

# Comprehensive analysis of additives in plastics ingested by seabirds: implication for exposure risk to seabirds

Kosuke Tanaka<sup>1\*</sup>, Hideshige Takada<sup>1</sup>, Jan A. van Franeker<sup>2</sup>

<sup>1</sup>Tokyo University of Agriculture and Technology, Fuchu-shi, Tokyo, Japan, <sup>2</sup>Wageningen-UR, PO Box 167, 1790AD Den Burg (Texel), The Netherlands

## Introduction

The consumption of plastics and resultant generation of wastes have increased globally over the last several decades.<sup>1</sup> Part of the plastic wastes end up in the marine environment,<sup>2</sup> where it is fragmented into smaller pieces over time. Many species of marine-based organisms, such as seabirds, ingest these plastics. The ingested plastics cause injuries and inhibit the digestion of food. On top of that, toxic chemicals contained in plastics as additives causes further concerns. .

In the previous study, polybrominated diphenyl ethers (PBDEs), a class of brominated flame retardants, were analyzed in the abdominal adipose tissue, liver, and ingested plastics of 30 short-tailed shearwaters.<sup>3,4</sup> Higher-brominated congeners BDE209 and BDE183 were detected in both the tissue and ingested plastics from the same bird in 5 cases of bird samples. This sporadic detection of higher-brominated congeners and the corresponding detection in both tissues and ingested plastic provided good evidence on the transfer of PBDEs from ingested plastics to seabirds.<sup>3,4</sup> Sporadic accumulation of high concentration of BDE209 was also detected in other seabird species such as the northern fulmar.<sup>5</sup> This occurrence could also be due to plastic ingestion. A study on the mechanisms of the transfer was also conducted.<sup>4</sup> PBDEs are supposedly compounded in the polymer matrix of plastics and are highly hydrophobic, making it difficult for PBDEs to leach out of plastics. Trace amounts can be found to leached into distilled water, seawater, and acidic pepsin solution.<sup>4</sup> However, more than 20 times the trace amount were discovered able to leached into stomach oil, which is made in the stomach of some seabirds.<sup>4</sup> It was suggested that oils in the stomach of seabirds could act as an organic solvent and accelerate leaching. These studies concluded that plastics retaining additives could be transported through the ocean, and chemicals in plastics could be exposed to seabirds through ingestion and the rapid extraction by stomach oils upon ingestion.

Marine plastics may also contain many other chemical additives apart from PBDEs. This suggested that chemical additives may also be transported through the marine environment and be exposed to seabirds through the ingestion of marine plastics, similarly to the pathway PBDEs. There is a growing concern on the bioaccumulation and toxicological risks of these chemicals towards seabirds. However, there have been little to no reports on additives in the range of mm- to cm-sized marine plastics, which are small enough to be ingested by seabirds. The objective of this study is to identify chemical additives in marine plastic debris, that could be exposed to seabirds, as a first step in examining plastic-associated multiple exposures of chemicals in seabirds and their toxicological risks. Comprehensive analysis of additives containing plastics ingested by three different species of seabirds, in which high frequency of plastic ingestion were observed, was conducted using gas chromatography–electron capture detector (GC–ECD) and gas chromatography–mass spectrometry (GC–MS) in full scan mode.

## Materials and methods

Fulmars chicks were harvested for human consumption at sea north of Vágoy (62°25'N 7°20'W), which is an island west of the Faroe Islands in 2010. Plastics were collected from their stomachs (Fig.1). Regurgitated boluses of undigestible material were collected from chicks consisting of 1 Laysan albatross and 5 black-footed albatross at Muko island (27°40'N 142°08'E). The regurgitated boluses were mainly composed of fishing filaments. Also found were plastic fragments, stones, and non-digestible parts of prey species, e.g., squid beaks and otoliths. Plastic pieces found in the stomach or regurgitated boluses were identified and sorted according to polymer type via near-infrared spectrometry (PlaScan-W, OPT Research Inc., Tokyo, Japan). Identification was done by

comparing the detected infrared spectra with those in recorded in a library<sup>e.g.6</sup>. For the northern fulmar samples, after sorting, several plastic pieces were randomly chosen from each polymer category of polyethylene (PE) fragments, polypropylene (PP) fragments, PE pellets, and PP pellets for analysis (Table 1).



Figure 1. Plastics found in the stomachs of northern fulmars.

Table 1. Number of analyzed plastics ingested by seabirds.

	Faroe islands (Norway)		Muko island (Japan)	
	Northern fulmar	Laysan albatross	Black-footed albatross	
PE fragment	88	7	14	
PP fragment	50	7	4	
PS fragment			2	
PE pellet	16			
PP pellet	3	1		

\* PE: polyethylene, PP: polypropylene, PS: polystyrene.

For both the Laysan albatross and black-footed albatross samples, all of the plastic fragments were analyzed. The number of samples in each category is listed in Table 1. The plastics were washed in water with neutral detergent, dried at room temperature, and weighed on an electronic balance (AB54, Shimadzu, Kyoto, Japan) with precision of 0.1 mg. All of the plastics were photographed. One piece of plastic and *n*-Hexane were placed in an amber vial at a liquid-to-solid ratio of 100 to 1 in volume. The vial was then continuously shaken (100 rpm) at 40 °C for more than 3 days. After the extraction period, 100 µL aliquot of each extract was passed through 5% H<sub>2</sub>O-deactivated silica gel packed in disposable glass pipettes (4 mm i.d. × 5 mm), followed by 5 mL of dichloromethane (DCM). The eluent was then evaporated just to dryness under gentle nitrogen stream and the residue was re-dissolved into 100 µL of *iso*-octane.

One microlitre aliquots were analyzed using gas chromatography. Comprehensive analysis of chemical additives were conducted via GC interfaced with a quadrupole MS (GC-MS; Agilent 5973 MSD with 6890 GC) in full-scan mode. Especially for the detection of halogenated compounds, analysis were also conducted using GC interfaced with an electron capture detector (GC-ECD). The GC settings were described in previous papers.<sup>7, 8</sup> Peaks detected in MS chromatogram were confirmed for identification of the compounds using NIST library. Standard solutions of chemicals suggested by MS spectra, related compounds, and some halogenated compounds, which could be contained in plastics were purchased for precise identification and quantification. The limit of detection (LOD) of chemicals in which, the standard solution was obtained is listed in Table 2.

## Results and discussion

Two kinds of brominated flame retardants (BFRs), 3 Ultraviolet (UV)-stabilizers, and styrene oligomers were detected and quantified against native standards (Table 3, Fig.2). One lubricant (squalene), and an oxidized antioxidant (Irganox 168) were also detected in more than one-third of plastic samples. However, they were not quantified because squalene could also be found in biota and is not specific to plastics, and standard solution of oxidized Irganox 168 was not available. In addition, one compound, which was estimated by MS spectra to belong to the Irganox type antioxidant, was detected in two plastic pieces from northern fulmar. Some chemicals were observed in most of the plastics samples, for example, cholesta-3,5-diene, and oleic acid. These chemicals were expected to derive from lipid adsorption of digested prey in the stomach.

Two major BFRs, i.e., hexabromocyclododecane (HBCDD) [25637-99-4] and deca-BDE, which is a commercial mixture of PBDEs mainly composed of BDE209 [1163-19-5], were detected. Both of these BFRs are well known to be toxic, persistent and bioaccumulative although there were differences in their application. Deca-BDE is used in various types of polymers, such as polycarbonates, polyester resin and polyolefins. HBCDD is mainly used as an additive in polystyrene foam for construction.<sup>9</sup> In the present study, HBCDD were detected in PE and PP fragments with higher frequency than deca-BDE. The sources of these plastics should be studied.

Table 2. Limit of detection (LOD) of contaminants.

	Detector	LOD concentration in plastic (ppm)
HBCD	ECD	0.3
BDE209	ECD	0.03
DBDPE	ECD	0.06
Tinuvin P	GC/MS	17
Tinuvin PS	GC/MS	20
Tinuvin 9	GC/MS	14
Tinuvin 320	GC/MS	5.3
Tinuvin 250	GC/MS	6.1
Tinuvin 326	GC/MS	4.8
Tinuvin 329	GC/MS	22
Tinuvin 328	GC/MS	5.1
Tinuvin 327	GC/MS	3.9
Tinuvin 234	GC/MS	13
Uvinul3008	GC/MS	170
ST5	GC/MS	1.2

Table 3. Concentration and amount in plastic of detected additives.

Sample ID	Polymer type*	chemical	Concentration (µg/g-plastics)	Amount (µg)
<b>Northern fulmar (n=157)</b>				
#87-7	PP	Deca-BDE	1070**	36**
#85-1	PE	HBCDD	3.0	0.1
#94-13	PP	HBCDD	13.7	0.2
#92-13	PE	HBCDD	28.6	0.5
#85-4	PE	UV-326	138	9.0
#94-5	PE	UV-327	1160	21
#85-8	PE	BP-12	566	33
<b>Black-footed albatross (n=20)</b>				
#96-16	PS	Styrene trimers	256***	22***
#96-19	PS	Styrene trimers	1670***	290***

\* PE: polyethylene, PP: polypropylene, PS: polystyrene.

\*\* Total of BDE-202, -197, -203, -196, -208, -207, -206, and -209.

\*\*\* Total of ST2, ST3, ST4, and ST5.

Three types of UV stabilizers were detected in plastics, UV-326 [3896-11-5], and UV-327 [3864-99-1] of the benzotriazole (BT) group, and BP-12 (octabenzene) [1843-05-6] of the benzophenone (BP) group. UV stabilizers are added in various plastic products to prevent discoloring and degradation of commercial products, generally at the concentration range of 0.05 to 2 % of the product's weight. There have been some reports on the toxicity of BT-UV stabilizer and BP-UV stabilizer, such as endocrine disruption<sup>10, 11</sup> resulting in the enactment of restrictions of some BT-UV stabilizer. European Chemicals Agency (ECHA) listed four BT-UV stabilizer, which included UV-327, as substances of very high concern (SVHC), due to the bioaccumulative and toxic, and/or very persistent and very bioaccumulative properties of the substances. One of the BT-UV stabilizer (UV-320) is prohibited from production, usage, and import in Japan since 2007.

Styrene oligomers were detected in polystyrene fragments. Four styrene trimers: ST-2 [1a-phenyl-4a-(1'-phenylethyl)tetralin], ST-3 [1a-phenyl-4e-(1'-phenylethyl)tetralin], ST-4 [1e-phenyl-4a-(1'-phenylethyl)tetralin], ST-5 [1e-phenyl-4e-(1'-phenylethyl)tetralin], were mainly observed and quantified. Styrene oligomers are incorporated into polystyrene resin as impurities during the course of manufacture.<sup>12</sup> They have been suggested to cause adverse effects on organisms, such as endocrine disruption.<sup>13</sup>

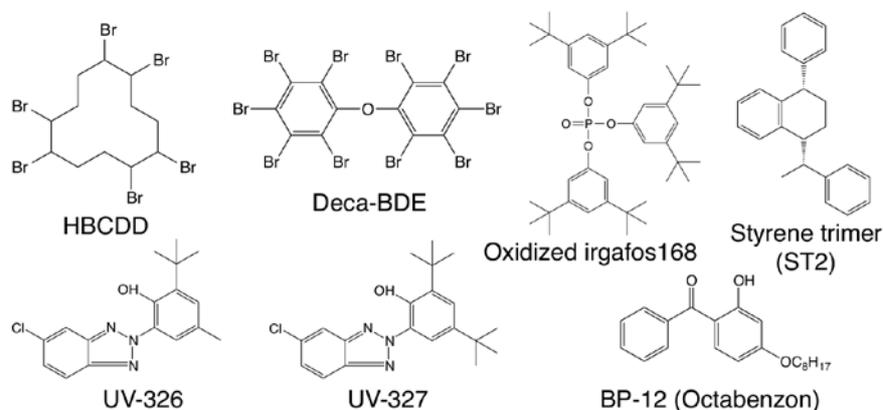


Figure 2. Chemical structures of additives detected in plastics ingested by seabirds.

Log Kow of the detected chemicals ranged from 5.6 for UV-326 and HBCDD to 10 for deca-BDE. Because plastic polymers are hydrophobic, chemicals with low log Kow can leach out rapidly from the polymer matrix into the marine environment. Our observation indicated that chemicals in which Kow was over 5.6, could be retained in plastics during the fragmentation and drifting period on the ocean surface, and could also be transferred to seabirds.

The ingestion frequency of plastics with additives in northern fulmar was estimated to be 27 % for HBCDD and 9 % for deca-BDE, UV-326, UV-327, and BP-12 respectively. In Laysan albatross, it was estimated to be 28% for styrene oligomers. These additives and impurities in plastics can be exposed to seabirds in the same way as PBDEs.<sup>4</sup> Exposure of additive-derived chemicals from plastics may overcome exposure from bioconcentration. The multiple contaminations of chemicals by plastic ingestion may cause toxic effects on seabirds, which have not been anticipated with the exposure through bioconcentration. This study indicates the necessity for further investigations on plastic-associated multiple exposures of chemical additives and their toxicological effects.

### Acknowledgements

The present study was supported by a Grant-in-Aid from the Ministry of Education and Culture of Japan (Projects No. 26-8120)

### References:

1. Jambeck, J. R.; Geyer, R.; Wilcox, C.; Siegler, T. R.; Perryman, M.; Andrady, A.; Narayan, R.; Lavender Law, K., Plastic waste inputs from land into the ocean. *Science* 2015, 347, (6223), 768–771.
2. Andrady, A. L., Microplastics in the marine environment. *Marine Pollution Bulletin* 2011, 62, (8), 1596-1605.
3. Tanaka, K.; Takada, H.; Yamashita, R.; Mizukawa, K.; Fukuwaka, M.; Watanuki, Y., Accumulation of plastic-derived chemicals in tissues of seabirds ingesting marine plastics. *Marine Pollution Bulletin* 2013, 69, (1–2), 219-222.
4. Tanaka, K.; Takada, H.; Yamashita, R.; Mizukawa, K.; Fukuwaka, M.-a.; Watanuki, Y., Facilitated Leaching of Additive-Derived PBDEs from Plastic by Seabirds' Stomach Oil and Accumulation in Tissues. *Environmental Science & Technology* 2015, 49, (19), 11799-11807.
5. Herzke, D.; Anker-Nilssen, T.; Nøst, T. H.; Götsch, A.; Christensen-Dalsgaard, S.; Langset, M.; Fangel, K.; Koelmans, A. A., Negligible Impact of Ingested Microplastics on Tissue Concentrations of Persistent Organic Pollutants in Northern Fulmars off Coastal Norway. *Environmental Science & Technology* 2016, 50, (4), 1924-1933.
6. Socrates, G., Infrared and Raman characteristic group frequencies: tables and charts. Third ed.; John Wiley & Sons: 2004.
7. Hirai, H.; Takada, H.; Ogata, Y.; Yamashita, R.; Mizukawa, K.; Saha, M.; Kwan, C.; Moore, C.; Gray, H.; Laursen, D.; Zettler, E. R.; Farrington, J. W.; Reddy, C. M.; Peacock, E. E.; Ward, M. W., Organic micropollutants in marine plastics debris from the open ocean and remote and urban beaches. *Marine Pollution Bulletin* 2011, 62, (8), 1683-1692.
8. Mizukawa, K.; Takada, H.; Takeuchi, I.; Ikemoto, T.; Omori, K.; Tsuchiya, K., Bioconcentration and biomagnification of polybrominated diphenyl ethers (PBDEs) through lower-trophic-level coastal marine food web. *Marine Pollution Bulletin* 2009, 58, (8), 1217-1224.
9. Alaei, M.; Arias, P.; Sjödin, A.; Bergman, Å., An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. *Environment International* 2003, 29, (6), 683-689.
10. Kunisue, T.; Chen, Z.; Buck Louis, G. M.; Sundaram, R.; Hediger, M. L.; Sun, L.; Kannan, K., Urinary Concentrations of Benzophenone-type UV Filters in U.S. Women and Their Association with Endometriosis. *Environmental Science & Technology* 2012, 46, (8), 4624-4632.
11. Ema, M.; Fukunishi, K.; Hirose, A.; Hirata-Koizumi, M.; Matsumoto, M.; Kamata, E., Repeated-Dose and Reproductive Toxicity of the Ultraviolet Absorber 2-(3',5'-Di-tert-butyl-2'-hydroxyphenyl)-5-chlorobenzotriazole in Rats. *Drug and Chemical Toxicology* 2008, 31, (3), 399-412.
12. Kitamura, S.; Ohmegi, M.; Sanoh, S.; Sugihara, K.; Yoshihara, S. i.; Fujimoto, N.; Ohta, S., Estrogenic activity of styrene oligomers after metabolic activation by rat liver microsomes. *Environmental Health Perspectives* 2003, 111, (3), 329-334.
13. Ohyama, K. I.; Nagai, F.; Tsuchiya, Y., Certain styrene oligomers have proliferative activity on MCF-7 human breast tumor cells and binding affinity for human estrogen receptor. *Environmental Health Perspectives* 2001, 109, (7), 699-703.