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SUPPLEMENTARY MATERIALS

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References (31–80)

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MARINE POLLUTION

Plastic waste inputs from land into the ocean

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Plastic debris in the marine environment is widely documented, but the quantity of plastic entering the ocean from waste generated on land is unknown. By linking worldwide data on solid waste, population density, and economic status, we estimated the mass of land-based plastic waste entering the ocean. We calculate that 275 million metric tons (MT) of plastic waste was generated in 192 coastal countries in 2010, with 4.8 to 12.7 million MT entering the ocean. Population size and the quality of waste management systems largely determine which countries contribute the greatest mass of uncaptured waste available to become plastic marine debris. Without waste management infrastructure improvements, the cumulative quantity of plastic waste available to enter the ocean from land is predicted to increase by an order of magnitude by 2025.

Reports of plastic pollution in the ocean first appeared in the scientific literature in the early 1970s, yet more than 40 years later, no rigorous estimates exist of the amount and origin of plastic debris entering the marine environment. In 1975, the estimated annual flux of litter of all materials to the ocean was 6.4 million tons [5.8 million metric tons (MT)], based only on discharges from ocean vessels, military operations, and ship casualties (1). The discharge of plastic from at-sea vessels has since been banned (2), but losses still occur. It is widely cited that 80% of marine debris originates from land; however, this figure is not well substantiated and does not inform the total mass of debris entering the marine environment from land-based sources.

Plastics have become increasingly dominant in the consumer marketplace since their commercial development in the 1930s and 1940s. Global plastic resin production reached 288 million MT in 2012 (3), a 620% increase since 1975. The largest market sector for plastic resins is packaging (3); that is, materials designed for immediate disposal. In 1960, plastics made up less than 1% of municipal solid waste by mass in the United States (4); by 2000, this proportion increased by an order of magnitude. By 2005, plastic made up at least 10% of solid waste by mass in 58% (61 out of 105) of countries with available data (5).

Plastics in the marine environment are of increasing concern because of their persistence and effects on the oceans, wildlife, and, potentially, humans (6). Plastic debris occurs on coastlines, in Arctic sea ice, at the sea surface, and on the sea floor (7, 8). Weathering of plastic debris causes fragmentation into particles that even small marine invertebrates may ingest (9). Its small size also renders this debris untraceable to its source and extremely difficult to remove from open ocean environments, suggesting that the most effective mitigation strategies must reduce inputs.

We estimated the annual input of plastic to the ocean from waste generated by coastal populations worldwide. We defined mismanaged waste as material that is either littered or inadequately disposed. Inadequately disposed waste is not formally managed and includes disposal in dumps or open, uncontrolled landfills, where it is not fully contained. Mismanaged waste could eventually enter the ocean via inland waterways, wastewater outflows, and transport by wind or tides. Estimates of the mass of plastic waste carried by particular waterways range from <<1 kg per day (Hilo, HI) to 4.2 MT (4200 kg) per day (Danube River) (10, 11). Because of their dependence on local watershed characteristics, these results cannot be easily extrapolated to a global scale.

Here we present a framework to calculate the amount of mismanaged plastic waste generated annually by populations living within 50 km of a coast worldwide that can potentially enter the ocean as marine debris. For each of 192 coastal countries with at least 100 permanent residents that border the Atlantic, Pacific, and Indian oceans and the Mediterranean and Black seas, the framework includes: (i) the mass of waste generated per capita annually; (ii) the percentage of waste that is plastic; and (iii) the percentage of plastic waste that is mismanaged and,

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therefore, has the potential to enter the ocean as marine debris (12) (data S1). By applying a range of conversion rates from mismanaged waste to marine debris, we estimated the mass of plastic waste entering the ocean from each country in 2010, used population growth data (13) to project the increase in mass to 2025, and predicted growth in the percentage of waste that is plastic. Lacking information on future

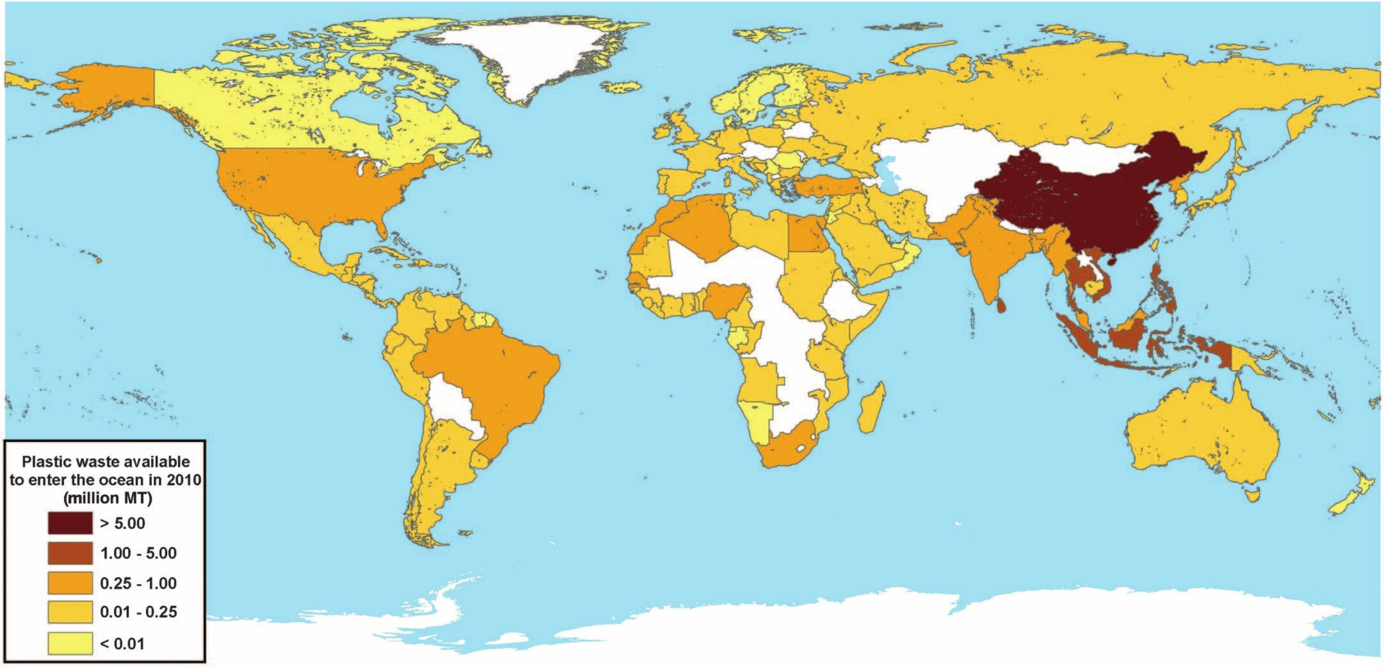

Fig. 1. Global map with each country shaded according to the estimated mass of mismanaged plastic waste [millions of metric tons (MT)] generated in 2010 by populations living within 50 km of the coast. We considered 192 countries. Countries not included in the study are shaded white.

Table 1. Waste estimates for 2010 for the top 20 countries ranked by mass of mismanaged plastic waste (in units of millions of metric tons per year). Econ classif., economic classification; HIC, high income; UMI, upper middle income; LMI, lower middle income; LI, low income (World Bank definitions on 2010 Gross National Income). Mismanaged waste is the sum of inadequately managed waste plus 2% littering. Total mismanaged plastic waste is calculated for populations within 50 km of the coast in the 192 countries considered. pop., population; gen., generation; ppd, person per day; MMT, m metric tons.

Rank	Country	Econ. classif.	Coastal pop. [millions]	Waste gen. rate [kg/ppd]	% plastic waste	% mismanaged waste	Mismanaged plastic waste [MMT/year]	% of total mismanaged plastic waste	Plastic marine debris [MMT/year]
1	China	UMI	262.9	1.10	11	76	8.82	27.7	1.32–3.53
2	Indonesia	LMI	187.2	0.52	11	83	3.22	10.1	0.48–1.29
3	Philippines	LMI	83.4	0.5	15	83	1.88	5.9	0.28–0.75
4	Vietnam	LMI	55.9	0.79	13	88	1.83	5.8	0.28–0.73
5	Sri Lanka	LMI	14.6	5.1	7	84	1.59	5.0	0.24–0.64
6	Thailand	UMI	26.0	1.2	12	75	1.03	3.2	0.15–0.41
7	Egypt	LMI	21.8	1.37	13	69	0.97	3.0	0.15–0.39
8	Malaysia	UMI	22.9	1.52	13	57	0.94	2.9	0.14–0.37
9	Nigeria	LMI	27.5	0.79	13	83	0.85	2.7	0.13–0.34
10	Bangladesh	LI	70.9	0.43	8	89	0.79	2.5	0.12–0.31
11	South Africa	UMI	12.9	2.0	12	56	0.63	2.0	0.09–0.25
12	India	LMI	187.5	0.34	3	87	0.60	1.9	0.09–0.24
13	Algeria	UMI	16.6	1.2	12	60	0.52	1.6	0.08–0.21
14	Turkey	UMI	34.0	1.77	12	18	0.49	1.5	0.07–0.19
15	Pakistan	LMI	14.6	0.79	13	88	0.48	1.5	0.07–0.19
16	Brazil	UMI	74.7	1.03	16	11	0.47	1.5	0.07–0.19
17	Burma	LI	19.0	0.44	17	89	0.46	1.4	0.07–0.18
18*	Morocco	LMI	17.3	1.46	5	68	0.31	1.0	0.05–0.12
19	North Korea	LI	17.3	0.6	9	90	0.30	1.0	0.05–0.12
20	United States	HIC	112.9	2.58	13	2	0.28	0.9	0.04–0.11

*If considered collectively, coastal European Union countries (23 total) would rank eighteenth on the list global infrastructure development, the projection represents a business-as-usual scenario.

We estimate that 2.5 billion MT of municipal solid waste was generated in 2010 by 6.4 billion people living in 192 coastal countries (93% of the global population). This estimate is broadly consistent with an estimated 1.3 billion MT of waste generated by 3 billion people in urban centers globally (5). Approximately 11% (275 million MT) of the waste generated by the total population of these 192 countries is plastic. We expect plastic waste to roughly track plastic resin production (270 million MT in 2010) (3), with differences resulting from the time lag in disposal of durable goods (lifetime of years to decades), for example. Scaling by the population living within 50 km of the coast (those likely to generate most of the waste becoming marine debris), we estimate that 99.5 million MT of plastic waste was generated in coastal regions in 2010. Of this, 31.9 million MT were classified as mismanaged and an estimated 4.8 to 12.7 million MT entered the ocean in 2010, equivalent to 1.7 to 4.6% of the total plastic waste generated in those countries.

Our estimate of plastic waste entering the ocean is one to three orders of magnitude greater than the reported mass of floating plastic debris in high-concentration ocean gyres and also globally (14–17). Although these ocean estimates represent only plastics that are buoyant in seawater (mainly polyethylene and polypropylene), in 2010 those resins accounted for 53% of plastic production in North America and 66% of plastic in the U.S. waste stream (4, 18). Because no global estimates exist for other sources of plastic into the ocean (e.g., losses from fishing activities or at-sea vessels, or input from natural disasters), we do not know what fraction of total plastic input our land-based waste estimate represents.

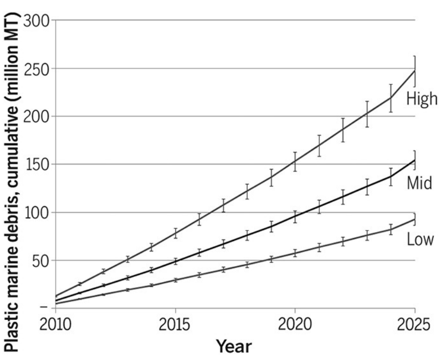

Fig. 2. Estimated mass of mismanaged plastic waste (millions of metric tons) input to the ocean by populations living within 50 km of a coast in 192 countries, plotted as a cumulative sum from 2010 to 2025. Estimates reflect assumed conversion rates of mismanaged plastic waste to marine debris (high, 40%; mid, 25%; low, 15%). Error bars were generated using mean and standard error from the predictive models for mismanaged waste fraction and percent plastic in the waste stream (12).

Our framework was designed to compute, from the best-available data, an order-of-magnitude estimate of the amount of mismanaged plastic waste potentially entering the ocean worldwide. It is also a useful tool to evaluate the factors determining the largest sources of mismanaged plastic waste. The amount of mismanaged plastic waste generated by the coastal population of a single country ranges from 1.1 MT to 8.8 million MT per year, with the top 20 countries' mismanaged plastic waste encompassing 83% of the total in 2010 (Fig. 1 and Table 1). Total annual waste generation is mostly a function of population size, with the top waste-producing countries having some of the largest coastal populations. However, the percentage of mismanaged waste is also important when assessing the largest contributors of waste that is available to enter the environment. Sixteen of the top 20 producers are middle-income countries, where fast economic growth is probably occurring but waste management infrastructure is lacking (the average mismanaged waste fraction is 68%). Only two of the top 20 countries have mismanaged fractions <15%; here, even a relatively low mismanaged rate results in a large mass of mismanaged plastic waste because of large coastal populations and, especially in the United States, high per capita waste generation.

Assuming no waste management infrastructure improvements, the cumulative quantity of plastic waste available to enter the marine environment from land is predicted to increase by an order of magnitude by 2025 (Fig. 2 and table S1). The predicted geographic distribution of mismanaged plastic waste in 2025 does not change substantially, although the disparity between developing and industrialized countries grows (table S2). For example, mismanaged plastic waste in the United States increases by 22%, whereas in the top five countries it more than doubles. The increase in these middle-income countries results from population growth, waste generation rates for 2025 that are consistent with economic growth (5), and a projected increase in plastic in the waste stream.

The analytical framework can also be used to evaluate potential mitigation strategies. For example, if the fraction of mismanaged waste were reduced by 50% (i.e., a 50% increase in adequate disposal of waste) in the 20 top-ranked countries, the mass of mismanaged plastic waste would decrease 41% by 2025. This falls to 34% if the reduction is only applied to the top 10 countries and to 26% if applied to the top 5. To achieve a 75% reduction in the mass of mismanaged plastic waste, waste management would have to be improved by 85% in the 35 top-ranked countries. This strategy would require substantial infrastructure investment primarily in low- and middle-income countries.

Alternatively, reduced waste generation and plastic use would also decrease the amount of mismanaged plastic waste. If per capita waste generation were reduced to the 2010 average (1.7 kg/day) in the 91 coastal countries that exceed it, and the percent plastic in the waste stream were capped at 11% (the 192-country average in 2010), a 26% decrease would be achieved by 2025. This strategy would target higher-income countries and might require smaller global investments. With a combined strategy, in which total waste management is achieved (0% mismanaged waste) in the 10 top-ranked countries and plastic waste generation is capped as described above, a 77% reduction could be realized, reducing the annual input of plastic waste to the ocean to 2.4 to 6.4 million MT by 2025 (table S3).

Sources of uncertainty in our estimates result from the relatively few measurements of waste generation, characterization, collection, and disposal, especially outside of urban centers. Even where data were available, methodologies were not always consistent, and some activities were not accounted for, such as illegal dumping (even in high-income countries) and ad hoc recycling or other informal waste collection (especially in low-income countries). In addition, we did not address international import and export of waste, which would affect national estimates but not global totals. Although national estimates are somewhat sensitive to the model predicting the percentage of mismanaged waste, the global estimate and ranking of top countries are not. The long-term projections are also sensitive to the model predicting growth of plastic in the waste stream; historical growth may not be a good indicator of future trends (12). The inclusion of the economic cost of implementation, as well as socio-cultural, environmental, and other factors that affect infrastructure development or behavioral change, would improve the evaluation of mitigation strategies (19).

We will not reach a global "peak waste" before 2100 (20). Our waste will continue to grow with increased population and increased per capita consumption associated with economic growth, especially in urban areas and developing African countries (see supplementary materials). Historically, waste management by burying or burning waste was sufficient for inert or biodegradable waste, but the rapid growth of synthetic plastics in the waste stream requires a paradigm shift. Long-term solutions will likely include waste reduction and "downstream" waste management strategies such as expanded recovery systems and extended producer responsibility (21, 22). Improving waste management infrastructure in developing countries is paramount and will require substantial resources and time. While such infrastructure is being developed, industrialized countries can take immediate action by reducing waste and curbing the growth of single-use plastics.

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SUPPLEMENTARY MATERIALS

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Fig. S1
Tables S1 to S6
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Data S1

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VIRAL REPLICATION

Structural basis for RNA replication by the hepatitis C virus polymerase

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Nucleotide analog inhibitors have shown clinical success in the treatment of hepatitis C virus (HCV) infection, despite an incomplete mechanistic understanding of NS5B, the viral RNA-dependent RNA polymerase. Here we study the details of HCV RNA replication by determining crystal structures of stalled polymerase ternary complexes with enzymes, RNA templates, RNA primers, incoming nucleotides, and catalytic metal ions during both primed initiation and elongation of RNA synthesis. Our analysis revealed that highly conserved active-site residues in NS5B position the primer for in-line attack on the incoming nucleotide. A b loop and a C-terminal membrane–anchoring linker occlude the active-site cavity in the apo state, retract in the primed initiation assembly to enforce replication of the HCV genome from the 3′ terminus, and vacate the active-site cavity during elongation. We investigated the incorporation of nucleotide analog inhibitors, including the clinically active metabolite formed by sofosbuvir, to elucidate key molecular interactions in the active site.

Hepatitis C virus (HCV) is a positive-sense, single-stranded RNA virus of the family Flaviviridae and genus Hepacivirus and is the cause of hepatitis C in humans (1). Long-term infection with HCV can lead to end-stage liver disease, including hepatocellular carcinoma and cirrhosis, making hepatitis C the leading cause of liver transplantation in the United States (2). Direct-acting antiviral drugs were approved in 2011, but they exhibited limited efficacy and had the potential for adverse side effects (3). The catalytic core of the viral replication complex, the NS5B RNA-dependent RNA polymerase (RdRp), supports a staggering rate of viral production, estimated to be 1.3×10^{12} virions produced per day in each infected patient (4). Because the NS5B polymerase active site is highly conserved, nucleotide analog inhibitors offer advantages over other classes of HCV drugs, including activity across different viral genotypes and a high barrier to the development of resistance (5, 6). The nucleotide prodrug sofosbuvir was recently approved for combination treatment of chronic HCV (7, 8).

One substantial obstacle for the rapid discovery of effective nucleotide-based drugs for HCV was the lack of molecular detail concerning substrate recognition during replication. NS5B contains several noncanonical polymerase elements, including a C-terminal membrane anchoring tail and a thumb domain b-loop insertion (9–11), that are implicated in RNA synthesis initiation (12). To gain insight into the mechanism of HCV RNA replication and its inhibition by nucleotide analog inhibitors, we determined atomic-resolution ternary structures of NS5B in both primed initiation and elongation states.

Because traditional approaches failed to yield ternary complexes (see the supplementary materials), we prepared multiple stalled enzyme-RNA-nucleotide ternary complex structures containing several designed features. First, we used NS5B from the JFH-1 genotype 2a isolate of HCV, which is extraordinarily efficient at RNA synthesis (13). Second, we exploited a conformational stabilization strategy that had been developed for structural analysis of G protein–coupled receptors (14). We hypothesized that a triple resistance NS5B mutant isolated under selective pressure of a guanosine analog inhibitor that exhibits 1.5 times the initiation activity of the wild type (15) might stabilize a specific conformational state along the initiation pathway. Indeed, this triple mutant exhibits a substantial structural rearrangement of the polymerase (15), which is consistent with the structural rearrangement observed in binary complexes of a b-loop deletion mutant bound to primer-template RNA (16). The triple mutant was able to incorporate native and nucleotide analog inhibitors with the RNA samples used in structure determination (fig. S1). The use of nucleotide diphosphate substrates rather than nucleotide triphosphates (fig. S2) generates stalled polymerase complexes in a catalytically relevant conformation. Ternary complexes could be obtained only with Mn^{2+}, which lowers the Michaelis constant (K_m) of the initiating nucleotide (17) and increases the activity of NS5B 20-fold relative to Mg^{2+} (18), and only with a nucleotide/Mn^{2+}/double-stranded RNA ratio of 1.0/0.6/0.2. These approaches designed to stabilize the incoming nucleotide allowed for soaking experiments targeting several distinct assemblies.

Hepatitis C virus NS5B initiates RNA synthesis by a primer-independent mechanism. Two slow steps in the catalytic pathway have been identified, including the formation of an initial dinucleotide

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