Figure 3.3A) and the leaching of PCB-28 and PCB-138 was about twice as high in both oils (Figure 3.3B). The higher affinity for oil compared to an aqueous solution can be explained by the hydrophobicity of these chemicals, illustrated by a high log K_{ow} of 9.97 (Bao, You and Zeng, 2011), 5.67 (Ballschmiter *et al.*, 2005) and 6.83 (Ballschmiter *et al.*, 2005) for PBDE-209, PCB-28 and PCB-138 respectively. Our results are consistent with other *in vitro* studies in seabirds showing that stomach oil significantly increases the leaching of PBDE-209 from plastics compared to water-based solutions such as distilled water, seawater and acidic pepsin solution (Tanaka *et al.*, 2015; Sun *et al.*, 2021).

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching



Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

Figure 3.3. Percentage of plastic additives (A) and PCBs (B) leached from HDPE and PVC in different solutions after 20h. HDPE and PVC pieces with 1 % PBDE-209 + PCB-28 and -138 or HDPE and PVC pieces with 1% BPS were incubated in hexane, salmon oil, calanus oil, an aqueous digestive solution made of albumin, pepsin, HCl and water (acid pep-alb solution), an acidic pepsin solution (acid pep solution), acidic water and water for 20h at 38°C (800 rpm ; n = 3). Panel A) the percentage of additives (PBDE-209 left panel and BPS right panel) leached from HDPE (light grey) and PVC (dark grey). Panel B) the percentage of PCBs (PCB-28 left panel and PCB-138 right panel) leached from HDPE (light grey) and PVC (dark grey). Values with different uppercase letters (A, B, C, D, E, F for PBDE-209 or PCB-28) or lowercase letters (a, b, c, d, e, f for BPS or PCB-138) show significant differences between treatments (p-val < 0.05). Asterisk (*) depict significant differences between polymers for each chemical and treatment (p-val < 0.05). Error bars represent standard deviation, n = 3.

BPS. The leaching of BPS from HDPE followed opposite trends compared to PBDE-209, with significantly higher concentrations leached in the aqueous solutions compared to the oils (Fig 3.3A). More specifically, the leaching of BPS from HDPE was 2.6-fold higher in the acidic albumin-pepsin solution than in salmon oil and 1.9-fold higher in the acidic albumin-pepsin solution than in calanus oil. This higher affinity of BPS for aqueous environments compared to PBDE-209 is consistent with the polar sulfonyl groups of BPS, which significantly increase its solubility in water (3518 mg/L), as illustrated by the lower log K_{ow} of the molecule (1.65) (Vasiljevic and Harner, 2021). Its positive log K_{ow} and ability to create hydrophobic interactions explain why it is also found in the lipid compartment (Broniatowski *et al.*, 2016). Surprisingly, the leaching of BPS from PVC did not support this pattern. A higher (1.6-fold) proportion was leached in the

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

calanus oil than in the aqueous solutions, followed by a lower (1.2-fold) proportion in the salmon oil than in the aqueous solutions (Fig 3.3A). These observations can partly be attributed to the distribution of BPS within the polymer matrices and the stronger influence of WEs on the leaching of chemicals (discussed below).

Overall, our findings indicate that the release of highly hydrophobic chemicals, such as PBDEs and PCBs, from plastic is primarily influenced by the presence of lipids. In contrast, the leaching of more water-soluble additives, like BPS, is affected by both lipids and aqueous components, with an affinity for one or the other depending on the type of lipid involved and the type of polymer.

3.3.2.2. Calanus versus salmon oil

The lipid composition of the diet (calanus or salmon oil) had a significant effect on the leaching of plastic-associated compounds with more additives and PCBs leached in calanus oil compared to salmon oil. For HDPE, calanus oil contained 1.3-fold more BPS and PCB-138 and 1.4-fold more PBDE-209 and PCB-28 than salmon oil (Figure 3.3A and B). For PVC, calanus oil contained 3.6-fold more PBDE-209, 3.9-fold more BPS and 1.2-fold more PCB-28 than salmon oil (Figure 3.3A and B). PCB-138 was entirely leached from PVC in both oils after 20h (Figure 3.3B). The observed differential influence between oils can be in part explained by the lipid composition of salmon versus calanus oil. Salmon oil is primarily made of TAGs (> 90%) while calanus oil is primarily made of WEs (> 90%) (Supplementary Table 3.1 and Supplementary Figure 3.1). As such, salmon oil is characterized by a higher concentration of polyunsaturated fatty acids

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

(PUFAs) whereas calanus oil contains more long-chain monounsaturated fatty acids and long-chain monounsaturated fatty alcohols, potentially creating a greater affinity for hydrophobic chemicals. Longer alkyl chains or lower number of unsaturated sites have been related to a higher hydrophobicity of fatty acids and fatty alcohols (Miura and Nonomura, 2019).

Procellariiform stomach oil is mainly made of TAGs and WE (70 – 90% total lipids) (Wang *et al.*, 2007; Connan *et al.*, 2014; Akanbi and Barrow, 2016). Connan *et al.* (2007) observed that TAG/WE ratio in stomach oil could be very different depending on the Procellariiform species, ranging from rich-WE and poor-TAG oil (short-tailed shearwater *Puffinus tenuirostris* and thin-billed prion *Pachyptila belcheri*) to rich-TAG and poor-WE oils (white-chinned petrel *Procellaria aequinoctialis*) with an intermediate group (Antarctic prion *Pachyptila desolata* and blue petrel *Halobaena caerulea*) varying with the dietary habits (Connan, Cherel and Mayzaud, 2007). The consumption of fish and squid will mainly provide TAGs while the consumption of crustaceans (krill, copepods) will mainly provide WEs (Connan, Cherel and Mayzaud, 2007). Accordingly, our data suggest a richer crustacean diet may result in a higher proportion of chemicals leached from ingested plastics in a Procellariiform's gastric model.

3.3.2.3. Acidity, enzymes and proteins

PBDE-209, PCB-28 and -138. Our results showed that acidity (HCl, pH 3) did not affect the leaching of PCBs from either HDPE or PVC (Figure 3.3B) and only slightly increased the leaching of PBDE-209 from HDPE (Figure 3.3A). However, the addition of an enzyme (pepsin) in solution with acidic water (pH 3) significantly increased the leaching of PBDE-209 and

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

PCBs from both polymers as compared to acidic water or water alone (Figure 3.3A and B). For HDPE, the leaching of PBDE-209 and PCB-28 was 3.3- and 13.6-fold higher in the acidic-pepsin solution compared to acidic water. For PCB-138, 4.6% were leached from HDPE in the acidic-pepsin solution while nothing was detected in acidic water (Figure 3.3B). For PVC, 0.11% PBDE-209 were leached in the acidic-pepsin solution while nothing was detected in acidic water (Figure 3.3A). The addition of pepsin had a particularly strong effect on the leaching of PCBs from PVC, where 60.9% PCB-138 were leached in acidic-pepsin solution while nothing was detected in acidic water (Figure 3.3B). For PCB-28, the leaching from PVC was 13.8-fold higher in the acidic pepsin-solution (Figure 3.3B). Our results are consistent with the observation made by Tanaka *et al.* (2015) where PBDE-209 leaching in the presence of an acidic pepsin solution was higher than in seawater or distilled water (Tanaka *et al.*, 2015).

The higher concentrations of PCBs detected in the leachates in the presence of pepsin may have been driven by the high affinity of PCBs for the hydrophobic part of the enzyme, as previously observed for PCB-126 and pepsin (Yue *et al.*, 2020). Additionally, proteins such as pepsin are known to form coronas at the surface of different types of structures including plastics (Stock *et al.*, 2020). Thus, PCB desorption in the presence of pepsin could have been driven by the surface-active behavior of the proteins, leading to a competition for the interface between both PCBs and the proteins, displacing the compound previously adsorbed (Absolom, Zingg and Neumann, 1987). The higher PCB desorption from PVC in the presence of pepsin was likely due to its glassy characteristic (discussed in the next section). The addition of albumin to the acidic pepsin solution slightly reduced the leaching of PBDE-

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

209 from HDPE by a factor of 1.3 (Figure 3.3A) and slightly increase the leaching of PCB-28 and -138 by a factor of 1.3 and 1.4 respectively (Figure 3.3B). The presence of albumin did not further influence the leaching of plastic-associated chemicals from PVC.

BPS. In general, BPS leaching was not influenced by the pH or the presence of pepsin and albumin, showing percentages of leaching similar to those found in water alone (about 2% ; Figure 3.3A). It is possible that BPS affinity for water dominated and concealed the lesser effects of pH, pepsin or albumin.

3.3.3. Influence of the type of polymer on chemical leaching

Polyethylene (PE) is the most abundant polymer found to be ingested by Procellariiformes, followed by polypropylene (PP) and PVC (Kühn *et al.*, 2021). We used HDPE, a rubbery semi-crystalline polymer similar to PP and characterized by a higher free volume and mobility as well as PVC, a glassy amorphous polymer characterized by a denser and more compact structure (Menéndez-Pedriz and Jaumot, 2020), in our model to assess exposure risk to plastic-associated chemicals. We found that the proportion of chemicals leached from plastic was dependent on the type of polymer (HDPE versus PVC).

PBDE-209. Significantly less PBDE-209 was leached from PVC compared to HDPE, ranging from 3.9-fold less in calanus oil (Figure 3.3A) to 15.9-fold less in acidic albumin-pepsin solution (Figure 3.3A). The polymer-dependent differences in chemical leaching might be explained by the

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

additive's distribution within the polymer matrices as well as by the polymer physico-chemical properties. Despite the homogenization step performed during plastic manufacture to obtain a homogeneous additive distribution, surface tension-driven migrations may possibly occur upon molding, leading to heterogeneity in the final plastic plates. Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) analyses performed using the depth-profiling mode revealed that PBDE-209 was enriched on the outermost surface of HDPE pieces, but not PVC (Figure 3.4), likely resulting in a higher percentage of PBDE-209 leached from HDPE. In addition, rubbery polymers such as HDPE are expected to demonstrate a great diffusion potential due to their large free volume and great flexibility and molecular mobility (Menéndez-Pedriza and Jaumot, 2020). Conversely, the large size and high molecular mass of PBDE-209 (959.17 g/mol) may have reduced its ability to diffuse out of the more compact PVC matrix.

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

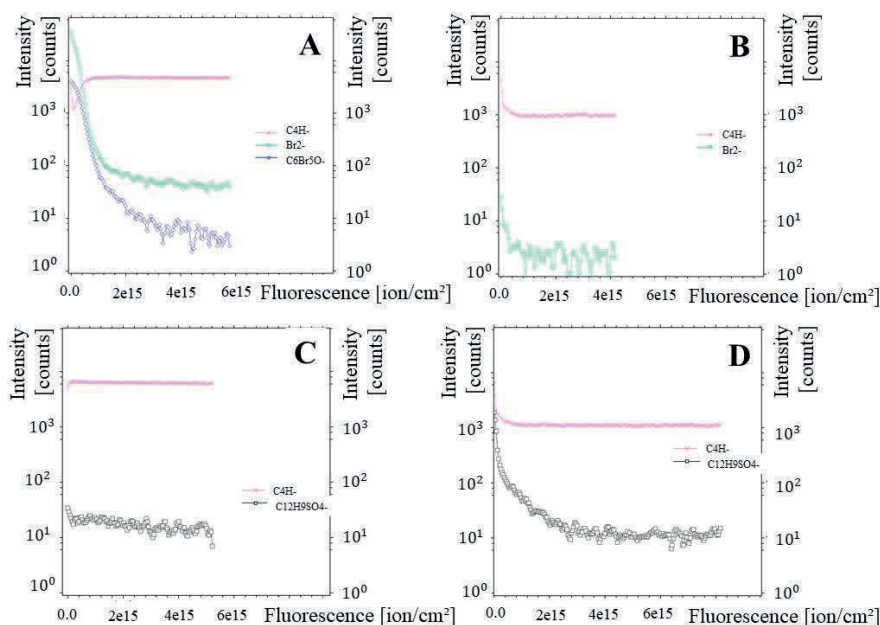


Figure 3.4. Intensity of selected negatively-charged secondary ions recorded using ToF-SIMS upon depth-profiling on A) HDPE + PBDE-209; B) PVC + PBDE-209; C) HDPE + BPS and D) PVC + BPS. C_4H^- is mostly representing the polymers (HDPE and PVC), Br_2^- and $C_6Br_5O^-$ are related to PBDE-209, $C_{12}H_9SO_4^-$ is related to BPS. Note that $C_6Br_5O^-$ was not found in PVC + PBDE-209. The conversion of fluence of the Argon GCIB used for surface erosion (see x-axis of the graphs) into a depth value is of the order of 58 nm/1015 ions/cm² for HDPE and of the order of 37 nm/1015 ions/cm² in the case of PVC. PBDE-209 is enriched in a surface layer of about 180 nm in HDPE, while BPS is enriched in a surface layer of about 100 nm in PVC.

BPS. The opposite was observed for the leaching of BPS in salmon and calanus oils, in which it was 1.7 and 2.6-fold more leached from PVC than from HDPE (Figure 3.3A). ToF-SIMS analysis revealed that BPS was

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

enriched in the superficial layers of PVC, while mostly absent from the surface and subsurface layers of HDPE, which may explain the larger quantities leached from PVC (Figure 3.4). In addition, the smaller size and lower molecular mass (250.28 g/mol) of BPS also facilitates its ability to diffuse out of the PVC matrix. On the other hand, no difference was observed between PVC and HDPE in the leaching of BPS in the different aqueous solutions. This lack of distinction may be explained by the pronounced affinity of BPS for water, which may have masked differences between polymers (Figure 3.3A).

The affinity of BPS for oils and aqueous environments was polymer-dependent. As previously discussed, its leaching from HDPE was greater in the aqueous environment than in lipids (calanus oil > salmon oil) (Figure 3.3A), while its leaching from PVC varied depending on the type of oil tested (calanus oil > aqueous solutions > salmon oil) (Figure 3.3A). Our findings indicate that BPS may exhibit a stronger affinity for calanus oil, rich in wax esters, compared to a water-rich medium when its leaching is facilitated by a higher distribution at the superficial layers of the plastic. To our knowledge, no study has reported the variation in affinity of BPS or its analogs for a lipid or aqueous environment as a function of the specific type of polymer and lipids involved. This observation warrants further investigation.

PCB-28 and -138 (sorbed). A higher proportion of both PCB-28 (1.7 to 6.4-fold) and -138 (1.8 to 8.7-fold) was leached from PVC compared to HDPE in oil as well as aqueous solutions after 20h (Figure 3.3B). The higher free volume and diffusivity of HDPE versus PVC resulted in sorption of chemicals deeper into the plastic matrix (Hüffer and Hofmann, 2016), which

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

in turn, is associated with a slower leaching of chemicals during leaching processes.

3.4. Conclusion

Our study explores the multifaceted interactions between polymer type, gastric content and chemical properties of plastic-associated chemicals (additives and sorbed POPs) on their leaching from plastics under different gastric conditions. Our results showed a strong influence of the type of polymer on the leaching behavior of chemicals, since higher proportions of PBDE-209 were leached from HDPE while higher proportions of PCBs and BPS were leached from PVC, which can be attributed to the chemical properties of plastic-associated compounds, their distribution within the polymer matrix as well as the diffusibility of the polymer. For BPS, this difference between polymer type was only observed in the presence of lipids, also highlighting the strong influence of lipids on the leaching behavior of chemicals. The leaching of highly hydrophobic chemicals (PBDE-209 and PCBs) was primarily enhanced by the presence of oils. However, the behavior of BPS was more complex, as its affinity for lipids depended on the type of polymer. When leached from HDPE, BPS exhibited a stronger affinity for the aqueous environment. However, when leached from PVC, calanus oil had a stronger impact than the aqueous environment, which can be partly attributed to the wide distribution of BPS at the surface of PVC and the stronger influence of calanus wax ester-rich oil on the release of plastic-associated chemicals. Indeed, the presence of calanus oil almost always resulted in higher proportions of leached chemicals compared to salmon oil, highlighting the

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

potentially stronger influence of a WEs-rich diet compared to TAGs-rich diet on the leaching of plastic-associated chemicals.

Our findings demonstrate the influence of both lipidic and aqueous elements present in a Procellariiform stomach on the leaching of additives and POPs from plastic, with an affinity depending on the properties of the chemicals, the type of lipids and the type of polymer. To accurately assess the risks associated with the leaching of both additives and sorbed POPs in a Procellariiform stomach, the temporal leaching of plastic-associated chemicals will be studied in the next chapter in a model that incorporates both aqueous and lipidic components.

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

Supplementary material

Supplementary Table 3.1. Lipidic composition of the salmon and calanus oil.

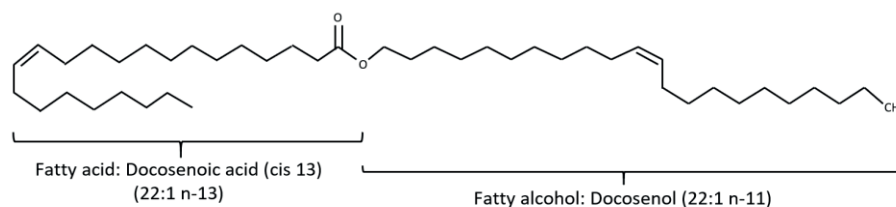
Parameters	Concentrations	
	Salmon oil	Calanus oil
Lipid class		
Triglycerides	> 900 mg/g	3 mg/g ⁽³⁾
Wax esters	NA	> 900 mg/g ⁽²⁾
Fatty acids		
Total fatty acids	893 mg/g ⁽¹⁾	550 mg/g ⁽²⁾
<i>Saturated fatty acids</i>	155 mg/g ⁽¹⁾	160 mg/g ⁽²⁾
Myristic acid 14:0	26 mg/g ⁽¹⁾	64 mg/g ⁽³⁾
Palmitoleic acid 16:0	94 mg/g ⁽¹⁾	45 mg/g ⁽³⁾
Stearic acid 18:0	24 mg/g ⁽¹⁾	2 mg/g ⁽³⁾
<i>Monounsaturated fatty acids</i>	425 mg/g ⁽¹⁾	150 mg/g ⁽²⁾
Palmitoleic acid + iso 16:1 n-7	32 mg/g ⁽¹⁾	17 mg/g ⁽³⁾
Oleic acid 18:1 n-9	326 mg/g ⁽¹⁾	16 mg/g ⁽³⁾
Eicosenoic acid + iso 20:1 Gondoic acid 20:1 n-9	33 mg/g ⁽¹⁾	24 mg/g ⁽³⁾
Docosenoic acid + iso 22:1 n- 13 Cetoleic acid 22:1 n-11	4 mg/g ⁽¹⁾	43 mg/g ⁽³⁾
<i>Polyunsaturated fatty acids</i>	313 mg/g	190 mg/g ⁽³⁾ - 240 mg/g ⁽²⁾
<u>n-3 fatty acids</u>	152 mg/g ⁽¹⁾	> 180 mg/g ^(2, 3)
A-linolenic acid (ALA) 18:3 n-3	44 mg/g ⁽¹⁾	14 mg/g ⁽³⁾
Stearidonic acid (SDA) 18:4 n-3	7 mg/g ⁽¹⁾	70 mg/g ⁽²⁾

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

Eicosapentanoic acid (EPA) 20:5 n-3	35 mg/g ⁽¹⁾	65 mg/g ⁽²⁾
Docosahexanoic acid (DHA) 22:6 n-3	43 mg/g ⁽¹⁾	50 mg/g ⁽²⁾
<u>n-6 fatty acids</u>	155 mg/g ⁽¹⁾	> 30 mg/g ⁽²⁾
Linoleic acid (LA) 18:2 n-6	140 mg/g	0.7 mg/g
Fatty alcohols		
Total fatty alcohols	NA	380 mg/g ⁽²⁾
Of which are policosanols (sum 22:1 n-11, C20:1 n-9)	NA	> 210 mg/g ⁽²⁾
Eicosenol 20:1 n-9	NA	188 mg/g ⁽³⁾
Docosenol 22:1 n-11	NA	129 mg/g ⁽³⁾

(1) Analysis performed in our laboratory, (2) Calanus oil technical sheet (Zooca lipids©), (3) (Schots *et al.*, 2020).

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching



Supplementary Figure 3.1. Example of wax ester found in the oil of *Calanus finmarchicus*. This wax ester is composed of a long-chain polyunsaturated fatty acid docosenoic acid (cis 13) (C22:1 n-13) bound to a long chain fatty alcohol docosenol (22:1 n-11).

Acknowledgements

The authors would like to thank Helene Dailly and Etienne Bodart from the Mineral & Organic Chemical Analysis (MOCA) lab (UCLouvain) for their help with chemical analysis. We also would like to thank Catherine Rasse from the Louvain Institute of Data Analysis and Modeling in economics and statistics (IDAM) (UCLouvain) for her help with statistical analysis.

References

Absolom, D. R., Zingg, W. and Neumann, A. W. (1987) ‘Protein adsorption to polymer particles: role of surface properties.’, *Journal of biomedical materials research*, 21(2), pp. 161–171. doi: 10.1002/jbm.820210202.

Adegoke, E. O., Rahman, M. S. and Pang, M. G. (2020) ‘Bisphenols Threaten Male Reproductive Health via Testicular Cells’, *Frontiers in Endocrinology*, 11(September), pp. 1–18. doi: 10.3389/fendo.2020.00624.

Akanbi, T. O. and Barrow, C. J. (2016) ‘Lipid profiles, in vitro digestion and oxidative stability of mutton bird oil’, *Journal of Food Science and Technology*, 53(2), pp. 1230–1237. doi: 10.1007/s13197-015-2106-7.

Aneck-Hahn, N. H. *et al.* (2018) ‘Estrogenic activity, selected plasticizers and potential health risks associated with bottled water in South Africa’, *Journal of Water and Health*, 16(2), pp. 253–262. doi: 10.2166/wh.2018.043.

Antunes, J. C. *et al.* (2013) ‘Resin pellets from beaches of the Portuguese coast and adsorbed persistent organic pollutants’, *Estuarine, Coastal and Shelf Science*, 130, pp. 62–69. doi: 10.1016/j.ecss.2013.06.016.

Bakir, A., Rowland, S. J. and Thompson, R. C. (2014) ‘Enhanced desorption of persistent organic pollutants from microplastics under simulated physiological conditions’, *Environmental Pollution*, 185, pp. 16–23. doi: 10.1016/j.envpol.2013.10.007.

Ballschmiter, K. *et al.* (2005) ‘High-resolution gas chromatography retention data as a basis for estimation of the octanol-water distribution coefficients (Kow) of PCB: The effect of experimental conditions’, *Analytical and*

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

Bioanalytical Chemistry, 382(8), pp. 1859–1870. doi: 10.1007/s00216-005-3307-0.

Banaderakhshan, R. *et al.* (2022) ‘Bisphenol A and its alternatives in Austrian thermal paper receipts, and the migration from reusable plastic drinking bottles into water and artificial saliva using UHPLC-MS/MS’, *Chemosphere*, 286(April 2021). doi: 10.1016/j.chemosphere.2021.131842.

Bao, L. J., You, J. and Zeng, E. Y. (2011) ‘Sorption of PBDE in low-density polyethylene film: Implications for bioavailability of BDE-209’, *Environmental Toxicology and Chemistry*, 30(8), pp. 1731–1738. doi: 10.1002/etc.564.

Barrick, A. *et al.* (2021) ‘Plastic additives: Challenges in ecotox hazard assessment’, *PeerJ*, 9, pp. 1–26. doi: 10.7717/peerj.11300.

‘Beltest I014-Rev3 Determination of polychlorinated biphenyls in animal feed, animal fat, egg and egg products, milk products and other foodstuffs. Beltest, Belgium’ (2000).

Bouhroum, R. *et al.* (2019) ‘Concentrations and fingerprints of PAHs and PCBs adsorbed onto marine plastic debris from the Indonesian Cilacap coast and the North Atlantic gyre’, *Regional Studies in Marine Science*, 29, p. 100611. doi: 10.1016/j.rsma.2019.100611.

Broniatowski, M. *et al.* (2016) ‘Studies on the interactions of bisphenols with anionic phospholipids of decomposer membranes in model systems’, *Biochimica et Biophysica Acta - Biomembranes*, 1858(4), pp. 756–766. doi: 10.1016/j.bbamem.2016.01.017.

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

Campos, P. De *et al.* (2019) ‘Maternal bisphenol A exposure disrupts spermatogenesis in adult rat offspring’, *Journal of Toxicology and Environmental Health, Part A: Current Issues*, 82(3), pp. 163–175. doi: 10.1080/15287394.2019.1572557.

Choi, Y., Cho, Y. M. and Luthy, R. G. (2013) ‘Polyethylene-water partitioning coefficients for parent- and alkylated-polycyclic aromatic hydrocarbons and polychlorinated biphenyls’, *Environmental Science and Technology*, 47(13), pp. 6943–6950. doi: 10.1021/es304566v.

Coffin, S. *et al.* (2019) ‘Fish and Seabird Gut Conditions Enhance Desorption of Estrogenic Chemicals from Commonly-Ingested Plastic Items’, *Environmental Science and Technology*, 53(8), pp. 4588–4599. doi: 10.1021/acs.est.8b07140.

Connan, M. *et al.* (2007) ‘Trophic relationships of white-chinned petrels from Crozet Islands: Combined stomach oil and conventional dietary analyses’, *Marine Biology*, 152(1), pp. 95–107. doi: 10.1007/s00227-007-0664-6.

Connan, M. *et al.* (2014) ‘Combined stomach content, lipid and stable isotope analyses reveal spatial and trophic partitioning among three sympatric albatrosses from the Southern Ocean’, *Marine Ecology Progress Series*, 497, pp. 259–272. doi: 10.3354/meps10606.

Connan, M., Cherel, Y. and Mayzaud, P. (2007) ‘Lipids from stomach oil of procellariiform seabirds document the importance of myctophid fish in the Southern Ocean’, *Limnology and Oceanography*, 52(6), pp. 2445–2455. doi: 10.4319/lo.2007.52.6.2445.

Curie-paris, P. M. *et al.* (2014) ‘Lipids from Stomach Oil of Procellariiform

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

Seabirds Document the Importance of Myctophid Fish in the Southern Ocean’, *Limnology and Oceanography*, 52(6), pp. 2445–2455.

Delaeter, C. *et al.* (2022) ‘Plastic leachates: Bridging the gap between a conspicuous pollution and its pernicious effects on marine life’, *Science of the Total Environment*, 826, p. 154091. doi: 10.1016/j.scitotenv.2022.154091.

Fry, M. D., Fefer, S. I. and Sileo, L. (1987) ‘Ingestion of Plastic Debris by Laysan Albatrosses and Wedge-tailed Shearwaters in the Hawaiian Islands’, *Marine Pollution Bulletin*, 18(61), pp. 339–343.

Furness, R. W. (1985) ‘Ingestion of plastic particles by seabirds at Gough Island, South Atlantic Ocean’, *Environmental Pollution. Series A, Ecological and Biological*, 38(3), pp. 261–272. doi: 10.1016/0143-1471(85)90131-X.

Gripon, L. *et al.* (2021) ‘Brominated flame retardants extraction from waste electrical and electronic equipment-derived ABS using supercritical carbon dioxide’, *Waste Management*, 131(July), pp. 313–322. doi: 10.1016/j.wasman.2021.06.020.

Gunaalan, K., Fabbri, E. and Capolupo, M. (2020) ‘The hidden threat of plastic leachates: A critical review on their impacts on aquatic organisms’, *Water Research*, 184, p. 116170. doi: 10.1016/j.watres.2020.116170.

Guo, H. *et al.* (2019) ‘The leaching of additive-derived flame retardants (FRs) from plastics in avian digestive fluids: The significant risk of highly lipophilic FRs’, *Journal of Environmental Sciences (China)*, 85, pp. 200–207. doi: 10.1016/j.jes.2019.06.013.

Hahladakis, J. N. *et al.* (2018) ‘An overview of chemical additives present in

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

plastics: Migration, release, fate and environmental impact during their use, disposal and recycling’, *Journal of Hazardous Materials*, 344, pp. 179–199. doi: 10.1016/j.jhazmat.2017.10.014.

Hilton, G. M., Furness, R. W. and Houston, D. C. (2000) ‘A comparative study of digestion in North Atlantic seabirds’, *Journal of Avian Biology*, 31(1), pp. 36–46. doi: 10.1034/j.1600-048X.2000.310106.x.

Howdeshell, K. L. *et al.* (2008) ‘Gestational and lactational exposure to ethinyl estradiol, but not bisphenol a, decreases androgen-dependent reproductive organ weights and epididymal sperm abundance in the male long evans hooded rat’, *Toxicological Sciences*, 102(2), pp. 371–382. doi: 10.1093/toxsci/kfm306.

Hüffer, T. and Hofmann, T. (2016) ‘Sorption of non-polar organic compounds by micro-sized plastic particles in aqueous solution’, *Environmental Pollution*, 214, pp. 194–201. doi: 10.1016/j.envpol.2016.04.018.

Jackson, S. (1992) ‘Do Seabird Gut Sizes and Mean Retention Times Reflect Adaptation to Diet and Foraging Method?’, *Physiological Zoology*, 65(3), pp. 674–697.

Jacobsen, J. K., Massey, L. and Gulland, F. (2010) ‘Fatal ingestion of floating net debris by two sperm whales (*Physeter macrocephalus*)’, *Marine Pollution Bulletin*, 60(5), pp. 765–767. doi: 10.1016/j.marpolbul.2010.03.008.

Koelmans, A. A. *et al.* (2016) ‘Microplastic as a Vector for Chemicals in the Aquatic Environment: Critical Review and Model-Supported Reinterpretation of Empirical Studies’, *Environmental Science and Technology*, 50(7), pp. 3315–3326. doi: 10.1021/acs.est.5b06069.

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

Kühn, S., Booth, Andy M, *et al.* (2020) ‘Transfer of Additive Chemicals From Marine Plastic Debris to the Stomach Oil of Northern Fulmars’, 8(August), pp. 1–14. doi: 10.3389/fenvs.2020.00138.

Kühn, S., Booth, Andy M., *et al.* (2020) ‘Transfer of Additive Chemicals From Marine Plastic Debris to the Stomach Oil of Northern Fulmars’, *Frontiers in Environmental Science*, 8. doi: 10.3389/fenvs.2020.00138.

Kühn, S. *et al.* (2021) ‘Polymer types ingested by northern fulmars (*Fulmarus glacialis*) and southern hemisphere relatives’, *Environmental Science and Pollution Research*, 28(2), pp. 1643–1655. doi: 10.1007/s11356-020-10540-6.

Kühn, S. and van Franeker, J. A. (2020) ‘Quantitative overview of marine debris ingested by marine megafauna’, *Marine Pollution Bulletin*, 151(110858). doi: 10.1016/j.marpolbul.2019.110858.

Li, X. *et al.* (2021) ‘BDE-209 and DBDPE induce male reproductive toxicity through telomere-related cell senescence and apoptosis in SD rat’, *Environment International*, 146(November 2020), p. 106307. doi: 10.1016/j.envint.2020.106307.

Li, Z. H. *et al.* (2012) ‘Effects of decabrominated diphenyl ether (PBDE-209) in regulation of growth and apoptosis of breast, ovarian, and cervical cancer cells’, *Environmental Health Perspectives*, 120(4), pp. 541–546. doi: 10.1289/ehp.1104051.

Mathieu-Denoncourt, J. *et al.* (2015) ‘Plasticizer endocrine disruption: Highlighting developmental and reproductive effects in mammals and non-mammalian aquatic species’, *General and Comparative Endocrinology*, 219,

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

pp. 74–88. doi: 10.1016/j.ygcen.2014.11.003.

Menéndez-Pedriza, A. and Jaumot, J. (2020) ‘Interaction of environmental pollutants with microplastics: A critical review of sorption factors, bioaccumulation and ecotoxicological effects’, *Toxics*, 8(2). doi: 10.3390/TOXICS8020040.

Miura, M. and Nonomura, Y. (2019) ‘Evaluation of hydrophobicity for fatty acids using reversed-phase thin layer chromatography’, *Journal of Oleo Science*, 68(7), pp. 665–670. doi: 10.5650/jos.ess19016.

Mohamed Nor, N. H. and Koelmans, A. A. (2019a) ‘Transfer of PCBs from Microplastics under Simulated Gut Fluid Conditions Is Biphasic and Reversible’, *Environmental Science and Technology*. doi: 10.1021/acs.est.8b05143.

Mohamed Nor, N. H. and Koelmans, A. A. (2019b) ‘Transfer of PCBs from Microplastics under Simulated Gut Fluid Conditions Is Biphasic and Reversible’, *Environmental Science and Technology*. doi: 10.1021/acs.est.8b05143.

Murata, M. and Kang, J. H. (2018) ‘Bisphenol A (BPA) and cell signaling pathways’, *Biotechnology Advances*, 36(1), pp. 311–327. doi: 10.1016/j.biotechadv.2017.12.002.

Olsen, M. A. *et al.* (2002) ‘The gastrointestinal tract of Adélie penguins - Morphology and function’, *Polar Biology*, 25(9), pp. 641–649. doi: 10.1007/s00300-002-0395-6.

Omeyer, L. C. M. *et al.* (2023) ‘Interactions between marine megafauna and

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

plastic pollution in Southeast Asia’, *Science of the Total Environment*, 874(March), p. 162502. doi: 10.1016/j.scitotenv.2023.162502.

Park, J. *et al.* (2019) ‘Assessment of endocrine-disrupting activities of alternative chemicals for bis(2-ethylhexyl)phthalate’, *Environmental Research*, 172, pp. 10–17. doi: 10.1016/j.envres.2019.02.001.

Perugini, M. (2009) ‘Environmental contaminants: Persistent organic pollutants’, *Handbook of Seafood and Seafood Products Analysis*, pp. 817–832.

Pfaendner, R. (2013) ‘Polymer Additives’, *Handbook of Polymer Synthesis, Characterization, and Processing*, pp. 225–247. doi: 10.1002/9781118480793.ch11.

Pierce, K. E. *et al.* (2004) ‘Obstruction and starvation associated with plastic ingestion in a Northern Gannet *Morus bassanus* and a Greater Shearwater *Puffinus gravis*’, *Marine Ornithology*, 32(2), pp. 187–189.

Pinto da Costa, J. *et al.* (2023) ‘Plastic additives and microplastics as emerging contaminants: Mechanisms and analytical assessment’, *TrAC - Trends in Analytical Chemistry*, 158. doi: 10.1016/j.trac.2022.116898.

Provencher, J. F. *et al.* (2017) ‘Quantifying ingested debris in marine megafauna: A review and recommendations for standardization’, *Analytical Methods*, 9(9), pp. 1454–1469. doi: 10.1039/C6AY02419J.

Roby, D., Brink, K. and Place, A. (1989) ‘Relative Passage Rates of Lipid and Aqueous Digesta in the Formation of Stomach Oils’, *The Auk: Ornithological Advances*, 106(2), pp. 303–313. doi: 10.1093/auk/106.2.303.

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

Roman, L., Lowenstine, L., *et al.* (2019) ‘Is plastic ingestion in birds as toxic as we think? Insights from a plastic feeding experiment’, *Science of the Total Environment*, 665, pp. 660–667. doi: 10.1016/j.scitotenv.2019.02.184.

Roman, L., Paterson, H., *et al.* (2019) ‘Size of marine debris items ingested and retained by petrels’, *Marine Pollution Bulletin*, 142, pp. 569–575. doi: 10.1016/j.marpolbul.2019.04.021.

Ryan, P. G. (2019) ‘Ingestion of Plastics by Marine Organisms’, *Handbook of Environmental Chemistry*, 78, pp. 235–266. doi: 10.1007/698_2016_21.

Santoro, A. *et al.* (2019) ‘Neuro-toxic and Reproductive Effects of BPA’, *Current Neuropharmacology*, 17(12), pp. 1109–1132. doi: 10.2174/1570159x17666190726112101.

Schots, P. C. *et al.* (2020) ‘Possible Health Effects of a Wax Ester Rich Marine Oil’, *Frontiers in Pharmacology*, 11(June), pp. 1–16. doi: 10.3389/fphar.2020.00961.

Staniszewska, M., Graca, B. and Nehring, I. (2016) ‘The fate of bisphenol A, 4-tert-octylphenol and 4-nonylphenol leached from plastic debris into marine water - experimental studies on biodegradation and sorption on suspended particulate matter and nano-TiO₂’, *Chemosphere*, 145, pp. 535–542. doi: 10.1016/j.chemosphere.2015.11.081.

Stock, V. *et al.* (2020) ‘Impact of artificial digestion on the sizes and shapes of microplastic particles’, *Food and Chemical Toxicology*, 135(October 2019), p. 111010. doi: 10.1016/j.fct.2019.111010.

Sun, B. *et al.* (2021) ‘Leaching of polybrominated diphenyl ethers from

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

microplastics in fish oil: Kinetics and bioaccumulation’, *Journal of Hazardous Materials*, 406(November 2020), p. 124726. doi: 10.1016/j.jhazmat.2020.124726.

Syberg, K. *et al.* (2020) ‘Case Studies in Chemical and Sorption of PCBs to environmental plastic pollution in the North Atlantic Ocean : Importance of size and polymer type’, *Case Studies in Chemical and Environmental Engineering*, 2(November), p. 100062. doi: 10.1016/j.cscee.2020.100062.

Tanaka, K. *et al.* (2013) ‘Accumulation of plastic-derived chemicals in tissues of seabirds ingesting marine plastics’, *Marine Pollution Bulletin*, 69(1–2), pp. 219–222. doi: 10.1016/j.marpolbul.2012.12.010.

Tanaka, K. *et al.* (2015) ‘Facilitated Leaching of Additive-Derived PBDEs from Plastic by Seabirds’ Stomach Oil and Accumulation in Tissues’, *Environmental Science and Technology*, 49(19), pp. 11799–11807. doi: 10.1021/acs.est.5b01376.

Tanaka, K. *et al.* (2019) ‘Piece-by-piece analysis of additives and manufacturing byproducts in plastics ingested by seabirds: Implication for risk of exposure to seabirds’, *Marine Pollution Bulletin*, 145(May), pp. 36–41. doi: 10.1016/j.marpolbul.2019.05.028.

Tanaka, K., Yamashita, R. and Takada, H. (2018) ‘Transfer of Hazardous Chemicals from Ingested Plastics to Higher-Trophic-Level Organisms’, in *Handbook of Environmental Chemistry*, pp. 267–280. doi: 10.1007/698_2018_255.

Vasiljevic, T. and Harner, T. (2021) ‘Bisphenol A and its analogues in outdoor and indoor air: Properties, sources and global levels’, *Science of the Total*

Chapter 3 – Influence of gastric components on plastic-associated chemical leaching

Environment, 789, p. 148013. doi: 10.1016/j.scitotenv.2021.148013.

Wang, S. W. *et al.* (2007) 'Fatty acid signatures of stomach oil and adipose tissue of northern fulmars (*Fulmarus glacialis*) in Alaska: Implications for diet analysis of Procellariiform birds', *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, 177(8), pp. 893–903. doi: 10.1007/s00360-007-0187-y.

Warham, J. (1977) 'The incidence, functions and ecological significance of petrel stomach oils', *Proceedings of the New Zealand Ecological Society*, 24, pp. 84–93.

Yue, Y. *et al.* (2020) 'Binding interactions between pepsin and 3,3',4,4',5-pentachlorobiphenyl', *Spectroscopy Letters*, 53(3), pp. 152–162. doi: 10.1080/00387010.2020.1715439.

Chapter 4

Dynamics of leaching of POPs and additives from plastic in a Procellariiform gastric model: implications for long-term exposure

Adapted from Van Hassel, L., Scholl, G., Eppe, G., Poleunisc, C., Dupont-Gillain, C., Finkelstein, M. and Debier, C. *Dynamics of leaching of POPs and additives from plastic in a Procellariiform gastric model : Diet- and polymer-dependent effects and implications for long-term exposure*. PLoS One 19, 1-19 (2024)

Table of Contents

Abstract.....	141
4.1. Introduction.....	142
4.2. Materials and methods	143
4.2.1. Leaching experiments	143
4.2.1.1. Procellariiform gastric model (PGM)	143
4.2.1.2. Influence of contact time with gastric solution.....	144
4.2.2. Statistical analyses	145
4.3. Results and discussion	146
4.3.1. PGM compared to individual gastric components	146
4.3.1.1. Oil versus aqueous solution	148
4.3.1.2. Calanus versus salmon oil.....	149
4.3.1.3. Influence of the type of polymer.....	152
4.3.2. Influence of contact time	152
4.3.3. Model limitations	156
4.3.4. Implications of plastic ingestion on seabird's health	156
Supplementary material	159
Acknowledgements.....	160
References.....	161

Abstract

Procellariiform seabirds, such as petrels, albatrosses, and shearwaters, are particularly vulnerable to plastic ingestion and accumulation, which may result in exposure to toxic chemicals for prolonged periods. This study investigates the temporal leaching of plastic-associated chemicals in a Procellariiform stomach using an *in vitro* Procellariiform gastric model (PGM). High-density polyethylene (HDPE) and polyvinyl chloride (PVC) pieces added with 1 % decabrominated diphenyl ether (PBDE-209) supplemented with 2,4,4'-trichloro-1,1'-biphenyl (PCB-28) and 2,2',3,4,4',5'-hexachlorobiphenyl (PCB-138) at their surface, or added with 1% bisphenol S (BPS) were leached in a gastric solution renewed every 20 hours for 100 hours. Two marine oils were tested to understand dietary influences. We saw that 100% of the sorbed POPs were leached from the plastic within 100 hours, while only 2-5% of the additives were leached from the matrix, suggesting that the remaining 95% of the additives could continue to be leached, posing a higher exposure risk compared to sorbed POPs.

4.1. Introduction

Procellariiform seabirds, including petrels, albatrosses, and shearwaters, rarely regurgitate indigestible items, resulting in the gastric accumulation of ingested plastics too large to be transferred into the intestine (> 2 mm) (Roman, Paterson, *et al.*, 2019; Ryan, 2019). In a Procellariiform stomach, plastics are continuously exposed to stomach oil and aqueous components. These gastric components have shown to individually enhance the leaching of additives and sorbed POPs in a diet- and polymer-dependent way (Chapter 3). However, their combined effects have never been studied. Moreover, plastics are suspected to remain in a Procellariiform stomach (proventriculus and/or gizzard chamber) for weeks or even months (Ryan, 2015; Van Franeker and Law, 2015), but the temporal leaching of these chemicals from ingested plastic has not been investigated in a complete gastric model.

In this chapter, we fill this gap by investigating the temporal leaching of plastic-associated chemicals using an *in vitro* Procellariiform gastric model (PGM). To simulate our PGM, plastic pieces (HDPE and PVC added with 1% PBDE-209 and having sorbed PCB-28 and PCB-138 and HDPE and PVC added with 1% BPS as in Chapter 3) were leached in a gastric solution renewed every 20 hours for a total of 100 hours. Two different marine oils (salmon and calanus oil) were tested to understand the influence of the diet. Our results highlight the exposure risk posed by additives compared to sorbed POPs present on the plastic surface.

4.2. Materials and methods

Plastic sample preparation and chemical analyses are explained in Chapter 3. Methodology used in this study closely follows that outlined in Chapter 3, with minor modifications as detailed below.

4.2.1. Leaching experiments

4.2.1.1. Procellariiform gastric model (PGM)

Our Procellariiform gastric model (PGM) was based on the conditions of the gizzard (second chamber) where the majority of the plastic remains trapped and is exposed to both chemical and mechanical digestion, increasing the potential leaching of chemicals (Tanaka, Yamashita and Takada, 2018). In this study, we considered that plastic pieces retained in the gizzard were exposed to a new gastric solution every 20 hours, based on the average time of gastric digestion in Procellariiformes (Roby, Brink and Place, 1989; Jackson, 1992; Hilton, Furness and Houston, 2000). The Procellariiform gastric solution was simulated with a mixture of 20 mL of marine oil and 20 mL of an aqueous solution made of milli-Q water, pepsin (10 g/L) and albumin (10 g/L) adjusted to a pH of 3 by adding first 37% and then 1 M HCl dropwise (Olsen *et al.*, 2002). Those conditions were chosen according to the volume of stomach oil and the composition of gastric juice in Procellariiformes (Kühn *et al.*, no date; Connan *et al.*, 2007; Coffin *et al.*, 2019). We investigated the effect of two marine oils: a salmon TAG-rich oil and a calanus WE-rich oil, reflecting the varied lipid composition of Procellariiform stomach oil (Wang *et al.*, 2007; Connan *et al.*, 2014; Akanbi and Barrow, 2016). In order to simulate the mechanical action of the gizzard, the Procellariiform gastric solution was continuously stirred with a glass magnetic stir bar at 800 rpm, at

Chapter 4 – Temporal leaching of chemicals in PGM

38°C [the body temperature of Procellariiformes (Warham, 1996)]. After incubation, samples were stored in pre-cleaned glass vials at -20°C in the dark prior to analyses.

4.2.1.2. Influence of contact time with gastric solution

Ten plastic pieces of HDPE (mass = $\pm$ 0.23 g) or ten plastic pieces of PVC (mass = $\pm$ 0.39 g) containing either 1% BPS or 1% PBDE-209 + PCB-28 and -138 (sorbed) were incubated in the PGM, combining the acidic albumin-pepsin solution and salmon (PGM-S) or calanus (PGM-C) oil for 100 hours [800 rpm; 10 pieces/40mL of solution; 50:50 (v/v)] to investigate the leaching and desorption behavior of chemicals over time (Figure 4.1). The gastric solution was replaced every 20 hours (Roby, Brink and Place, 1989; Jackson, 1992; Hilton, Furness and Houston, 2000). The solid-to-liquid ratio was based on the average number of plastic pieces and the digestive fluid volume in the stomach of Procellariiformes (Connan *et al.*, 2007; J F Provencher *et al.*, 2017).

Chapter 4 – Temporal leaching of chemicals in PGM

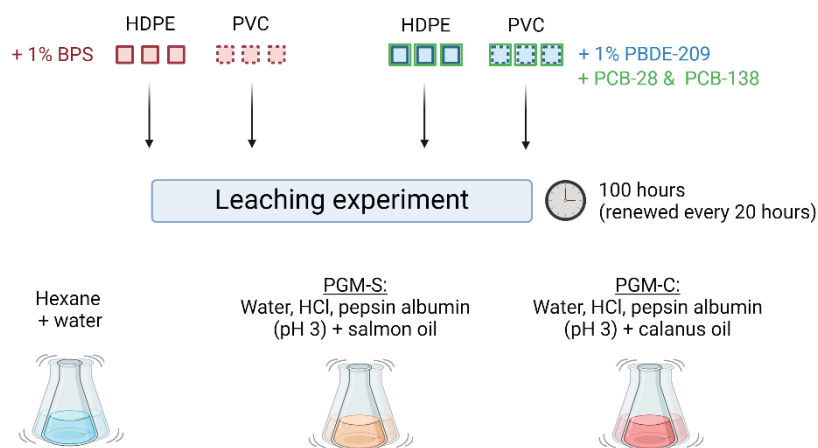


Figure 4.1. Experimental design. To study the influence of contact time on the release of chemicals, 10 plastic pieces containing either 1% BPS or 1% PBDE-209 + PCB-28 and -138 were incubated in the Procarriniform gastric model (PGM), containing an acidic albumin-pepsin solution mixed either with salmon oil or calanus oil for 100 hours. This gastric solution was replaced every 20 hours. Controls of water mixed with hexane were run in parallel. Created with BioRender.com

4.2.2. Statistical analyses

The percentage of additive leached in each solution was calculated as the ratio of the mean additive concentration measured in the digestive medium ($n=3$) and the total concentration of additive added to the polymer (10,000 $\mu\text{g/g}$). The percentage of PCBs leached in each medium was calculated as the ratio of the mean PCB concentrations (PCB-28 or PCB-138) measured in the digestive medium ($n=3$) and the mean PCB concentrations measured in hexane (positive control; $n=3$).

Welch ANOVA was employed to assess the differences in chemical leaching over time between the two treatments (PGM-S and PGM-C) while

Chapter 4 – Temporal leaching of chemicals in PGM

accounting for potential heterogeneity of variances between the treatment groups. Following the Welch ANOVA, we performed individual t-tests at specific time intervals to explore where statistically significant differences existed. The Bonferroni correction was applied to adjust for multiple comparisons, reducing the risk of Type I errors (false positives). To further investigate the differences in the percentage of chemicals leaching over time within each treatment group while accounting for the repeated measurements, a Tukey test was conducted. This test allowed us to identify specific time points where significant differences in chemical leaching occurred, providing a more comprehensive understanding of the temporal dynamics within each treatment group.

All statistical analyses were conducted using JMP Pro 16 (JMP Software), and significance levels were set at $p < 0.05$.

4.3. Results and discussion

4.3.1. PGM compared to individual gastric components

In Chapter 3, we studied the influence of isolated gastric components on the leaching of PBDE-209, BPS and PCBs from HDPE and PVC. We observed that chemical leaching was influenced by the presence of both lipidic and aqueous elements with a stronger affinity for one or the other depending on the chemical properties, the type of polymer and the type of lipid. We found similar patterns in the PGM for the leaching of plastic-associated chemicals. A comprehensive overview of experimental factors influencing the leaching

Chapter 4 – Temporal leaching of chemicals in PGM

of plastic-associated chemicals in the presence of individual gastric components and in the PGM can be found in Table 4.1.

Table 4.1. Comprehensive overview of the influence of digestive conditions and the polymer type on the leaching of PBDE-209, PCBs and BPS. Three questions are summarized in this table: (i) the influence of lipids compared to the aqueous environment, (ii) the influence of the type of oil and (iii) the influence of the polymer type on the leaching of plastic-associated chemicals. The table is divided according to the type of polymer involved, and whether the oil and aqueous components were studied individually (noted as “individual”) or combined (noted as “PGM”). For the PGM, the influence of the lipidic and aqueous environment, type of oil, and polymer is reported for the 100-hour period, based on observations made for each 20-hour interval. If the influence of a factor differs between the first 20 hours and subsequent periods, it is specified in the table.

<i>Influence lipidic and aqueous environment</i>				
	HDPE		PVC	
	Individual	PGM	Individual	PGM
PBDE-209	Lipidic > aqueous environment	Only found in lipidic phase	Lipidic > aqueous environment	Only found in lipidic phase
PCBs	Lipidic > aqueous environment	Only found in lipidic phase	Lipidic > aqueous environment	Only found in lipidic phase
BPS	Aqueous > lipidic environment	Found in both phases Aqueous > lipidic phase	Calanus oil > Aqueous solution > Salmon oil	Found in both phases <u>PGM-C:</u> Lipidic > aqueous phase during first 20 hours <u>PGM-S:</u> Lipidic = aqueous phase
<i>Influence type of oil</i>				
	HDPE		PVC	
	Individual	PGM	Individual	PGM
PBDE-209	Calanus oil > salmon oil	PGM-C > PGM-S	Calanus oil > salmon oil	PGM-C = PGM-S
PCBs	Calanus oil > salmon oil	PGM-C > PGM-S	Calanus oil > salmon oil	<u>PCB-28:</u> PGM-C > PGM-S

Chapter 4 – Temporal leaching of chemicals in PGM

				first 20 hours PCB-138: PGM-C = PGM-S
BPS	Calanus oil > salmon oil	PGM-C > PGM-S	Calanus oil > salmon oil	PGM-C = PGM-S
<i>Influence type of polymer</i>				
	Individual		PGM	
PBDE-209	HDPE > PVC		HDPE > PVC	
PCBs	HDPE < PVC		HDPE < PVC	
BPS	<u>Oils</u> HDPE < PVC <u>Aqueous solutions</u> HDPE = PVC		HDPE < PVC during first 20 hours	

4.3.1.1. Oil versus aqueous solution

PBDE-209, PCB-28 and – 138. In the complete PGM, including both oil and aqueous solution, PBDE-209 and PCBs were only detected in the lipidic phase (salmon or calanus oil), regardless of the polymer type, confirming their higher affinity for lipids over the aqueous phase (Table 4.1).

BPS. BPS exhibited an affinity for both lipidic and aqueous environments, as it was found in both phases (Figure 4.2). BPS preference for one phase over the other also depended on the type of polymer and lipids present (Table 4.1). For HDPE, higher concentrations of BPS (1.2 to 1.7-fold) were found in the aqueous phase of the gastric solution compared to the lipid phase (salmon or calanus oil) (Figure 4.2). For PVC, a higher proportion of BPS was found in the calanus lipid phase compared to the aqueous phase during the first 20 hours, whereas no difference was found in the distribution of BPS between the salmon lipid phase and the aqueous phase (Figure 4.2). These observations confirmed that the leaching of BPS is affected by both lipids and aqueous components, with an affinity for one or the other depending

Chapter 4 – Temporal leaching of chemicals in PGM

on whether the oil and aqueous components are combined, the type of lipid involved and the type of polymer (Table 4.1).

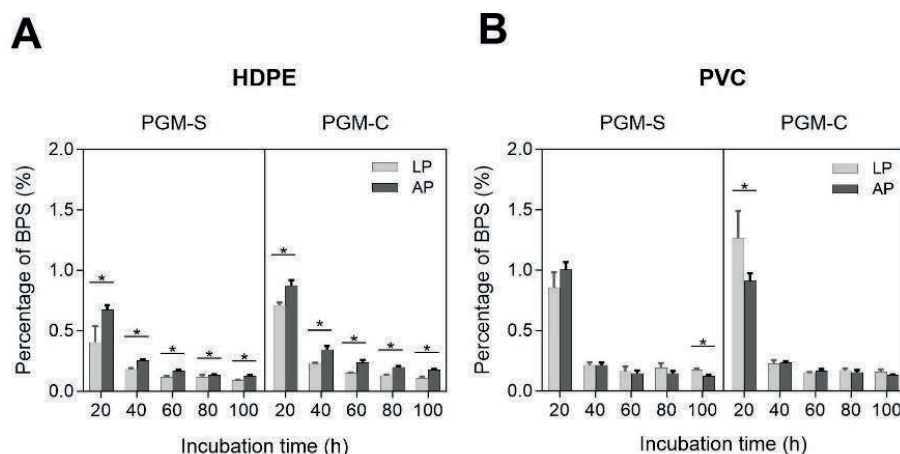


Figure 4.2. Percentage of BPS leached from HDPE (A) and PVC (B) in each phase of Procellariiform gastric model (PGM) over time. HDPE and PVC pieces with 1% BPS were incubated in the full PGM containing an acidic albumin-pepsin solution (pH 3) mixed with salmon oil (PGM-S) or calanus oil (PGM-C) at 38°C for 100h (800 rpm ; n = 3). The mixture was renewed every 20h. Lipidic phases are shown in light grey (LP) and aqueous phases are shown in dark grey (AP). Asterisk symbols (*) show significant differences between phases per hour (p-val < 0.05). Error bars represent standard deviation.

4.3.1.2. Calanus versus salmon oil

Similar patterns were observed in the PGM as compared to isolated gastric components (Table 4.1). For HDPE, the stronger impact of calanus oil compared to salmon oil was confirmed as PGM-C contained levels of PBDE-209, BPS, PCB-28 and -138 that were 1.4-, 1.5-, 1.3- and 1.4-fold higher than in PGM-S (Figure 4.3A and C). The stronger affinity for calanus oil compared to salmon oil was also observed for the leaching of PCB-28 from PVC (1.5-

Chapter 4 – Temporal leaching of chemicals in PGM

fold higher in PGM-C than in PGM-S) (Figure 4.3D). On the other hand, the leaching of PBDE-209, BPS and PCB-138 from PVC did not differ between PGM-S and PGM-C (Figure 4.3B and D), which can be attributed to the differential leaching behavior of chemicals between polymers (Table 4.1).

Chapter 4 – Temporal leaching of chemicals in PGM

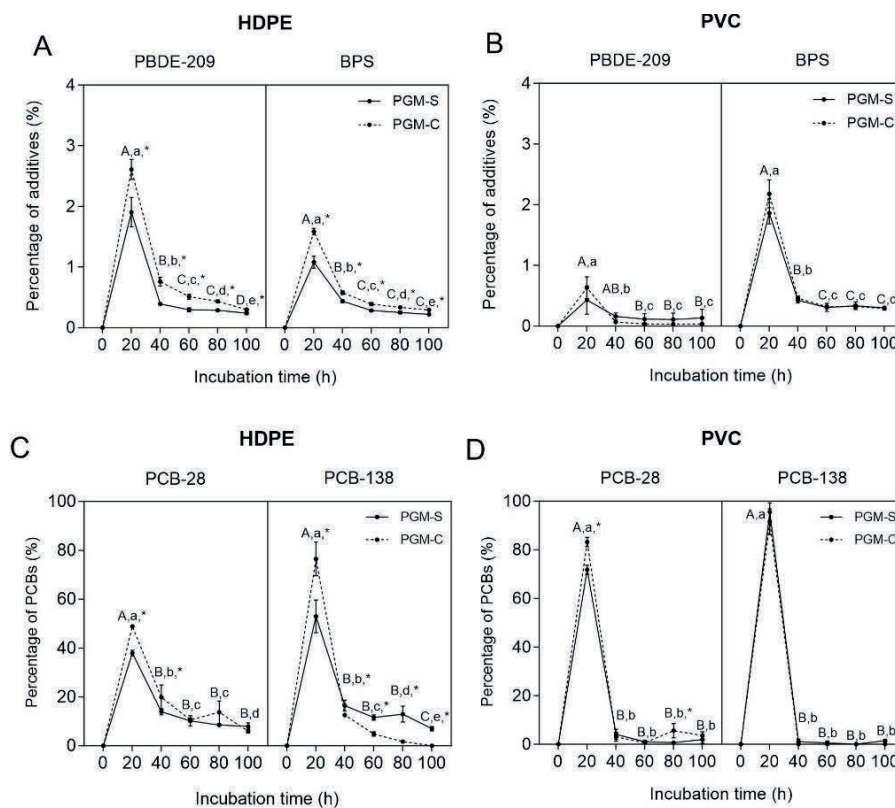


Figure 4.3. Percentage of plastic additives and PCBs leached from HDPE and PVC in salmon or calanus gastric fluid over time. HDPE and PVC pieces with 1% PBDE-209 + PCB-28 and -138 or HDPE and PVC pieces with 1% BPS were incubated in the full PGM containing an acidic albumin-pepsin solution (pH 3) mixed with salmon oil (PGM-S) or calanus oil (PGM-C) at 38°C for 100h (800 rpm ; n = 3). The mixture was renewed every 20h. Aqueous and lipidic phases were analyzed separately and concentrations were summed to give the total concentration leached in the digestive mixtures. Concentrations were below the detection limit in the aqueous phases for PBDE-209 and PCBs. Panels A and B) the percentage of PBDE-209 and BPS leached from HDPE and PVC. Panels C and D) the percentage of PCB-28 and PCB-138 desorbed from HDPE and PVC. Values with different uppercase letters (A, B, C, D, E for PGM-S) and lowercase (a, b, c, d, e for PGM-C) show significant differences in

Chapter 4 – Temporal leaching of chemicals in PGM

the percentage of chemicals released (additives or PCBs) over time for each treatment (p-val < 0.05). Asterisk symbols (*) show significant differences between salmon and calanus gastric fluid for each chemical at each timepoint (p-val < 0.05). Error bars represent standard deviation, n = 3.

4.3.1.3. Influence of the type of polymer

Following the same pattern as in Chapter 3, higher proportions of PBDE-209 were leached from HDPE (~ 4.3-fold after the first 20 hours), while higher proportions of BPS (~1.5-fold after the first 20 hours) and PCBs (~1.4-fold for PCB-28 and ~1.5-fold for PCB-138 after the first 20 hours) were leached from PVC (Table 4.1, Fig 4.3 and Table S1).

4.3.2. Influence of contact time

Plastic pieces were incubated in the PGM combining the lipidic and aqueous phases for 100 hours. We found a peak in the leaching of chemicals during the first 20h followed by a significant decrease for the remaining 80 hours (Figure 4.3).

PBDE-209 and BPS (additives). For HDPE, $1.9 \pm 0.2\%$ and $2.6 \pm 0.2\%$ of PBDE-209 were leached in PGM-S and PGM-C respectively, during the first 20h, while only $0.4 \pm 0.03\%$ and $0.8 \pm 0.1\%$ were leached between 20 and 40 hours (Figure 4.3A). The percentage leached continued to decrease slowly but significantly until the end of the experiment (100 hours). A similar dynamic was observed for BPS, with a leaching of $1.8 \pm 0.1\%$ and $1.6 \pm 1.0\%$ in PGM-S and PGM-C, during the first 20 hours, followed by $0.4 \pm 0.02\%$ and $0.6 \pm 0.03\%$ between 20 and 40 hours (Figure 4.3A).

For PVC, the peak leaching of PBDE-209 was lower than HDPE, with $0.4 \pm 0.2\%$ and $0.6 \pm 0.2\%$ of PBDE-209 leached in PGM-S and PGM-C

Chapter 4 – Temporal leaching of chemicals in PGM

during the first 20 hours of contact (Figure 4.3B). A significant drop in the proportions leached was observed between 20 and 40 hours, with $0.2 \pm 0.1\%$ and $0.1 \pm 0.01\%$ of PBDE-209 in PGM-S and PGM-C. BPS was leached at $1.9 \pm 0.2\%$ and $2.2 \pm 0.2\%$ in PGM-S and PGM-C over the first 20 hours before dropping at $0.4 \pm 0.04\%$ and $0.5 \pm 0.04\%$ between 20 and 40 hours (Figure 3.3B). In both cases, levels continued to drop between 40 and 60 hours and then remained constant.

The higher proportion of additives leached during the first 20 hours of digestion (Figure 4.3A and B) could be attributed to the higher leachability of additives in the superficial layers of the plastic, which are readily accessible to the surrounding solution. The heterogeneous distribution of additives within the polymer matrices identified by ToF-SIMS supports this observation as higher proportions of PBDE-209 and BPS were present in the superficial layers of HDPE and PVC respectively (Fig 3.4 in Chapter 3). These observations are consistent with other studies showing an exponential decrease in the leaching of additives from PE in oil and water over time (Tanaka *et al.*, 2015; Xia and Rubino, 2015).

PCB-28 and -138 (sorbed). The proportion of PCBs leached from HDPE was much higher than the one of PBDE-209 and BPS, with peaks of $38.0 \pm 1.2\%$ and $48.9 \pm 0.3\%$ of PCB-28 and $52.9 \pm 6.7\%$ and $76.5 \pm 7.0\%$ of PCB-138 leached in PGM-S and PGM-C during the first 20 hours of contact (Figure 4.3C). The proportions then dropped to $13.9 \pm 1.3\%$ and $19.9 \pm 5.0\%$ of PCB-28 and $16.4 \pm 2.2\%$ and $12.6 \pm 0.2\%$ of PCB-138 between 20 and 40 hours. Levels continued to decline over time until the end of the experiment (100 hours). PCBs were also leached from PVC in much higher proportions than PBDE-209 and BPS with peaks of $71.8 \pm 1.9\%$ and $48.9 \pm 0.3\%$ of PCB-

Chapter 4 – Temporal leaching of chemicals in PGM

28 and $95.6 \pm 3.7\%$ and $91.5 \pm 5.4\%$ of PCB-138 in salmon and calanus gastric solution, respectively (Figure 4.3D). Proportions leached then dramatically dropped between 20 and 40 hours of contact, with levels below 1%.

Additives versus sorbed PCBs. The majority (79 – 100%) of sorbed PCBs were leached after 100 hours while only a small percentage of additives leached out (less than 5%). When considering total $\mu\text{g/g}$, 100 – 450 times more additives were leached compared to PCBs (Table 4.2) which can be attributed to the higher concentration of additives present within the plastic (10,000 μg additive/g of plastic versus 925 – 1700 ng PCBs/g plastic), representative of what happens in the environment (Barrick *et al.*, 2021). Our findings suggest that after ~ 5 days in a Procellariiform stomach, plastics could leach all the sorbed chemicals, but a substantial amount of additives remain in the plastic matrix and could potentially be leached out in the following days or weeks.

Table 4.2. Total percentage (%) and concentration ($\mu\text{g/g}$) of PBDE-209, BPS, PCB-28 and PCB-138 leached after 100 hours in the PGM. HDPE and PVC pieces with 1% PBDE-209 + PCB-28 and -138 (sorbed) or HDPE and PVC pieces with 1% BPS were incubated in the complete PGM containing an acidic albumin-pepsin solution (pH 3) mixed with salmon oil (PGM-S) or calanus oil (PGM-C) at 38°C for 100h (800 rpm ; n = 3). The total percentage (%) or concentration ($\mu\text{g/g}$) of chemical leached after 100 hours were measured by summing the percentage or concentration of chemicals leached from each polymer after 20, 40, 60, 80 and 100 hours in the PGM-S or PGM-C.

Polymer	Oil	Chemical leached							
		PBDE-209		BPS		PCB-28		PCB-138	
		% $\pm$ SD	[$\mu\text{g/g}$] $\pm$ SD	% $\pm$ SD	[$\mu\text{g/g}$] $\pm$ SD	% $\pm$ SD	[$\mu\text{g/g}$] $\pm$ SD	% $\pm$ SD	[$\mu\text{g/g}$] $\pm$ SD
HDPE	PGM-S	3.1 $\pm$ 0.3	311.2 $\pm$ 28.2	2.3 $\pm$ 0.07	226.9 $\pm$ 6.6	78.6 $\pm$ 2.5	1.3 $\pm$ 0.07	100.9 $\pm$ 10.0	1.4 $\pm$ 0.17
	PGM-C	4.6 $\pm$ 0.3	461.0 $\pm$ 30.2	3.2 $\pm$ 0.02	317.4 $\pm$ 2.1	99.0 $\pm$ 3.2	1.7 $\pm$ 0.17	107.1 $\pm$ 10.6	1.5 $\pm$ 0.39
PVC	PGM-S	0.9 $\pm$ 0.4	94.6 $\pm$ 43.3	3.2 $\pm$ 0.29	323.1 $\pm$ 29.4	79.5 $\pm$ 16.0	0.9 $\pm$ 0.02	98.7 $\pm$ 15.3	0.9 $\pm$ 0.05
	PGM-C	0.8 $\pm$ 0.2	80.0 $\pm$ 18.4	3.6 $\pm$ 0.17	357.1 $\pm$ 17.1	96.1 $\pm$ 19.3	1.0 $\pm$ 0.05	91.5 $\pm$ 14.2	0.8 $\pm$ 0.05

4.3.3. Model limitations

While the Procellariiform gastric model used in the present study has many advantages, such as the absence of experimental animals and the ability to analyze the impact of multiple factors under controlled conditions, it is important to highlight that it also has limitations. First, the use of porcine pepsin, rather than a Procellariiform-specific pepsin, may not fully replicate the digestive environment of these birds. Several studies have observed species-specific differences in the activity of pepsin, as well as variations in their optimal pH and temperature conditions (Kamegashira *et al.*, 1977; Chu and Nakagawa, 1982; Nurhayati *et al.*, 2019). Similarly, bovine serum albumin (BSA) was used as a protein source, which differs from the types of proteins found in seabird prey (e.g., fish collagen). Marine proteins might interact with pepsin and influence the leaching of chemicals from ingested plastics differently than bovine albumin. For example, BSA is known to preferentially adsorb onto hydrophobic surfaces while collagen preferentially adsorbs onto hydrophilic surfaces (Ying *et al.*, 2003). Additionally, the mechanical stress applied in the model was limited to a stirring bar, which may not accurately mimic the pressure and mechanical forces within the gizzard that contribute to the weathering of plastic and likely increase the leaching of chemicals *in vivo*.

4.3.4. Implications of plastic ingestion on seabird's health

Out of 226 seabird species studied for plastic ingestion, 80% have been found with plastics in their stomach, with Procellariiforms accounting for half of this percentage (Kühn and van Franeker, 2020). In addition to their surface-

Chapter 4 – Temporal leaching of chemicals in PGM

feeding behavior making them more likely to ingest floating plastics than diving seabird species (Jackson, 1990; Bond *et al.*, 2014; Jennifer F. Provencher *et al.*, 2017), Procellariiformes rarely regurgitate indigestible items, which increases their propensity to accumulate plastics in their stomach for extended periods of time (van Franeker and Law, 2015).

PCBs were completely leached after 100 hours in the PGM. However, in the environment, it is possible that POPs may leach over longer periods of time. Indeed, plastics in sea water can be in contact with POPs for months or even years, which can lead to a deeper sorption of POPs inside the plastic at equilibrium (Rochman *et al.*, 2013; Koelmans *et al.*, 2016). A deeper sorption may in turn lead to a slower release in the digestive tract. Regardless of the desorption time, concentrations of PCBs leached from the plastic are marginal compared to the exposure resulting from POPs that bioaccumulate in bird prey (Herzke *et al.*, 2016), highlighting the negligible impact of sorbed POPs compared with other pathways of exposure. On the other hand, the continued leaching of additives was a concerning finding. Only a small fraction of additives (< 5%) was leached out during the residence time in the gastric model, suggesting that the remaining > 95% could continue to leach over time, subjecting Procellariiformes to chronic exposure to these chemicals for weeks or even months. Since additives are present at considerably higher concentrations than sorbed contaminants (0.05 – 70% of additive per plastic weight (Hahladakis *et al.*, 2018) compared to on average < 0.003% of POP per plastic weight (Wang *et al.*, 2021)), they could be released at levels thousand times higher than sorbed POPs. Moreover, many plastic additives are non-persistent pollutants (e.g. bisphenol A and its analogous, phthalates, alkylphenols) which are found at higher concentrations in plastic than in natural prey (Gallo *et al.*, 2018). Seabird exposure to this type of molecule is

Chapter 4 – Temporal leaching of chemicals in PGM

therefore mainly due to plastic ingestion, unlike exposure to POPs. This chronic exposure to plastic-associated chemicals raises concerns about potential health risks to seabirds, as most of these compounds are known as endocrine disruptors (Hauser and Calafat, 2005; Diamanti-Kandarakis *et al.*, 2009; Rochester, 2013; Marlatt *et al.*, 2022). Indeed, many plastic additives have been associated with negative reproductive effects such as delays in sexual maturity, decrease of fertility, gonadal abnormalities (feminization/masculinization) and development of cancer (Hauser and Calafat, 2005; Rochester, 2013; Roman, Lowenstine, *et al.*, 2019; Marlatt *et al.*, 2022).

Our study underscores that plastic ingestion by seabirds may pose a substantial health risk, primarily due to the leaching of significant concentrations of additives. Based on these findings, future research should prioritize investigating the potential health effects arising from the release of plastic additives when ingested by seabirds.

Supplementary material

Table S1. Comparison of additive percentage leached over time between polymers for PGM-S and PGM-C: p-value analysis. Welch ANOVA was employed to assess the differences in chemical leaching over time between the two treatments (PGM-S and PGM-C) while accounting for potential heterogeneity of variances between the treatment groups. Following the Welch ANOVA, we performed individual t-tests at specific time intervals to explore where statistically significant differences existed. The Bonferroni correction was applied to adjust for multiple comparisons, reducing the risk of Type I errors (false positives). P-value are shown for each chemical and PGM.

Time	PBDE-209		BPS	
	PGM-S	PGM-C	PGM-S	PGM-C
20	<0,0001*	<0,0001*	<0,0001*	<0,0001*
40	0,1766	<0,0001*	>0,9999	0,2729
60	0,4927	<0,0001*	>0,9999	>0,9999
80	0,5317	<0,0001*	0,6801	>0,9999
100	>0,9999	0,0008*	0,7230	>0,9999

Acknowledgements

The authors would like to thank Helene Dailly and Etienne Bodart from the Mineral & Organic Chemical Analysis (MOCA) lab (UCLouvain) for their help with chemical analysis. We also would like to thank Catherine Rasse from the Louvain Institute of Data Analysis and Modeling in economics and statistics (IDAM) (UCLouvain) for her help with statistical analysis.

References

- Akanbi, T. O. and Barrow, C. J. (2016) 'Lipid profiles, in vitro digestion and oxidative stability of mutton bird oil', *Journal of Food Science and Technology*, 53(2), pp. 1230–1237. doi: 10.1007/s13197-015-2106-7.
- Barrick, A. *et al.* (2021) 'Plastic additives: Challenges in ecotox hazard assessment', *PeerJ*, 9, pp. 1–26. doi: 10.7717/peerj.11300.
- Bond, A. L. *et al.* (2014) 'Plastic ingestion by fulmars and shearwaters at Sable Island, Nova Scotia, Canada', *Marine Pollution Bulletin*, 87(1), pp. 68–75. doi: 10.1016/j.marpolbul.2014.08.010.
- Chu, S. S. T. and Nakagawa, Y. (1982) 'Comparison of pepsins isolated from porcine, bovine and *Penicillium jathiuellum*', *Comparative Biochemistry and Physiology -- Part B: Biochemistry and*, 72(4), pp. 625–629. doi: 10.1016/0305-0491(82)90516-8.
- Coffin, S. *et al.* (2019) 'Fish and Seabird Gut Conditions Enhance Desorption of Estrogenic Chemicals from Commonly-Ingested Plastic Items', *Environmental Science and Technology*, 53(8), pp. 4588–4599. doi: 10.1021/acs.est.8b07140.
- Connan, M. *et al.* (2007) 'Trophic relationships of white-chinned petrels from Crozet Islands: Combined stomach oil and conventional dietary analyses', *Marine Biology*, 152(1), pp. 95–107. doi: 10.1007/s00227-007-0664-6.
- Connan, M. *et al.* (2014) 'Combined stomach content, lipid and stable isotope analyses reveal spatial and trophic partitioning among three sympatric albatrosses from the Southern Ocean', *Marine Ecology Progress Series*, 497, pp. 259–272. doi: 10.3354/meps10606.

Chapter 4 – Temporal leaching of chemicals in PGM

Diamanti-Kandarakis, E. *et al.* (2009) ‘Endocrine-disrupting chemicals: An Endocrine Society scientific statement’, *Endocrine Reviews*, 30(4), pp. 293–342. doi: 10.1210/er.2009-0002.

van Franeker, J. A. and Law, K. L. (2015) ‘Seabirds, gyres and global trends in plastic pollution’, *Environmental Pollution*, 203, pp. 89–96. doi: 10.1016/j.envpol.2015.02.034.

Van Franeker, J. A. and Law, K. L. (2015) ‘Seabirds, gyres and global trends in plastic pollution’, *Environmental Pollution*, 203, pp. 89–96. doi: 10.1016/j.envpol.2015.02.034.

Gallo, F. *et al.* (2018) ‘Marine litter plastics and microplastics and their toxic chemicals components: the need for urgent preventive measures’, *Environmental Sciences Europe*, 30(1). doi: 10.1186/s12302-018-0139-z.

Hahladakis, J. N. *et al.* (2018) ‘An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling’, *Journal of Hazardous Materials*, 344, pp. 179–199. doi: 10.1016/j.jhazmat.2017.10.014.

Hauser, R. and Calafat, A. M. (2005) ‘Phthalates and human health’, *Occupational and Environmental Medicine*, 62(11), pp. 806–818. doi: 10.1136/oem.2004.017590.

Herzke, D. *et al.* (2016) ‘Negligible Impact of Ingested Microplastics on Tissue Concentrations of Persistent Organic Pollutants in Northern Fulmars off Coastal Norway’, *Environmental Science and Technology*, 50(4), pp. 1924–1933. doi: 10.1021/acs.est.5b04663.

Hilton, G. M., Furness, R. W. and Houston, D. C. (2000) ‘A comparative study

Chapter 4 – Temporal leaching of chemicals in PGM

of digestion in North Atlantic seabirds’, *Journal of Avian Biology*, 31(1), pp. 36–46. doi: 10.1034/j.1600-048X.2000.310106.x.

Jackson, S. (1990) ‘Seabird digestive physiology in relation to foraging ecology’, PhD, p. 210 pp.

Jackson, S. (1992) ‘Do Seabird Gut Sizes and Mean Retention Times Reflect Adaptation to Diet and Foraging Method ?’, *Physiological Zoology*, 65(3), pp. 674–697.

Kamegashira, M. *et al.* (1977) ‘Role of Pepsin in Species Differences in Erosive Gastritis’, *Archives of Surgery*, 112(2), pp. 193–197. doi: 10.1001/archsurg.1977.01370020087009.

Koelmans, A. A. *et al.* (2016) ‘Microplastic as a Vector for Chemicals in the Aquatic Environment: Critical Review and Model-Supported Reinterpretation of Empirical Studies’, *Environmental Science and Technology*, 50(7), pp. 3315–3326. doi: 10.1021/acs.est.5b06069.

Kühn, S. *et al.* (no date) ‘Transfer of additive chemicals from marine plastic debris to the stomach oil of northern fulmars’.

Kühn, S. and van Franeker, J. A. (2020) ‘Quantitative overview of marine debris ingested by marine megafauna’, *Marine Pollution Bulletin*, 151(110858). doi: 10.1016/j.marpolbul.2019.110858.

Marlatt, V. L. *et al.* (2022) ‘Impacts of endocrine disrupting chemicals on reproduction in wildlife and humans’, *Environmental Research*, 208. doi: 10.1016/j.envres.2021.112584.

Nurhayati, T. *et al.* (2019) ‘Pepsin activity from gastric of milkfish and catfish in Indonesian Waters’, *IOP Conference Series: Earth and Environmental*

Chapter 4 – Temporal leaching of chemicals in PGM

Science, 404(1). doi: 10.1088/1755-1315/404/1/012060.

Olsen, M. A. *et al.* (2002) ‘The gastrointestinal tract of Adélie penguins - Morphology and function’, *Polar Biology*, 25(9), pp. 641–649. doi: 10.1007/s00300-002-0395-6.

Provencher, J F *et al.* (2017) ‘Quantifying ingested debris in marine megafauna: A review and recommendations for standardization’, *Analytical Methods*, 9(9), pp. 1454–1469. doi: 10.1039/C6AY02419J.

Provencher, Jennifer F. *et al.* (2017) ‘Quantifying ingested debris in marine megafauna: A review and recommendations for standardization’, *Analytical Methods*, 9(9), pp. 1454–1469. doi: 10.1039/c6ay02419j.

Roby, D., Brink, K. and Place, A. (1989) ‘Relative Passage Rates of Lipid and Aqueous Digesta in the Formation of Stomach Oils’, *The Auk: Ornithological Advances*, 106(2), pp. 303–313. doi: 10.1093/auk/106.2.303.

Rochester, J. R. (2013) ‘Bisphenol A and human health: A review of the literature’, *Reproductive Toxicology*, 42, pp. 132–155. doi: 10.1016/j.reprotox.2013.08.008.

Rochman, C. M. *et al.* (2013) ‘Long-term field measurement of sorption of organic contaminants to five types of plastic pellets: Implications for plastic marine debris’, *Environmental Science and Technology*, 47(3), pp. 1646–1654. doi: 10.1021/es303700s.

Roman, L., Lowenstine, L., *et al.* (2019) ‘Is plastic ingestion in birds as toxic as we think? Insights from a plastic feeding experiment’, *Science of the Total Environment*, 665, pp. 660–667. doi: 10.1016/j.scitotenv.2019.02.184.

Roman, L., Paterson, H., *et al.* (2019) ‘Size of marine debris items ingested

Chapter 4 – Temporal leaching of chemicals in PGM

and retained by petrels’, *Marine Pollution Bulletin*, 142, pp. 569–575. doi: 10.1016/j.marpolbul.2019.04.021.

Ryan, P. G. (2015) ‘How quickly do albatrosses and petrels digest plastic particles?’, *Environmental Pollution*, 207, pp. 438–440. doi: 10.1016/j.envpol.2015.08.005.

Ryan, P. G. (2019) ‘Ingestion of Plastics by Marine Organisms’, *Handbook of Environmental Chemistry*, 78, pp. 235–266. doi: 10.1007/698_2016_21.

Tanaka, K. *et al.* (2015) ‘Facilitated Leaching of Additive-Derived PBDEs from Plastic by Seabirds’ Stomach Oil and Accumulation in Tissues’, *Environmental Science and Technology*, 49(19), pp. 11799–11807. doi: 10.1021/acs.est.5b01376.

Tanaka, K., Yamashita, R. and Takada, H. (2018) ‘Transfer of Hazardous Chemicals from Ingested Plastics to Higher-Trophic-Level Organisms’, in *Handbook of Environmental Chemistry*, pp. 267–280. doi: 10.1007/698_2018_255.

Wang, L. C. *et al.* (2021) ‘The sorption of persistent organic pollutants in microplastics from the coastal environment’, *Journal of Hazardous Materials*, 420(June), p. 126658. doi: 10.1016/j.jhazmat.2021.126658.

Wang, S. W. *et al.* (2007) ‘Fatty acid signatures of stomach oil and adipose tissue of northern fulmars (*Fulmarus glacialis*) in Alaska: Implications for diet analysis of Procellariiform birds’, *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, 177(8), pp. 893–903. doi: 10.1007/s00360-007-0187-y.

Warham, J. (1996) ‘Physiology and Energetics’, in *The Behaviour, Population*

Chapter 4 – Temporal leaching of chemicals in PGM

Biology and Physiology of the Petrels, pp. 317–363.

Xia, Y. and Rubino, M. (2015) ‘Kinetic study of bisphenol a migration from low-density polyethylene films into food simulants’, *Industrial and Engineering Chemistry Research*, 54(14), pp. 3711–3716. doi: 10.1021/acs.iecr.5b00088.

Ying, P. *et al.* (2003) ‘Competitive protein adsorption studied with atomic force microscopy and imaging ellipsometry’, *Colloids and Surfaces B: Biointerfaces*, 32(1), pp. 1–10. doi: 10.1016/S0927-7765(02)00133-9.

Chapter 5

Hormonal disruption from plastic
ingestion in northern fulmar:
activation and inhibition of
estrogen receptors

Table of Contents

Abstract.....	170
5.1. Introduction.....	172
5.2. Materials and methods	174
5.2.1. Chemicals and materials	174
5.2.2. Human and fulmar ER cloning	175
5.2.3. Plastic-associated chemicals for human and fulmar ER comparison.....	176
5.2.4. Plastic collection from northern fulmar stomachs	176
5.2.5. Plastic leaching	177
5.2.5.1. Solvents.....	177
5.2.5.2. Leaching experiment.....	178
5.2.6. Cell culture and receptor activation/inhibition assay	180
5.2.7. Plastic identification.....	181
5.2.8. Qualitative assessment of plastic leachates with mass spectrometry	182
5.2.9. Statistical analysis.....	183
5.2.9.1. Human and northern fulmar ER comparison	183
5.2.9.2. Fulmar ER response to plastic leachates.....	183
5.3. Results.....	184
5.3.1. Fulmar ER exhibits similar response to human ER, with chemical and receptor type-specific sensitivity differences.....	184

Chapter 5 – Hormonal disruption from plastic ingestion

5.3.2.	Plastic leachates activated and inhibited fulmar ERs.....	187
5.3.2.1.	One, five and fourteen day ER activation and inhibition	187
5.3.2.2.	Plastic characteristics	189
5.3.2.2.1.	Mass of plastic ingested.....	189
5.3.2.2.2.	Polymer identification of ingested plastics	190
5.3.2.3.	Qualitative assessment of plastic leachates.....	191
5.4.	Discussion.....	192
5.4.1.	Fulmar ERs exhibit a similar response to human ERs, with chemical and receptor type-specific sensitivity differences	193
5.4.2.	Plastic leachates activate and inhibit fulmar ERs	194
5.4.3.	Biological significance.....	195
5.5.	Conclusion	197
	Supplementary material	198
	Acknowledgments	231
	References.....	232

Abstract

Procellariiformes such as northern fulmars (*Fulmarus glacialis*) have high levels of plastic ingestion, yet the implication of plastic-associated chemical exposure is poorly understood. Our study investigates potential harmful effects of plastic ingestion on northern fulmar hormonal function by examining the activation and inhibition of fulmar estrogen receptors (ERs) after exposure to plastic-associated chemicals. Human ERs are commonly used to assess estrogenic activity, yet, species-specific ER sensitivity differences exist. Thus, we investigated potential differences between fulmar and human ER responses to plastic-associated chemicals using a luciferase-reporter gene assay. ERs from both species were activated by BPA, BPS, 4-OP, and BBP, and inhibited by TBBPA, PCB-52, and PCB-138, although we observed species-specific differences in sensitivity. Additionally, to assess if ingested plastic had the potential to alter hormone function, plastics recovered from fulmar stomachs (proventriculus, gizzard) were leached for 14 days and exposed to fulmar ERs. More than half (55%) of the fulmar exhibited ER activation and/or inhibition after one day of leaching, with more than a third (37%) of the birds showing a subsequent ER response after 5 and/or 14 days of leaching. The polymer composition of plastic samples was assessed with infrared spectroscopy, identifying polyethylene (PE) (60%) as the most abundant polymer followed by polypropylene (PP) (35.2%) and polyethylene terephthalate (PET) (2.8%). The type of polymer did not correlate with ER response, suggesting that plastic leachates interacting with ER are not specifically linked to polymer composition. To our knowledge, we provide the first data on how seabird ERs respond to plastic-associated chemicals. Our findings highlight the potential for exposure to xenoestrogens from plastic

Chapter 5 – Hormonal disruption from plastic ingestion

ingestion to disrupt hormone function. Overall, our results provide important information to understand the potential health risks associated with plastic ingestion in Procellariiformes.

5.1. Introduction

Plastic pollution has become a pervasive environmental concern, particularly in marine ecosystems where an estimated 8 million tons of plastic enter the ocean annually, posing significant risks to wildlife through plastic ingestion (Kühn and van Franeker, 2020; Pilapitiya and Ratnayake, 2024). Seabirds, especially Procellariiformes (i.e., albatrosses, petrels, shearwaters) are particularly at risk due to their infrequent regurgitation and distinctive gastric morphology (Ryan, 2019). Indeed, 63.2% of Procellariiform species have been documented with ingested plastic (Kühn and van Franeker, 2020) and studies suggest plastic can be retained in their stomachs for periods of weeks to months (Ryan, 2015; Nania and Shugart, 2021). Given the threatened status of many Procellariiform species, resource managers need a better understanding of the impacts of plastic ingestion to inform conservation efforts (Dias *et al.*, 2019; *IUCN Red List of Threatened Species*, 2023).

Plastic ingestion in Procellariiformes poses a dual threat, causing physical harm, such as obstruction or perforation (Fry, Fefer and Sileo, 1987; Pierce *et al.*, 2004) and exposure to plastic-associated chemicals (Tanaka *et al.*, 2015; Coffin *et al.*, 2019; Kühn, Andy M. Booth, *et al.*, 2020). Once ingested, plastic can leach toxic chemicals such as the monomers from the polymer matrix, additives (e.g., plasticizers, flame retardants, antioxidants) added during plastic manufacture to modify properties, and persistent organic pollutants (POPs) that sorb onto the surface of plastic in the ocean (Kühn, Andy M Booth, *et al.*, 2020; Van Hassel *et al.*, 2024). Plastic-associated chemicals can be absorbed by the intestinal barrier and circulate within the

Chapter 5 – Hormonal disruption from plastic ingestion

organism where they may cause physiological harm such as endocrine disruption (Bodziach *et al.*, 2021; Maddela *et al.*, 2023).

Many plastic-associated chemicals are known as xenoestrogens [i.e., able to bind or block estrogen receptors (ERs)] and in mammals have been associated with reproductive dysfunction such as the development of gonadal abnormalities, decrease of fertility and development of cancer (Hauser and Calafat, 2005; Rochester, 2013; Marlatt *et al.*, 2022). In birds, studies investigating the impact of plastic ingestion on the quail reproductive system found an increase in reproductive abnormalities (e.g., cysts) in male and reduced estrogen levels in females (Roman *et al.*, 2019; Monclús *et al.*, 2022). Researchers emphasized the importance of understanding how chemical leaching contributes to the endocrine disruptive effects of plastic ingestion.

In vitro ER reporter assays, predominantly using human ERs, is a common method used for assessing the estrogenicity of chemicals (Rogers and Denison, 2000). A recent study demonstrated that leachates of microplastics recovered from common murre (*Uria aalge*) digestive tracts could activate human ERs (Michishita *et al.*, 2023). However, variations in ER sequences across species can influence their sensitivity to xenoestrogens (Matthews *et al.*, 2000; Rider *et al.*, 2010; Tubbs *et al.*, 2012; Asnake, Modig and Olsson, 2019; Felton *et al.*, 2020). For example, disparities in the transactivation domain (A/B) and C-terminal domain (F) of the ER in two rhino species showed two distinct responses to phytoestrogens, resulting in different impacts on fertility (Tubbs *et al.*, 2012). Similarly, variations in human and northern fulmar (*Fulmarus glacialis*, fulmar) – a Procellariiform species widely documented with plastic ingestion (Kühn and van Franeker, 2020) – ER sequences suggest potential divergent responses to plastic-

Chapter 5 – Hormonal disruption from plastic ingestion

associated chemicals (Supplementary Figure 5.1). Thus, assessing the response of seabird specific ERs to xenoestrogens is important for understanding the consequences of plastic ingestion in these birds.

The goal of our study was to investigate whether differences exist between human and fulmar ER response to xenoestrogens, and whether plastic recovered from the stomach of fulmars has the potential to induce hormonal disruption. We compared the activation and inhibition of fulmar and human estrogen receptors in the presence of plastic-associated chemicals with the luciferase-reporter assay. We also studied the response of fulmar ERs to plastics recovered from fulmar stomachs and leached for 14 days. The polymer composition of plastic was assessed to investigate whether the type of plastic influenced the response of ERs. To our knowledge, we provide the first assessment of how a Procellariiform seabird ER responds to plastic-associated chemicals. Our study helps to provide information on the potential health risks posed by plastic ingestion in Procellariiformes.

5.2. Materials and methods

5.2.1. Chemicals and materials

17 β -estradiol (E2) was purchased from Steraloids (Newport, RI). Analytical-grade ($\geq 98\%$ purity) hexanes, methanol, dimethylsulfoxide (DMSO), 4-octylphenol (4-OP), benzyl n-butyl phthalate (BBP), decabromodiphenyl ether (PBDE-209), tetrabromobisphenol A (TBBPA) were purchased from Thermo Fisher Scientific (Scotts Valley, CA, USA). Analytical-grade ($\geq 98\%$ purity) bisphenol A (BPA), bisphenol S (BPS), 2,2',3,4,4',5'-hexachlorobiphenyl (PCB-138) were purchased from Millipore

Chapter 5 – Hormonal disruption from plastic ingestion

Sigma (St. Louis, MO, USA). Analytical-grade ($\geq 98\%$ purity) 2,2',5,5'-tetrachlorobiphenyl (PCB-52) was purchased from Santa Cruz Biotechnologies, Inc. (Dallas, TX, USA).

Glass Erlenmeyers (50 mL) sealed with foil-lined rubber caps and parafilm were used to incubate plastics in solvents. Round glass stirring bars were purchased from Sigma-Aldrich (Overijse, Belgium). Leaching extracts were collected with Pasteur pipets and stored in glass vials with polypropylene caps lined with aluminum foil. To avoid chemical contamination, glassware was baked in a muffle furnace at 450 °C for 4 h prior to use.

5.2.2. Human and fulmar ER cloning

Fulmar estrogen receptor (fER α and fER β) sequences (fESR1 and fESR2) and human estrogen receptor (hER α and hER β) sequences (hESR1 and hESR2) were cloned into four pcDNA3.1+ expression vectors using IDT custom gene synthesis (<https://www.idtdna.com>). Fulmar ESR2 was obtained from GeneBank: [XM_009572778.1](https://www.ncbi.nlm.nih.gov/nuclot/XM_009572778.1). Fulmar ESR1 was found by doing a BLAST on the fulmar genome (NCBI:txid30455) using chicken ESR1. Human ESR1 and ESR2 in pcDNA3.1+ were purchased from the cDNA resource center (<https://www.cdna.org/>). Plasmids were reconstituted and transformed into TOP10 competent *Escherichia coli*, cultured and finally purified using a ZymoPureII Maxi Prep Kit (<https://www.zymoresearch.com/>). Receptor sequences were verified by sending out purified plasmids for sanger sequencing (<https://retrogen.net/>).

Chapter 5 – Hormonal disruption from plastic ingestion

5.2.3. Plastic-associated chemicals for human and fulmar ER comparison

Plasticizers, flame retardants and stabilizers (e.g., antioxidants) are among the most common additives used in plastics (Hahladakis *et al.*, 2018). To compare the response of human and northern fulmar estrogen receptors in the presence of plastic-associated chemicals, we selected representative additives detected in marine plastic (Staniszewska, Graca and Nehring, 2016; Aminot *et al.*, 2020; Dhavamani *et al.*, 2022) and known to impair reproduction (Van der Ven *et al.*, 2008; European Chemicals Agency (ECHA), 2014; Y. Y. Liu *et al.*, 2018; Santoro *et al.*, 2019; Adegoke, Rahman and Pang, 2020; Thoene, Dzika and Gonkowski, 2020; Li *et al.*, 2021): BPA, found as a residual monomer of polycarbonate and also used as an antioxidant, bisphenol S (BPS), a BPA substitute, 4-octylphenol (4-OP) found as a monomer of phenolic resins and used as an antioxidant (DEFRA, 2006), benzyl n-butyl phthalate (BBP) used as a plasticizer and PBDE-209 and tetrabromobisphenol A (TBBPA) used as flame retardants. In addition, 2,2',3,4,4',5'-hexachlorobiphenyl (PCB-138) and 2,2',5,5'-tetrachlorobiphenyl (PCB-52) were investigated as POPs commonly adsorb to plastic (Antunes *et al.*, 2013; Bouhroum *et al.*, 2019). Stock solutions were concentrated at 100 mM in DMSO. Estradiol (E2) was used as a control and concentrated at 10 mM in DMSO.

5.2.4. Plastic collection from northern fulmar stomachs

Plastic samples were recovered from the stomachs of 27 northern fulmars (14 females and 13 males) incidentally caught as bycatch in U.S.

Chapter 5 – Hormonal disruption from plastic ingestion

North Pacific groundfish and halibut fisheries in Alaska in 2016 and 2017. Standard methods for bird necropsies and dissection followed those described in van Franeker, 2004. After removal of the stomach, contents were carefully rinsed in a sieve with a 1 mm mesh and left to air-dry in open glass vials for several days. Plastic recovered from each chamber (proventriculus and gizzard) was kept separate. In total, 25 birds had plastic in their gizzard, 1 bird had plastic in its proventriculus and 1 bird had plastic in both chambers, giving a total of 28 plastic samples (Supplementary Table 5.1). The total mass of plastic, the type of plastic items (industrial pellets, pellets other than industrial, sheet, threads, foam, fragment and “other”), the number of plastic items and their combined mass by type was recorded (Supplementary Table 5.1).

5.2.5. Plastic leaching

5.2.5.1. Solvents

Plastics were leached into a 40 ml mixture of methanol-hexane (1:1). Methanol is often used as a polar solvent for the extraction of a broad range of low hydrophobic chemicals such as bisphenol A and its analogous from plastics and aqueous matrices (Bonon *et al.*, 2016; Coffin *et al.*, 2019; Zimmermann *et al.*, 2019; Nissy, Myneni and Vangalapati, 2022; Wang and Kannan, 2023) while hexane is commonly employed as a non-polar solvent to leach high hydrophobic chemicals from plastics such as phthalates (Chao, Huang and Wei, 2013), brominated flame retardants (Tanaka *et al.*, 2015, 2019; Gripon *et al.*, 2021), polychlorinated biphenyls (Endo *et al.*, 2005; Syberg *et al.*, 2020) or UV-stabilizers (Tanaka *et al.*, 2020).

Chapter 5 – Hormonal disruption from plastic ingestion

5.2.5.2. Leaching experiment

Plastic samples were leached in a methanol-hexane mixture renewed every 24 hours for 14 days (stirred at 600 rpm), and the methanol and hexane fractions of each 24-hour leachate were recovered separately (Figure 5.1). Leaching was conducted in two stages, with half of the samples (n=14) incubated initially (set 1), followed by the second half (n=14) (set 2). Vehicle controls without plastics (n=3) were run in parallel without solvent replacement and recovered after 14 days for sets 1 and 2 (Figure 5.1). Each fraction of the leachates was filtered with a Whatman™ glass microfiber filter and then evaporated under a gentle nitrogen stream until almost complete dryness to avoid the loss of volatile chemicals. Then, 50 µl of DMSO was added to the remaining leachate (hexane or methanol) and the mixture was evaporated, leaving only the DMSO. Day 1 leachates were tested for estrogenic activity and samples showing a response were subsequently evaluated on days 5 and 14 for potential long-term effects (Figure 5.1). To assess mass loss during leaching, plastic samples were weighed before the leaching experiment and then weighed again 24 hours after collecting the final leachates from day 1 (Supplementary Table 5.1).

Chapter 5 – Hormonal disruption from plastic ingestion

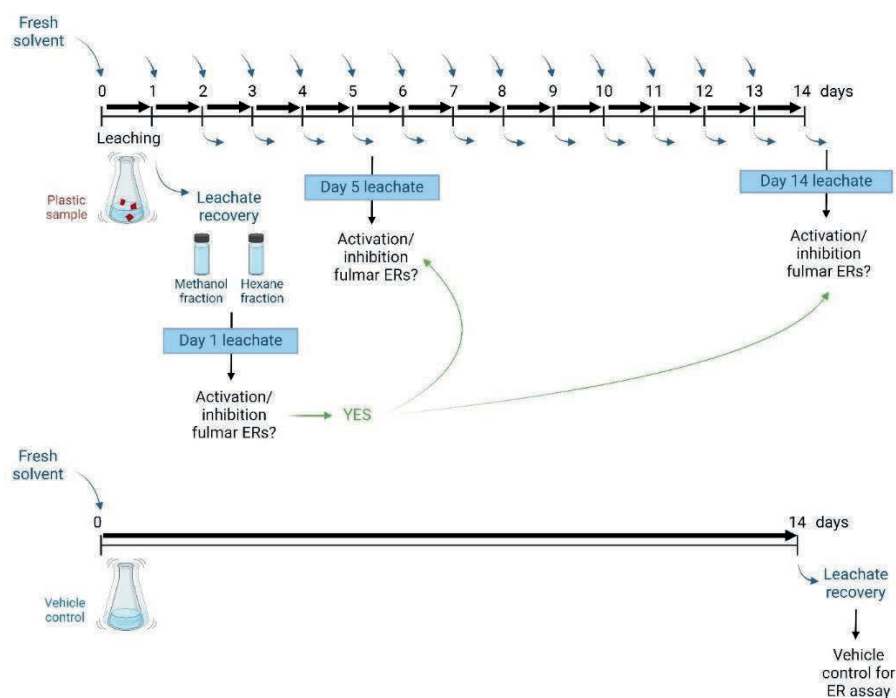


Figure 5.1. Experimental design for the leaching of plastics recovered from northern fulmar stomachs. Plastic samples were leached in a new methanol-hexane mixture every 24 hours for 14 days and the methanol and hexane fractions of each 24-hour leachate were recovered separately. Day 1 leachates were tested for estrogenic activity and samples showing a response were subsequently evaluated on days 5 and 14 for potential long-term effects. Vehicle controls without plastics were run in parallel without solvent replacement and recovered after 14 days. Created with BioRender.com

Chapter 5 – Hormonal disruption from plastic ingestion

5.2.6. Cell culture and receptor activation/inhibition assay

Human embryonic kidney (HEK293) cells were maintained in MEM (Corning) supplemented with 10% fetal bovine serum at 37°C and 5% CO₂. Cells were split into a new culture flask two times per week. For receptor assays, 1x10⁵ cells were added to each well of a 96-well plate. After 24 hours, cells were co-transfected using TransIT-2020 transfection reagent (Mirus Bio LLC) with 3 plasmids: 5-μg pCMX-β-galactosidase (β-gal), 5-μg pGL2–3xERE luciferase reporter plasmid containing three copies of chicken vitellogenin estrogen response element (Addgene plasmid 11354) and human or fulmar ESR1 or ESR2 0.5-μg ESR-pcDNA3.1 (+). After 24 hours, transfection medium was removed and replaced with MEM supplemented with 10% charcoal resin-stripped fetal bovine serum containing 10⁻¹⁴ M – 10⁻⁷ M endogenous hormone E2 (0.1% DMSO), 10⁻⁹ M – 10⁻⁴ M test chemical (0.1% DMSO), leaching extracts (0.25% DMSO) or vehicle controls (0.1% and 0.25% DMSO). We used 0.25% DMSO for the leachates to ensure sufficient concentration for inducing a potential ER response, given the unknown chemical concentration in the samples. For receptor inhibition, transfection medium was replaced with MEM supplemented with 10% charcoal resin-stripped fetal bovine serum containing 10⁻¹⁰ M E2 and 10⁻⁹ M – 10⁻⁴ M test chemical or the leaching extracts. We chose an E2 concentration of 10⁻¹⁰ M for the inhibition assay to ensure partial receptor activation (~80%), providing sufficient room for competition between the hormone and the test chemicals (Plíšková *et al.*, 2005). After 24 hours, cells were lysed and each well assayed for luciferase and β-gal activity. Luciferase activity per well was standardized to β-gal activity, and fold activation was determined from the

Chapter 5 – Hormonal disruption from plastic ingestion

normalized luciferase values relative to the vehicle control. Sigmoidal dose-response curves for ER α and ER β activation by 10^{-12} M – 10^{-7} M E2, which was run in triplicate in each assay, were fit using GraphPad Prism software and used to calculate maximal activation by E2. Fold activation of ERs for each test chemical and leachate was then normalized to maximal E2 activation of ER α or ER β . Chemicals and leachates were tested in triplicate for each assay. Assays were conducted three times for plastic-associated chemicals (results are presented as the average) and once for plastic leachates. Plastic leachates from day 1 that showed receptor activation or inhibition were further tested in a second assay for validation. Additionally, leachates from day 5 and day 14 were tested once to investigate potential long-term effects of the plastic samples that activated or inhibited on day 1 (Figure 5.1).

To assure low cytotoxicity, the β -gal signal obtained for treatments was compared to the one obtained for E2. No treatment showed any cytotoxicity, except for 4-OP which killed all the cells at 10^{-4} M.

5.2.7. Plastic identification

A total of 142 pieces recovered from fulmar stomachs were analyzed for polymer identification. One hundred twenty-nine pieces were analyzed using hyperspectral near-infrared spectroscopy (HIS) using a Specim FX-17 hyperspectral camera (line-scan mode, 900 – 1700 nm) (Bruker, Belgium). Data were predicted using a discrimination model based on the chemometric tool partial least squares discriminant analysis (PLS-DA). This model was built using a set of reference polymers covering spectra of seven types [polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polyethylene terephthalate (PET), polystyrene (PS), nylon and nitrile butadiene rubber (NBR)]. PLS-DA results were validated by visual

Chapter 5 – Hormonal disruption from plastic ingestion

observation of the spectra analysis, which was also used to differentiate between HDPE and LDPE due to limitations of the discrimination model. Plastic pieces with unidentifiable spectra (n=9) were further analyzed using near-infrared microscopy (NIRM) via a Bruker Hyperion 3000 near-infrared spectrometer (Bruker, Belgium). Due to complete light absorption in near-infrared, black samples (n=4) were analyzed using mid-infrared spectroscopy (MIR) with a Bruker Vertex 70 spectrometer equipped with an attenuated total reflectance (ATR) system (X, Gembloux, Belgium). The NIRM and MIR spectra were visually examined to ensure accurate identification. Specific details regarding the spectroscopic methods, chemometric approaches, and corresponding polymer type for each plastic piece are provided in Supplementary Table 5.2. Data were analyzed using Matlab 7.5 software with the PLS toolbox version 7.5.2.

5.2.8. Qualitative assessment of plastic leachates with mass spectrometry

A subset of leachates (n = 9) were qualitatively assessed using mass spectrometry to investigate the presence of distinct compounds potentially responsible for ER responses (Supplementary Table 5.1). Hexane fractions were evaporated and resuspended in methanol for analysis. Samples were analyzed by direct injection from a syringe needle with 10 μ L/min flow rate on a Thermo Electron Finnigan LTQ mass spectrometer (Thermo, Santa Cruz, United States) in positive and negative ion mode over a full scan range of m/z 150 – 2000. The mass spectrometry electrospray voltage was set to 5.0 kV and the capillary temperature was 275°C. Data were analyzed by using the XCalibur software (Thermo).

Chapter 5 – Hormonal disruption from plastic ingestion

5.2.9. Statistical analysis

5.2.9.1. Human and northern fulmar ER comparison

Comparison of human and fulmar estrogen receptors response in the presence plastic-associated chemicals was performed with a two-way ANOVA using GraphPad PRISM with a Bonferroni correction to account for risk of type 1 error. Inhibition of receptors was assessed using a two-way ANOVA with a Bonferroni correction by comparing the ER activation by 10^{-10} M E2 alone to the activation by 10^{-10} M E2 in the presence of different chemical concentrations.

5.2.9.2. Fulmar ER response to plastic leachates

Receptors were considered activated when the percentage of maximum E2 activation was greater than the average of the vehicle controls plus twice the standard deviation of the vehicle controls. Inhibition of the receptors by plastic leachates was considered significant when the percentage of maximum E2 activation fell below the average of the vehicle controls minus twice the standard deviation of the vehicle controls. This method was used to reduce the likelihood of Type I errors (false positives) given the variability observed within the vehicle controls. We performed a Pearson correlation test to assess the linear relationship between the mass loss of the plastics and the ER response using JM Pro 17. We conducted a Fisher's exact test to assess the potential correlation between polymer type and the ER response using JM Pro 17.

5.3. Results

5.3.1. Fulmar ER exhibits similar response to human ER, with chemical and receptor type-specific sensitivity differences.

Human and fulmar ERs were activated by BPA, BPS, 4-OP and BBP (Figure 5.2) and inhibited by TBBPA, PCB-52 and PCB-138 (Figure 5.3), while no response was observed for PBDE-209 (Supplementary Figure 5.2).

Activation – ER α . Human ER α showed a higher activation than fulmar ER α by BPA at 10^{-6} and 10^{-5} M (up to 1.5-fold) and BPS at 10^{-5} M (1.5-fold) (Figure 5.2A). 4-OP at 10^{-6} M and 10^{-5} M activated fulmar ER α at a higher level than human ER α (1.4-fold) (Figure 5.2). BBP activated ER α with comparable levels between fulmar and human (Figure 5.2).

– **ER β .** Fulmar ER β showed a higher activation than human ER β by BPA at 10^{-7} , 10^{-6} and 10^{-5} M (up to 1.9-fold), BPS at 10^{-6} and 10^{-5} M (1.3-fold), 4-OP at 10^{-7} M (3.4-fold) and BBP at 10^{-5} M (4.6-fold) (Figure 5.2). No difference was observed at higher concentrations of 4-OP.

Inhibition – ER α . For TBBPA at 10^{-4} M, comparable levels of inhibition were observed between human and fulmar ER α . Fulmar ER α showed a higher inhibition than human ER α by PCB-52 and PCB-138 at 10^{-4} M (1.8-fold) (Figure 5.3).

– **ER β .** TBBPA at 10^{-4} M inhibited ERs at comparable levels between human and fulmar. Human ER β showed a higher inhibition than fulmar ER β by PCB-52 at 10^{-4} M and PCB-138 at 10^{-5} and 10^{-4} M (Figure 5.3).

Chapter 5 – Hormonal disruption from plastic ingestion

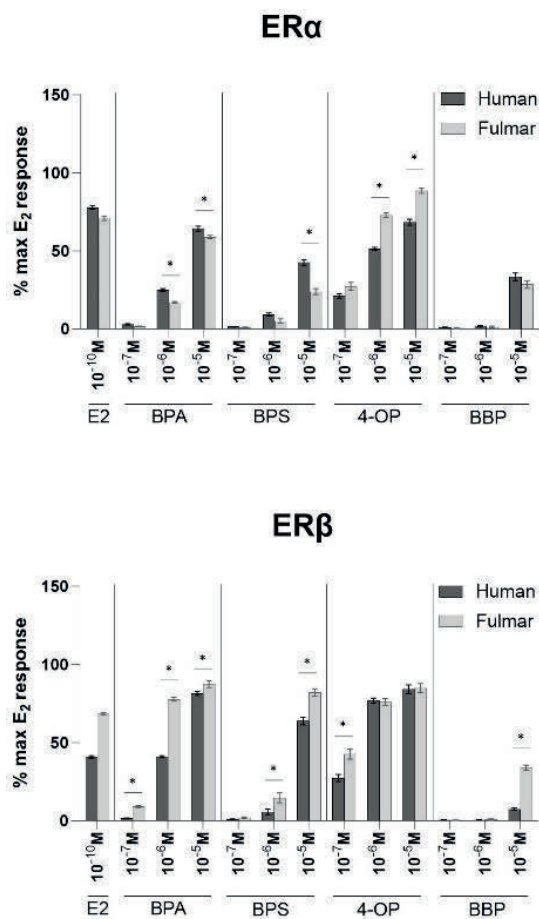


Figure 5.2. Activation of human and northern fulmar ERα and ERβ by plastic-associated chemicals. HEK293 cells expressing ESRs were treated in triplicate with increasing concentrations (10⁻⁹ M – 10⁻⁴ M) of BPA, BPS, 4-OP and BBP resuspended in 0.1% DMSO. Bars represent mean ± SEM of the fold activation of each treatment relative to maximal E2 activation. Significant differences between human and fulmar were determined using a two-way ANOVA followed by individual t-test with Bonferroni correction (p < 0.05) (asterisk).

Chapter 5 – Hormonal disruption from plastic ingestion

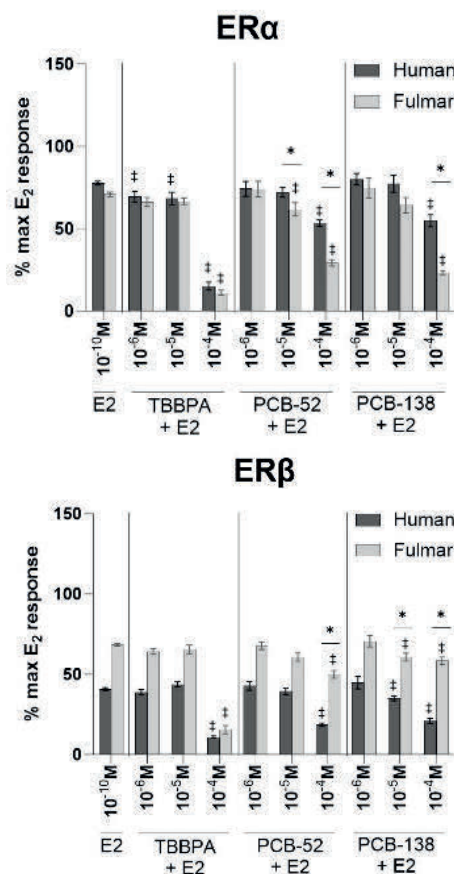


Figure 5.3. Inhibition of human and northern fulmar ER α and ER β by plastic-associated chemicals. HEK293 were treated in triplicate with increasing concentrations (10^{-7} M – 10^{-4} M) of TBBPA, PCB-52 and PCB-138 resuspended in 10^{-10} M E2 (0.1% DMSO). Bars represent mean $\pm$ SEM of the fold activation of each treatment relative to maximal E2 activation. Significant differences between human and fulmar were determined using a two-way ANOVA followed by individual t-test with Bonferroni correction ($p < 0.05$) (asterisk). Significant inhibition was determined using a two-way ANOVA with a Bonferroni correction by comparing the activation by 10^{-10} M E2 alone to the activation by 10^{-10} M E2 in the presence of the chemical ($p < 0.05$) (dagger).

Chapter 5 – Hormonal disruption from plastic ingestion

5.3.2. Plastic leachates activated and inhibited fulmar ERs

5.3.2.1. One, five and fourteen day ER activation and inhibition

Activation – Day 1. Out of 27 fulmars evaluated, 6 plastic leachates (22%) activated fulmar ER α (Supplementary Table 5.1 and Supplementary Figure 5.3). Five leachates activated the receptors from the hexane fraction at 1.1 to 17-fold over the threshold (Table 5.1). One leachate activated the receptors from both the methanol and hexane fractions at 4.3- and 2.1-fold over the threshold respectively. Fulmar ER β were activated by the same leachates, with methanol fractions from two additional birds also showing moderate activation (Supplementary Figure 5.3).

– **Day 5 and 14.** Activation of fulmar ERs was investigated with plastic samples that exhibited a measurable (e.g., above threshold) response for day 1 leachates. Five leachates activated fulmar ER α from the hexane fraction on day 5 and day 14 at 1.7- to 2.5-fold over threshold (Table 5.1). Two of these five leachates also activated the receptors from the methanol fraction on day 5 and 14 respectively. Fulmar ER β exhibited similar activation patterns (Supplementary Figure 5.4).

Inhibition – Day 1. Nine plastic leachates (33% of northern fulmars) inhibited fulmar ER α from the methanol fraction at 1.1- to 1.4-fold below the threshold (Table 1 and Supplementary Figure 5.5). One of the 9 leachates also inhibited ER α from the hexane fraction (Table 5.1). Fulmar ER β were

Chapter 5 – Hormonal disruption from plastic ingestion

inhibited by 5 plastic leachates out of the 9 samples that inhibited fulmar ER α (Supplementary Figure 5.5).

– **Day 5 and 14.** Inhibition of fulmar ERs was investigated with plastic samples that showed a measurable inhibition (e.g., below threshold) for day 1 leachates. Two leachates inhibited fulmar ER α on day 5 and two leachates inhibited fulmar ER α on day 5 and 14 (Table 5.1). Fulmar ER β were inhibited by one leachate on day 5 and one leachate on day 14 (Supplementary Figure 5.6).

Chapter 5 – Hormonal disruption from plastic ingestion

Table 5.1. Leachates that activated or inhibited fulmar ERs. Sample ID (“bird”), the chamber where plastic was recovered (“sto. cha.”), the number of plastic pieces found per individual (“n pieces”), the polymer composition of each sample [HDPE, LDPE, PP, PET and NI (nonidentified)] and the estrogenicity of plastic leachates measured on days 1, 5, and 14 for each leachate fraction (hexane or methanol) are listed. Estrogenicity was expressed as the fold change above (for activation) or below (for inhibition) the threshold. Thresholds are defined as the average vehicle control response plus (activation) or minus (inhibition) twice the standard deviation.

Bird	Sto. cha.	n pieces	Mass (g)	Fold change above/below the threshold					
				Day 1		Day 5		Day 14	
				Hex	Met	Hex	Met	Hex	Met
<i>Samples that activated ERs</i>									
F13	G	1	0.018	17	NA	1.8	3.4	1.8	NA
F14	P	1	0.014	10	NA	NA	NA	NA	1.7
F15	G	4	0.010	1.6	NA	2.5	NA	2.2	NA
F17	G	2	0.023	1.2	1.7	2.0	NA	1.9	3.4
F18	G	3	0.042	1.7	NA	1.8	NA	1.8	1.2
F27	G	10	0.051	1.1	NA	1.8	NA	1.7	NA
<i>Samples that inhibited ERs</i>									
F9	G	1	0.008	NA	1.1	NA	NA	NA	NA
F18	G	3	0.042	NA	1.2	NA	NA	NA	NA
F19	G	5	0.069	NA	1.4	NA	1.5	NA	NA
F20	G	2	0.089	NA	1.3	NA	1.1	NA	1.1
F22	G	4	0.090	NA	1.2	NA	NA	NA	NA
F24	G	21	0.058	NA	1.1	NA	NA	NA	NA
F25	G	1	0.010	1.1	NA	NA	NA	NA	NA
F26	G	1	0.023	NA	1.1	NA	1.1	NA	NA
F27	G	10	0.051	NA	1.1	NA	1.1	NA	1.1

5.3.2.2. Plastic characteristics

5.3.2.2.1. Mass of plastic ingested

The mass of plastic recovered from northern fulmar stomachs did not appear to be correlated to the response of fulmar ERs. For example, sample F7 had the highest mass (0.5548 g) but did not induce any response of fulmar

Chapter 5 – Hormonal disruption from plastic ingestion

ERs (Supplementary Table 5.1). After 14 days of leaching, the mass from 86% of plastic samples decreased (range = 0.001 to 0.066 grams, 1.4% to 94% of initial weight) (Supplementary Table 5.1). This decrease showed no correlation with the ER response to the leachates ($R^2 = 0.03$, $r = -0.169$ and $p = 0.39$) (Supplementary Figure 5.7).

5.3.2.2.2. Polymer identification of ingested plastics

Out of 142 pieces, HDPE and LDPE account for 25% and 35% of the total respectively (one PE piece could not be distinguished between HDPE and LDPE). PP was the second most frequent polymer identified, representing 35.2% of the total, followed by PET, detected at 2.8%. Three plastic pieces could not be identified as any specific polymer type (2.1%). There was no correlation between the type of polymer ingested and the response of fulmar ERs ($p = 0.96$) (Figure 5.4).

Chapter 5 – Hormonal disruption from plastic ingestion

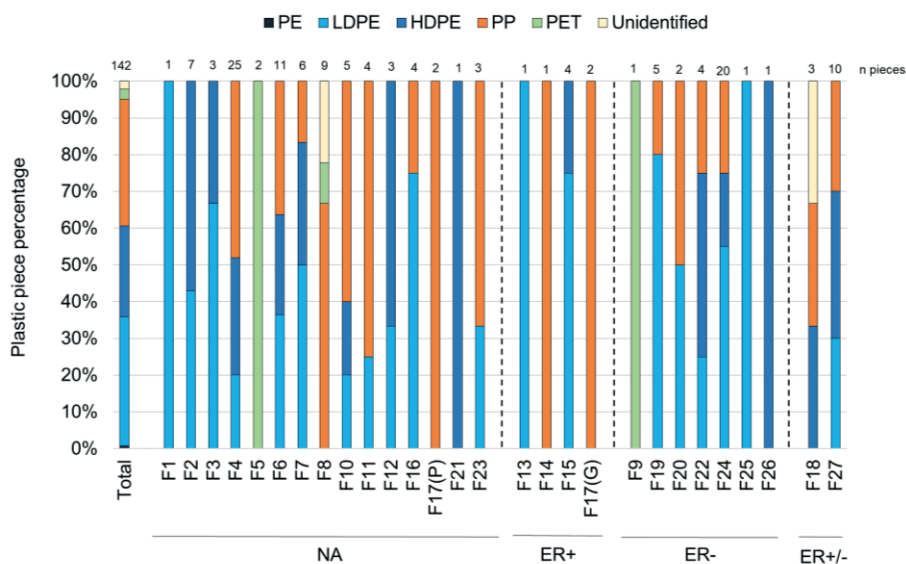


Figure 5.4. Plastic polymer type. Distribution in percentage of polymer types identified among the total plastic pieces analyzed (n = 142) and among subgroups categorized by their effect on fulmar ERs: non-responsive (NA), activating (ER+), inhibiting (ER-), and both activating and inhibiting (ER+/-). The number of plastic pieces found per bird is indicated at the top of each bar. PE: black (when not identified as HDPE or LDPE), LDPE: light blue, HDPE: dark blue, PP: orange, PET: green, and unidentified: yellow.

5.3.2.3. Qualitative assessment of plastic leachates

Hexane fractions. Hexane fractions from day 3 were analyzed for samples F15, F17 (G), F18, and F27 that exhibited ER activation on day 1, 5, and 14 (Table 5.1). A distinct peak was observed at 217 m/z in positive ion mode for the four samples (Supplementary Figure 5.8). This peak was not observed in the vehicle controls and non-responsive samples (Supplementary Figure 5.8).

Chapter 5 – Hormonal disruption from plastic ingestion

Methanol fractions. Methanol fractions from day 3 were analyzed for sample F19 which inhibited ERs on day 1 and 5, and sample F20 and F27 which inhibited ERs on day 1, 5 and 14. Distinct peaks were observed at 359 m/z and 588 m/z for F19 and at 648 m/z for F20 in positive ion mode (Supplementary Figure 5.9). These peaks were not found in non-responsive samples. In negative ion mode, a distinct peak was also observed at 486 m/z for F19 (Supplementary Figure 5.9).

These findings indicate that distinct compounds are present in the responsive samples that are not observed in the non-responsive samples and vehicle controls. The peaks observed in mass spectrometry were not identified because it typically requires coupling with chromatographic techniques (LC-MS or GC-MS) for better separation and resolution, particularly in complex matrices such as plastic leachates (Li *et al.*, 2022).

5.4. Discussion

Our study demonstrates the endocrine disruption potential of plastic ingestion in northern fulmars. Both human and fulmar estrogen receptors (ERs) responded similarly to plastic-associated chemicals, though with species-specific differences in sensitivity. Plastics ingested by fulmars leached chemicals that activated or inhibited fulmar ERs, with 55% showing a response after one day of leaching and 37% exhibiting effects on day 5 and/or 14. Our findings highlight potential risks posed by chemicals leached from ingested plastics.

Chapter 5 – Hormonal disruption from plastic ingestion

5.4.1. Fulmar ERs exhibit a similar response to human ERs, with chemical and receptor type-specific sensitivity differences

Our study revealed that both human and northern fulmar estrogen receptors (ERs) exhibited similar responses to plastic-associated chemicals. Bisphenol A (BPA), BPS, 4-OP, and BBP acted as potent activators of both human and fulmar ERs while TBBPA, PCB-53, and PCB-138 acted as potent inhibitors of ERs. These findings align with existing literature on human ERs (Paris *et al.*, 2002; Plíšková *et al.*, 2005; Molina-Molina *et al.*, 2013; Krivoschiev *et al.*, 2016; Park *et al.*, 2019), except for PCB-52, which has been documented as an ER agonist (Plíšková *et al.*, 2005; Zhang *et al.*, 2014).

Differences in ER sensitivity were observed between human and fulmar depending on the chemical, the chemical's concentration and the type of receptor (alpha or beta). ER sensitivity differences to xenoestrogens have been documented between species including humans, mice, chickens, green anoles, and rainbow trout (Matthews *et al.*, 2000) and within different species of rhino, with sensitivity varying based on the chemical and receptor type (Tubbs *et al.*, 2012). Differential activation between species is often attributed to disparities in the transactivation domains (A/B and F) (Tubbs *et al.*, 2012; Felton *et al.*, 2020) and ligand-binding domains (E) (Matthews *et al.*, 2000; Katsu *et al.*, 2013; Asnake, Modig and Olsson, 2019) of ERs. The A/B (86% and 72% similarity) and F (84% similarity) domains displayed the most sequence variability between human and fulmar (Supplementary Figure 5.10), suggesting potential differences in coregulator recruitment and receptor dimerization (Arao and Korach, 2018, 2021). Minor distinctions in the E

Chapter 5 – Hormonal disruption from plastic ingestion

domain for ER β (95% similarities) suggests potential differences in receptor affinity for the chemicals tested (Arao and Korach, 2021). Notably, 4-OP activated fulmar ERs at concentrations documented in the blood of razorbill (*Alca torda*) seabirds (Bodziach *et al.*, 2021), indicating that environmental exposure to 4-OP could result in endocrine disruption via ER activation.

5.4.2. Plastic leachates activate and inhibit fulmar ERs

Plastic ingested by northern fulmars can leach plastic-associated chemicals capable of activating or inhibiting northern fulmar ERs *in vitro*. Overall, more than half of the fulmars (55%) had ingested plastic that induced a response of fulmar estrogen receptors. Our findings are consistent with the Michishita *et al.* (2023) study which observed human ER activation by leachates from microplastics ingested by common murrelets (*Uria aalge*) (Michishita *et al.*, 2023). The type of polymer did not correlate with ER response, suggesting that the endocrine-disrupting effects of plastic leachates are not specifically related to polymer composition. The variations in the types of additives, their initial concentrations in the polymer, as well as the residence time of plastics in the ocean and/or the bird stomach likely contributed to the observed differences in ER response among individuals (Koelmans *et al.*, 2016).

We previously showed that high concentrations of additives can be released in an *in vitro* Procellariiform stomach model for prolonged periods of time (Van Hassel *et al.*, 2024). The ER activation or inhibition observed on day 5 and/or 14 for 37% of fulmars also suggests that high concentrations of chemicals from ingested plastic continue to be released after several days or

Chapter 5 – Hormonal disruption from plastic ingestion

weeks. Although POPs have also been shown to be ER disruptors (Bonefeld-Jørgensen *et al.*, 2014), we suspect that POPs sorbed to plastic in the ocean are not primarily responsible for the ER response, given that these sorbed chemicals are released within 5 day following ingestion (Van Hassel *et al.*, 2024) and that the plastics extracted from fulmar stomachs in our study had likely been there for more than one day.

The widespread use of plastic additives poses a challenge in fully characterizing plastic leachates, with approximately 450 compounds lacking standardized identification protocols (Ong, Samsudin and Soto-Valdez, 2022). Coffin *et al.* (2019) saw that estrogenicity of leachates from commercial plastic was not attributed to the presence of bisphenol A and phthalates, indicating the leaching of other uncharacterized plastic additives (Coffin *et al.*, 2019). Our findings showing that both hexane and methanol fractions induced a response suggest that pollutants with different levels of hydrophobicity were leached, which is consistent with the wide range of physico-chemical properties that characterize plastic additives (Ellington, 1999). Interestingly, two samples activated ERs in the hexane fraction after the first day of leaching and in the methanol fraction on day 5 or 14 (Table 5.1), suggesting that different chemicals, located deeper within the plastic matrix may be released over time. The qualitative assessment of leachates confirms the presence of distinct compounds responsible for ER activation and inhibition (Supplementary Figure 5.8 and 5.9).

5.4.3. Biological significance

Our work shows that ingested plastic has the ability to disrupt northern fulmar hormone function *in vitro*. Using the same *in vitro* expression system, Tubbs *et al.* (2016) established a link between the response of southern white

Chapter 5 – Hormonal disruption from plastic ingestion

rhino to phytoestrogens and their poor reproductive success, suggesting that the *in vitro* fulmar ER response to leachates might predict a physiological response at the individual level (Tubbs *et al.*, 2016).

While solvents were used in our study, our previous research demonstrated that plastic-associated chemicals can leach under Procellariiform gastric conditions (Van Hassel *et al.*, 2024). Nevertheless, the use of solvents may have influenced leachate concentrations differently than gastric conditions. For example, PBDE-209 and BPS were released at different concentrations in solvents compared to digestive conditions (Van Hassel *et al.*, 2024). It is also important to acknowledge that plastics were in the birds' stomachs for unknown time periods before they were caught as fisheries bycatch. Thus, our results should be a cautious estimate of risk to fulmars as the most biologically available chemicals might have already been released from the ingested plastic. Moreover, our study did not include plastic < 1 mm (e.g., microplastic), which could also contribute to additional estrogenic activity (Michishita *et al.*, 2023).

Bird exposure to ER α and ER β agonists and antagonists has shown to induce brain demasculinization in male and disturb differentiation of the reproductive tract in female (Mattsson, Olsson and Brunström, 2011; Court *et al.*, 2020). The observed activation or inhibition of fulmar ERs suggests that plastic-associated chemicals could interfere with hormonal signaling in birds and potentially contribute to reproductive disruption and changes in sexual behavior (Roman *et al.*, 2019; Monclús *et al.*, 2022). The detection of plastic-associated chemicals such as phthalates in seabird egg yolk also highlight the ability of toxic chemicals to be maternally transferred to the offspring, which

Chapter 5 – Hormonal disruption from plastic ingestion

could also be detrimental to reproduction by directly impacting embryo development (Allen *et al.*, 2021).

5.5. Conclusion

Our study underscores the potential impact of plastic ingestion on northern fulmars. We found that both human and fulmar estrogen receptors (ERs) respond similarly to plastic-associated chemicals, albeit with sensitivity differences depending on the chemical, concentration, and receptor type. More than half northern fulmars had plastic that activated or inhibited fulmar ERs, likely due to the leaching of additives. ER responses observed on subsequent plastic leaching after 5 or 14 days suggest a potential prolonged exposure to xenoestrogens which could have a negative impact on an individual's reproductive function. Overall, our findings highlight the potential negative consequences from plastic pollution on Procellariiform health, emphasizing the need for effective measures to reduce plastic waste in marine environments.

Supplementary material

ER α

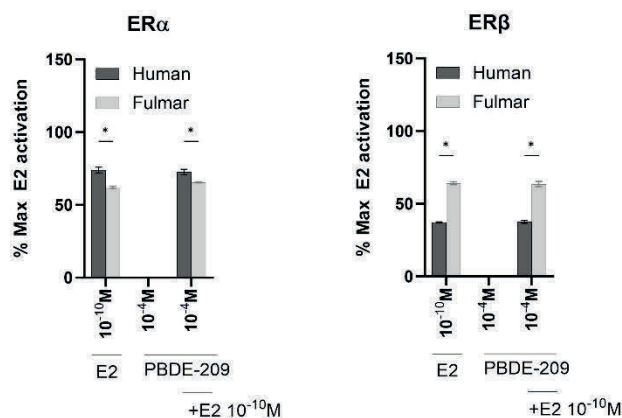
	1	183	249	350	543	594
Human	A/B	C	D	E	F	
Fulmar	86	100	91	100	84	

ER β

	1	148	214	303	495	520
Human	A/B	C	D	E	F	
Fulmar	72	100	93	95	84	

Supplementary Figure 5.1. Comparison of human and northern fulmar ER sequences. Values above ER diagrams correspond to the number of amino acid residues in ER functional domains A/B-F. Values within northern fulmar ER diagrams represent percent similarity of predicted amino acid sequences of individual functional domains compared with human ER domains. A/B: N-terminal domain (NTD), C: DNA-binding domain (DBD), D: hinge region, E: ligand-binding domain (LBD) and F: C-terminal domain (CTD).

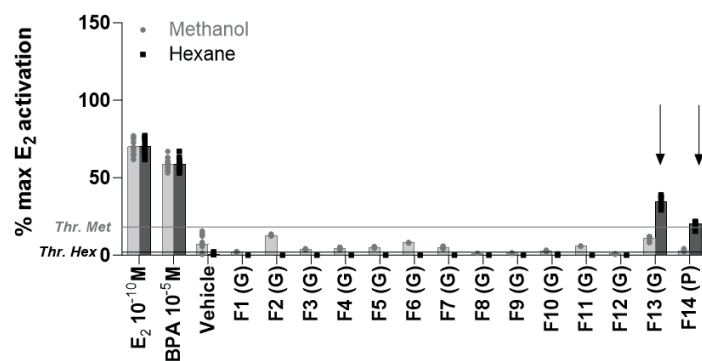
Chapter 5 – Hormonal disruption from plastic ingestion



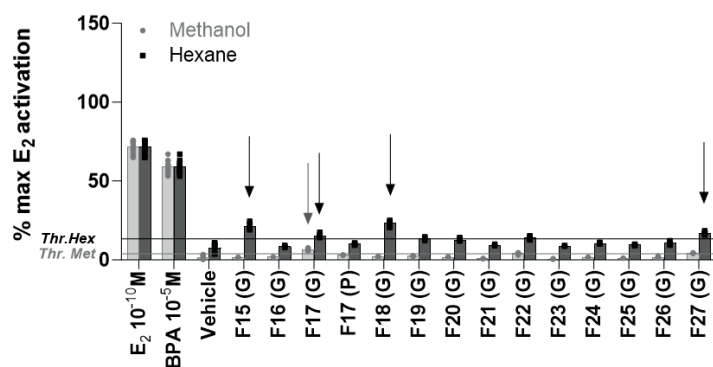
Supplementary Figure 5.2. Absence of activation and inhibition of human and northern fulmar ER α and ER β by PBDE-209. HEK293 cells expressing ESRs were treated in triplicate with 10^{-4} M of PBDE-209 resuspended in 0.1% DMSO (activation) or resuspended in 10^{-10} M of 17 β -E2 (0.1% DMSO) (inhibition). Bars represent mean of the fold activation of each treatment relative to maximal E2 activation. Error bars are $\pm$ SEM of triplicate wells. Significant differences between human and fulmar were determined using a two-way ANOVA followed by individual t-test with Bonferroni correction ($p < 0.05$) (asterisk). Significant inhibition was assessed using a two-way ANOVA with a Bonferroni correction by comparing the activation by 10^{-10} M E2 alone to the activation by 10^{-10} M E2 in the presence of the chemical ($p < 0.05$) (no inhibition here).

Chapter 5 – Hormonal disruption from plastic ingestion

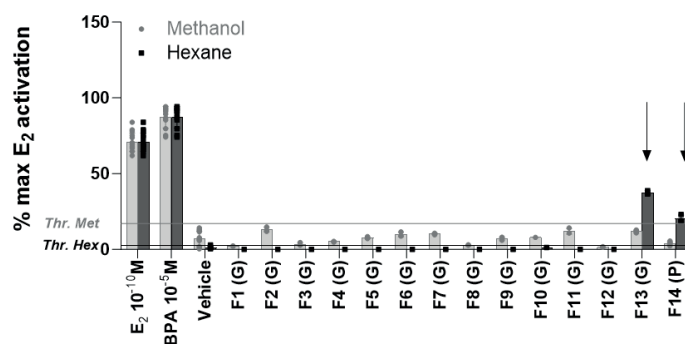
A



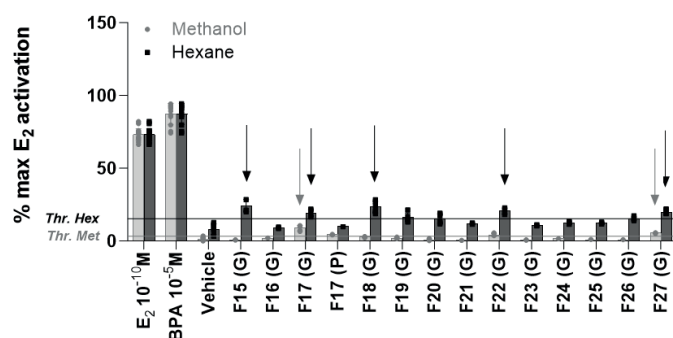
B



C

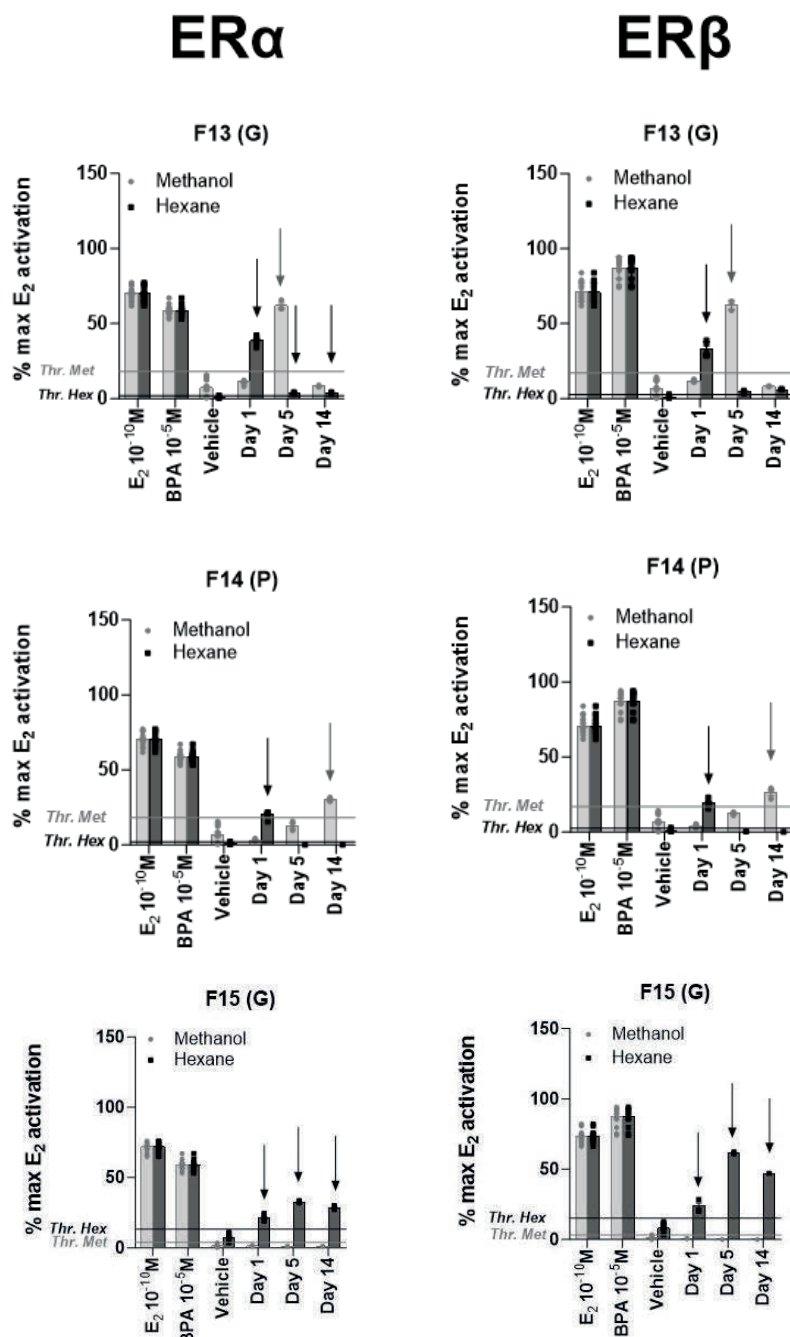


D

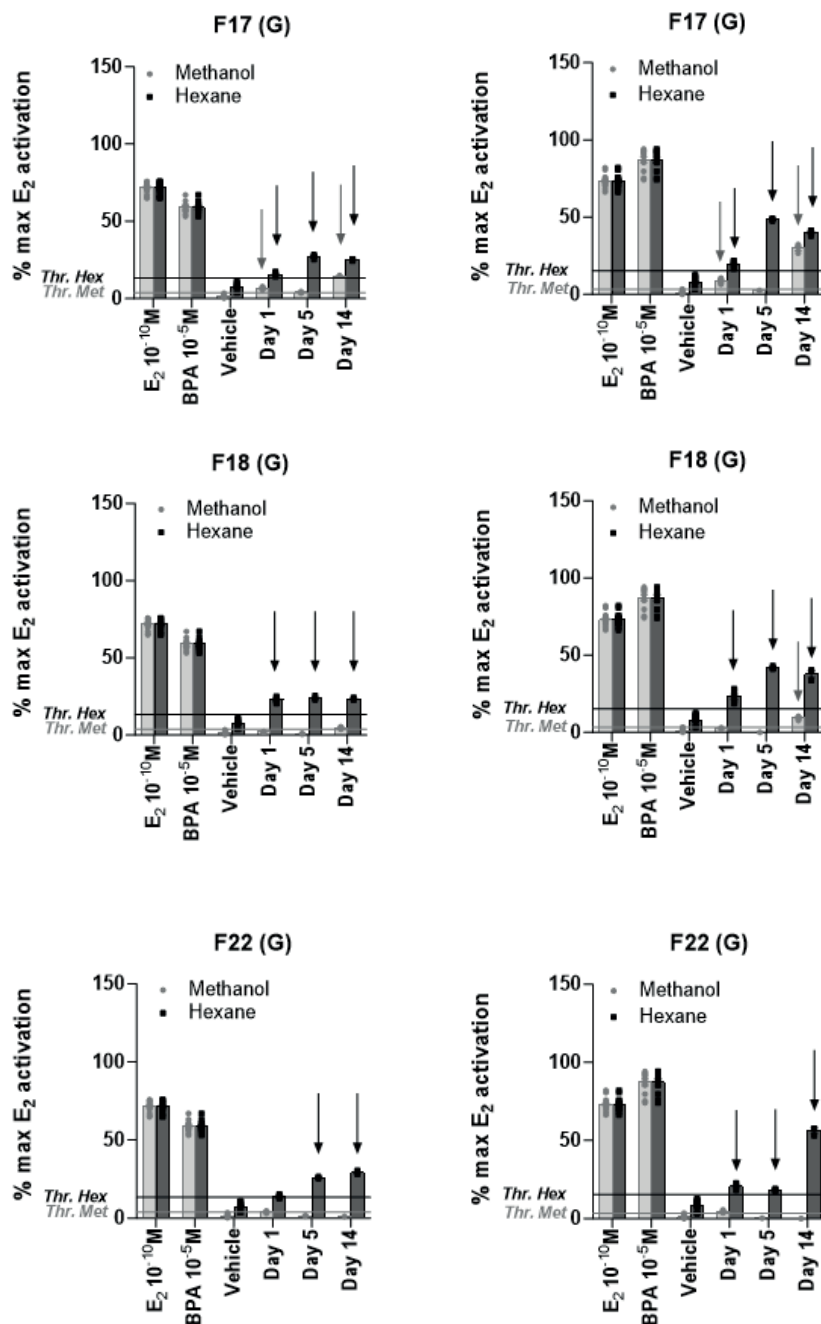


Supplementary Figure 5.3. Activation of northern fulmar ER α (A) and ER β (B) to leachates of plastics recovered from northern fulmar stomach after 24 hours. HEK293 cells expressing ESRs were treated in triplicate with leachates resuspended in 0.25% DMSO. Panel A and C show leachates of plastics recovered from fulmar 1 – 14 (set 1) and panel B and D show leachates of plastics recovered fulmar 15 – 27 (set 2). Methanol fraction: light gray, hexane fraction: dark gray. Data are represented as mean $\pm$ SEM of the fold activation of each treatment relative to maximal E₂ activation. Significant activation (arrow) was determined using a threshold defined as the mean of the vehicle controls plus two times standard deviation.

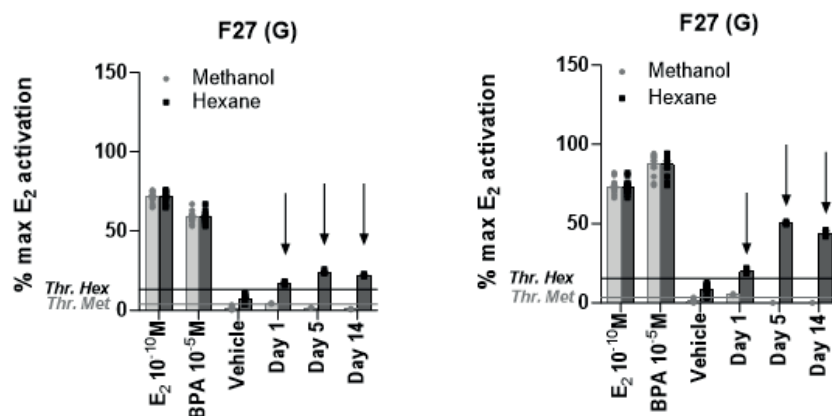
Chapter 5 – Hormonal disruption from plastic ingestion



Chapter 5 – Hormonal disruption from plastic ingestion



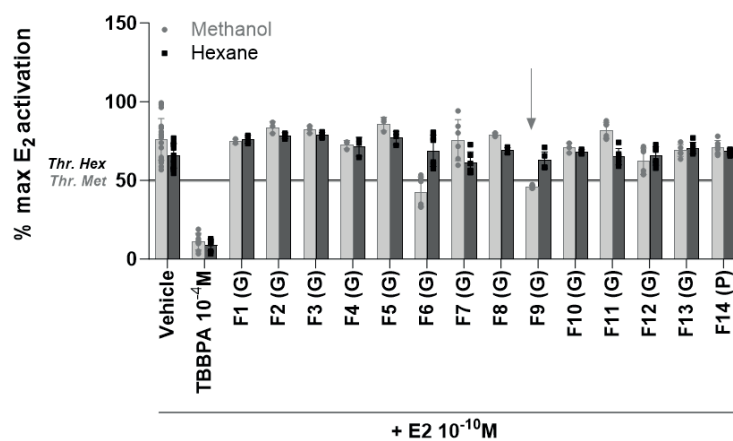
Chapter 5 – Hormonal disruption from plastic ingestion



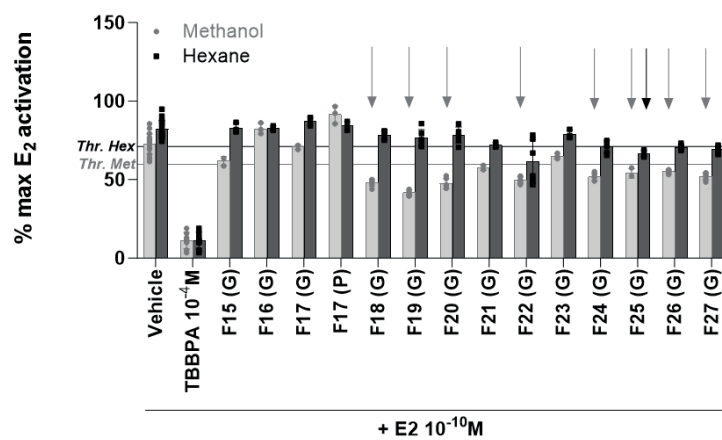
Supplementary Figure 5.4. Activation of northern fulmar ER α (left) and ER β (right) to leachates from day 1, 5 and 14. HEK293 cells expressing ESRs were treated in triplicate with leachates resuspended in 0.25% DMSO. The methanol and hexane fraction of each leachate are showed in gray and black, respectively. Data are represented as mean $\pm$ SEM of the fold activation of each treatment relative to maximal E2 activation. Significant activation (arrow) was determined using a threshold defined as the mean of the vehicle controls plus two times standard deviation.

Chapter 5 – Hormonal disruption from plastic ingestion

A

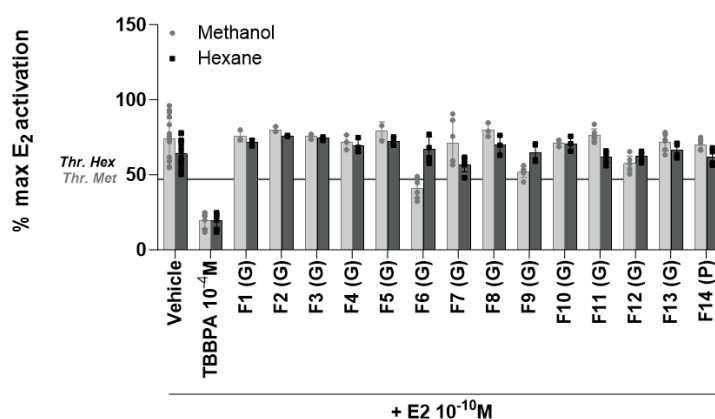


B

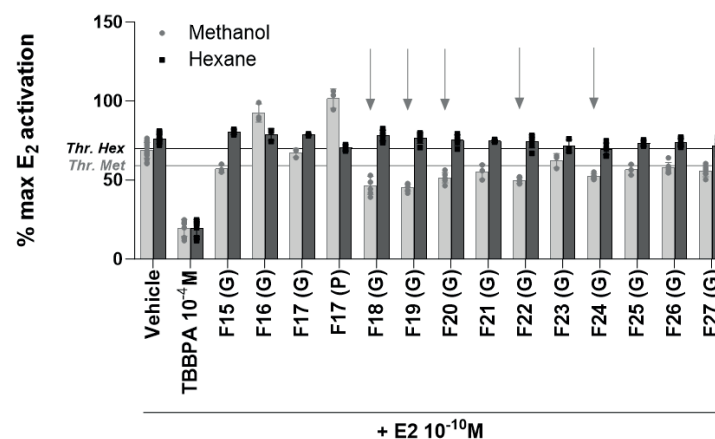


Chapter 5 – Hormonal disruption from plastic ingestion

C



D

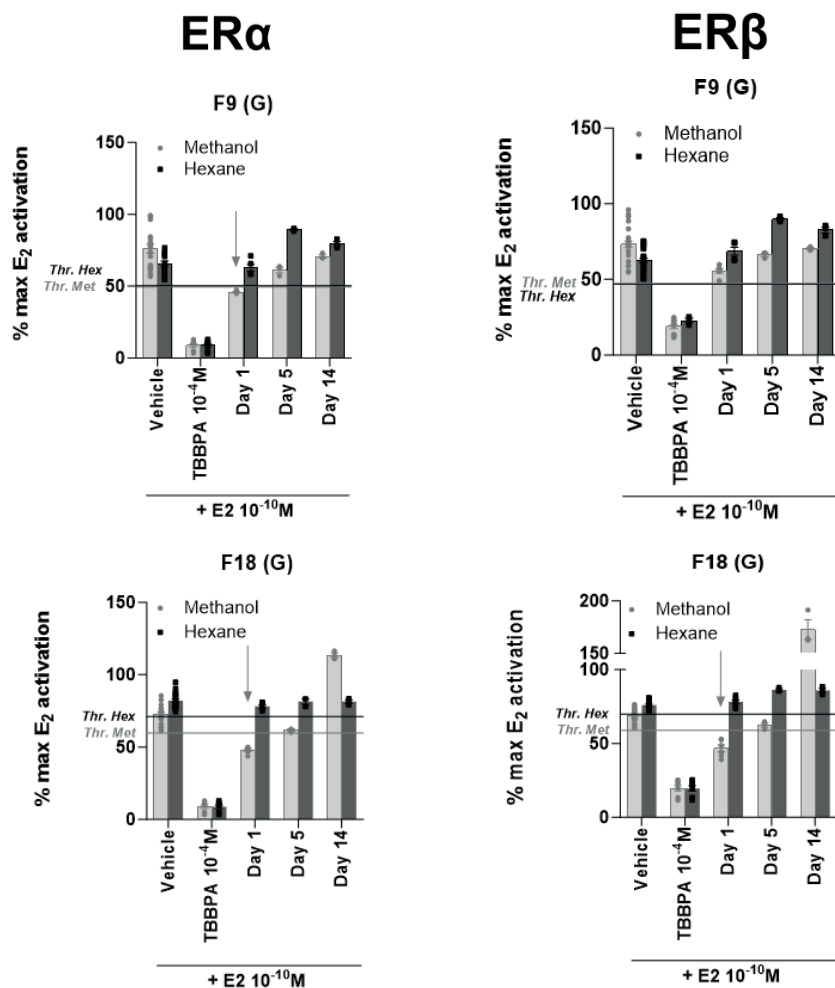


Supplementary Figure 5.5. Inhibition of northern fulmar $ER\alpha$ (A) and $ER\beta$ (B) to leachates of plastics recovered from northern fulmar stomach after 24 hours. HEK293 cells expressing ESRs were treated in triplicate with leachates resuspended in E_2 10^{-10} M (0.25% DMSO). Panel A and C show leachates of plastics recovered from fulmar n°1 – 14 (set 1) and panel B and D show leachates of plastics recovered fulmar n°15 – 27 (set 2). Methanol fraction: light gray, hexane fraction: dark gray. Data are

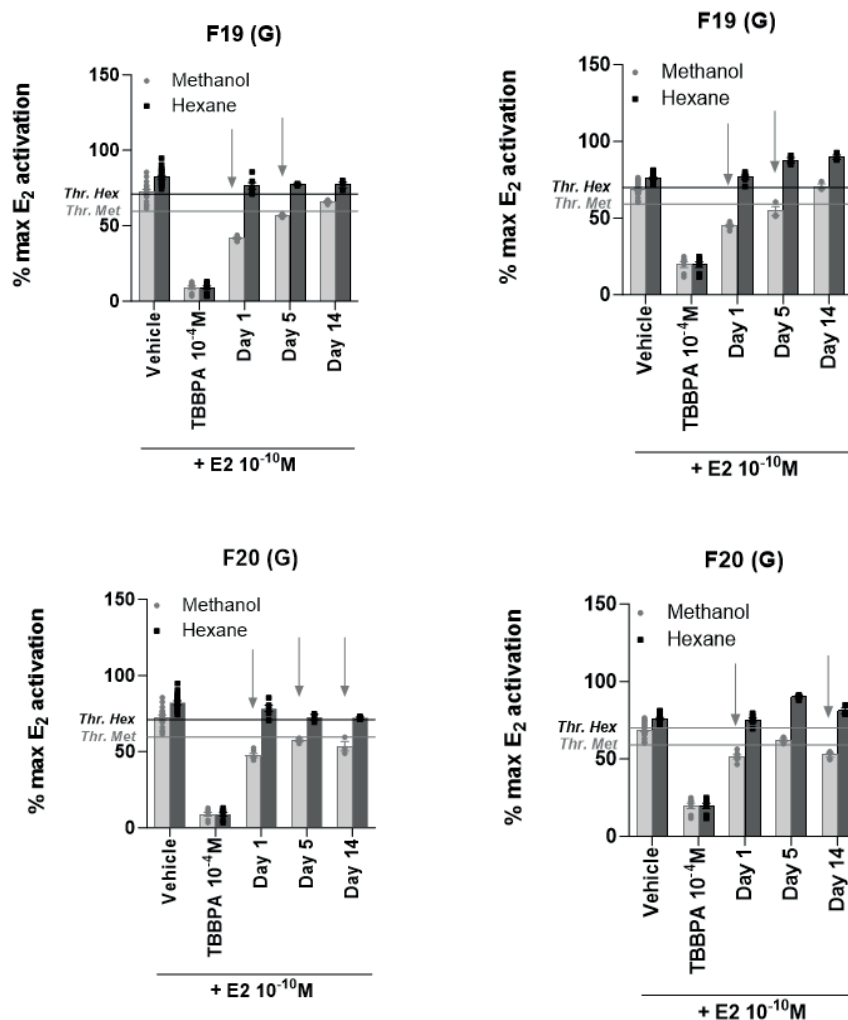
Chapter 5 – Hormonal disruption from plastic ingestion

represented as mean $\pm$ SEM of the fold activation of each treatment relative to maximal E2 activation. Significant inhibition (arrow) was determined using a threshold defined as the mean of the vehicle controls minus two times standard deviation.

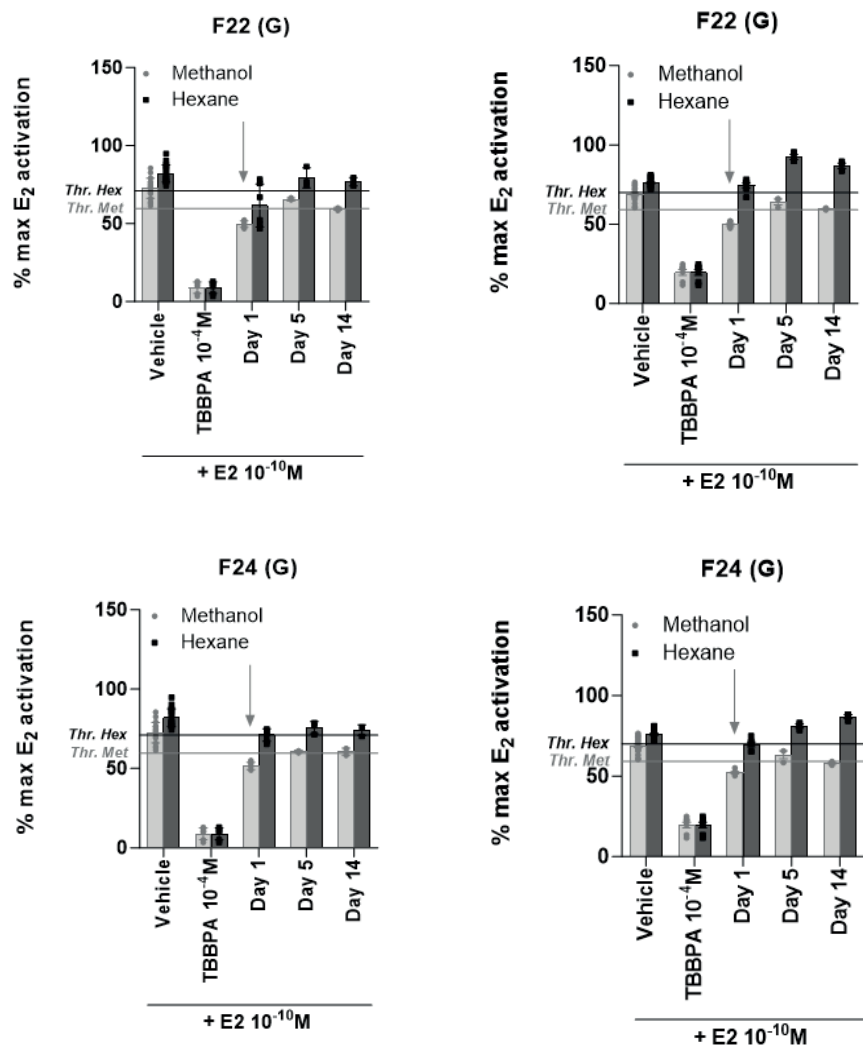
Chapter 5 – Hormonal disruption from plastic ingestion



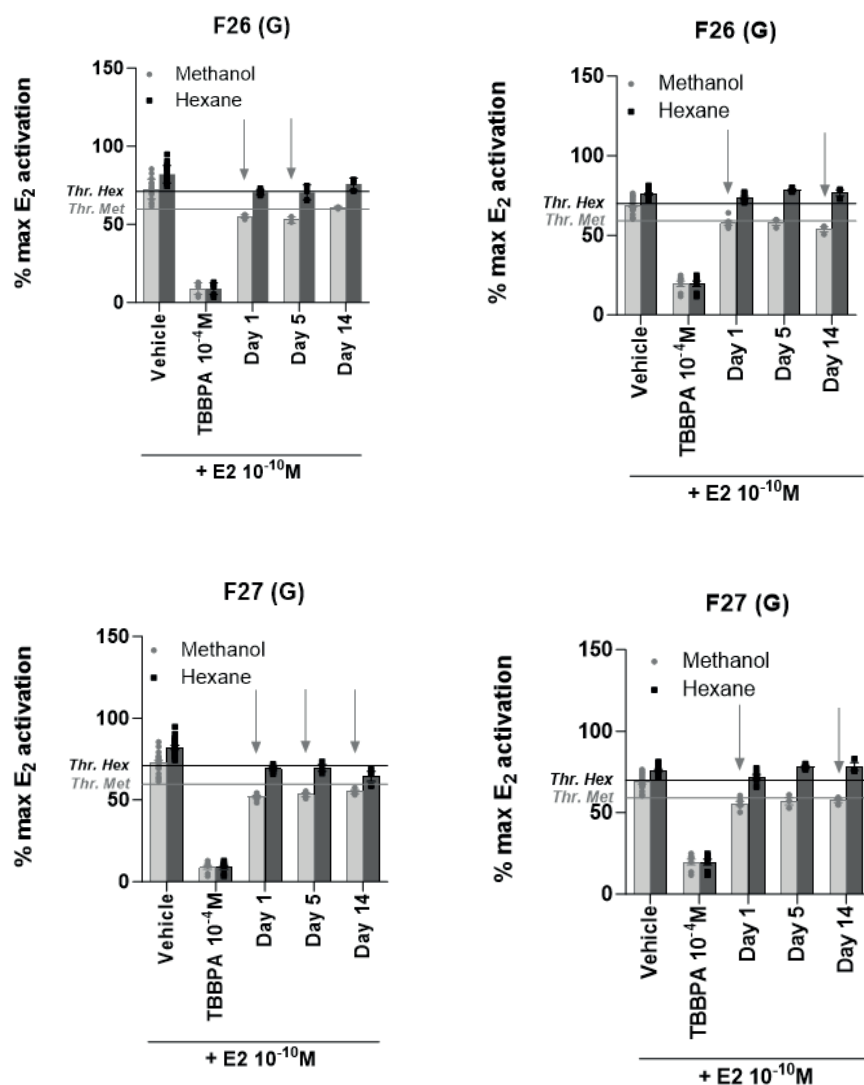
Chapter 5 – Hormonal disruption from plastic ingestion



Chapter 5 – Hormonal disruption from plastic ingestion



Chapter 5 – Hormonal disruption from plastic ingestion

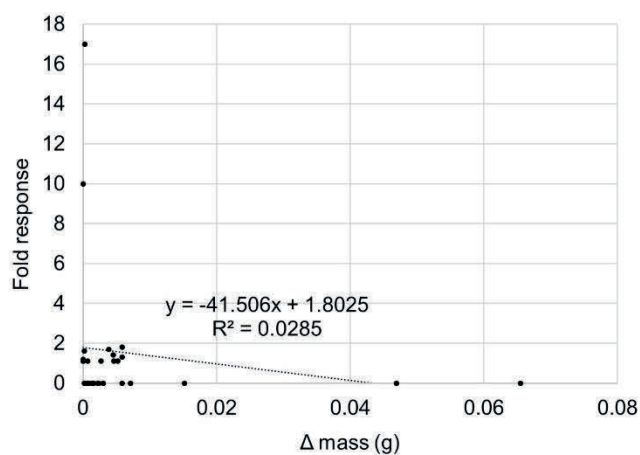


Supplementary Figure 5.6. Inhibition of northern fulmar ER α and ER β to leachates from day 1, 5 and 14. HEK293 cells expressing ESRs were treated in triplicate with leachates resuspended in E2 10⁻¹⁰ M (0.25% DMSO). The methanol and hexane fraction of each leachate are showed in gray and black, respectively. Data are represented as mean $\pm$ SEM of the fold activation of each treatment relative to

Chapter 5 – Hormonal disruption from plastic ingestion

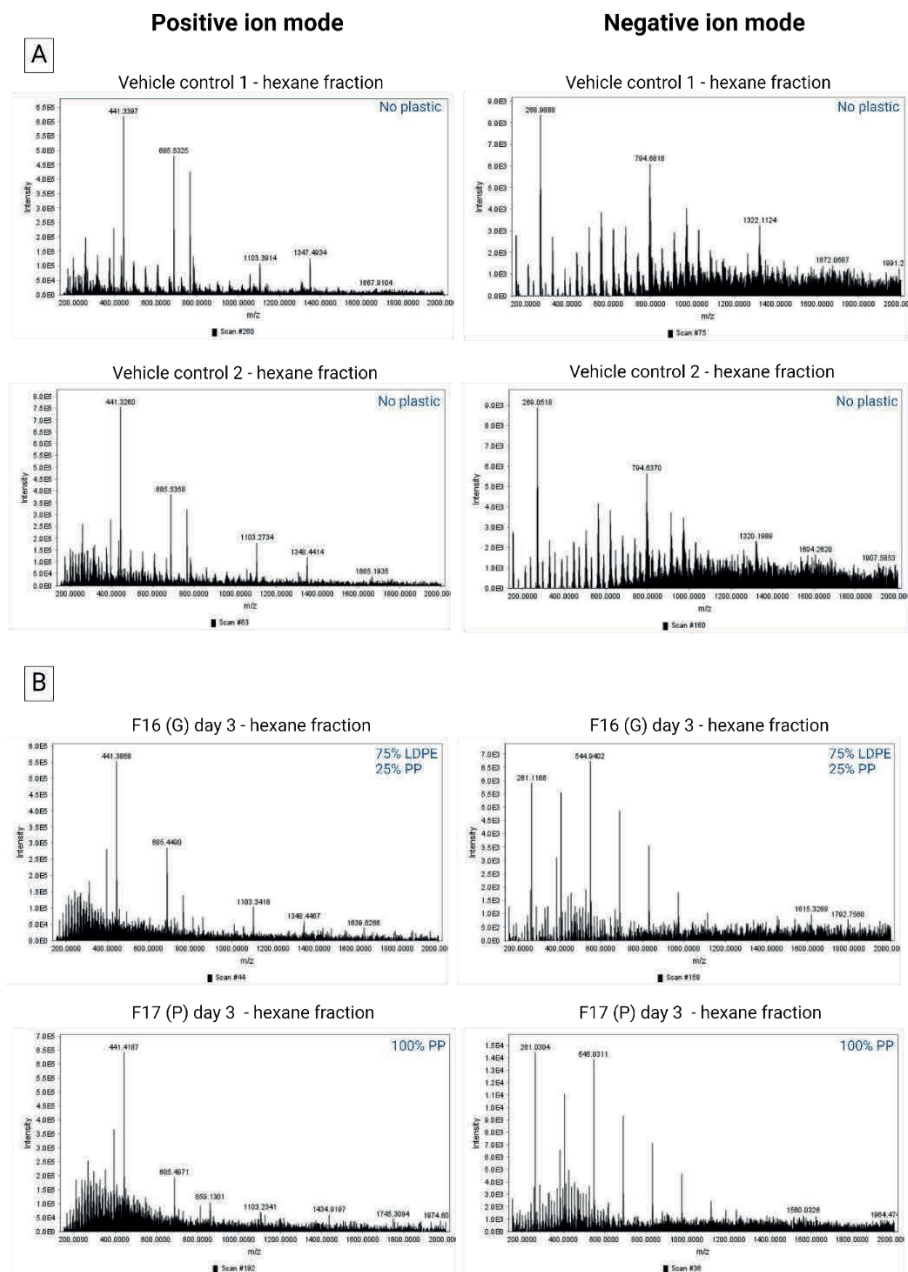
maximal E2 activation. Significant inhibition (arrow) was determined using a threshold defined as the mean of the vehicle control minus two times standard deviation.

Chapter 5 – Hormonal disruption from plastic ingestion

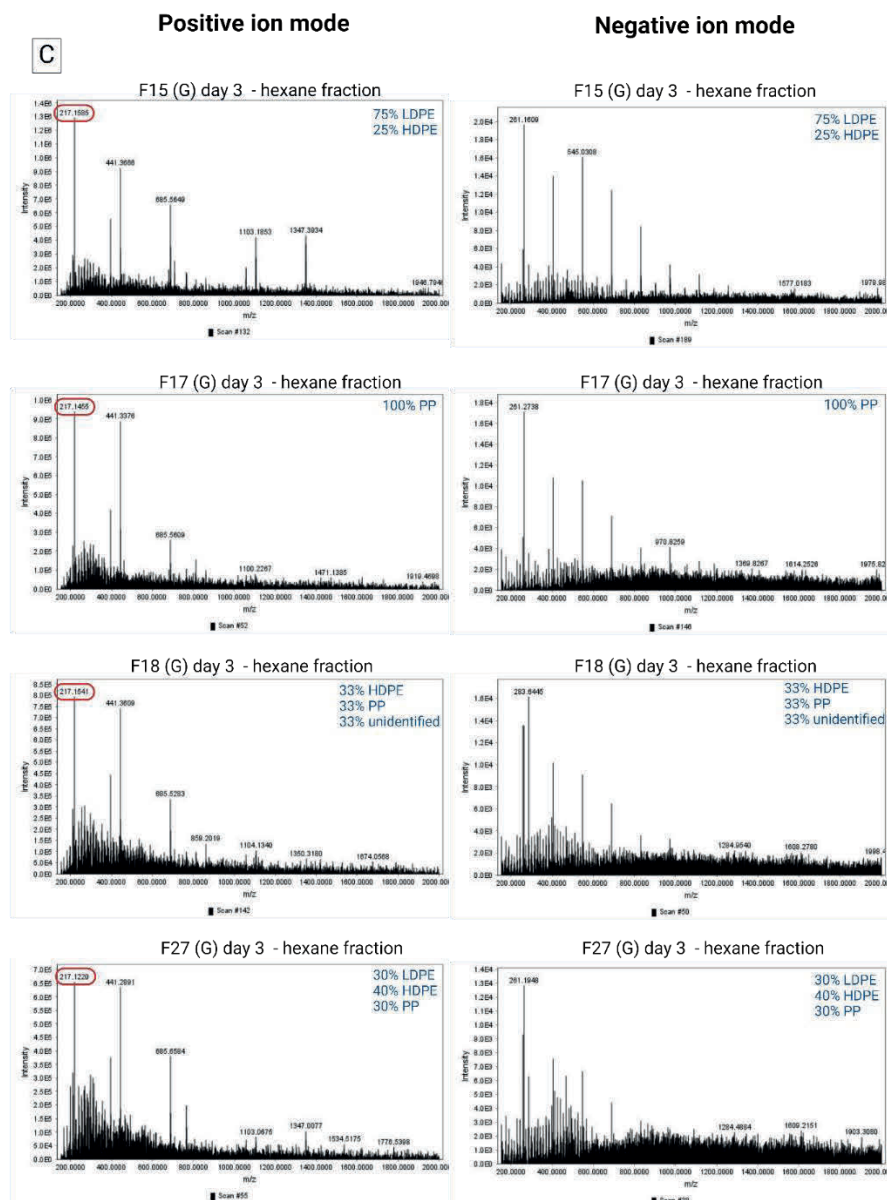


Supplementary Figure 5.7. Correlation between the mass of plastic loss during the leaching experiment with the fulmar ER fold response over or below the threshold. Estrogenicity was expressed as the fold change above (for activation) or below (for inhibition) the threshold. Thresholds are defined as the average vehicle control response plus (activation) or minus (inhibition) twice the standard deviation. Delta mass represent the mass of the plastic sample (in gram) before the leaching experiment minus the mass of the plastic sample (in gram) after 14 days of leaching.

Chapter 5 – Hormonal disruption from plastic ingestion



Chapter 5 – Hormonal disruption from plastic ingestion

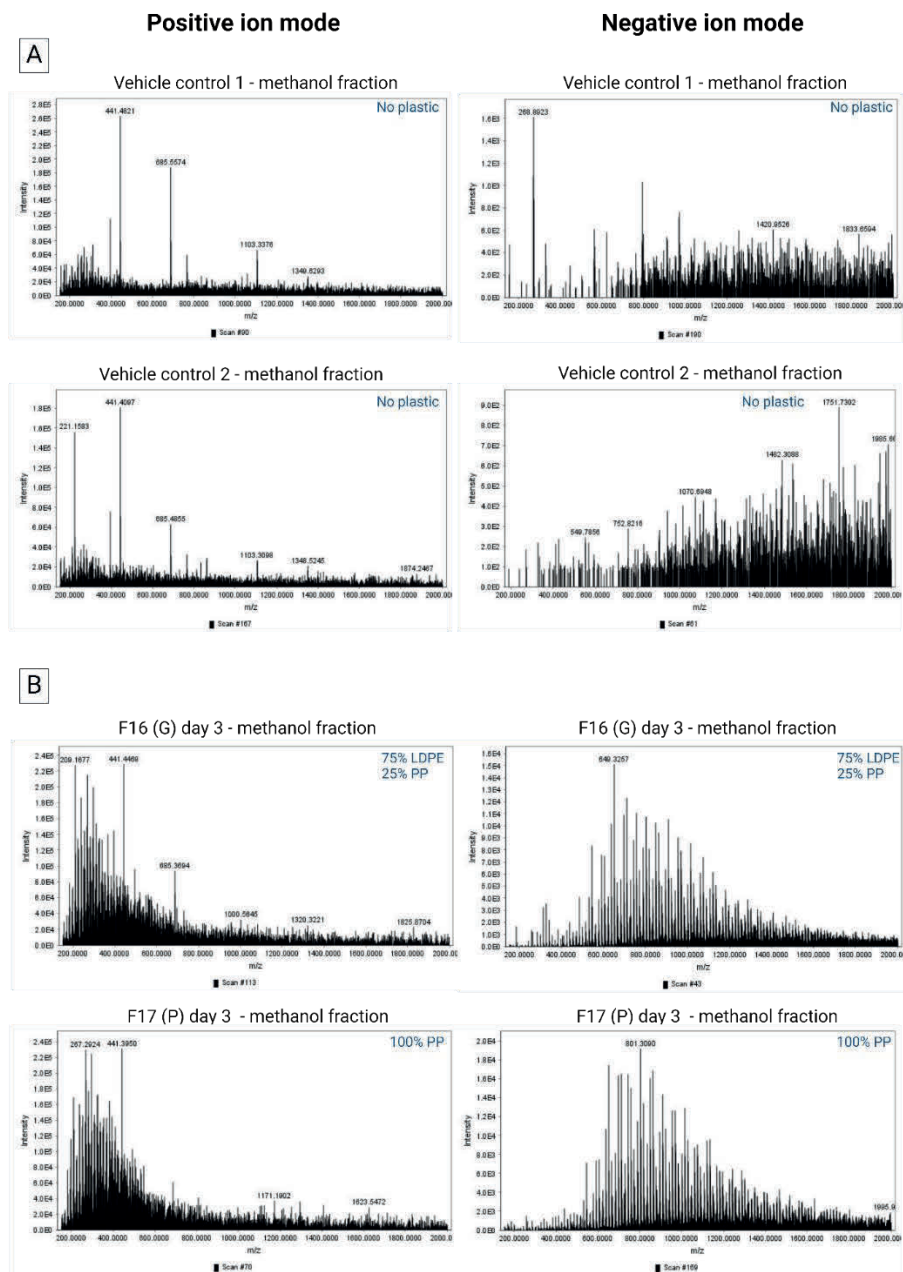


Supplementary Figure 5.8. Mass spectra of the hexane fraction of two vehicle controls, two non-responsive samples and 4 activating samples from day 3. Panel A

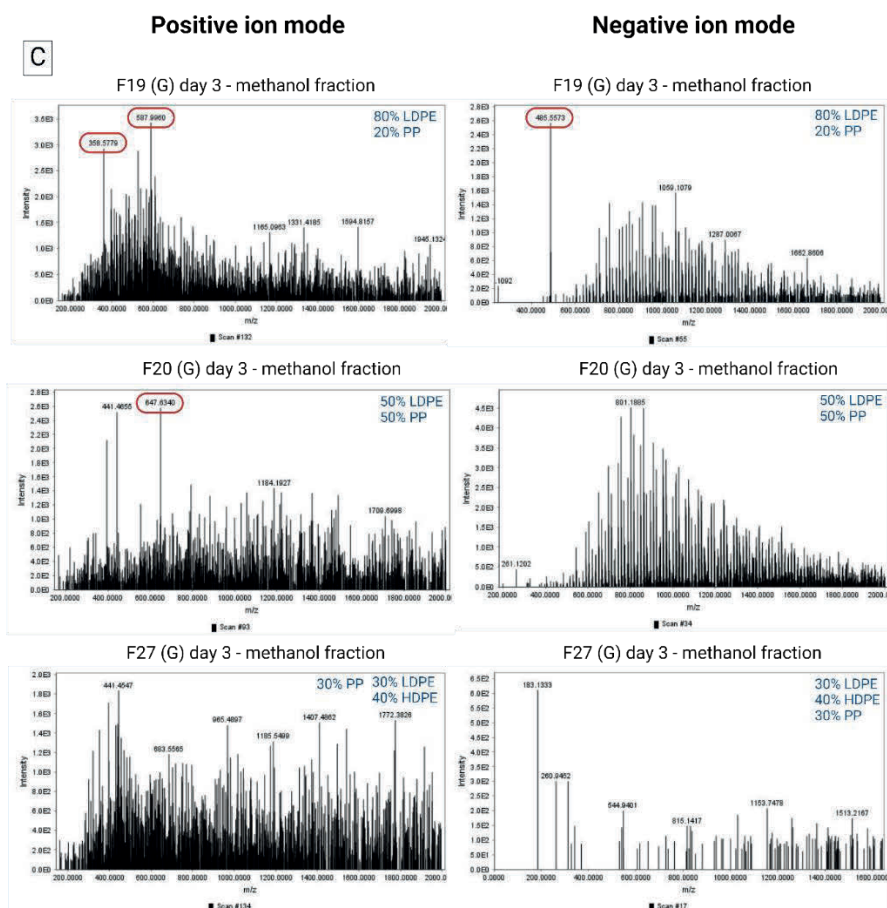
Chapter 5 – Hormonal disruption from plastic ingestion

shows the mass spectra of the hexane fraction of two vehicle controls (no plastic). Panel B shows the mass spectra of the day 3 hexane fraction from sample F16 (G) and F17 (P) which did not induce a response (activation or inhibition) of fulmar ERs on day 1. Panel C shows the mass spectra of the day 3 hexane fraction from sample F15 (G), F17 (G), F18 (G) and F27 (G) which activated fulmar ERs on day 1, 5 and 14. The mass-to-charge ratio (m/z) of peaks only present in samples that activated ERs are circled in red.

Chapter 5 – Hormonal disruption from plastic ingestion



Chapter 5 – Hormonal disruption from plastic ingestion



Supplementary Figure 5.9. Mass spectra of the methanol fraction of two vehicle controls, two non-responsive samples and 4 activating samples from day 3. Panel A shows the mass spectra of the methanol fraction of two vehicle controls (no plastic). Panel B shows the mass spectra of the day 3 methanol fraction from sample F16 (G) and F17 (P) which did not induce a response (activation or inhibition) of fulmar ERs on day 1. Panel C shows the mass spectra of the day 3 methanol fraction from sample F19 (G) which inhibited fulmar ERs on day 1, 5 and sample F20 (G) and F27 (G) which inhibited fulmar ERs on day 1, 5 and 14. The mass-to-charge ratio (m/z) of peaks only present in samples that inhibited ERs are circled in red.

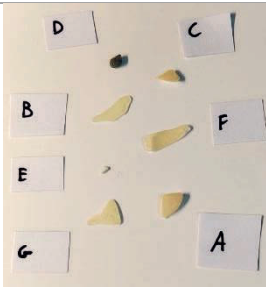
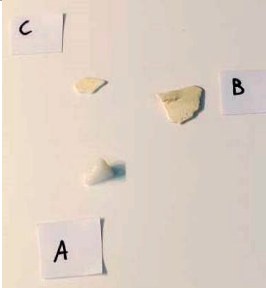
Chapter 5 – Hormonal disruption from plastic ingestion

Supplementary Table 5.1. Summary Details of Plastic collected from Northern Fulmars. Information includes the date of collection, the sex of the bird (F: female, M: male), the stomach chamber in which the plastic was found (G: gizzard, P: proventriculus), the number of plastic pieces recovered in the chamber, the weight of the plastic sample (in gram) before and after leaching, and the type of plastic. Leachates of samples analyzed for qualitative mass spectrometry are marked with an asterisk.

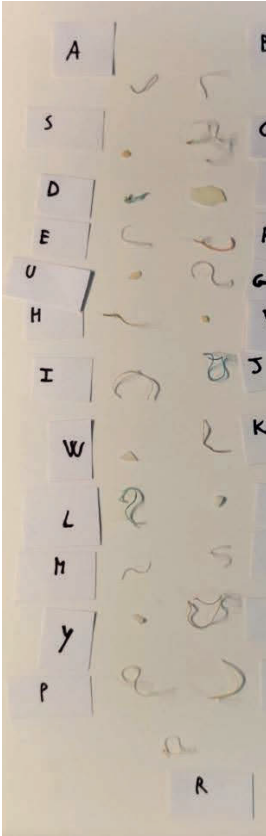
Bird	Bird ID	Year	Sex	Chamber	Total number of pieces	Weight before (g)	Weight after (g)	Plastic type
F1	18-0124	2016	F	G	1	0.011	0.008	Thread
F2	18-0075	2016	F	G	7	0.088	0.085	Fragment
F3	19-0078	2017	F	G	3	0.094	0.092	Fragment
F4	18-0129	2016	F	G	25	0.037	0.031	Thread (n=20) Fragment (n=7)
F5	18-0128	2016	F	G	2	0.022	0.021	Thread
F6	18-0329	2017	F	G	11	0.155	0.140	Fragment
F7	19-0053	2017	F	G	6	0.554	0.507	Fragment
F8	18-0377	2017	F	G	9	0.069	0.069	Threads (n=6) Other (n=3)
F9	18-0122	2016	F	G	1	0.006	0.005	Thread
F10	19-0062	2017	F	G	5	0.171	0.164	Industrial pellet (n=1) Fragment (n=4)
F11	19-0065	2017	F	G	4	0.093	0.090	Fragment
F12	19-0066	2017	F	G	3	0.069	0.069	Fragment
F13	18-0118	2016	F	G	1	0.018	0.018	Industrial pellet
F14	18-0120	2016	M	P	1	0.014	0.014	Industrial pellet
F15*	18-0332	2017	M	G	4	0.010	0.010	Fragment
F16*	18-0382	2017	M	G	4	0.006	0.005	Thread (n=1) Fragment (n=3)
F17 (G)*	19-0010	2017	M	G	2	0.023	0.019	Fragment
F17 (P)*				P	2	0.015	0.012	Fragment
F18*	18-0130	2016	M	G	3	0.042	0.036	Fragment
F19*	18-0336	2017	M	G	5	0.069	0.064	Fragment
F20*	19-0058	2017	M	G	2	0.089	0.083	Fragment
F21	19-0052	2017	M	G	1	0.035	0.035	Fragment
F22	19-0014	2017	M	G	4	0.090	0.090	Industrial pellet (n=1) Fragment (n=3)
F23	19-0055	2017	M	G	3	0.004	0.003	Threads
F24	19-0074	2017	M	G	21	0.058	0.053	Industrial pellet (n=1) Threads (n=4) Fragment (n=16)
F25	19-0064	2017	M	G	1	0.010	0.009	Industrial pellet
F26	18-0127	2016	M	G	1	0.023	0.023	Fragment
F27*	18-0327	2017	F	G	10	0.051	0.048	Fragment

Chapter 5 – Hormonal disruption from plastic ingestion

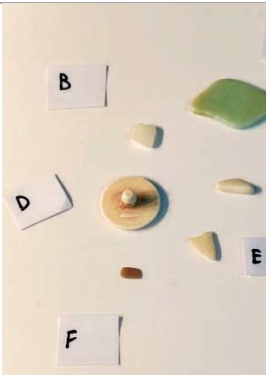
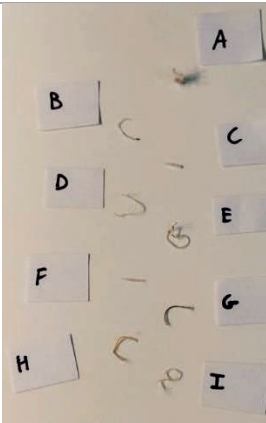
Supplementary Table 5.2. Polymer identification of plastic samples. Information includes the name of the bird, the number of plastic pieces recovered from its stomach chamber, a picture of the sample with associated letter for each plastic piece, the polymer composition in percentage of the whole sample, the method used to identify each piece of plastic (“Ident. method”) [hyperspectral near-infrared spectroscopy (HIS), near-infrared microscopy (NIRM) and mid-infrared spectroscopy (MIR)] and the type of polymer identified [high-density polyethylene (HDPE), low-density polyethylene (LDPE), polypropylene (PP), polyethylene terephthalate (PET) and non-identified (NI)].

Bird	n pieces	Sample photo	Polymer composition	Piece letter	Ident. method	Polymer type
F1	1		100% LDPE	NA	HIS	LDPE
F2	7		57% HDPE 43% LDPE	A B C D E F G	HIS HIS HIS HIS HIS HIS HIS	HDPE HDPE HDPE LDPE LDPE HDPE LDPE
F3	3		66.6% LDPE 33.4% HDPE	A B C	HIS HIS HIS	LDPE LDPE HDPE
F4	25			A B	HIS NIRM	LDPE PP

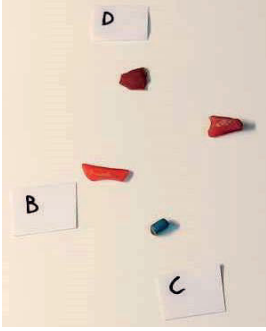
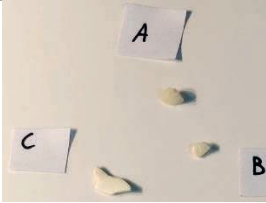
Chapter 5 – Hormonal disruption from plastic ingestion

				C	HIS	PP
				D	HIS	LDPE
				E	HIS	PP
				F	HIS	LDPE
				G	HIS	PP
				H	HIS	LDPE
				I	HIS	PP
				J	NIRM	PP
				K	HIS	LDPE
				L	HIS	HDPE
				M	HIS	PP
				N	HIS	PP
				O	HIS	PP
				P	HIS	PP
				Q	HIS	PP
				R	HIS	PP
				S	HIS	HDPE
				T	HIS	HDPE
				U	HIS	HDPE
				V	HIS	HDPE
				W	HIS	HDPE
				X	HIS	HDPE
				Y	HIS	HDPE
F5	2		100% PET	A	HIS	PET
				B	HIS	PET
F6	11		36.4% PP 36.4% LDPE 27.2% HDPE	A	HIS	LDPE
				B	HIS	LDPE
				C	HIS	PP
				D	HIS	LDPE
				E	HIS	HDPE
				F	HIS	HDPE
				G	HIS	HDPE
				H	HIS	LDPE
				I	HIS	PP

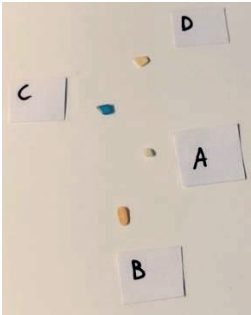
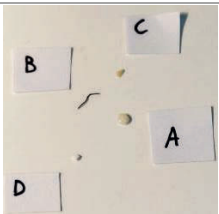
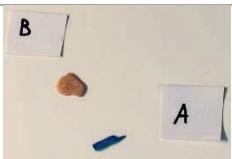
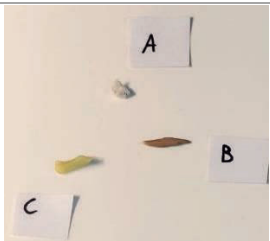
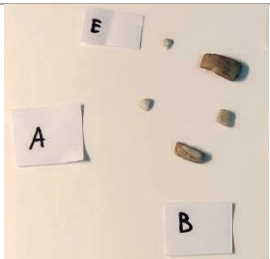
Chapter 5 – Hormonal disruption from plastic ingestion

				J K	HIS HIS	PP PP
F7	6		50% LDPE 33.4% HDPE 16.6% PP	A B C D E F	HIS HIS HIS HIS HIS HIS	LDPE HDPE LDPE PP HDPE LDPE
F8	9		66.6% PP 22.2% NI 11.2% PET	A B C D E F G H I	HIS HIS NIRM HIS HIS NIRM HIS HIS HIS	PET PP Unidentified PP PP Unidentified PP PP PP

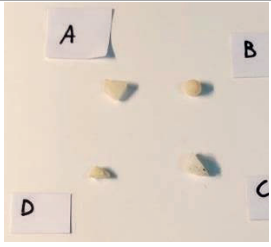
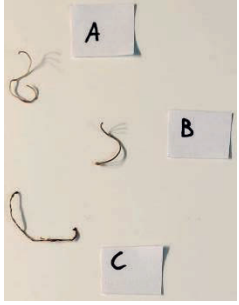
Chapter 5 – Hormonal disruption from plastic ingestion

F9	1		100% PET	/	HIS	PET
F10	5		60% PP 20% HDPE 20% LDPE	A	HIS	PP
				B	HIS	PP
				C	HIS	HDPE
				D	HIS	LDPE
				E	MIR	PP
F11	4		75% PP 25% LDPE	A	HIS	PP
				B	HIS	PP
				C	HIS	LDPE
				D	HIS	PP
F12	3		66.6% HDPE 33.3% LDPE	A	HIS	LDPE
				B	HIS	HDPE
				C	HIS	HDPE
F13	1		100% LDPE	NA	MIR	LDPE
F14	1		100% PP	NA	HIS	PP
F15	4		75% LDPE 25% HDPE	A	HIS	HDPE
				B	HIS	LDPE
				C	HIS	LDPE

Chapter 5 – Hormonal disruption from plastic ingestion

				D	HIS	LDPE
F16	4		75% LDPE 25% PP	A B C D	HIS NIRM HIS HIS	LDPE PP LDPE LDPE
F17 (G)	2		100 % PP	A B	NIRM HIS	PP PP
F17 (P)	2		100% PP	A B	HIS HIS	PP PP
F18	3		33% PP 33% HDPE 33% NI	A B C	HIS NIRM HIS	PP Unidentifi ed HDPE
F19	5		80% LDPE 20% PP	A B C D E	HIS HIS HIS HIS HIS	LDPE PP LDPE LDPE LDPE

Chapter 5 – Hormonal disruption from plastic ingestion

F20	2		50% PP 50% LDPE	A	HIS	LDPE
				B	HIS	PP
F21	1		100% HDPE	NA	HIS	HDPE
F22	4		50% HDPE 25% LDPE 25% PP	A	HIS	HDPE
				B	HIS	PP
				C	HIS	LDPE
				D	HIS	HDPE
F23	3		66.6% PP 33.3% LDPE	A	HIS	PP
				B	HIS	PP
				C	HIS	LDPE
F24	21		52.4% LDPE 23.8% PP 19% HDPE	A	MIR	PP
				B	NIRM	PP
				C	HIS	LDPE
				D	NIRM	PP
				E	MIR	PE
				F	HIS	LDPE
				G	HIS	HDPE
				H	HIS	LDPE
				I	HIS	LDPE
				K	HIS	HDPE
				L	HIS	HDPE
				M	HIS	LDPE

Chapter 5 – Hormonal disruption from plastic ingestion



N	HIS	LDPE
O	HIS	PP
P	HIS	LDPE
Q	HIS	HDPE
R	HIS	LDPE
S	HIS	LDPE
T	HIS	LDPE
U	HIS	PP
V	HIS	LDPE

F25	1		100% LDPE	NA	HIS	LDPE
F26	1		100% HDPE	NA	HIS	HDPE
F27	10		40% HDPE 30% LDPE 30% PP	A	HIS	LDPE
				B	HIS	LDPE
				C	HIS	HDPE
				D	HIS	PP
				E	HIS	LDPE
				F	HIS	HDPE
				G	HIS	HDPE
				H	HIS	PP
				I	HIS	PP
				J	HIS	HDPE

Chapter 5 – Hormonal disruption from plastic ingestion

Supplementary Method 5.1 – Assessing estrogenic activity from extracts of artificial gastric fluid leachates

To measure the estrogenic activity of northern fulmar estrogen receptor in the presence of chemicals released from ingested plastic, we initially aimed to leach plastics with our Procellariiform gastric model (PGM) that we previously developed (Van Hassel et al. 2024). To do so, plastics would be leached into a mixture of salmon oil and acidic pepsin-albumin solution (pH 3) and plastic-associated chemicals would be extracted from the leachates to assess their estrogenic activity on fulmar ER transfected HEK293 cells. Four extraction methods were tested (QuEChERS Sample Preparation Kits, liquid chromatography, solid phase extraction (SPE) and lipid hydrolysis with acids) to assess the viability of HEK293 cells in the presence of non-contaminated extracts. Viability was assessed via visual inspection.

QuEChERS extraction

QuEChERS Sample Preparation Kits have been used in several studies to extract plastic-associated chemicals (e.g. POPs, phthalates, bisphenols, etc.) from large volumes of aqueous solutions and oils (Cunha *et al.*, 2007; Cunha and Fernandes, 2013; Fasano *et al.*, 2015; Dualde *et al.*, 2019; Verma *et al.*, 2021; Sun *et al.*, 2022). QuEChERS procedure requires a number of simple analytical steps and is thus considered fast and easy to perform with a low likelihood of errors, in addition to being inexpensive. QuEChERS is expected to provide high recoveries and clean products for liquid- and gas-chromatography analysis.

Briefly, 10 mL of acetonitrile was added to each phase of the gastric fluid and the mixture was shaken for 1 min with a vortex and introduced in an

Chapter 5 – Hormonal disruption from plastic ingestion

ice bath. A pouch of QuEChERS salt packet containing 4 g anhydrous MgSO_4 , 0.5 g disodium citrate, 1 g trisodium citrate, 1 g NaCl was added. The content was shaken for 1 min and the tube was introduced in the ice bath again. Then, the mixture was centrifuged at 3000 rpm and 20 °C during 10 min. The total acetonitrile layer was taken and transferred into a 15 mL QuEChERS dispersive-SPE (d-SPE) AOAC kit (900 mg MgSO_4 , 150 mg PSA, 150 mg C18) polypropylene tube. The mixture was shaken for 1 min and centrifuged for 15 min at 3000 rpm at 15 °C. The supernatant was then filtered (0.2 μm), transferred into a 15 mL glass tube, evaporated until almost complete dryness and resuspended into 50 μL methanol. Methanol controls were run in parallel. QuEChERS Sample Preparation Kits did not provide a 100% clean product (oil residue and white precipitate), leading to the death of cells exposed to the extract.

Liquid chromatography

Both oily and aqueous phases were separately tested by loading 20 ml of each on a glass column packed with C18. The oily phase was conditioned and eluted with hexane and the aqueous phase was conditioned and eluted with methanol. Both extracts obtained were very cloudy and had a high amount of suspended particles. For this reason, this extraction method was not considered further.

Solid phase extraction (SPE)

Aqueous phase - Coffin et al. (2019), extracted estrogenic chemicals from an acidic pepsin solution by using C18 SPE cartridges (Coffin *et al.*, 2019). Sigma-Aldrich customer service recommended the use of a polymeric bounded material, which provides more complete coverage of the silica

Chapter 5 – Hormonal disruption from plastic ingestion

surface, and a higher carbon loading resulting in a better affinity for hydrophobic compounds containing hydrophobic functionality (e.g. phenols). Accordingly, purification of the aqueous phase was tested on HyperSep Retain PEP Cartridges. Cartridges were first conditioned with 10 ml of methanol and 10 ml of milliQ-water. The sample was loaded and washed with 10 ml of water before eluted with 10 ml of methanol. Throughout the SPE process, the flow rate was ≤ 1 ml/min. The eluate obtained was cloudy, and a white precipitate was present at the bottom the collection tube after complete evaporation.

Because the precipitate was likely due to the presence of residual proteins and salts, a protein precipitation was performed on the aqueous solution before loading onto the cartridge by adding acetonitrile to the aqueous phase at a ratio of 3:1 ('Instructions Protein Precipitation Plates', no date). The mixture was vortexed and centrifuged at 3000 rpm for 10 minutes and the supernatant was loaded on a HyperSep Retain PEP Cartridges. This method provided a cleaner eluate. The extract was then evaporated and resuspended in 50 μ l DMSO prior incubation with HEK293 cells at 0.1%. With this method, cells did not die.

Lipid phase - Sigma-Aldrich customer service recommended to perform a liquid-liquid extraction with acetonitrile prior to load the extract on a HyperSep Retain PEP Cartridges. Accordingly, acetonitrile was added to the oily phase at a ratio of 1:1. The solution was vortexed, centrifuged and the supernatant was transferred on a HyperSep Retain PEP Cartridges. The eluate obtained after SPE still contained a high amount of lipids. Accordingly, an additional step called "freezing-lipid precipitation" was added after the liquid-liquid extraction, where the acetonitrile supernatant was frozen at -20°C overnight to induce the precipitation of lipids in the bottom of the tube(Y. E.

Chapter 5 – Hormonal disruption from plastic ingestion

Liu *et al.*, 2018). Although this step significantly reduced lipid concentrations, the extract obtained after SPE extraction still presented visible oil residues. A final cleaning step was added to eliminate lipid residues with 200 mg of Z-Sep/C18 (1:1) according to Liu et al. (2018). Unfortunately, this 4-step method (liquid-liquid extraction, freezing-lipid precipitation, SPE and clean-up with Z-Sep/C18 dispersant) did not provide an extract clean enough, and HEK293 cells died after being incubated with the eluate (suspended in 0.1% DMSO) after 24 hours.

Lipid hydrolysis with acids

Another method tested to extract chemicals from the oily phase was to hydrolyze lipids with acids, and recover the aqueous supernatant. Such a method would prevent the loss of hydrophobic pollutants during the liquid-liquid extraction and subsequent clean-up. Tests were performed with two different acids of different strength: formic acid (HF, pH = 3.25) and sulfuric acid (H₂SO₄; pH = 1). According to protocols described by our collaborators from the Center for Analytical Research and Technology (ULiège, Belgium), acids were added at a ratio of 1:1 to the oil. HF was not strong enough to solubilize and hydrolyze the oil but H₂SO₄ was. However, its addition to the oil created a very thick dark precipitate that solidified once the reaction cooled down, making it impossible to filter or centrifuge to eliminate the hydrolysis byproducts.

Acknowledgments

The authors would like to thank Oikonos Ecosystem Knowledge for providing the plastic samples recovered from northern fulmar stomachs. We thank the NOAA North Pacific Observer Program for collecting northern fulmars, and the CDFW-OSPR Marine Wildlife Veterinary Care and Research Center for volunteer and facility support. We would also like to thank Nicaise Kayoka, Quentin Arnould and Delphine Delhotte from the Walloon Agricultural Research Centre (CRA-W) (Gembloux, Belgium) for the HIS, NIRM and MIR analysis and data treatment. Thank you to Qiangli (Li) Zhang from the Mass Spectrometry Facility (University of California, Santa Cruz) for her support in running mass spectrometry analysis on plastic leachates. This work was funded by the F.R.I.A. fellowship and research credits awarded by the FNRS (Fonds de la Recherche Scientifique, Belgium).

Chapter 5 – Hormonal disruption from plastic ingestion

References

- Adegoke, E. O., Rahman, M. S. and Pang, M. G. (2020) 'Bisphenols Threaten Male Reproductive Health via Testicular Cells', *Frontiers in Endocrinology*, 11(September), pp. 1–18. doi: 10.3389/fendo.2020.00624.
- Allen, S. F. *et al.* (2021) 'Phthalate diversity in eggs and associations with oxidative stress in the European herring gull (*Larus argentatus*)', *Marine Pollution Bulletin*, 169(May), p. 112564. doi: 10.1016/j.marpolbul.2021.112564.
- Aminot, Y. *et al.* (2020) 'Leaching of flame-retardants from polystyrene debris: Bioaccumulation and potential effects on coral', *Marine Pollution Bulletin*, 151(December 2019), pp. 1–7. doi: 10.1016/j.marpolbul.2019.110862.
- Antunes, J. C. *et al.* (2013) 'Resin pellets from beaches of the Portuguese coast and adsorbed persistent organic pollutants', *Estuarine, Coastal and Shelf Science*, 130, pp. 62–69. doi: 10.1016/j.ecss.2013.06.016.
- Arao, Y. and Korach, K. S. (2018) 'The F domain of estrogen receptor is involved in species-specific, tamoxifen-mediated transactivation', *Journal of Biological Chemistry*, 293(22), pp. 8495–8507. doi: 10.1074/jbc.RA117.001212.
- Arao, Y. and Korach, K. S. (2021) 'The physiological role of estrogen receptor functional domains', *Essays in Biochemistry*, 65(6), pp. 867–875. doi: 10.1042/EBC20200167.
- Asnake, S., Modig, C. and Olsson, P. E. (2019) 'Species differences in ligand interaction and activation of estrogen receptors in fish and human', *Journal of*

Chapter 5 – Hormonal disruption from plastic ingestion

Steroid Biochemistry and Molecular Biology, 195(August). doi: 10.1016/j.jsbmb.2019.105450.

Bodziach, K. *et al.* (2021) ‘Gastrointestinal and respiratory exposure of water birds to endocrine disrupting phenolic compounds’, *Science of the Total Environment*, 754. doi: 10.1016/j.scitotenv.2020.142435.

Bonefeld-Jørgensen, E. C. *et al.* (2014) ‘Biomonitoring and hormone-disrupting effect biomarkers of persistent organic pollutants in vitro and ex vivo’, *Basic and Clinical Pharmacology and Toxicology*, 115(1), pp. 118–128. doi: 10.1111/bcpt.12263.

Bonon, A. J. *et al.* (2016) ‘Physicochemical characterization of three fiber-reinforced epoxide-based composites for dental applications’, *Materials Science & Engineering C*, 69, pp. 905–913. doi: 10.1016/j.msec.2016.07.002.

Bouhroum, R. *et al.* (2019) ‘Concentrations and fingerprints of PAHs and PCBs adsorbed onto marine plastic debris from the Indonesian Cilacap coast and the North Atlantic gyre’, *Regional Studies in Marine Science*, 29, p. 100611. doi: 10.1016/j.rsma.2019.100611.

Chao, K. P., Huang, C. S. and Wei, C. Y. (2013) ‘Extraction and percolation of PAEs from chemical protective gloves’, *Polymer Testing*, 32(8), pp. 1551–1557. doi: 10.1016/j.polymertesting.2013.10.004.

Coffin, S. *et al.* (2019) ‘Fish and Seabird Gut Conditions Enhance Desorption of Estrogenic Chemicals from Commonly-Ingested Plastic Items’, *Environmental Science and Technology*, 53(8), pp. 4588–4599. doi: 10.1021/acs.est.8b07140.

Court, L. *et al.* (2020) ‘Key role of estrogen receptor β in the organization of

Chapter 5 – Hormonal disruption from plastic ingestion

brain and behavior of the Japanese quail’, *Hormones and Behavior*, 125(August), p. 104827. doi: 10.1016/j.yhbeh.2020.104827.

Cunha, S. C. *et al.* (2007) ‘Evaluation of the QuEChERS sample preparation approach for the analysis of pesticide residues in olives’, *Journal of Separation Science*, 30(4), pp. 620–632. doi: 10.1002/jssc.200600410.

Cunha, S. C. and Fernandes, J. O. (2013) ‘Assessment of bisphenol A and bisphenol B in canned vegetables and fruits by gas chromatography-mass spectrometry after QuEChERS and dispersive liquid-liquid microextraction’, *Food Control*, 33(2), pp. 549–555. doi: 10.1016/j.foodcont.2013.03.028.

DEFRA (2006) *Risk reduction strategy and analysis of advantages and drawbacks for 4-tert-octylphenol*, Department for environment, food and rural affairs.

Dhavamani, J. *et al.* (2022) ‘Phthalate esters and plastic debris abundance in the Red Sea and Sharm Obhur and their ecological risk level’, *Environmental Pollution*, 315(May), p. 120447. doi: 10.1016/j.envpol.2022.120447.

Dias, M. P. *et al.* (2019) ‘Threats to seabirds: A global assessment’, *Biological Conservation*, 237(April), pp. 525–537. doi: 10.1016/j.biocon.2019.06.033.

Dualde, P. *et al.* (2019) ‘Determination of four parabens and bisphenols A, F and S in human breast milk using QuEChERS and liquid chromatography coupled to mass spectrometry’, *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, 1114–1115(March), pp. 154–166. doi: 10.1016/j.jchromb.2019.03.004.

Ellington, J. J. (1999) ‘Octanol/water partition coefficients and water solubilities of phthalate esters’, *Journal of Chemical and Engineering Data*,

Chapter 5 – Hormonal disruption from plastic ingestion

44(6), pp. 1414–1418. doi: 10.1021/je990149u.

Endo, S. *et al.* (2005) ‘Concentration of polychlorinated biphenyls (PCBs) in beached resin pellets: Variability among individual particles and regional differences’, *Marine Pollution Bulletin*, 50(10), pp. 1103–1114. doi: 10.1016/j.marpolbul.2005.04.030.

European Chemicals Agency (ECHA) (2014) *Substance Name : Benzyl butyl phthalate (BBP) EC Number : 201-622-7 CAS Number : 85-68-7 SUPPORT DOCUMENT TO THE OPINION OF THE MEMBER STATE COMMITTEE FOR IDENTIFICATION OF BENZYL BUTYL PHTHALATE (BBP) AS A SUBSTANCE OF VERY HIGH CONCERN BECAUSE OF IT.*

Fasano, E. *et al.* (2015) ‘Migration of monomers and plasticizers from packed foods and heated microwave foods using QuEChERS sample preparation and gas chromatography/mass spectrometry’, *Lwt*, 64(2), pp. 1015–1021. doi: 10.1016/j.lwt.2015.06.066.

Felton, R. G. *et al.* (2020) ‘Identification of California condor (*Gymnogyps californianus*) estrogen receptor variants and their activation by xenoestrogens’, *General and Comparative Endocrinology*, 289, p. 113392. doi: 10.1016/j.ygcen.2020.113392.

van Franeker, J. A. (2004) ‘Save the North Sea e Fulmar Study Manual 1- Collection and Dissection Procedures.’, *Wageningen Alterra*, (January 2004).

Fry, M. D., Fefer, S. I. and Sileo, L. (1987) ‘Ingestion of Plastic Debris by Laysan Albatrosses and Wedge-tailed Shearwaters in the Hawaiian Islands’, *Marine Pollution Bulletin*, 18(61), pp. 339–343.

Gripon, L. *et al.* (2021) ‘Brominated flame retardants extraction from waste

Chapter 5 – Hormonal disruption from plastic ingestion

electrical and electronic equipment-derived ABS using supercritical carbon dioxide’, *Waste Management*, 131(July), pp. 313–322. doi: 10.1016/j.wasman.2021.06.020.

Hahladakis, J. N. *et al.* (2018) ‘An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling’, *Journal of Hazardous Materials*, 344, pp. 179–199. doi: 10.1016/j.jhazmat.2017.10.014.

Van Hassel, L. *et al.* (2024) ‘Dynamics of leaching of POPs and additives from plastic in a Procellariiform gastric model : Diet- and polymer-dependent effects and implications for long-term exposure’, *PLoS ONE*, 19(3), pp. 1–19. doi: 10.1371/journal.pone.0299860.

Hauser, R. and Calafat, A. M. (2005) ‘Phthalates and human health’, *Occupational and Environmental Medicine*, 62(11), pp. 806–818. doi: 10.1136/oem.2004.017590.

‘Instructions Protein Precipitation Plates’ (no date) *Thermo Scientific*, 0747(90036).

IUCN Red List of Threatened Species (2023) *International Union for Conservation of Nature*. Available at: <https://www.iucnredlist.org/>.

Katsu, Y. *et al.* (2013) ‘Cloning, expression and functional characterization of carp, *Cyprinus carpio*, estrogen receptors and their differential activations by estrogens’, *Journal of Applied Toxicology*, 33(1), pp. 41–49. doi: 10.1002/jat.1707.

Koelmans, A. A. *et al.* (2016) ‘Microplastic as a Vector for Chemicals in the Aquatic Environment: Critical Review and Model-Supported Reinterpretation

Chapter 5 – Hormonal disruption from plastic ingestion

of Empirical Studies’, *Environmental Science and Technology*, 50(7), pp. 3315–3326. doi: 10.1021/acs.est.5b06069.

Krivoshiev, B. V. *et al.* (2016) ‘Assessing in-vitro estrogenic effects of currently-used flame retardants’, *Toxicology in Vitro*, 33, pp. 153–162. doi: 10.1016/j.tiv.2016.03.006.

Kühn, S., Booth, Andy M., *et al.* (2020) ‘Transfer of Additive Chemicals From Marine Plastic Debris to the Stomach Oil of Northern Fulmars’, *Frontiers in Environmental Science*, 8. doi: 10.3389/fenvs.2020.00138.

Kühn, S., Booth, Andy M., *et al.* (2020) ‘Transfer of Additive Chemicals From Marine Plastic Debris to the Stomach Oil of Northern Fulmars’, *Frontiers in Environmental Science*, 8(August), pp. 1–14. doi: 10.3389/fenvs.2020.00138.

Kühn, S. and van Franeker, J. A. (2020) ‘Quantitative overview of marine debris ingested by marine megafauna’, *Marine Pollution Bulletin*, 151(110858). doi: 10.1016/j.marpolbul.2019.110858.

Li, X. *et al.* (2021) ‘Influence of octylphenol on gene expression of gonadotropins and their receptors, testicular structure and mating behavior of male *Rana chensinensis*’, *Environmental Toxicology and Pharmacology*, 87(October 2020), p. 103694. doi: 10.1016/j.etap.2021.103694.

Li, Y. *et al.* (2022) ‘Non-targeted analysis for organic components of microplastic leachates’, *Science of the Total Environment*, 816, p. 151598. doi: 10.1016/j.scitotenv.2021.151598.

Liu, Y. E. *et al.* (2018) ‘Determination of organophosphorus flame retardants in fish by freezing-lipid precipitation, solid-phase extraction and gas chromatography-mass spectrometry’, *Journal of Chromatography A*, 1532,

Chapter 5 – Hormonal disruption from plastic ingestion

pp. 68–73. doi: 10.1016/j.chroma.2017.12.001.

Liu, Y. Y. *et al.* (2018) ‘Identification, contribution, and estrogenic activity of potential EDCs in a river receiving concentrated livestock effluent in Southern Taiwan’, *Science of the Total Environment*, 636, pp. 464–476. doi: 10.1016/j.scitotenv.2018.04.031.

Maddela, N. R. *et al.* (2023) ‘Additives of plastics: Entry into the environment and potential risks to human and ecological health’, *Journal of Environmental Management*, 348(October), p. 119364. doi: 10.1016/j.jenvman.2023.119364.

Marlatt, V. L. *et al.* (2022) ‘Impacts of endocrine disrupting chemicals on reproduction in wildlife and humans’, *Environmental Research*, 208. doi: 10.1016/j.envres.2021.112584.

Matthews, J. *et al.* (2000) ‘Differential estrogen receptor binding of estrogenic substances: a species comparison’, *Journal of Steroid Biochemistry & Molecular Biology*, 74, pp. 223–234.

Mattsson, A., Olsson, J. A. and Brunström, B. (2011) ‘Activation of estrogen receptor alpha disrupts differentiation of the reproductive organs in chicken embryos’, *General and Comparative Endocrinology*, 172(2), pp. 251–259. doi: 10.1016/j.ygcen.2011.03.010.

Michishita, S. *et al.* (2023) ‘Microplastic in northern anchovies (*Engraulis mordax*) and common murrelets (*Uria lomvia*) from the Monterey Bay, California USA - Insights into prevalence, composition, and estrogenic activity’, *Environmental Pollution*, 316(P2), p. 120548. doi: 10.1016/j.envpol.2022.120548.

Molina-Molina, J. M. *et al.* (2013) ‘In vitro study on the agonistic and

Chapter 5 – Hormonal disruption from plastic ingestion

antagonistic activities of bisphenol-S and other bisphenol-A congeners and derivatives via nuclear receptors', *Toxicology and Applied Pharmacology*, 272(1), pp. 127–136. doi: 10.1016/j.taap.2013.05.015.

Monclús, L. *et al.* (2022) 'Microplastic Ingestion Induces Size-Specific Effects in Japanese Quail', *Environmental Science and Technology*, 56(22), pp. 15902–15911. doi: 10.1021/acs.est.2c03878.

Nania, T. G. and Shugart, G. W. (2021) 'Are plastic particles reduced in size in seabirds ' stomachs ?', *Marine Pollution Bulletin*, 172(June), p. 112843. doi: 10.1016/j.marpolbul.2021.112843.

Nissy, S. M., Myneni, V. R. and Vangalapati, M. (2022) 'Wrenching of Bisphenol A from plastics: Response surface methodology', *Materials Today: Proceedings*, 62, pp. 3869–3877. doi: 10.1016/j.matpr.2022.04.539.

Ong, H. T., Samsudin, H. and Soto-Valdez, H. (2022) 'Migration of endocrine-disrupting chemicals into food from plastic packaging materials: an overview of chemical risk assessment, techniques to monitor migration, and international regulations', *Critical Reviews in Food Science and Nutrition*, 62(4), pp. 957–979. doi: 10.1080/10408398.2020.1830747.

Paris, F. *et al.* (2002) 'Phenylphenols, biphenols, bisphenol-A and 4-tert-octylphenol exhibit α and β estrogen activities and antiandrogen activity in reporter cell lines', *Molecular and Cellular Endocrinology*, 193(1–2), pp. 43–49. doi: 10.1016/S0303-7207(02)00094-1.

Park, C. *et al.* (2019) 'The effects of bisphenol A, benzyl butyl phthalate, and di(2-ethylhexyl) phthalate on estrogen receptor alpha in estrogen receptor-positive cells under hypoxia', *Environmental Pollution*, 248, pp. 774–781.

Chapter 5 – Hormonal disruption from plastic ingestion

doi: 10.1016/j.envpol.2019.02.069.

Pierce, K. E. *et al.* (2004) ‘Obstruction and starvation associated with plastic ingestion in a Northern Gannet *Morus bassanus* and a Greater Shearwater *Puffinus gravis*’, *Marine Ornithology*, 32(2), pp. 187–189.

Pilapitiya, P. G. C. N. T. and Ratnayake, A. S. (2024) ‘The world of plastic waste: A review’, *Cleaner Materials*, 11(August 2023), p. 100220. doi: 10.1016/j.clema.2024.100220.

Plíšková, M. *et al.* (2005) ‘Impact of polychlorinated biphenyls contamination on estrogenic activity in human male serum’, *Environmental Health Perspectives*, 113(10), pp. 1277–1284. doi: 10.1289/ehp.7745.

Rider, C. V. *et al.* (2010) ‘Differences in sensitivity but not selectivity of xenoestrogen binding to alligator versus human estrogen receptor alpha’, *Environmental Toxicology and Chemistry*, 29(9), pp. 2064–2071. doi: 10.1002/etc.233.

Rochester, J. R. (2013) ‘Bisphenol A and human health: A review of the literature’, *Reproductive Toxicology*, 42, pp. 132–155. doi: 10.1016/j.reprotox.2013.08.008.

Rogers, J. and Denison, M. (2000) ‘Recombinant cell bioassays for endocrine disruptors: Development of a stably transfected human ovarian cell line for the detection of estrogenic and anti-estrogenic chemicals’, *In vitro & molecular toxicology*, 13, pp. 67–82.

Roman, L. *et al.* (2019) ‘Is plastic ingestion in birds as toxic as we think? Insights from a plastic feeding experiment’, *Science of the Total Environment*, 665, pp. 660–667. doi: 10.1016/j.scitotenv.2019.02.184.

Chapter 5 – Hormonal disruption from plastic ingestion

Ryan, P. G. (2015) 'How quickly do albatrosses and petrels digest plastic particles?', *Environmental Pollution*, 207, pp. 438–440. doi: 10.1016/j.envpol.2015.08.005.

Ryan, P. G. (2019) 'Ingestion of Plastics by Marine Organisms', *Handbook of Environmental Chemistry*, 78, pp. 235–266. doi: 10.1007/698_2016_21.

Santoro, A. *et al.* (2019) 'Neuro-toxic and Reproductive Effects of BPA', *Current Neuropharmacology*, 17(12), pp. 1109–1132. doi: 10.2174/1570159x17666190726112101.

Staniszewska, M., Graca, B. and Nehring, I. (2016) 'The fate of bisphenol A, 4-tert-octylphenol and 4-nonylphenol leached from plastic debris into marine water - experimental studies on biodegradation and sorption on suspended particulate matter and nano-TiO₂', *Chemosphere*, 145, pp. 535–542. doi: 10.1016/j.chemosphere.2015.11.081.

Sun, L. *et al.* (2022) 'Rapid and simultaneous extraction of phthalates, polychlorinated biphenyls and polycyclic aromatic hydrocarbons from edible oil for GC–MS determination', *Journal of Food Composition and Analysis*, 114(August), p. 104827. doi: 10.1016/j.jfca.2022.104827.

Syberg, K. *et al.* (2020) 'Case Studies in Chemical and Sorption of PCBs to environmental plastic pollution in the North Atlantic Ocean : Importance of size and polymer type', *Case Studies in Chemical and Environmental Engineering*, 2(November), p. 100062. doi: 10.1016/j.cscee.2020.100062.

Tanaka, K. *et al.* (2015) 'Facilitated Leaching of Additive-Derived PBDEs from Plastic by Seabirds' Stomach Oil and Accumulation in Tissues', *Environmental Science and Technology*, 49(19), pp. 11799–11807. doi:

Chapter 5 – Hormonal disruption from plastic ingestion

10.1021/acs.est.5b01376.

Tanaka, K. *et al.* (2019) ‘Piece-by-piece analysis of additives and manufacturing byproducts in plastics ingested by seabirds: Implication for risk of exposure to seabirds’, *Marine Pollution Bulletin*, 145(May), pp. 36–41. doi: 10.1016/j.marpolbul.2019.05.028.

Tanaka, K. *et al.* (2020) ‘In Vivo Accumulation of Plastic-Derived Chemicals into Seabird Tissues’, *Current Biology*, 30(4), pp. 723-728.e3. doi: 10.1016/j.cub.2019.12.037.

Thoene, M., Dzika, E. and Gonkowski, S. (2020) ‘Thoene M., Dzika E., Gonkowski S., Wojtkiewicz J., Bisphenol S in food causes hormonal and obesogenic effects comparable to or worse than bisphenol A: A literature review, *Nutrients*, 2020, 12 (2): 532’, *Nutrients*, 12(2), p. 532.

Tubbs, C. *et al.* (2012) ‘Activation of southern white rhinoceros (*Ceratotherium simum simum*) estrogen receptors by phytoestrogens: Potential role in the reproductive failure of captive-born females?’, *Endocrinology*, 153(3), pp. 1444–1452. doi: 10.1210/en.2011-1962.

Tubbs, C. W. *et al.* (2016) ‘Estrogenicity of captive southern white rhinoceros diets and their association with fertility’, *General and Comparative Endocrinology*, 238, pp. 32–38. doi: 10.1016/j.ygcen.2016.05.004.

Van der Ven, L. T. M. *et al.* (2008) ‘Endocrine effects of tetrabromobisphenol-A (TBBPA) in Wistar rats as tested in a one-generation reproduction study and a subacute toxicity study’, *Toxicology*, 245(1–2), pp. 76–89. doi: 10.1016/j.tox.2007.12.009.

Verma, J. *et al.* (2021) ‘QuEChERS based analysis of multiple pesticides and

Chapter 5 – Hormonal disruption from plastic ingestion

phthalates in packaged food products’, *Microchemical Journal*, 171(October), p. 106882. doi: 10.1016/j.microc.2021.106882.

Wang, W. and Kannan, K. (2023) ‘Leaching of Phthalates from Medical Supplies and Their Implications for Exposure’, *Environmental Science & Technology*. doi: 10.1021/acs.est.2c09182.

Zhang, Q. *et al.* (2014) ‘Characterization of estrogen receptor α activities in polychlorinated biphenyls by in vitro dual-luciferase reporter gene assay’, *Environmental Pollution*, 189, pp. 169–175. doi: 10.1016/j.envpol.2014.03.001.

Zimmermann, L. *et al.* (2019) ‘Benchmarking the in Vitro Toxicity and Chemical Composition of Plastic Consumer Products’, *Environmental Science & Technology*, 53, p. 11467–11477. doi: 10.1021/acs.est.9b02293.

Chapter 6

General discussion and perspectives

Table of Contents

6.1.	General discussion	247
6.1.1.	Leaching dynamic of plastic-associated chemicals in a Procellariiform stomach.....	247
6.1.2.1.	Factors influencing the leaching of plastic-associated chemicals.....	248
6.1.2.1.	Limitations of the Procellariiform gastric model.....	253
6.1.2.	Health risks associated with exposure to plastic associated-chemicals	255
6.1.3.	Conservation implications of plastic ingestion	260
6.1.4.	Plastic pollution: current situation and future directions	261
6.2.	Conclusion	263
6.3.	Perspectives	266
	References.....	270

6.1. General discussion

The aim of this thesis was to investigate the toxicological impact of plastic ingestion in Procellariiformes. Specifically, we focused on (i) understanding the leaching dynamics of plastic-associated chemicals in a Procellariiform stomach and (ii) investigating the potential hormonal disruption caused by the leaching of chemicals from ingested plastic. The major outcomes of this research, along with broader implications and future directions are discussed below.

6.1.1. Leaching dynamic of plastic-associated chemicals in a Procellariiform stomach

A seabird stomach is divided into two chambers: the proventriculus, where the chemical digestion occurs through enzyme and acid secretion, and the gizzard, where mechanical digestion via food grinding takes place in addition to chemical digestion. Unlike most birds, which secrete gastric lipase during digestion, Procellariiformes do not (Tanaka, Yamashita and Takada, 2018). The absence of gastric lipase combined with low muscular contractions results in the formation of two distinct phases in the proventriculus: a lower aqueous phase created from the breakdown of proteins and carbohydrates in gastric secretions, and an upper lipid phase known as “stomach oil.” In the gizzard, these phases are mixed before being transferred to the intestine (Tanaka, Yamashita and Takada, 2018).

Plastics ingested by Procellariiformes become mainly trapped in the gizzard, where they are exposed to both aqueous and lipid phases and subjected to mechanical grinding, potentially fragmenting them into smaller

Chapter 6 – General discussion and perspectives

pieces (Tanaka, Yamashita and Takada, 2018). In this first section, we discuss the factors that influence the leaching of chemicals from plastics in a Procellariiform stomach and the limitation of the Procellariiform gastric model developed.

6.1.2.1. Factors influencing the leaching of plastic-associated chemicals

OUTCOME 1 - The leaching of chemicals is influenced by the lipid and aqueous phase in a Procellariiform stomach

Marine plastic contains a broad range of chemicals ranging from plastic additives to monomers and sorbed environmental pollutants (e.g. POPs and heavy metals) characterized by different degrees of hydrophobicity ranging from low ($\log K_{ow} < 3$) [e.g. bisphenol S (BPS), dimethyl phthalate (DMP), tris(2-chloroethyl) phosphate (TCEP)] to moderate ($3 < \log K_{ow} < 5$) (e.g. bisphenol A (BPA), butylated hydroxyanisole (BHA), triphenyl phosphate (TPP)] and high ($\log K_{ow} > 5$) (e.g. di(2-ethylhexyl) phthalate (DEHP), 4-tert-octylphenol (4-OP), polybrominated diphenyl ethers (PBDEs)].

Our Procellariiform gastric model (PGM) showed that the leaching of highly hydrophobic chemicals (e.g. PBDEs and PCBs) is primarily induced by the presence of lipids (i.e. stomach oil) while the leaching of slightly hydrophobic chemicals (e.g. BPS) is induced by both lipid and aqueous components. This dual-phase leaching shows that a broad spectrum of chemicals can be released from ingested plastics.

Chapter 6 – General discussion and perspectives

OUTCOME 2 - Chemicals are leached faster in a wax ester-rich oil

Stomach oil, unique to Procellariiformes, is primarily composed of wax esters (WEs) and triacylglycerols (TAGs) (> 70%). The proportions of WEs and TAGs vary depending on the species studied, reflecting differences in diet (Connan, Cherel and Mayzaud, 2007). For example, the stomach oil of white-chinned petrel (*Procellaria aequinoctia*) and black-browed albatross (*Thalassarche melanophris*) is characterized by high proportions of TAGs (> 60%) compared to WEs (< 20%) while the stomach oil of short-tailed shearwater (*Puffinus tenuirostris*) and blue petrel (*Halobaena caerulea*) is predominantly composed of WEs (> 70%) due to the consumption of lantern fish and copepods (Clarke and Prince, 1976; Connan, Cherel and Mayzaud, 2007). In Chapters 3 and 4, we observed higher concentrations of chemicals in WE-rich copepod oil compared to TAG-rich salmon oil. These findings suggest that chemicals could leach faster in the stomach of individuals with a diet rich in WE. To help determine which individuals or species are more at risk, further studies should investigate whether a correlation exists between the lipid composition of stomach oil and the level of exposure to plastic-associated chemical (e.g., chemicals concentration in tissue and stomach oil).

OUTCOME 3 - The concentrations of leached chemicals depend on the polymer type

Polyethylene (PE) and polypropylene (PP) are the most common polymers found in the stomach of Procellariiformes (Tanaka *et al.*, 2019; Kühn *et al.*, 2021) (Chapter 5). This prevalence is due to the surface-feeding behavior of most Procellariiformes (Hamer, 2019), which leads them to ingest buoyant plastics like PE and PP that dominate the ocean's surface (Erni-

Chapter 6 – General discussion and perspectives

Cassola *et al.*, 2019). Other polymers such as PVC and PET are also found but at smaller proportions (Kühn *et al.*, 2021) (Chapter 5).

Rubbery polymers such as PE and PP possess a higher free volume and polymer chain mobility than glassy polymer (e.g., PVC, PET) which increases the ability for additives to migrate out of the polymer matrix (Rodrigues *et al.*, 2019) (observed with PBDE-209 in Chapter 3 and 4). The higher free volume also allows PE and PP to sorb higher concentrations of POPs in the ocean compared to glassy polymer (Rochman *et al.*, 2013), which could result in higher concentrations of POPs leached in the stomach compared to PVC and PET (Rodrigues *et al.*, 2019). In our gastric model, HDPE and PVC sorbed all the PCBs introduced into salt water. To highlight any difference in sorbing potential between the two polymers, it would have been necessary to carry out several incubations in salt water containing PCBs (Koelmans *et al.*, 2016). We also saw that sorbed PCBs leached faster from PVC because they are majorly present at the surface of the plastic (adsorption) (Chapter 3 and 4).

Although this suggests that PE and PP could leach higher concentrations of additives than PVC or PET from their matrix, the actual leaching concentrations will also depend on the initial additive levels within the plastic (Bridson *et al.*, 2023). Different additives are used at different concentrations depending on the application field. For example, common additives found in PE and PP include antioxidants, stabilizers, flame retardants, and antistatic compounds, typically present at concentrations of less than 3% of the plastic weight (Hahladakis *et al.*, 2018). In contrast, plasticizers (i.e., phthalates) are commonly used in PVC at 10% to 70% of the plastic weight. Thus, PE and PP added with antioxidants could potentially

Chapter 6 – General discussion and perspectives

leach smaller concentrations of additives than PVC added with phthalates (Bridson *et al.*, 2023).

The additive compatibility with the polymer may also result in different leaching dynamics. While additives are usually homogeneously mixed with the polymer during manufacture, they can subsequently migrate to the surface through a mechanism called “blooming” due to their poor compatibility with the polymer (Nouman *et al.*, 2017). Blooming has been reported to occur with antioxidants and flame retardants in PE and PP (Nouman *et al.*, 2017). In medical devices, antioxidants blooming at the surface of plastic has shown to increase their leaching in the surrounding media (Saunier *et al.*, 2012). In Chapter 3 and 4, higher concentrations of BPS were found at the surface of PVC compared to HDPE, resulting in higher concentrations leached from PVC, particularly during the first hours following ingestion. In contrast, a localized concentration of PBDE-209 was observed at the surface of HDPE but not PVC, contributing to higher concentrations leached from HDPE. Thus, the concentrations released from the plastic may also depend on the affinity and distribution of the additive with the polymer. The dynamics of release from the polymer may also depend on the size of the additive, with larger additives having greater difficulty in being released from glassy polymers such as PVC (Hahladakis *et al.*, 2018).

In Chapter 5, no pattern was observed between the type of polymer ingested by northern fulmar (PE, PP and PET) and the estrogenicity of the chemicals leached. The types of additives used, their differing initial concentrations within the polymer and the duration plastics have spent in the ocean (Koelmans *et al.*, 2016) likely contributed to different levels of exposure between individuals.

Chapter 6 – General discussion and perspectives

OUTCOME 4 - Potential prolonged leaching and exposure to plastic additives

Plastic additives and POPs that sorb at the surface of plastic in the ocean are the most commonly studied plastic-associated chemicals susceptible to harm marine animals (Pinto da Costa *et al.*, 2023). Two decades ago, sorbed POPs used to be the major concern associated with plastic ingestion due to their ability to concentrate 10-fold more at the surface of plastic than in seawater (Mato *et al.*, 2001). However, today, studies show that the level of exposure to POPs via plastic ingestion is negligible compared to the level of exposure via prey consumption (Koelmans *et al.*, 2016). Nevertheless, there is a growing concern regarding the risks associated with plastic additives due to their presence within the plastic matrix at concentrations sometimes up to 10^5 higher than sorbed POPs (Jang *et al.*, 2024).

In Chapter 4, we saw that only small proportions of additives were leached from plastic within 5 days in our PGM, suggesting that additives can continue to leach in the bird's stomach for prolonged periods (e.g., weeks to months). This observation was further supported in Chapter 5, where we showed that 37% of the northern fulmars sampled had plastics in their stomach that were still releasing xenoestrogens after up to 14 days in stomach fluid-mimicking solvents. This result confirms that ingested plastics can leach chemicals over extended periods. We suspect that these xenoestrogens were rather additives leached from plastic matrix than POPs desorbed from its surface, given that plastic had been sitting in the bird's stomach for unknown but potentially long periods prior to our study.

Our PGM suggests that individuals will be exposed to higher concentrations of additives in the first hours following ingestion, and then to

Chapter 6 – General discussion and perspectives

lower – but still significant – levels over longer periods of time. This burst of leaching can be explained by the contact of the gastric solution with additives present near the surface of plastic. The remaining additives take more time to migrate as they are located deeper within the matrix, not directly in contact with the gastric solution (Hahladakis *et al.*, 2018). The weathering of plastic in the stomach (e.g., cracking) may facilitate the penetration of the gastric solution within the polymer over time, which could potentially later increase the leaching rate of an additive (Luo *et al.*, 2022). Bridson *et al.* (2023) observed that the leaching of the antioxidant AO-1076 from LDPE exhibited a slower rate in the first 16 days of leaching, followed by an increase in the next 48 days due to the diffusion of water in the polymer matrix (Bridson *et al.*, 2023). In Chapter 5, two northern fulmars had plastics that leached highly hydrophobic chemicals (i.e. found in hexane) on day 1, followed by weaker hydrophobic chemicals (i.e. found methanol) on day 5 and 14. It demonstrates that the leaching profile may show multiple stages if the plastic contains additives with different chemical properties.

6.1.2.1. Limitations of the Procellariiform gastric model

The gastric model developed represents a significant advancement to understand the leaching behavior of chemicals from plastic ingested by Procellariiformes. One benefit of using an *in vitro* gastric model is the controlled environment, enabling a clear understanding of how different conditions affect chemical leaching from plastic. The model also benefits from its non-invasive nature, preventing the use of experimental seabirds which avoid stress on the individual and ethical concerns. Nonetheless, this method has limitations that must be considered.

Chapter 6 – General discussion and perspectives

The Procellariiform gastric model developed does not perfectly mimic the complex conditions of the bird stomach. The pepsin used in the model is derived from porcine source, which does not fully replicate the enzymatic activity of an avian pepsin (Kamegashira *et al.*, 1977; Chu and Nakagawa, 1982; Nurhayati *et al.*, 2019). Similarly, the use of a commercial marine oil and bovine serum albumin in the model does not accurately represent the digesta of prey found in a Procellariiform stomach (e.g., fish, squid, crustacean) (Wang *et al.*, 2007). The mechanical stress applied to the plastic in the model, simulated with a magnetic stirring bar might also underrepresent the level of strength and pressure exerted by the gizzard *in vivo*, resulting in a potential smaller leaching of plastic-associated chemicals.

The gizzard chamber was studied because it is where plastic primarily remains trapped in the stomach and cannot be regurgitated. However, plastics are also found in the proventriculus, where they encounter different conditions, including the absence of mechanical stress and the presence of whole prey being digested. Incorporating this chamber into the model could enhance our understanding of chemical leaching under these conditions. Additionally, complementing gastric digestion with a phase of intestinal digestion would provide a more comprehensive view. Plastics that pass through the pyloric junction encounter a distinct environment, with factors like bile salts and lipase breaking down lipids, altering the hydrophobicity of the digestive solution, and affecting the leaching of hydrophobic chemicals (Mohamed Nor *et al.*, 2023). The higher pH, presence of bile salts, and pancreatic juice (containing proteolytic and lipolytic enzymes) in the intestine are likely to influence the leaching behavior of chemicals differently than in the gastric environment.

Chapter 6 – General discussion and perspectives

To address these limitations, future research could incorporate a multi-stage approach. For example, conducting an initial digestion phase with common prey species followed by exposure to plastics could more closely simulate natural conditions. Subsequent exposure to conditions mimicking those in the intestine would provide a more comprehensive understanding of the overall impact of digestion on chemical leaching.

6.1.2. Health risks associated with exposure to plastic associated-chemicals

Once released in the stomach, plastic-associated chemicals are transferred into the intestines where they can be absorbed by the intestinal barrier and circulate in the organism (Maddela *et al.*, 2023). During fledging season, stomach oil can also be regurgitated from the proventriculus to the chicks as a source of energy, potentially transferring chemicals to the chick (Foster *et al.*, 2010). Once plastic-associated chemicals have reached the bloodstream, their fate depends on their chemical properties, such as their hydrophobicity and affinity for certain tissues.

Hydrophobic chemicals (e.g., PBDEs, PCBs) will tend to accumulate in lipid-rich tissues such as adipose tissues and preen glands (glands that produce oil to lubricate the feathers) (Tanaka *et al.*, 2020) which can slowly release the chemicals back into the bloodstream over time. Other chemicals such as bisphenols, phthalates and alkylphenols are easily metabolized and excreted in the urine (Rochester, 2013). Nevertheless, continuous exposure can lead to sustained levels in the bloodstream and tissues, potentially causing chronic effects (Rochester, 2013). Moreover, chemicals such as POPs and phthalates can be maternally transferred to the yolk during the egg incubation period,

Chapter 6 – General discussion and perspectives

which could be particularly detrimental to the embryo development (Allen *et al.*, 2021; van der Schyff *et al.*, 2021).

Most plastic-associated chemicals are known as endocrine disrupting chemicals (EDCs) (Hermabessiere *et al.*, 2017). Accordingly, accumulation and continuous exposure to plastic compounds (e.g., additives) could negatively impact seabird's health. In this section, we discuss the potential risk of hormonal disruption associated with exposure to plastic-associated chemicals in Procellariiformes.

OUTCOME 5 – Fulmar and human ERs exhibit similar response with different sensitivity to plastic-associated chemicals

One of the most studied forms of endocrine disruption involves the binding of chemicals to ERs (Shanle and Xu, 2011). To date, studies investigating the estrogenicity of chemicals leached from plastics in the context of seabird toxicity have not used seabird ERs (Coffin *et al.*, 2019; Michishita *et al.*, 2023). Yet, studies show that differences in the ER sequences between species can result in different sensitivity to xenoestrogens (Matthews *et al.*, 2000; Tubbs *et al.*, 2012). In Chapter 5, we observed species-specific sensitivity differences between human and fulmar ER due to differences in the transactivation domain of their ER sequences.

The response of Procellariiform estrogen receptors (ERs) to plastic-associated chemicals may also vary between more closely related species, potentially contributing to differences in vulnerability to these chemicals. For example, differences in ER sensitivity to phytoestrogens between two rhino species have been shown to be responsible for the impaired fertility observed in one of them (Tubbs *et al.*, 2012, 2016). Similarly, variability in ER response

Chapter 6 – General discussion and perspectives

between different species of Procellariiformes could have implications for real-world exposure scenarios. Some Procellariiform species may be more susceptible to the endocrine-disrupting effects of plastic-associated chemicals, potentially leading to greater impacts on their health. The expression level of ERs and the ERE sequences may also vary between species, which may result in different sensitivity to environmental chemicals (Bourdeau *et al.*, 2004). This sensitivity could be particularly critical for species already facing other environmental stresses, such as invasive species, climate change, and fisheries, increasing their vulnerability (Phillips, Fort and Dias, 2022).

OUTCOME 6 - Ingested plastic leach chemicals able to activate and inhibit fulmar estrogen receptors

In this thesis, we found that plastics ingested by more than half (55%) of the northern fulmars studied could leach chemicals able to activate and inhibit fulmar ER, sometimes over several days. ERs regulate the expression of a wide array of genes involved in various physiological processes such as development and function of reproductive organs, egg formation, reproductive cycle, cell proliferation, lipid metabolism, bone formation and inflammatory response (Kumar *et al.*, 2019; Alva-Gallegos, Carazo and Mladěnka, 2023). The ability of chemicals to both activate and inhibit ERs suggests that plastic-associated chemicals could exert complex and multifaceted toxicological effects.

Several studies suggest that bird exposure to xenoestrogen could alter reproductive and developmental processes. For example, early exposure to ER β agonists showed to alter male quail sexual behavior by demasculinizing their brain (Court *et al.*, 2020). Exposure to ER α agonists during developmental stage has also shown to cause ovotestis formation in male and

Chapter 6 – General discussion and perspectives

malformation of the Müllerian ducts (embryonic oviducts) in female (Mattsson, Olsson and Brunström, 2011). Embryonic exposure to xenoestrogens has also shown to result in loss of copulatory behavior in male quail later in life (Panzica *et al.*, 2005). These findings suggest that exposure to xenoestrogen leached from ingested plastics could potentially contribute to detrimental effects on an individual's reproductive system and mating behavior, particularly if it occurs during embryonic development (via maternal transfer to the egg yolk) and early-age exposure.

Plastic ingestion has been associated with negative health effects in quails such as increased reproductive abnormalities (e.g., cysts) (Roman, Lowenstine, *et al.*, 2019), growth alteration (Roman, Lowenstine, *et al.*, 2019; Monclús *et al.*, 2022), decreased estrogen levels in female (Monclús *et al.*, 2022), lipid metabolism disruption (e.g., alteration of fatty acid composition) and increased oxidative stress (de Souza *et al.*, 2022). Beyond ER binding, endocrine disruption involves many other processes which could also be responsible for the toxicity reported in the literature. For example, BPA estrogenic effects observed at low doses have been proposed to be mainly mediated by membrane receptors in a non-genomic way, in particular through the binding to G-protein coupled estrogen receptor 30 (GPR30) (Cimmino *et al.*, 2020). BPA can also exert antiandrogenic effects through AR binding, contributing to male reproductive toxicity via inhibition of Sertoli cell proliferation (Wang *et al.*, 2017). Phthalates have also shown to exert hormone disruption through activation of peroxisome proliferator-activated receptors (PPARs), which plays a major role in lipid metabolism and homeostasis (Meling *et al.*, 2022). The aryl-hydrocarbon receptor (AhR) has also shown to be a target of plastic additives such as phthalates, BPA, nonylphenol (NP) (Krüger, Long and Bonefeld-Jørgensen, 2008) and POPs such as benzo-a-

Chapter 6 – General discussion and perspectives

pyrene (BaP), dioxins and dioxin-like PCBs, which activation can lead to an increase in the production of reactive oxygen species (ROS), causing oxidative stress (Grishanova and Perepechaeva, 2022). Phthalates have also shown to reduce levels of estradiol in cells by inhibiting the aromatase enzyme converting androgen into estrogens (Lovekamp and Davis, 2001). Accordingly, it is important to highlight that our study shows only one potential way for ingested plastic to disrupt the hormonal system among many other potential forms of endocrine disruption.

It should be emphasized that other compounds can be released from plastic in addition to additives and sorbed POPs. As plastic experiences abiotic and physical stresses in the stomach, they may break down and release monomers, oligomers and micro- or nanoplastics which may also exert additional toxicity on the organism (Sorasan *et al.*, 2022; Shi *et al.*, 2023). For example, BPA and BPS can be leached from PC and polyphenylsulfone (PPSU) respectively as monomer degradation products (Li *et al.*, 2022). PET has also shown to release oligomers classified as toxic (Dhaka *et al.*, 2022). Moreover, exposure to micro- and nanoplastics released from larger plastic pieces in the stomach or originating from contaminated preys may also induce additional toxicity such as tissue and cell damage, oxidative stress and inflammation (Wang *et al.*, 2022; Yang *et al.*, 2022; Kadac-czapska and Knez, 2024).

Seabirds are also exposed to a variety of other chemicals, including persistent organic pollutants (POPs) (Campioni *et al.*, 2024), heavy metals (Ishii *et al.*, 2017) and oil pollutants [e.g., polycyclic aromatic hydrocarbons (PAHs)] (Troisi, Barton and Bexton, 2016), through ingestion and to a lesser extent inhalation. Chemicals leached from plastics add another layer to this

Chapter 6 – General discussion and perspectives

existing burden, potentially leading to additive or synergistic effects where the combined impact of multiple chemicals is greater than the sum of their individual effects (Lagunas-Rangel *et al.*, 2022). Competition between two or more chemicals with opposite effects may also lead to antagonism where their combined effect is less than what would be expected if each acted independently (Lagunas-Rangel *et al.*, 2022) .

6.1.3. Conservation implications of plastic ingestion

Procellariiformes are among the most threatened groups of seabirds, with 25% of species listed as endangered and 19.6% as vulnerable on the International Union for Conservation of Nature red list of threatened species (IUCN Red List of Threatened Species, 2023). Procellariiformes face a myriad of environmental stressors, including climate change, overfishing, habitat loss, and pollution (Dias *et al.*, 2019). Among these, plastic ingestion has emerged as a significant threat, especially in species with a high frequency of plastic ingestion (e.g., northern fulmar) (Wilcox *et al.*, 2015; Dias *et al.*, 2019). Yet, the overall impact of this threat on the populations remains unknown.

The direct physical impacts of plastic ingestion in Procellariiformes is well-documented [gastrointestinal blockages, reduced food intake, and injury to internal organs (Ryan, 2019)] with an estimated mortality risk of 20.5% following ingestion of a single debris item (Roman, Hardesty, *et al.*, 2019). Directly correlating the chemical impact of plastic ingestion with seabird conservation status is complex, as wild birds are also exposed to a multitude of other environmental pollutants, which can similarly cause endocrine disruption and complicate the assessment of plastic-related effects.

Chapter 6 – General discussion and perspectives

Nevertheless, the leaching of EDCs from ingested plastic, as observed in this thesis, and the documented endocrine disruption (e.g., estradiol level decrease, growth alteration) (Monclús *et al.*, 2022) in quail fed with plastic, suggest that plastic ingestion could have a non-negligible impact on individuals in the wild. These effects, though not immediately fatal, could reduce seabird ability to cope with other environmental stressors such as climate change, invasive alien species, diseases and fisheries (Phillips, Fort and Dias, 2022).

Procellariiformes could be more vulnerable than other seabird species because their gastric morphology enables them to retain plastic in their stomach for weeks to months (Ryan, 2015), increasing the risk for chronic exposure. Moreover, the presence of stomach oil – unique to Procellariiformes – plays an important role in exposure to plastic-associated chemicals (Chapter 3 and 4). Nonetheless, other marine species could experience similar risks of endocrine disruption, given that plastic ingestion has been documented in many other marine species such as turtles, fish and marine mammals (Kühn and van Franeker, 2020). Accordingly, action needs to be taken to reduce plastic pollution and subsequent exposure of wildlife.

6.1.4. Plastic pollution: current situation and future directions

Efforts to address plastic pollution and associated toxicity have been implemented at various levels, from local to global initiatives. Devices such as river interceptors and ocean cleanup projects aim to capture and remove plastic waste before it reaches the open ocean (Kibria *et al.*, 2023). The Ocean Cleanup project, for instance, targets the removal of large debris from ocean gyres (Gonzalez Jimenez *et al.*, 2023). Many countries have also introduced

Chapter 6 – General discussion and perspectives

bans or restrictions on single-use plastics, such as plastic bags, straws, and cutlery (Knoblauch and Mederake, 2021). The use of microplastic beads in personal care products has also been restricted in several countries (Knoblauch and Mederake, 2021). Nevertheless, despite being banned, many of these products are still found on the market (Anagnosti *et al.*, 2021; Knoblauch and Mederake, 2021). Moreover, while the use of chemicals documented as toxic tends to be regulated, these chemicals are often replaced by new ones that also turn out to be toxic [e.g., BPA substitutes (Adamovsky *et al.*, 2024), BFRs substitutes (Qadeer *et al.*, 2024)]. For example, BPS has been used as a substitute for BPA and can also activate ERs, as observed with human and fulmar ERs in Chapter 5.

Solutions to reduce plastic waste must focus on improving and developing recycling and waste management infrastructure, particularly in countries with poor waste management systems (Kibria *et al.*, 2023). The exponential increase of plastic production must also be halted by promoting the use of alternative material less toxic. Filters should be considered at many level (e.g., washing machine, wastewater) to help reduce the entry of microplastics into the environment (Kim *et al.*, 2024). International cooperation is also essential to address plastic pollution on a global scale. Stronger enforcement of existing regulations and the development of new international agreements can help reduce plastic waste (Dauvergne, 2023). For that, continuing research into the impacts of plastic on ecosystems and human health is necessary to inform policy and management decisions.

6.2. Conclusion

This thesis provides insights about the digestive fate and potential hormone disruption of plastic ingestion in Procellariiform seabirds. A schematic summary of the main outcomes of this thesis is shown in figure 6.1. Our findings demonstrated that both lipid and aqueous gastric components enhance the leaching of chemicals from plastic trapped in a Procellariiform stomach, which dynamic is influenced by the chemical properties, the diet (i.e., lipids) composition and the type of polymer. Plastic additives could represent a significant risk of chronic exposure to toxic chemicals, which could result in potential hormone disruption. Our findings showed that 55% of the northern fulmars studied had ingested plastic that leached estrogenic chemicals, suggesting that plastic ingestion could contribute to reproductive toxicity in Procellariiformes. Other potential forms of endocrine disruption and toxicity may also occur from chemical and microplastic exposure. Overall, plastic ingestion may represent an additional threat faced by seabirds, particularly Procellariiformes, but also other marine organisms, in addition to exposure to other environmental pollutants, climate change, habitat change, etc. which may contribute to population vulnerability. Therefore, it is imperative to reduce plastic pollution to mitigate the risk to marine wildlife.

Chapter 6 – General discussion and perspectives

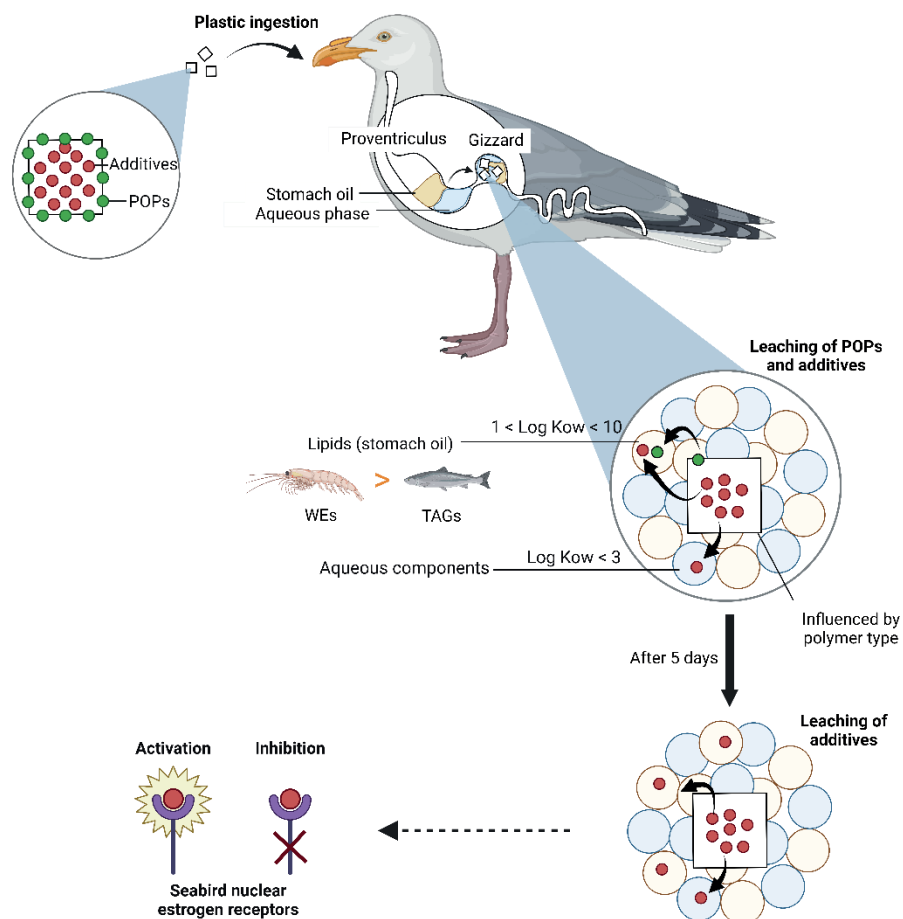


Figure 6.1. Leaching and toxicity of additives and POPs from plastics ingested by a Procellariiform. Plastic ingested by Procellariiformes contain additives (red circles) and POPs (green circles) that sorbed on the plastic in the ocean. Plastic trapped in the gizzard will leach both POPs and additives. Stomach oil will induce the leaching of low to high hydrophobic chemicals ($1 < \text{Log Kow} < 10$). WE-rich copepod oil induces a higher leaching of chemicals than TAG-rich oil. The aqueous compounds (i.e., water, pepsin, albumin, HCl) will primarily induce the leaching of low hydrophobic chemicals ($\text{log Kow} < 3$). The type of polymer also influences the leaching dynamic of chemicals. After 5 days of residency in the gizzard, all the POPs

Chapter 6 – General discussion and perspectives

sorbed from the ocean are expected to be leached, while additives will continue to leach for prolonged periods. The chemical mixture leached from ingested plastics can activate and inhibit fulmar ERs *in vitro*. HDPE, High-density polyethylene; POPs, persistent organic pollutants; PVC, polyvinyl chloride; TAGs, triacylglycerols; WEs, wax esters.

6.3. Perspectives

The present thesis aimed to understand the impact of plastic ingestion on Procellariiformes. While our research provides insight about the factors influencing the leaching of chemicals in a Procellariiform stomach, and the potential hormonal disruption associated with exposure to plastic-associated chemicals, other parameters should be considered to better understand how plastic ingestion impacts Procellariiformes – and to a greater extent, other seabird species – health.

In this thesis, we focused on the leaching of chemicals (i.e., additives and POPs) leached from plastic trapped in a Procellariiform stomach. However, we can also expect for microplastics (including nanoplastics) to be released from fragmentation of larger pieces. These particles could potentially represent an important additional risk of toxicity. Accordingly, in the future, the amount of microplastics leached from larger plastic pieces should be quantified in the PGM. Moreover, seabirds are also exposed to microplastics via food consumption. Thus, exposure to microplastics from preys should also be taken into consideration. Comparing the concentrations of microplastics found in prey with concentrations released from larger plastic pieces will help clarify the role of food consumption and plastic ingestion in microplastic exposure. Moreover, the leaching of chemicals from these microplastics should also be considered for toxicity assessment.

In Chapter 3 and 4, we highlighted the importance of stomach oil – unique to Procellariiformes – on the leaching of chemicals. It would be interesting to investigate whether other seabird species (e.g., seagulls), not having this unique gastric characteristic (i.e., absence of gastric lipase) are exposed to

Chapter 6 – General discussion and perspectives

similar concentrations of chemicals. Preys (e.g., fish) could be digested in the PGM and the influence of the presence of gastric lipase on the leaching of plastic-associated chemicals should be investigated. It would provide information about the potential higher risk associated with plastic ingestion in Procellariiformes compared to other seabirds.

Further studies should investigate the fate of plastic-associated chemicals and microplastics throughout the digestive tract, with a focus on the intestines where chemicals and nanoplastics can be absorbed. A multi-stage approach could be incorporated to the Procellariiform gastric model by doing a subsequent exposure of plastics to intestinal conditions which would involve adding bile salts, pancreatin (which contains pancreatic enzymes such as lipase, co-lipase, trypsin), and adjusting the pH to 6.5 (Guo *et al.*, 2019). Additionally, the absorption mechanism of plastic-associated chemicals and nanoplastics could be studied by using avian intestinal cell line. Nevertheless, most avian epithelial cell lines are patented and not available for research purposes (patent WO/2020/157076) (Nash and Vervelde, 2022). Accordingly, the use of avian epithelial cells would require the establishment of a primary cell culture (Nash and Vervelde, 2022). Absorbed chemicals and microplastics could be quantified in cell and media using mass spectroscopy and electron microscopy (Bodziach *et al.*, 2021; Cassano *et al.*, 2023).

Different Procellariiform species may also experience different sensitivity to endocrine disruptors, which could result in species-specific vulnerabilities. Accordingly, the sensitivity of ER from different species of Procellariiformes to plastic-associated chemicals should be investigated. By examining whether these differences in ER sensitivity correlate with the conservation status of these species, we can better understand the impact of plastic-associated

Chapter 6 – General discussion and perspectives

chemicals on their populations and identify species that may be at greater risk due to their heightened sensitivity to endocrine disruptors.

Moreover, we should expand future research on other forms of endocrine disruption, such as interactions with different hormone receptors, as well as other types of cellular toxicity like immune responses and oxidative stress. The luciferase-reporter assay could be used to assess the activation and inhibition of other nuclear receptors such as AR, TR, AhR, retinoid X receptor (RXR) and retinoid acid receptor (RAR) by plastic leachates. To investigate for immune toxicity and oxidative stress, chicken macrophage cells (HD11) could be exposed to plastic leachates, and cell viability, oxidative index, apoptosis and proinflammatory secretion levels could be analyzed (Zhang *et al.*, 2020). Chicken leghorn male hepatoma (LMH) cell line could also be used to study cytotoxicity and expression of genes involved in xenobiotic metabolism [e.g., cytochromes P450 (CYPs)], oxidative stress (e.g., superoxide dismutase) and immune response (e.g., interleukins) (Sharin, Crump and O'Brien, 2022). Investigating these effects will provide a more comprehensive understanding of the potential health risks posed by plastic ingestion in seabirds.

To better understand the potential endocrine disruption observed *in vitro*, future studies should include non-target analysis on the leachates to identify the specific chemicals responsible. This approach will help pinpoint the substances causing hormonal effects and provide a clearer picture of the potential risks associated with plastic ingestion. Additionally, spectroscopy analysis of ingested plastics showing endocrine disruption *in vitro* could be performed, as visual inspection of the spectra may help to detect the presence

Chapter 6 – General discussion and perspectives

of plastic-associated chemicals responsible for the observed endocrine disruption.

Finally, further studies should be conducted in wild birds to help understand the chemical impact of plastic ingestion on individuals and populations. We could investigate whether a correlation exists between the plasma concentration of endocrine disrupting plastic-associated chemicals (e.g., phthalates, PBDE-209, UV-stabilizers, bisphenols) and different forms of endocrine disruption in wild seabirds (e.g., hormone levels, altered gene expression). The presence of physical abnormalities (e.g., reproductive system abnormalities) could also be correlated to the hormonal disruption assessed from leachates of plastics recovered from the bird stomachs (e.g., estrogenic activity of the leachates). By studying real-world cases of seabirds exposed to plastic-associated chemicals and linking observed physiological changes to laboratory findings, we can better understand the actual impact of plastic ingestion on seabird health and populations.

References

- Adamovsky, O. *et al.* (2024) ‘Exploring BPA Alternatives – Environmental Levels and Toxicity Review’, *Environment International*, p. 108728. doi: 10.1016/j.envint.2024.108728.
- Allen, S. F. *et al.* (2021) ‘Phthalate diversity in eggs and associations with oxidative stress in the European herring gull (*Larus argentatus*)’, *Marine Pollution Bulletin*, 169(May), p. 112564. doi: 10.1016/j.marpolbul.2021.112564.
- Alva-Gallegos, R., Carazo, A. and Mladěnka, P. (2023) ‘Toxicity overview of endocrine disrupting chemicals interacting in vitro with the oestrogen receptor’, *Environmental Toxicology and Pharmacology*, 99(February). doi: 10.1016/j.etap.2023.104089.
- Anagnosti, L. *et al.* (2021) ‘Worldwide actions against plastic pollution from microbeads and microplastics in cosmetics focusing on European policies. Has the issue been handled effectively?’, *Marine Pollution Bulletin*, 162(January), pp. 1–6. doi: 10.1016/j.marpolbul.2020.111883.
- Bodziach, K. *et al.* (2021) ‘Gastrointestinal and respiratory exposure of water birds to endocrine disrupting phenolic compounds’, *Science of the Total Environment*, 754. doi: 10.1016/j.scitotenv.2020.142435.
- Bourdeau, V. *et al.* (2004) ‘Genome-wide identification of high-affinity estrogen response elements in human and mouse’, *Molecular Endocrinology*, 18(6), pp. 1411–1427. doi: 10.1210/me.2003-0441.
- Bridson, J. H. *et al.* (2023) ‘Release of additives and non-intentionally added

Chapter 6 – General discussion and perspectives

substances from microplastics under environmentally relevant conditions’, *Environmental Advances*, 12(March), p. 100359. doi: 10.1016/j.envadv.2023.100359.

Campioni, L. *et al.* (2024) ‘Exposure of an endangered seabird species to persistent organic pollutants: Assessing levels in blood and link with reproductive parameters’, *Science of the Total Environment*, 930(April). doi: 10.1016/j.scitotenv.2024.172814.

Cassano, D. *et al.* (2023) ‘Investigating the Cellular Uptake of Model Nanoplastics by Single-Cell ICP-MS’, *Nanomaterials*, 13(3). doi: 10.3390/nano13030594.

Chu, S. S. T. and Nakagawa, Y. (1982) ‘Comparison of pepsins isolated from porcine, bovine and *Penicillium jathiuellum*’, *Comparative Biochemistry and Physiology -- Part B: Biochemistry and*, 72(4), pp. 625–629. doi: 10.1016/0305-0491(82)90516-8.

Cimmino, I. *et al.* (2020) ‘Potential mechanisms of bisphenol a (BPA) contributing to human disease’, *International Journal of Molecular Sciences*, 21(16), pp. 1–22. doi: 10.3390/ijms21165761.

Clarke, A. and Prince, P. A. (1976) ‘The origin of stomach oil in marine birds: Analyses of the stomach oil from six species of subantarctic procellariiform birds’, *Journal of Experimental Marine Biology and Ecology*, 23(1), pp. 15–30. doi: 10.1016/0022-0981(76)90082-4.

Coffin, S. *et al.* (2019) ‘Fish and Seabird Gut Conditions Enhance Desorption of Estrogenic Chemicals from Commonly-Ingested Plastic Items’, *Environmental Science and Technology*, 53(8), pp. 4588–4599. doi:

Chapter 6 – General discussion and perspectives

10.1021/acs.est.8b07140.

Connan, M., Cherel, Y. and Mayzaud, P. (2007) ‘Lipids from stomach oil of procellariiform seabirds document the importance of myctophid fish in the Southern Ocean’, *Limnology and Oceanography*, 52(6), pp. 2445–2455. doi: 10.4319/lo.2007.52.6.2445.

Court, L. *et al.* (2020) ‘Key role of estrogen receptor β in the organization of brain and behavior of the Japanese quail’, *Hormones and Behavior*, 125(August), p. 104827. doi: 10.1016/j.yhbeh.2020.104827.

Dauvergne, P. (2023) ‘The necessity of justice for a fair, legitimate, and effective treaty on plastic pollution’, *Marine Policy*, 155(July 2023), p. 105785. doi: 10.1016/j.marpol.2023.105785.

Dhaka, V. *et al.* (2022) ‘Occurrence, toxicity and remediation of polyethylene terephthalate plastics. A review’, *Environmental Chemistry Letters*, 20(3), pp. 1777–1800. doi: 10.1007/s10311-021-01384-8.

Dias, M. P. *et al.* (2019) ‘Threats to seabirds: A global assessment’, *Biological Conservation*, 237(April), pp. 525–537. doi: 10.1016/j.biocon.2019.06.033.

Erni-Cassola, G. *et al.* (2019) ‘Distribution of plastic polymer types in the marine environment; A meta-analysis’, *Journal of Hazardous Materials*, 369(February), pp. 691–698. doi: 10.1016/j.jhazmat.2019.02.067.

Foster, K. L. *et al.* (2010) ‘Preliminary assessment of avian stomach oils: A vector of contaminants to chicks and potential for diet analysis and biomonitoring’, *Environmental Science and Technology*, 44(17), pp. 6869–6874. doi: 10.1021/es1009983.

Gonzalez Jimenez, M. A. *et al.* (2023) ‘On the Digital Twin of The Ocean

Chapter 6 – General discussion and perspectives

Cleanup Systems—Part I: Calibration of the Drag Coefficients of a Netted Screen in OrcaFlex Using CFD and Full-Scale Experiments’, *Journal of Marine Science and Engineering*, 11(10). doi: 10.3390/jmse11101943.

Grishanova, A. Y. and Perepechaeva, M. L. (2022) ‘Aryl Hydrocarbon Receptor in Oxidative Stress as a Double Agent and Its Biological and Therapeutic Significance’, *International Journal of Molecular Sciences*, 23(12). doi: 10.3390/ijms23126719.

Guo, H. *et al.* (2019) ‘The leaching of additive-derived flame retardants (FRs) from plastics in avian digestive fluids: The significant risk of highly lipophilic FRs’, *Journal of Environmental Sciences (China)*, 85, pp. 200–207. doi: 10.1016/j.jes.2019.06.013.

Hahladakis, J. N. *et al.* (2018) ‘An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling’, *Journal of Hazardous Materials*, 344, pp. 179–199. doi: 10.1016/j.jhazmat.2017.10.014.

Hamer, K. C. (2019) ‘Procellariiformes’, *Encyclopedia of Ocean Sciences*, (April 2018), pp. 39–48. doi: 10.1016/B978-0-12-409548-9.04307-4.

Hermabessiere, L. *et al.* (2017) ‘Occurrence and effects of plastic additives on marine environments and organisms: A review’, *Chemosphere*, 182, pp. 781–793. doi: 10.1016/j.chemosphere.2017.05.096.

Ishii, C. *et al.* (2017) ‘Contamination status and accumulation characteristics of heavy metals and arsenic in five seabird species from the central Bering Sea’, *Journal of Veterinary Medical Science*, 79(4), pp. 807–814. doi: 10.1292/jvms.16-0441.

Chapter 6 – General discussion and perspectives

Jang, M. *et al.* (2024) ‘Hazardous chemical additives within marine plastic debris and fishing gear: Occurrence and implications’, *Journal of Cleaner Production*, 442(January), p. 141115. doi: 10.1016/j.jclepro.2024.141115.

Kadac-czapska, K. and Knez, E. (2024) ‘Microplastics and Oxidative Stress — Current Problems and Prospects’, *Antioxidant*, 13(579).

Kamegashira, M. *et al.* (1977) ‘Role of Pepsin in Species Differences in Erosive Gastritis’, *Archives of Surgery*, 112(2), pp. 193–197. doi: 10.1001/archsurg.1977.01370020087009.

Kibria, M. G. *et al.* (2023) *Plastic Waste: Challenges and Opportunities to Mitigate Pollution and Effective Management*, *International Journal of Environmental Research*. Springer International Publishing. doi: 10.1007/s41742-023-00507-z.

Kim, S. *et al.* (2024) ‘Ceramic membranes as a potential high-performance alternative to microplastic filters for household washing machines’, *Separation and Purification Technology*, 344(January), p. 127278. doi: 10.1016/j.seppur.2024.127278.

Knoblauch, D. and Mederake, L. (2021) ‘Government policies combatting plastic pollution’, *Current Opinion in Toxicology*, 28, pp. 87–96. doi: 10.1016/j.cotox.2021.10.003.

Koelmans, A. A. *et al.* (2016) ‘Microplastic as a Vector for Chemicals in the Aquatic Environment: Critical Review and Model-Supported Reinterpretation of Empirical Studies’, *Environmental Science and Technology*, 50(7), pp. 3315–3326. doi: 10.1021/acs.est.5b06069.

Krüger, T., Long, M. and Bonefeld-Jørgensen, E. C. (2008) ‘Plastic

Chapter 6 – General discussion and perspectives

components affect the activation of the aryl hydrocarbon and the androgen receptor', *Toxicology*, 246(2–3), pp. 112–123. doi: 10.1016/j.tox.2007.12.028.

Kühn, S. *et al.* (2021) 'Polymer types ingested by northern fulmars (*Fulmarus glacialis*) and southern hemisphere relatives', *Environmental Science and Pollution Research*, 28(2), pp. 1643–1655. doi: 10.1007/s11356-020-10540-6.

Kumar, N. *et al.* (2019) 'Steroid receptors and their regulation in avian extraembryonic membranes provide a novel substrate for hormone mediated maternal effects', *Scientific Reports*, 9(1), pp. 1–6. doi: 10.1038/s41598-019-48001-x.

Lagunas-Rangel, F. A. *et al.* (2022) 'Role of the Synergistic Interactions of Environmental Pollutants in the Development of Cancer', *GeoHealth*, 6(4), pp. 1–17. doi: 10.1029/2021GH000552.

Li, Y. *et al.* (2022) 'Photoaging of Baby Bottle-Derived Polyethersulfone and Polyphenylsulfone Microplastics and the Resulting Bisphenol S Release', *Environmental Science and Technology*, 56(5), pp. 3033–3044. doi: 10.1021/acs.est.1c05812.

Lovekamp, T. N. and Davis, B. J. (2001) 'Mono-(2-ethylhexyl) phthalate suppresses aromatase transcript levels and estradiol production in cultured rat granulosa cells', *Toxicology and Applied Pharmacology*, 172(3), pp. 217–224. doi: 10.1006/taap.2001.9156.

Luo, H. *et al.* (2022) 'Effects of aging on environmental behavior of plastic additives: Migration, leaching, and ecotoxicity', *Science of the Total*

Chapter 6 – General discussion and perspectives

Environment, 849(July), p. 157951. doi: 10.1016/j.scitotenv.2022.157951.

Maddela, N. R. *et al.* (2023) ‘Additives of plastics: Entry into the environment and potential risks to human and ecological health’, *Journal of Environmental Management*, 348(October), p. 119364. doi: 10.1016/j.jenvman.2023.119364.

Mato, Y. *et al.* (2001) ‘Plastic resin pellets as a transport medium for toxic chemicals in the marine environment’, *Environmental Science and Technology*, 35(2), pp. 318–324. doi: 10.1021/es0010498.

Matthews, J. *et al.* (2000) ‘Differential estrogen receptor binding of estrogenic substances: A species comparison’, *Journal of Steroid Biochemistry and Molecular Biology*, 74(4), pp. 223–234. doi: 10.1016/S0960-0760(00)00126-6.

Mattsson, A., Olsson, J. A. and Brunström, B. (2011) ‘Activation of estrogen receptor alpha disrupts differentiation of the reproductive organs in chicken embryos’, *General and Comparative Endocrinology*, 172(2), pp. 251–259. doi: 10.1016/j.ygcen.2011.03.010.

Meling, D. D. *et al.* (2022) ‘Phthalate monoesters act through peroxisome proliferator-activated receptors in the mouse ovary’, pp. 113–123. doi: 10.1016/j.reprotox.2022.04.002.Phthalate.

Michishita, S. *et al.* (2023) ‘Microplastic in northern anchovies (*Engraulis mordax*) and common murrelets (*Uria lomvia*) from the Monterey Bay, California USA - Insights into prevalence, composition, and estrogenic activity’, *Environmental Pollution*, 316(P2), p. 120548. doi: 10.1016/j.envpol.2022.120548.

Mohamed Nor, N. H. *et al.* (2023) ‘How Digestive Processes Can Affect the

Chapter 6 – General discussion and perspectives

Bioavailability of PCBs Associated with Microplastics: A Modeling Study Supported by Empirical Data', *Environmental Science and Technology*, 57(31), pp. 11452–11464. doi: 10.1021/acs.est.3c02129.

Monclús, L. *et al.* (2022) 'Microplastic Ingestion Induces Size-Specific Effects in Japanese Quail', *Environmental Science and Technology*, 56(22), pp. 15902–15911. doi: 10.1021/acs.est.2c03878.

Nash, T. and Vervelde, L. (2022) 'Advances, challenges and future applications of avian intestinal in vitro models', *Avian Pathology*, 51(4), pp. 317–329. doi: 10.1080/03079457.2022.2084363.

Nouman, M. *et al.* (2017) 'Additive blooming in polymer materials: Consequences in the pharmaceutical and medical field', *Polymer Degradation and Stability*, 143, pp. 239–252. doi: 10.1016/j.polymdegradstab.2017.07.021.

Nurhayati, T. *et al.* (2019) 'Pepsin activity from gastric of milkfish and catfish in Indonesian Waters', *IOP Conference Series: Earth and Environmental Science*, 404(1). doi: 10.1088/1755-1315/404/1/012060.

Panzica, G. C. *et al.* (2005) 'Early embryonic administration of xenoestrogens alters vasotocin system and male sexual behavior of the Japanese quail', *Domestic Animal Endocrinology*, 29(2), pp. 436–445. doi: 10.1016/j.domaniend.2005.02.010.

Phillips, R. A., Fort, J. and Dias, M. P. (2022) *Conservation status and overview of threats to seabirds*, *Conservation of Marine Birds*. doi: 10.1016/B978-0-323-88539-3.00015-7.

Pinto da Costa, J. *et al.* (2023) 'Plastic additives and microplastics as emerging

Chapter 6 – General discussion and perspectives

contaminants: Mechanisms and analytical assessment’, *TrAC - Trends in Analytical Chemistry*, 158. doi: 10.1016/j.trac.2022.116898.

Qadeer, A. *et al.* (2024) ‘Global environmental and toxicological impacts of polybrominated diphenyl ethers versus organophosphate esters: A comparative analysis and regrettable substitution dilemma’, *Journal of Hazardous Materials*, 466(January). doi: 10.1016/j.jhazmat.2024.133543.

Rochester, J. R. (2013) ‘Bisphenol A and human health: A review of the literature’, *Reproductive Toxicology*, 42, pp. 132–155. doi: 10.1016/j.reprotox.2013.08.008.

Rodrigues, J. P. *et al.* (2019) ‘Significance of interactions between microplastics and POPs in the marine environment: A critical overview’, *TrAC - Trends in Analytical Chemistry*, 111, pp. 252–260. doi: 10.1016/j.trac.2018.11.038.

Roman, L., Hardesty, B. D., *et al.* (2019) ‘A quantitative analysis linking seabird mortality and marine debris ingestion’, *Scientific Reports*, 9(1), pp. 1–7. doi: 10.1038/s41598-018-36585-9.

Roman, L., Lowenstine, L., *et al.* (2019) ‘Is plastic ingestion in birds as toxic as we think? Insights from a plastic feeding experiment’, *Science of the Total Environment*, 665, pp. 660–667. doi: 10.1016/j.scitotenv.2019.02.184.

Ryan, P. G. (2015) ‘How quickly do albatrosses and petrels digest plastic particles?’, *Environmental Pollution*, 207, pp. 438–440. doi: 10.1016/j.envpol.2015.08.005.

Ryan, P. G. (2019) ‘Ingestion of Plastics by Marine Organisms’, *Handbook of Environmental Chemistry*, 78, pp. 235–266. doi: 10.1007/698_2016_21.

Chapter 6 – General discussion and perspectives

Saunier, J. *et al.* (2012) ‘Blooming of Irganox 3114® antioxidant onto a medical grade elastomer. Impact of the recrystallization conditions on the antioxidant polymorphism, on the film wettability and on the antioxidant leachability’, *International Journal of Pharmaceutics*, 437(1–2), pp. 89–99. doi: 10.1016/j.ijpharm.2012.07.060.

van der Schyff, V. *et al.* (2021) ‘Persistent organic pollutants in sea bird eggs from the Indian Ocean’s Mascarene Basin’, *Science of the Total Environment*, 771, p. 145348. doi: 10.1016/j.scitotenv.2021.145348.

Shanle, E. K. and Xu, W. (2011) ‘Endocrine disrupting chemicals targeting estrogen receptor signaling: Identification and mechanisms of action’, *Chem Res Toxicol*, 24(1), pp. 6–19. doi: 10.1021/tx100231n.Endocrine.

Sharin, T., Crump, D. and O’Brien, J. M. (2022) ‘Toxicity screening of bisphenol A replacement compounds: cytotoxicity and mRNA expression in LMH 3D spheroids’, *Environmental Science and Pollution Research*, 29(29), pp. 44769–44778. doi: 10.1007/s11356-022-18812-z.

Shi, C. *et al.* (2023) ‘Oligomers from the Synthetic Polymers: Another Potential Iceberg of New Pollutants’, *Environment & Health*, 1(4), pp. 228–235. doi: 10.1021/envhealth.3c00086.

Sorasan, C. *et al.* (2022) ‘Ageing and fragmentation of marine microplastics’, *Science of the Total Environment*, 827(2022). doi: 10.1016/j.scitotenv.2022.154438.

de Souza, S. S. *et al.* (2022) ‘Toxicity induced via ingestion of naturally-aged polystyrene microplastics by a small-sized terrestrial bird and its potential role

Chapter 6 – General discussion and perspectives

as vectors for the dispersion of these pollutants’, *Journal of Hazardous Materials*, 434(March). doi: 10.1016/j.jhazmat.2022.128814.

Tanaka, K. *et al.* (2019) ‘Piece-by-piece analysis of additives and manufacturing byproducts in plastics ingested by seabirds: Implication for risk of exposure to seabirds’, *Marine Pollution Bulletin*, 145(May), pp. 36–41. doi: 10.1016/j.marpolbul.2019.05.028.

Tanaka, K. *et al.* (2020) ‘In Vivo Accumulation of Plastic-Derived Chemicals into Seabird Tissues’, *Current Biology*, 30(4), pp. 723-728.e3. doi: 10.1016/j.cub.2019.12.037.

Tanaka, K., Yamashita, R. and Takada, H. (2018) ‘Transfer of Hazardous Chemicals from Ingested Plastics to Higher-Trophic-Level Organisms’, in *Handbook of Environmental Chemistry*, pp. 267–280. doi: 10.1007/698_2018_255.

Troisi, G., Barton, S. and Bexton, S. (2016) ‘Impacts of oil spills on seabirds: Unsustainable impacts of non-renewable energy’, *International Journal of Hydrogen Energy*, 41(37), pp. 16549–16555. doi: 10.1016/j.ijhydene.2016.04.011.

Tubbs, C. *et al.* (2012) ‘Activation of southern white rhinoceros (*Ceratotherium simum simum*) estrogen receptors by phytoestrogens: Potential role in the reproductive failure of captive-born females?’, *Endocrinology*, 153(3), pp. 1444–1452. doi: 10.1210/en.2011-1962.

Tubbs, C. W. *et al.* (2016) ‘Estrogenicity of captive southern white rhinoceros diets and their association with fertility’, *General and Comparative Endocrinology*, 238, pp. 32–38. doi: 10.1016/j.ygcen.2016.05.004.

Chapter 6 – General discussion and perspectives

Wang, Hui *et al.* (2017) ‘Anti-androgenic mechanisms of Bisphenol A involve androgen receptor signaling pathway’, *Toxicology*, 387(June), pp. 10–16. doi: 10.1016/j.tox.2017.06.007.

Wang, S. W. *et al.* (2007) ‘Fatty acid signatures of stomach oil and adipose tissue of northern fulmars (*Fulmarus glacialis*) in Alaska: Implications for diet analysis of Procellariiform birds’, *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, 177(8), pp. 893–903. doi: 10.1007/s00360-007-0187-y.

Wang, W. *et al.* (2022) ‘Effects of polyethylene microplastics on cell membranes: A combined study of experiments and molecular dynamics simulations’, *Journal of Hazardous Materials*, 429(December 2021), p. 128323. doi: 10.1016/j.jhazmat.2022.128323.

Wilcox, C. *et al.* (2015) ‘Threat of plastic pollution to seabirds is global, pervasive, and increasing’, *Proceedings of the National Academy of Sciences of the United States of America*, 112(38), pp. 11899–11904. doi: 10.1073/pnas.1502108112.

Yang, W. *et al.* (2022) ‘Impacts of microplastics on immunity’, *Frontiers in Toxicology*, 4(September), pp. 1–14. doi: 10.3389/ftox.2022.956885.

Zhang, S. *et al.* (2020) ‘Cytotoxicity studies of Fe₃O₄ nanoparticles in chicken macrophage cells’, *Royal Society Open Science*, 7(4). doi: 10.1098/rsos.191561.