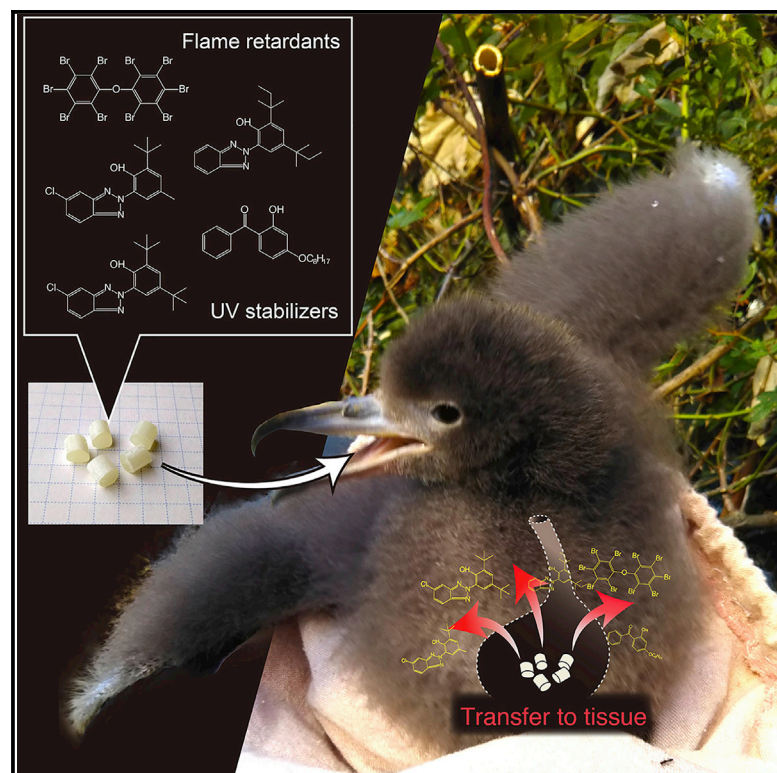


Current Biology

In Vivo Accumulation of Plastic-Derived Chemicals into Seabird Tissues

Graphical Abstract



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In Brief

Tanaka et al. show that feeding additive-laced plastic pellets to seabirds results in the accumulation of chemical additives in liver and adipose tissue at 10^1 – 10^5 times above baseline. These findings demonstrate seabird exposure to plastic additives and additives' importance as emerging pollution sources.

Highlights

- UV-stabilizers and BDE-209 were industrially compounded into plastic resin pellets
- The pellets were fed to seabird chicks under environmentally relevant conditions
- The additives were detected in liver and adipose at 10^1 – 10^5 times above controls
- This study provides evidence of transfer and accumulation of plastic additives

In Vivo Accumulation of Plastic-Derived Chemicals into Seabird Tissues

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SUMMARY

Plastic debris is ubiquitous and increasing in the marine environment [1]. A wide range of marine organisms ingest plastic, and its impacts are of growing concern [2]. Seabirds are particularly susceptible to plastic pollution because of high rates of ingestion [3]. Because marine plastics contain an array of hazardous compounds, the chemical impacts of ingestion are concerning. Several studies on wild seabirds suggested accumulation of plastic-derived chemicals in seabird tissues [4–7]. However, to date, the evidence has all been indirect [4–7], and it is unclear whether plastic debris is the source of these pollutants. To obtain direct evidence for the transfer and accumulation of plastic additives in the tissues of seabirds, we conducted an *in vivo* plastic feeding experiment. Environmentally relevant exposure of plastics compounded with one flame retardant and four ultraviolet stabilizers to streaked shearwater (*Calonectris leucomelas*) chicks in semi-field conditions resulted in the accumulation of the additives in liver and adipose fat of 91 to 120,000 times the rate from the natural diet. Additional monitoring of six seabird species detected these chemical additives only in those species with high plastic ingestion rates, suggesting that plastic debris can be a major pathway of chemical pollutants into seabirds. These findings provide direct evidence of seabird exposure to plastic additives and emphasize the role of marine debris ingestion as a source of chemical pollution in marine organisms.

RESULTS AND DISCUSSION

Inputs of plastic wastes into the ocean reached ~8 million tonnes per year in 2010 and continue to increase [1]. As a result, plastic debris is ubiquitously distributed in marine environments, and its

potential impacts on marine organisms raise serious concerns [8]. The number of species that ingest marine plastic debris continues to grow [2] and is expected to increase [3]. Seabirds, in particular, have a high rate of plastic ingestion, with at least 45% and up to 78% of all species having been documented ingesting plastics since the 1960s [2]. Plastic ingestion can lead to physical impacts, such as blockage and injury of the digestive tract, and it also can lead to exposure to associated hazardous chemicals. Marine plastic debris contains both additives compounded during manufacturing and chemicals sorbed from ambient seawater [9]. The many toxic chemicals present and their adverse effects on those organisms that ingest plastics raise concerns about individual health and population-level impacts. Among studies of the accumulation of plastic-derived chemicals in seabirds' tissues [10], results found in short-tailed shearwater (*Ardenna tenuirostris*) were notable for the sporadic detection of a class of flame retardants (polybrominated diphenyl ethers: PBDEs) in both tissues and ingested plastics, with a consistent pattern between the two [4], suggesting the transfer of chemical additives from plastics to tissues. Although these correlational studies indicate the transfer of additives from plastics to tissues, these results provide indirect evidence. To collect direct evidence of this transfer, we conducted a feeding experiment under environmentally relevant conditions, in which we fed plastic resin pellets compounded with additives to streaked shearwater (*Calonectris leucomelas*) chicks and measured concentrations of additives in their liver, abdominal adipose, and preen gland oil.

Whereas many shearwater species frequently ingest marine plastic debris and often contain large loads of plastics in their stomachs, streaked shearwater rarely do, despite sharing similar morphological features and foraging ecology [11]. Interestingly, the closely related Cory's shearwaters (*Calonectris diomedea*) contained plastic loads lower than other petrel species [12]. In fact, other than the resin pellets we administered, we did not find any plastics in the stomach of 21 streaked shearwater examined as part of this study. Thus, additional supplement of plastics from their parents is not likely and, therefore, streaked shearwater chicks are suitable for this experiment.

We prepared polyethylene pellets compounded with five plastic additives; each pellet was cylindrical (diameter 5 mm, length

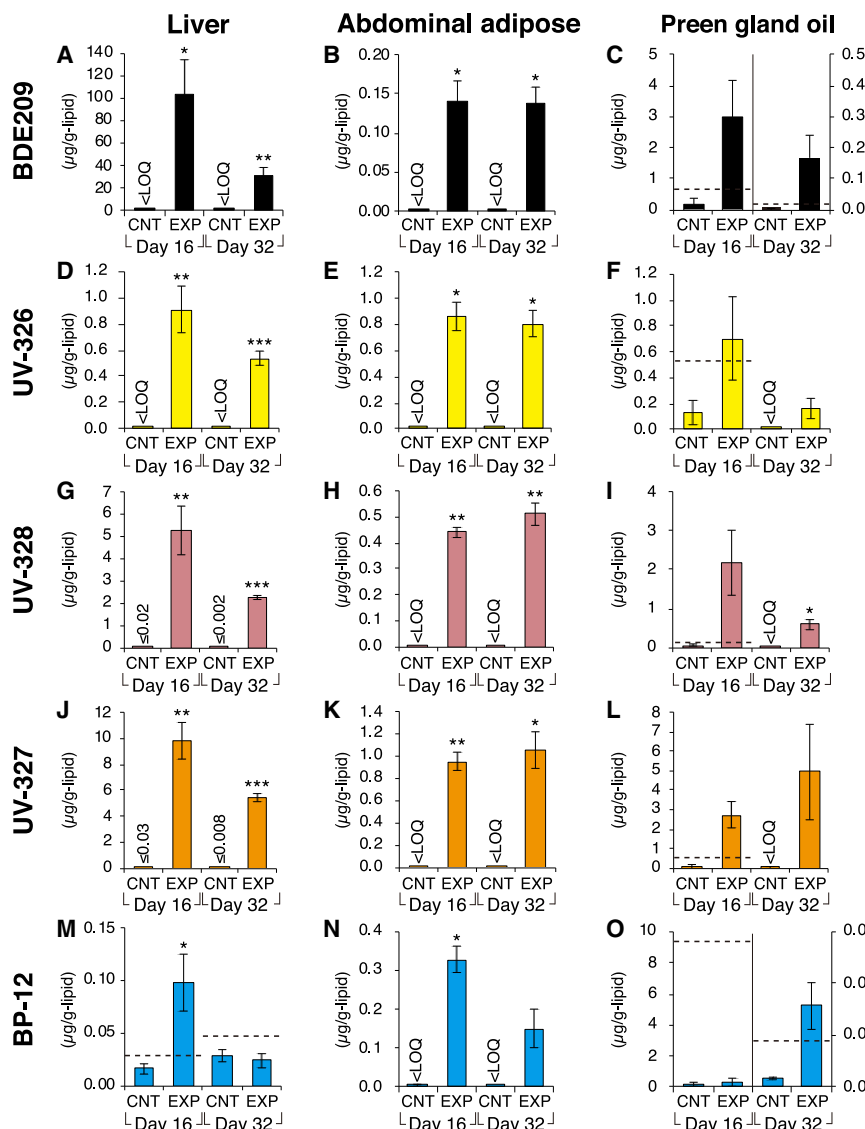


Figure 1. Concentrations of Chemical Additives in Tissues of Seabirds in Plastic Exposure Group and Control Group

Mean ($\pm$ SE) concentrations of BDE209, UV-326, UV-328, UV-327, and BP-12, respectively, in the (A, D, G, J, and M) liver (plastic exposure group [EXP] $n = 6$, control group [CTL] $n = 5$ at day 15–16; EXP $n = 5$, CTL $n = 5$ at day 32), (B, E, H, K, and N) abdominal adipose (EXP $n = 3$, CTL $n = 3$ at day 16; EXP $n = 3$, CTL $n = 3$ at day 32), and (C, F, I, L, and O) preen gland oil (EXP $n = 3$, CTL $n = 3$ at day 16; EXP $n = 3$, CTL $n = 2$ at day 32, one bird from control group [bird ID: #5] was excluded from calculation of mean values because high LOD and LOQ values on lipid base due to small sample volume was available).

“<LOQ” (dashed line) indicates lower than limit of quantification in all samples. “ $\leq$ ” indicates maximum concentration among the samples. Statistical significance at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. Transfer of chemical additives from plastics and accumulation in the tissues of birds exposed to plastics were clearly observed. See also Table S2, S3, and S4, and Data S1A–F.

The additives and their concentrations are thus relevant to environmental conditions.

We fed the plastics to 37-day-old chicks in a natural colony on a cliff on Awashima Island, Japan, in 2017. The parent birds fed their chicks a natural diet, mainly composed of pelagic fish such as Japanese anchovy (*Engraulis japonicus*) and Pacific saury (*Cololabis saira*) [16, 17]. We haphazardly chose 11 exposed chicks and 10 control chicks (without feeding pellets), and we orally administered 5 pellets (i.e., 0.4 g) to the exposed chicks. While there have been many reports of plastic ingestion by species of the family Procel-

ariidae, the occurrence and loads vary widely [18]. Among the species with a similar body size to the streaked shearwater [19], the flesh-footed shearwater (*Ardenna carneipes*) has one of the highest plastic loads, with fledglings containing, on average, 3.2 g and 21 pieces (range: 0–37 g, 0–263 pieces [20]). Thus, the amount of administered plastics, (i.e., 5 pellets of 0.08 g each, or 0.4 g in total), was within those ranges. Chicks were euthanized and dissected on day 15 or 16 after administration (exposed: 6 birds, control: 5 birds) and again on day 32 (exposed: 5 birds, control: 5 birds), to collect the liver, abdominal adipose, and preen gland oil for analysis of additives. All exposed chicks retained all administered pellets in their proventriculus or gizzard.

There has been debate about wearing of ingested plastics in seabirds' stomachs [21]. In the present study, comparing weight of the pellets in the stomach before and after experiment in each bird did not show clear decreasing trend in mass. That is, the change in weight of individual pellets was $-0.14\% \pm 0.2\%$ (range: -0.5% – 0.6%) at day 16 and $-0.44\% \pm 0.2\%$

5 mm) and weighed 0.08 g. Polyethylene is one of the most common polymers in marine environments and is the one most frequently ingested by seabirds [5]. The five chemical additives were chosen from those detected in a screening analysis of plastics found in the stomach of seabirds ($n = 194$) [13]: a flame retardant, deca-BDE, which is composed of several PBDE congeners, dominated by 2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether (BDE209); three benzotriazole ultraviolet (UV) stabilizers, specifically 2-(2-hydroxy-3-tert-butyl-5-methylphenyl)-5-chlorobenzotriazole (UV-326), 2-(3,5-di-tert-amyl-2-hydroxyphenyl) benzotriazole (UV-328), and 2-(3,5-di-tert-butyl-2-hydroxyphenyl)-5-chlorobenzotriazole (UV-327); and one benzophenone UV stabilizer, 2-hydroxy-4-octyloxybenzophenone (BP-12). Industrially, deca-BDE is mixed with polyolefins at a concentration of 5% to 8% by weight [14], and benzotriazole and benzophenone UV stabilizers are mixed at 0.05% to 2% by weight [15]. We set the concentration of each additive in the pellets at 0.4% by weight (Table S1), which is of the same order of magnitude as in plastics found in the stomach of seabirds [13].

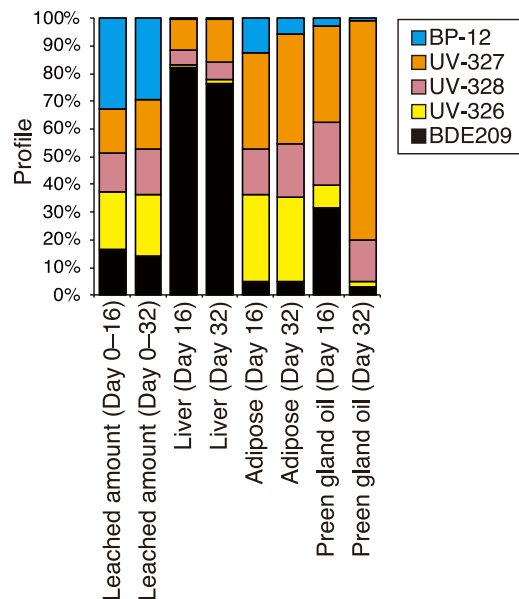


Figure 2. Profiles of Chemical Additives Leached Out from Plastics and Those Accumulated in Tissues of Plastic-Exposed Birds

The relative composition of the five additives in the seabird tissues differed from that in the plastics fed and varied among tissues, indicating that the additives were metabolized. See also [Table S1](#) and [Data S1A–F](#) and [S2A](#). Because concentrations of the additives for control birds were mostly insignificant (< LOQ), no profiles were available.

(range: -1.2% – 0.1%) at day 32. In 3 of the 11 birds, the mass of the pellets increased slightly, likely due to the swelling of polyethylene pellets by contact with the stomach oil. Various polymers are known to absorb oils and increase in weight [22]. Tubenose seabirds (order Procellariiformes) accumulate oils derived from their diet, which is composed mainly of wax esters or triacylglycerol (>70% of total lipids) [23, 24]. All of the chicks in our experiment held tens of milliliters of oil in the stomach. Thus, we hypothesize that the ingested pellets could have absorbed the oils and swelled, resulting in a net increase in weight or a smaller net loss of weight by wearing.

In the plastic-exposed chicks, all of the five additives were detected in liver, abdominal adipose, and preen gland oil, except BP-12 in preen gland oil at day 16 ([Figure 1](#); [Data S1A–S1F](#)). The concentrations in all the tissues were significantly higher in the exposed group than in the control group, except for BP-12 in liver and adipose at day 32. These results are solid evidence of the transfer and accumulation of plastic additives in the tissues of seabirds. From day 16 to day 32, the concentrations of the five additives in the liver of the exposed chicks decreased by up to half (50%), whereas in adipose tissue, only BP-12 decreased and the four other additives showed no change (BDE209, UV-326, UV-328, and UV-327) ([Figure 1](#); [Data S1A–S1D](#)). Although the increasing body mass in growing chicks may dilute and decrease the concentration of contaminants in their organs and/or tissues, the weight of the liver in the exposure group did not increase from day 16 ($18.1 \text{ g} \pm 1.9 \text{ g}$) to day 32 ($17.5 \text{ g} \pm 3.6 \text{ g}$), indicating that the dilution was not likely. Thus, the decrease of additives in liver may be caused by their metabolism and/or their redistribution to the other organs in the

body. Although all five additives were significantly detected in preen gland oil, over limit of quantification (LOQ) values at day 32, two additives (UV-326 and BP-12) were mostly under LOQ in the exposed group at day 16. This change can be explained by the limited amount of preen oil sampled from chicks at day 16 ($\sim 1 \text{ mg}$, [Data S1E](#) and [S1F](#)), which was probably because the preen gland was not fully developed and oil excretion was low in 53-day-old chicks.

The accumulation profiles of the five additives were clearly different among tissues ([Figure 2](#)). One reason might be the high metabolic activity of the liver. In particular, the proportion of BP-12 was trace in liver, which can be explained by the susceptible nature of BP-12 to hepatic metabolism, as suggested for derivatives of benzophenones [25]. However, all five additives, including BP-12, were substantially accumulated in abdominal adipose ([Figure 2](#)). This can be explained by the mechanism in which all exposed additives are absorbed from the gut and distributed throughout the birds' bodies.

Because these five chemicals occur in the prey species of seabirds [26–28], the shearwater chicks are likely exposed to chemicals from sources other than the ingested plastic. In fact, we found UV-328 and UV-327 in some liver samples and UV-326 and UV-328 in preen gland oil samples from the control group at concentrations over the LOQ, which thus could be derived from natural diet ([Data S1A](#), [S1B](#), and [S1E](#)). To estimate the ratio of exposure from ingested plastics to that from environmental sources, we calculated the ratios of the concentrations of additives in tissues in the exposed group to those in the control group ([Table S2](#)). The highest ratios among tissues for each chemical were 1.2×10^5 for BDE209, 1.4×10^3 for UV-326, 1.9×10^3 for UV-328, 1.9×10^3 for UV-327, and 9.1×10^1 for BP-12 ([Table S2](#)). Thus, these shearwaters were subjected to much higher chemical exposure from ingested plastics than from their diet. As for the additives, plastic ingestion can be the most important pathway to seabirds.

Percent leaching of additives was calculated based on the amounts of additives retained in the plastics sampled from the stomach, compared to those in the administered pellets ([Data S2A](#)). By day 15–16 ($n = 30$: 5 pieces/individual $\times$ 6 birds), $45\% \pm 0.6\%$ of BDE209, $57\% \pm 0.6\%$ of UV-326, $42\% \pm 0.6\%$ of UV-328, $44\% \pm 0.7\%$ of UV-327, and $88\% \pm 0.4\%$ of BP-12 were leached out, and by day 32 ($n = 25$: 5 pieces/individual $\times$ 5 birds), $47\% \pm 0.5\%$ of BDE209, $76\% \pm 0.5\%$ of UV-326, $60\% \pm 0.6\%$ of UV-328, $63\% \pm 0.5\%$ of UV-327, and $97\% \pm 0.2\%$ of BP-12 were leached out from the plastics. The leaching of hydrophobic chemicals from plastics is usually slow [29, 30]. Especially for BDE209, leaching rate has been estimated based on the diffusion coefficient, and reported significant leaching is not likely from millimeter-size polymers [29, 31]. We also confirmed that no significant leaching of these five additives occurred (percent leaching was less than 0.02% for each additive) by soaking in distilled water for 16 days at room temperature ($\sim 25^\circ\text{C}$). The significantly larger leaching of the hydrophobic additives (e.g., 47% for BDE209) in the present exposure experiment can be explained by facilitation of contaminant diffusion within the polymer matrix. This facilitation may be due to swelling of the polymer by exposure to stomach oil, as evidenced by the changing mass of the pellets exposed. Previously, the acceleration of contaminant diffusion in polyethylene as a result of

Table 1. Concentrations of Additives in Preen Gland Oil from Wild Seabirds in Hawaiian Islands

	Bird Individual ID	Sampling year	BDE209 (ng/g lipid weight)	UV-326 (ng/g lipid weight)	UV-328 (ng/g lipid weight)	UV-327 (ng/g lipid weight)	BP-12 (ng/g lipid weight)	Plastic ingestion frequency reported in [35].
Black footed albatross (<i>Phoebastria nigripes</i> , Tern Island)	#1	2012	<LOQ	77	4.8	<LOQ	<LOQ	82.2%(n = 45)
	#2	2012	<LOQ	24	4.5	1.2	<LOQ	
	#3	2012	<LOQ	17	2.8	<LOQ	85	
Laysan albatross (<i>Phoebastria immutabilis</i> , Oahu Island)	#1	2015	<LOQ	22	<LOQ	<LOQ	<LOQ	96.0%(n = 126)
	#2	2015	<LOQ	14	<LOQ	3.4	44	
	#3	2015	<LOQ	1.9	<LOQ	<LOQ	47	
Brown booby (<i>Sula leucogaster</i> , Oahu Island)	#1	2014	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	33.3%(n = 3)
	#2	2015	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
	#3	2015	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
Red footed booby (<i>Sula sula</i> , Oahu Island)	#1	2014	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5.3%(n = 19)
	#2	2014	2.8	<LOQ	<LOQ	<LOQ	<LOQ	
	#3	2014	<LOQ	<LOQ	<LOQ	29	<LOQ	
Brown noddy (<i>Anous stolidus</i> , Oahu Island)	#1	2011	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5.6%(n = 18)
	#2	2011	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
	#3	2011	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
Sooty tern (<i>Onychoprion fuscatus</i> , Oahu Island)	#1	2011	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0%(n = 14)
	#2	2011	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
	#3	2011	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	

<LOQ, lower than limit of quantification.

swelling by triacylglycerol has been documented [32]. Also in our previous study, leaching of BDE209 from high-density polyethylene (HDPE), in which BDE209 was industrially compounded, was studied in various solutions, and around 15% of leaching was observed in stomach oil collected from wild streaked shearwater, whereas no significant leaching was observed in distilled water [33]. The higher leaching in the present study (45–47%) could be explained by the use of low-density polyethylene (LDPE) pellets for this exposure experiment.

The amounts of additives accumulated in the liver were calculated by multiplying the weight of liver by the concentrations of the additives in the liver and compared with those in the administered plastics (Data S2B). The amount of additives in the liver ranged across chemicals, from 0.04 μg for BP-12 to 46 μg for BDE209 (Data S2B). These values represent substantial amounts compared to those originally administered in the plastics, with the highest values at day 16 (4.0% for BDE209, 0.03% for UV-326, 0.22% for UV-328, 0.37% for UV-327, and 0.004% for BP-12) and lower values at day 32 (Data S2B). Moreover, they correspond to a high proportion (9.0% for BDE209, 0.06% for UV-326, 0.52% for UV-328, 0.85% for UV-327, and 0.004% for BP-12) of the amounts leached out from the administered plastics (Data S2B). These results demonstrate that the amounts of additives transferred to the birds are detectable via their decreasing concentration in the pellets. However, the amounts measured in the liver account for only a portion of the absorbed additives during the experiment, with the remainder being distributed into other organs, metabolized, and excreted through feces and preen gland oil. Thus, future analysis involving detailed mass balance could track the fates of the additives derived from the plastics by quantifying their concentrations in other organs and excretions.

To assess the transfer and bioaccumulation of these five additives in wild birds, we analyzed preen gland oil collected from seabirds in the field (six species from the Hawaiian Islands), because preen gland oil offers a noninvasive tool for monitoring the accumulation of chemicals in wild seabirds' tissues [34]. UV-326, UV-328, UV-327, and BP-12 were present in two albatross species, which had the highest levels of plastic ingestion, but were almost absent in the other species, which ingest little or no plastics (Table 1). These observations may provide field evidence of the transfer of additives from ingested plastics and their accumulation in tissues. However, wild seabirds can also be exposed to these contaminants from their diet. Although there are limited data on the bioaccumulation of these additives, future studies should evaluate this exposure from the diet and assess the relative importance of diet and plastic-mediated exposure.

BDE209 has been sporadically detected in tissues of field-caught seabirds: 0.032 $\mu\text{g/g}$ lipid weight in liver of short-tailed shearwater [33], a few $\mu\text{g/g}$ lipid weight in liver of northern fulmar (*Fulmarus glacialis*) [36], and 0.106 $\mu\text{g/g}$ lipid weight in abdominal adipose of short-tailed shearwater [4]; plastics were suggested as a source. The concentration in liver was several orders of magnitude lower than we observed, but that in adipose was in the same range as we observed. The results of these studies suggest that similar levels of exposure to those in our experiment are occurring in the environment.

Globally, seabirds have suffered pervasive population declines over recent decades, with monitored populations declining, on average, by 70% between 1950 and 2010 [37]. Currently, nearly half of the world's species are experiencing population declines, and 28% are classified as globally threatened [38]. Given the worsening conservation status of species, chemical pollution represents a pervasive and growing

threat [39]. In particular, because chemical additives found in marine plastic debris include endocrine disruptors, adverse reproductive and developmental effects are possible [40, 41]. Based on the detection frequency (2%) of these additives in plastics (10 out of 194 pieces [13]), for species ingesting >20 pieces of plastic per individual (e.g., northern fulmar [42] and short-tailed shearwater [33]), >65% of individuals can be exposed to any of the five additives, which can be accumulated in their tissues. Furthermore, given the present trends, it is estimated that 99% of seabirds will have ingested plastic debris by 2050 [3]. Our findings provide direct evidence of plastic-derived chemical exposure in seabirds and underscore the importance of marine plastic debris as a growing source of pollutants in seabirds.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.cub.2019.12.037>.

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AUTHOR CONTRIBUTIONS

K.T., Y.W., H.T., and M.I. designed the study with input from R.Y., H.M., Y.I., and S.M.M.N.; K.T., Y.W., H.T., R.Y., M.K., K.M., and H.M. performed the exposure experiment; D.H. and M.H. provided field samples; K.T., N.H., and F.K. conducted chemical analysis; and K.T. and H.T. wrote the paper with input from all authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological Samples		
Black-footed albatross (<i>Phoebastria nigripes</i>)	Tern Island	N/A
Laysan albatross (<i>Phoebastria immutabilis</i>)	Oahu Island	N/A
Brown booby (<i>Sula leucogaster</i>)	Oahu Island	N/A
Red-footed booby (<i>Sula sula</i>)	Oahu Island	N/A
Brown noddy (<i>Anous stolidus</i>)	Oahu Island	N/A
Sooty tern (<i>Onychoprion fuscatus</i>)	Oahu Island	N/A
Chemicals, Peptides, and Recombinant Proteins		
Isoflurane	Wako Pure Chemical Industries	Cat# 099-06571
Polybrominated diphenyl ethers (BDE197, 203, 196, 208, 207, 206, and 209)	Wellington Laboratories	Cat# BDE-197, BDE-203, BDE-196, BDE-208, BDE-207, BDE-206, BDE-209
2-(2-hydroxy-3-tert-butyl-5-methylphenyl)-5-chlorobenzotriazole	AccuStandard	Cat# PLAS-UV-009N
2-(3,5-di-tert-amyl-2-hydroxyphenyl) benzotriazole	AccuStandard	Cat# PLAS-UV-012N
2-(3,5-di-tert-butyl-2-hydroxyphenyl)-5-chlorobenzotriazole	AccuStandard	Cat# PLAS-UV-011N
2-hydroxy-4-octyloxybenzophenone	AccuStandard	Cat# PLAS-UV-002S
4'-fluoro-2,2',3,3',4,5,5',6,6'-nonabromo-diphenylether	AccuStandard	Cat# FBDE-9001S
2-(2-hydroxy-3-tert-butyl-5-methylphenyl)-5-chlorobenzotriazole-d ₃	Toronto Research Chemicals	Cat# B689682
2-(3,5-di-tert-amyl-2-hydroxyphenyl) benzotriazole- ¹³ C ₆	Hayashi Pure Chemical Ind.	Cat# 99053228
2-(3,5-di-tert-butyl-2-hydroxyphenyl)-5-chlorobenzotriazole-d ₂₀	Toronto Research Chemicals	Cat# D428017
2-hydroxy-4-octyloxybenzophenone-d ₁₇	Hayashi Pure Chemical Ind.	Cat# 99052780
Acenaphthene-d ₈	Sigma-Aldrich	Cat# 452459
Chrysene-d ₁₂	Sigma-Aldrich	Cat# 442523
Silica-gel (Wakogel Q-22, through 75 μm)	Wako Pure Chemical Industries	Cat# 231-00115
Florisil (75–150 μm)	Wako Pure Chemical Industries	Cat# 066-05265
Experimental Models: Organisms/Strains		
streaked shearwater (<i>Calonectris leucomelas</i>)	Awashima Island	N/A
Software and Algorithms		
R version 3.5.3	R Foundation for Statistical Computing	https://www.r-project.org
Other		
Polyethylene pellets	DJK Corporation	N/A

LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Hideshige Takada (shige@cc.tuat.ac.jp). This study did not generate new unique reagents.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

We selected streaked shearwater (*Calonectris leucomelas*) chicks in a colony on Awashima Island (Niigata pref.), where all the materials are administered and controlled by Niigata prefecture. Approximately 84,000 streaked shearwaters breed in the colony [43]. We focused on the chick period because the amount of plastics found in the stomach is usually the highest before fledging [2]. To make the exposure experiment environmentally relevant, we conducted it entirely in the wild. Before starting the exposure experiment, we numbered 52 chicks with #1 to #52 and estimated their ages by wing length and bill length [44], and selected 21 chicks in the same stage of development and age (hatched from 17 to 24 August 2017) for the exposure experiment. Then, the birds were randomly assigned to one of two treatments: experimental and control. Because these were wild birds, the sample size was set 5 – 6 birds per treatment group, which was sufficient to perform statistical comparisons.

All procedures were approved by the Animal Care and Use Committee of the Graduate School of Veterinary Medicine, Hokkaido University (approval number 17-0095).

METHOD DETAILS

Preparation of plastics with additives

We used cylindrical low density polyethylene (LDPE) pellets (diameter 5 mm, length 5 mm; DJK Corporation, Chiba, Japan) compounded with 2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether (BDE209, CAS no. 1163-19-5), 2-(2-hydroxy-3-tert-butyl-5-methylphenyl)-5-chlorobenzotriazole (UV-326, CAS no. 3896-11-5), 2-(3,5-di-tert-amyl-2-hydroxyphenyl) benzotriazole (UV-328, CAS no. 25973-55-1), 2-(3,5-di-tert-butyl-2-hydroxyphenyl)-5-chlorobenzotriazole (UV-327, CAS no. 3864-99-1), and 2-hydroxy-4-octyloxybenzophenone (BP-12, CAS no. 1843-05-6). PE powder (Flo-Thene, FG701N, Sumitomo Seika Chemicals Co., Ltd., Osaka, Japan) and authentic standards of the additives in powder form (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were mixed well and molded into pellets in a co-rotating twin-screw kneading extruder (HK-25D; Parker Corporation, Inc., Tokyo, Japan). The pellets were then melted and re-extruded twice to obtain a uniform distribution of the constituents. The mean weight of the pellets was $0.084 \text{ g} \pm 0.0009 \text{ g}$. The concentrations of the chemicals were quantified (Table S1).

Experimental design

We used twenty-one 37-day-old chicks, which were partly feathered and less than half-way through their development stage, from hatching to fledging at approximately 80 days [43]. We fed 11 chicks 5 pellets on day 0. The pellets were put on the throat of the chicks by using tweezers and were smoothly ingested by the chicks. Every 5 days, we measured body weight, bill length, bill depth, head length, wing length, tarsus length, and tail length, and collected feces. On day 15 or 16, we euthanized 11 chicks (exposed: 6 birds, control: 5 birds), and on day 32 (day 29–33 in control group), euthanized another 10 (exposed: 5 birds, control: 5 birds;) with an overdose of isoflurane. We dissected them immediately. Bird #38 died of illness on day 15 but was included in the analysis. The chicks were dissected with stainless-steel tools rinsed with methanol and acetone three times each. During the dissection, the tools were cleaned with water and methanol before each tissue. Preen gland oil was sampled by wiping the preen gland with a glass fiber filter (Whatman GF/F, Whatman International Ltd., Maidstone, Kent, UK). The abdominal adipose, liver were removed, put in pre-baked (at 550°C for 4 h) glass vials, and stored in a freezer at -30°C until analysis. In all treated birds, all 5 pellets were retrieved from the stomach and showed no apparent changes. No other plastic fragments were found in the stomach. The body weights of the birds were 490–780 g at administration, 460–840 g at the first dissection, and 550–1050 g at the second dissection. Body weight change ranged from no clear change to gradual increase, as seen in streaked shearwaters on Awashima Island [44].

Sampling of preen gland oil from wild seabirds

We sampled preen gland oil from 18 freshly-dead salvaged seabirds representing 6 species sampled opportunistically in the Hawaiian Islands, i.e., 3 adult black-footed albatrosses (*Phoebastria nigripes*) from Tern Island in 2012, 3 adult Laysan albatrosses (*Phoebastria immutabilis*) from Oahu Island in 2015, 3 adult brown boobies (*Sula leucogaster*) from Oahu Island in 2014–2015, 1 adult and 2 juvenile red-footed boobies (*Sula sula*) from Oahu Island in 2014, 3 adult brown noddies (*Anous stolidus*) from Oahu Island in 2011, and 3 adult sooty terns (*Onychoprion fuscatus*) from Oahu Island in 2011. We took preen gland oil (~50 mg) by wiping with pre-baked glass fiber filter, and stored in a freezer at -30°C until analysis.

Materials for chemical analysis

Seven congeners of polybrominated diphenyl ethers (octa- to deca-brominated; BDE197, 203, 196, 208, 207, 206, and 209) were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada). Three benzotriazole ultraviolet (UV) stabilizers (UV-326, UV-328, and UV-327) and one benzophenone UV stabilizer (BP-12) were purchased from AccuStandard, Inc. (New Haven, CT, USA). As surrogate standard for PBDEs, 4'-fluoro-2,2',3,3',4,5,5',6,6'-nonabromo-diphenylether (F-BDE208) was purchased from AccuStandard. As surrogate standards for UV stabilizers, UV-326- d_3 and UV-327- d_{20} were purchased from Toronto Research Chemicals, Inc. (North York, ON Canada), and UV-328- $^{13}\text{C}_6$ and BP-12- d_{17} were purchased from Hayashi Pure Chemical Ind., Ltd. (Osaka, Japan). As internal injection standards, acenaphthene- d_8 and chrysene- d_{12} were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hexane, acetone, 2,2,4-trimethylpentane (iso-octane), methanol, pyridine, acetic anhydride, hydrochloric acid, anhydrous sodium sulfate, silica-gel (Wakogel Q-22, through $75 \mu\text{m}$), and Florisil ($75\text{--}150 \mu\text{m}$) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Dichloromethane (DCM) was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Hexane and acetone were distilled in glass. All glass and stainless steel equipment was rinsed with methanol, acetone, and distilled hexane three times each, or pre-baked at 550°C for 4 h.

Chemical analysis of tissues

We first weighed ~1 g (wet weight) of liver (from the right lobe) and ~0.1 g (wet weight) of abdominal adipose tissue and then extracted them in a Polytron PT2000 homogenizer with DCM and anhydrous sodium sulfate. Preen gland oil was extracted from the glass fiber filters by ultrasonication in DCM. The extracts were spiked with surrogate standards (25 ng F-BDE208, UV-326- d_3 , UV-328- $^{13}\text{C}_6$, and UV-327- d_{20} , and 50 ng BP-12- d_{17}), and then reduced in volume to 10 mL on a rotary evaporator and centrifuged (737 g for 30 min). Half of the extract was subjected to further chemical analysis of the additives, whereas the rest was used to measure lipid contents.

The extracts for the chemical analysis was rotary-evaporated just to dryness and transferred onto a 10%-H₂O-deactivated silica gel column (1 cm i.d. × 1.5 cm). Elution with 10 mL hexane/DCM (3:1, v/v) eluted PBDEs, UV-326, UV-328, and UV-327 (first fraction). Elution with 10 mL DCM eluted BP-12 (second fraction). The first fraction was rotary-evaporated just to dryness and transferred onto a 10%-H₂O-deactivated Florisil column (1 cm i.d. × 9 cm). It was eluted with 10 mL hexane/DCM (3:1, v/v), rotary-evaporated to ~0.5 mL, and transferred to a 1-mL amber glass ampoule. The solvent was evaporated just to dryness under a gentle nitrogen stream and the residue was re-dissolved in 200 μ L *iso*-octane containing internal injection standards (acenaphthene-d₈ and chrysene-d₁₂, 1.25 ppm). The second fraction was reduced to ~0.5 mL on a rotary evaporator and transferred to 1.5-mL clear vials. The solvent was evaporated to ~50 μ L under a gentle nitrogen stream. To each vial we added 50 μ L each of pyridine and acetic anhydride. After holding at room temperature for > 8 h, the reaction was stopped with the addition of 200 μ L 4 N HCl. Acetylates, including the target analyte BP-12, were extracted with *n*-hexane. The hexane extract was passed through anhydrous sodium sulfate for dehydration and collected in 2-mL amber glass ampoules. The hexane was evaporated just to dryness under a gentle nitrogen stream, and the residue was transferred onto a 10%-H₂O-deactivated silica gel column (1 cm i.d. × 1.5 cm). Following elution with 20 mL hexane/DCM (3:1, v/v), elution with 30 mL DCM eluted BP-12. This second fraction was rotary-evaporated just to dryness, and the residue was redissolved in ~2 mL DCM and separated by gel permeation chromatography (2 cm i.d. × 30 cm, CLNpak PAE-2000; Showa-Denko, Tokyo, Japan) in DCM at 4 mL/min. The fraction with a retention time of 11 to 15 min was collected, rotary-evaporated to ~0.5 mL, and transferred to a 1-mL amber glass ampoule. The solvent was evaporated just to dryness under a gentle nitrogen stream and the residue was redissolved in 200 μ L of *iso*-octane containing internal injection standards (acenaphthene-d₈ and chrysene-d₁₂, 1.25 ppm).

Aliquots of 1 μ L were analyzed by GC-ECD for PBDEs and by GC-ion-trap mass spectrometry (GC-IT-MS) for benzotriazole and benzophenone UV stabilizers. Concentrations of the contaminants in the samples were corrected against the recovery of the surrogates. All results are presented in [Data S1A–F](#).

Repeatability and recovery of the analytical procedures for the tissue samples were confirmed in advance through 4 replicate analyses of liver tissue extracts with and without spiking of native standards. The relative standard deviations (RSDs) of the concentrations of target contaminants were $\leq$ 4% and the recoveries were 95% to 108%. Those of surrogates and individual compounds are listed in [Table S3](#). A procedural blank was run with every batch of 7 samples. The limit of detection (LOD) was set at 3 × the signal-to-noise ratio on the detector. The limit of quantification (LOQ) was set at 3 × the amount detected in the procedural blank. LOD and LOQ values for the analysis of each tissue are listed in [Table S4](#).

By using the other half of the extracts, weight of lipid was measured gravimetrically or an instrument, i.e., Iatroscan (MLK-6, LSI Medience Corporation, Tokyo, Japan) where aliquots of dried lipid are combusted and amounts of generated CO₂ are quantified by flame ionization detector (FID). To calibrate for quantification, gravimetrically-measured-preen grand oil from short-tailed shearwater (*Puffinus tenuirostris*) was used.

Chemical analysis of plastics

For efficient extraction, each plastic pellet was cut into approximately 15 pieces < 0.5 mm thick and put into hexane at a liquid-to-solid ratio of 100:1 by volume at 40°C for 72 h. An aliquot of 100 μ L of the extract was evaporated just to dryness under a nitrogen stream and the residue was redissolved in *iso*-octane. After surrogate standards and internal injection standards were added, 1 μ L was injected into the GC equipped with ECD or IT-MS as for tissue analysis.

Repeatability and recovery of the analytical procedures for the plastic samples were confirmed through 4 replicate analyses of plastic extracts with and without spiking of native standards. The RSDs of concentrations of BDE209, UV-326, UV-328, UV-327, and BP-12 were all < 4%, and their recoveries were 91%, 105%, 100%, 99%, and 97%, respectively. The extraction efficiency was confirmed by successive extraction as follows: After the first extraction, the pieces were taken out, cut further into > 70 pieces, and then extracted again with new hexane at 40°C for 72 h. The second extracts had < 1% of the contents of target additives found in the first extracts, indicating an extraction efficiency of > 99%. Five replicate analyses were conducted to quantify the concentrations of the additives in the pellets ([Table S1](#)). Concentrations of additives in plastic pellets recovered from chicks' digestive tracts are presented in [Data S2A](#).

QUANTIFICATION AND STATISTICAL ANALYSIS

Data were analyzed using R version 3.5.3 (R Foundation for Statistical Computing, Vienna, Austria). The censored values (under detection limit) were substituted by 1/2 LOD. Data are presented as mean $\pm$ standard error. Two-sided Welch's *t* test was used to compare concentrations in tissues between plastic exposure and control groups, whereby variances were not assumed to be equal. Significant difference assessed at *p* < 0.05. Statistical details are noted in the legend of [Figure 1](#).

DATA AND CODE AVAILABILITY

The published article includes all datasets generated or analyzed during this study.