# **PLASTIC WASTE**

# Mixed plastics waste valorization through tandem chemical oxidation and biological funneling

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Mixed plastics waste represents an abundant and largely untapped feedstock for the production of valuable products. The chemical diversity and complexity of these materials, however, present major barriers to realizing this opportunity. In this work, we show that metal-catalyzed autoxidation depolymerizes comingled polymers into a mixture of oxygenated small molecules that are advantaged substrates for biological conversion. We engineer a robust soil bacterium, Pseudomonas putida, to funnel these oxygenated compounds into a single exemplary chemical product, either β-ketoadipate or polyhydroxyalkanoates. This hybrid process establishes a strategy for the selective conversion of mixed plastics waste into useful chemical products.

lastics have revolutionized modern life because of their low cost and utility in a vast range of applications. However, the accumulation of synthetic polymers in landfills and the environment has created a global pollution crisis that the existing reclamation and recycling infrastructure is not equipped to resolve (1-3). This challenge has accelerated investigation into new chemical recycling technologies that could enable the conversion of plastic waste streams into valuable chemicals and support the creation of a circular plastics economy (3-11). Many chemical recycling approaches focus on selective depolymerization of single plastic streams. However, plastics chemistry is diverse in both monomer and bond type, and plastics in postconsumer waste are physically mixed and include complex materials, such as multilayer packaging. These features complicate and increase the cost of separation methods required to isolate individual polymers for recycling (3). Chemical recycling processes that enable the deconstruction of mixed plastics into valuable products without requiring sorting could bypass this limitation and substantially enhance plastics reclamation and recycling infrastructure.

mixed plastics are converted into single products through a tandem catalytic and biological pro-

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cess (Fig. 1). The initial catalytic step uses metalpromoted autoxidation to depolymerize mixed plastics into oxygenated intermediates that represent advantaged substrates for subsequent bioconversion (12). The biological step uses a robust engineered bacterial strain to funnel the mixed oxygenates into the target product, in this case illustrated by either  $\beta$ -ketoadipate or polyhydroxyalkanoates. This approach is demonstrated with mixtures of high-density polyethylene (HDPE), polystyrene (PS), and poly(ethylene terephthalate) (PET), which are among the most abundant components of postconsumer plastics waste.

Autoxidation is a complex chemical process in which initiation reactions generate organic radicals that react with O2 and undergo chain propagation to generate oxygenated products. A notable example of industrial autoxidation is the conversion of p-xylene to terephthalic acid in the Amoco process, which operates at ~80 million metric tons per year, where acetate salts of Co and Mn are used as catalysts with Br radicals to promote reaction of the hydrocarbon with  $O_2$  (13). Partenheimer has shown that similar conditions support depolymerization of several pristine polymers (14). Autoxidation of polymers is initiated by H-atom transfer from C-H bonds in the polymer backbone to generate alkyl radicals, which react at diffusion-controlled rates with O2 to form peroxide intermediates (Fig. 2A). Homolytic cleavage of the O-O bond, aided by the Co and Mn catalysts, forms alkoxyl radicals that can undergo C-C cleavage through β-scission steps. This collection of H-atom transfer, radical trapping by O2, and C-C cleavage steps ultimately affords a mixture of low-molecular weight oxygenated products (14). This precedent from Partenheimer, among other studies (15-17), prompted us to explore a similar approach as the initial stage of our mixed plastics valorization process.

Autoxidation studies began with singlecomponent commercial polymer resins including HDPE beads, PS beads, or PET powder. Use of bromide as a cocatalyst was avoided in our studies because it is corrosive and requires specialized reactor materials. N-hydroxyphthalimide (NHPI) has precedent as an initiator and cocatalyst in autoxidation (18) and proved to be an effective alternative to bromide.

Under autoxidation conditions, depolymerization of PS yields benzoic acid (62.9  $\pm$  1.1 mol %), HDPE yields a distribution of  $C_4$  to  $C_{22}$   $\alpha,\omega$ dicarboxylic acids (34.2 ± 0.7 mol %), and PET generates terephthalic acid (67.6 ± 1.4 mol %) (Fig. 2B, figs. S1 to S12, and tables S1 to S3). To compare results more easily between different polymers, molar yields were determined in terms of moles of carbon in the isolated product relative to the starting polymer, such that the maximum theoretical yield is 87.5% for PS, 100% for PE, and 80% for PET. This difference accounts for the loss of the methylene carbon in PS and the ethylene glycol carbons in PET. The molecular weight of the starting polymer had little influence on the product yields (fig. S3A and fig. S8A), which suggests that depolymerization occurs through random chain scission. The yields obtained for the single-component substrates in this study are comparable to those observed previously by Partenheimer when using bromidecontaining systems (14) and generated the expected products from the polymer precursor. Depolymerization of PS and PET affords a single major product-benzoic acid and terephthalic acid, respectively—whereas HDPE depolymerization led to a more complex product mixture, including dicarboxylic acids and fivemembered lactone-containing products (figs. S13 to S16 and tables S3 and S4). Experiments with PS and PET demonstrate that product yields are similar over a range of 1 to 10 wt % loadings, whereas higher HDPE loadings afford lower yields (fig. S17) that are attributed to in situ degradation of the dicarboxylic acid products.

We then shifted our efforts to reactions of mixed plastics, including binary mixtures of PS and HDPE and ternary mixtures of PS, HDPE, and PET, for both commercial resins and postconsumer plastics. The latter were derived from expanded PS (EPS) cups (e.g., Styrofoam), HDPE bottles (e.g., milk containers), or PET bottles (e.g., single-use beverage bottles). Depolymerization of PS and PET is favored at higher temperatures (180° and 210°C, respectively) and longer reaction times (5.5 hours) relative to HDPE (160°C, 2.5 hours) (Fig. 2B and figs. S1, S6, and S9) because HDPE generates products that degrade at higher temperatures (14, 19). Despite these differences, we identified conditions that support effective autoxidative depolymerization of mixed PS and HDPE as well as mixed PS, HDPE, and PET. Treatment of the mixed PS and HDPE commercial resins at 180°C for 5.5 hours led to benzoic acid (68.9  $\pm$  0.6 mol %) and dicarboxylic acids  $(22.5 \pm 0.5 \text{ mol }\%)$  (Fig. 2C and figs. S18 to S22). Very similar results were obtained from reactions of mixed PS and HDPE postconsumer plastics, showing a slight increase in benzoic acid  $(71.0 \pm 0.1 \text{ mol }\%)$  and similar dicarboxylic acid  $(22.1 \pm 0.3 \text{ mol }\%)$  yields. Analogous results were obtained from the PS, HDPE, and PET mixture of commercial resins and postconsumer plastics (Fig. 2C). These reactions were conducted at elevated temperature  $(210^{\circ}\text{C})$  to facilitate PET depolymerization, but despite this adjustment, autoxidation of these mix-

tures afforded the same products in similar (benzoic acid,  $59.5 \pm 2.3$  mol %) or moderately reduced (dicarboxylic acids,  $20.4 \pm 1.1$  mol %; terephthalic acid,  $62.9 \pm 1.4$  mol %) yields relative to those observed from depolymerization of the individual polymers (Fig. 2, B and C).

The mixture of oxygenates obtained from autoxidation of mixed plastics is sufficiently complex that it would require advanced separation methods to isolate and purify individual products suitable for downstream application. This challenge is akin to that encountered in thermal

treatments of mixed plastics waste, such as pyrolysis. The autoxidation products are appealing, however, because they have enhanced water solubility that make them advantaged feedstocks for biological funneling, wherein an engineered microbe converts diverse chemicals to a single product (20). To pursue this opportunity, we engineered two strains of *Pseudomonas putida* (21, 22): first, to convert acetate,  $C_4$  to  $C_{17}$  dicarboxylates, benzoate, and terephthalate to polyhydroxyalkanoates, a natural polyester with growing industrial applications (23), and second,

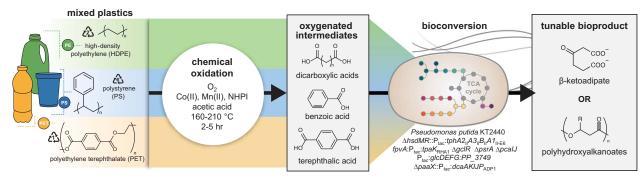
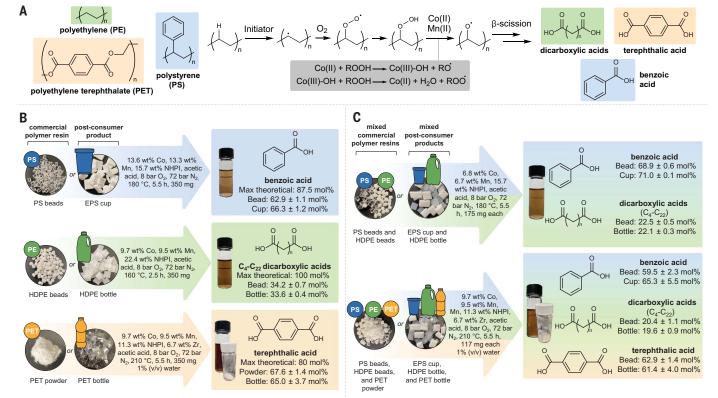


Fig. 1. Concept for the upcycling of mixed plastic waste through tandem chemical oxidation and bioconversion. Metal-promoted autoxidation simultaneously deconstructs multiple polymers, generating a mixture of oxygenated

intermediates that are advantaged substrates for bioconversion. An engineered *P. putida* strain funnels the heterogeneous mixture of oxygenates into a single target product. TCA cycle, tricarboxylic acid cycle.



**Fig. 2. Oxidative depolymerization of mixed plastics. (A)** Autoxidation scheme, illustrated for HDPE, with primary products for all polymers shown. **(B)** Oxidation of single-component commercial polymer resins and postconsumer plastics. **(C)** Oxidation of mixed plastic substrates. Experimental details are

in the supplementary materials. Data are means  $\pm$  SDs; N=3 trials. Mole % values indicate mole % carbon in isolated products relative the total carbon in the starting polymer, and weight % values are relative to the total plastics loading. Tabulated values are included in data S1.

to use acetate and dicarboxylates for growth while converting benzoate and terephthalate to β-ketoadipate, a monomer for performance-advantaged polymers (24, 25) (Fig. 3).

P. putida natively uses acetate, benzoate, and C4 and C5 dicarboxylates as carbon sources, and we had previously engineered a strain of P. putida to use terephthalate and ethylene glycol (26), which was used a base strain for further engineering in the present study (figs. S23 and S24). Ackermann et al. have enabled adipate (C<sub>6</sub>) utilization in P. putida by combining the native phenylacetate pathway with select transport and  $\beta$ -oxidation reactions from Acinetobacter baylyi ADP1 (fig. S25) (27). We took a similar approach by heterologously expressing the A. baylyi dcaAKIJP operon (28) and deleting genes encoding two native repressors of phenylacetate catabolism and β-oxidation, PaaX and PsrA, respectively (29, 30). The resulting strain, AW162, grew on C<sub>4</sub> to C<sub>10</sub> dicarboxvlates when each was tested as a sole carbon source and simultaneously used C4 to C14 dicarboxylates when tested in a mixture (figs. S24 to S31 and tables S5 to S8). Additionally, the strain was not inhibited by trace catalyst, initiator, or lactone acid species (figs. S32 to S37).

Metal catalysts were recovered from crude, deconstructed polymers by suspension in water, NaOH addition, and filtration to remove the precipitated metal hydroxides (fig. S38). The resulting filter-sterilized solutions contained substrates congruent with the feedstocks, trace (<5  $\mu$ M) Co and Mn metals, and acetate (tables S9 to S12). These so-called effluent streams were used directly in microbial cultivations.

We next engineered and demonstrated biological funneling of intermediates from both commercial polymer resins and postconsumer plastics to polyhydroxyalkanoates and, separately, to  $\beta$ -ketoadipate. AW162 grew on effluent from mixed PS and HDPE commercial polymer resin as well as effluent from mixed EPS and HDPE postconsumer products, with simultaneous utilization of benzoate and  $C_4$  to  $C_{17}$  dicarboxylates (Fig. 4, A to C, and fig. S39). In effluent from mixed PS, HDPE, and PET commercial polymer resin, AW162 cultivations simultaneously used the aromatic and aliphatic substrates during growth (Fig. 4, D and E, and fig. S40).

AW162 was also used for polyhydroxyalkanoates production by cultivation in nitrogen-limited medium: Polyhydroxyalkanoates were produced from both effluent from mixed PS beads and HDPE beads as well as effluent from mixed postconsumer EPS cups and HDPE bottles; polyhydroxyalkanoates primarily comprised 3hydroxydodecanoic acid and 3-hydroxydecanoic acid (Fig. 4F and figs. S41 and S42). In previous work, deletion of pcalJ in P. putida has enabled the conversion of 4-hydroxybenzoate to β-ketoadipate at 41 g/liter, 0.8 g/liter per hour, and quantitative yield in bioreactors (24). In this case, pcalJ was deleted in strain AW162, resulting in strain AW307, to enable the conversion of benzoate and terephthalate to β-ketoadipate (Fig. 3 and Fig. 4G). β-ketoadipate production by AW307 was evaluated, with aliphatic substrates supporting growth and conversion of aromatic substrates to  $\beta$ -ketoadipate. Yields are reported as mole β-ketoadipate per mole aromatic monomer, where the theoretical yield is 1 mol/mol (see table S13 for consideration of aliphatic carbon that is used as the substrate for cellular growth). In effluent from mixed PS and HDPE commercial

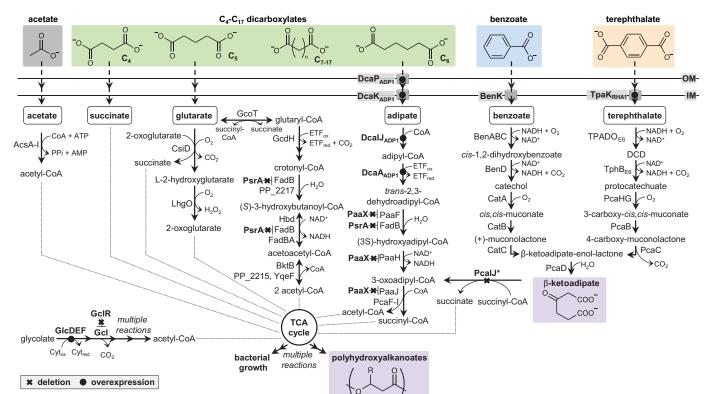
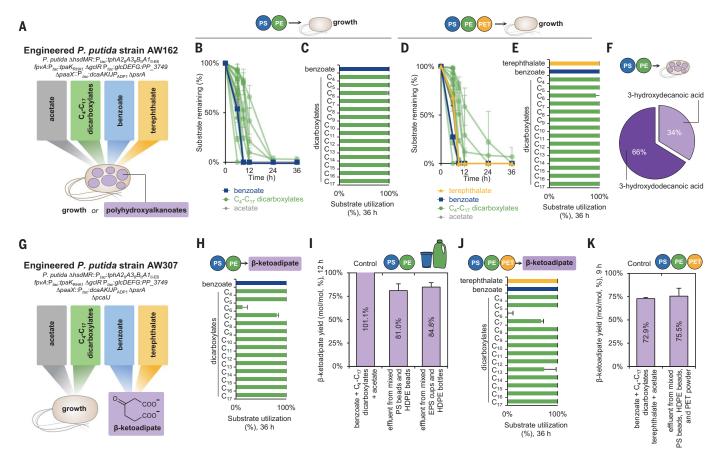


Fig. 3. Engineered metabolic pathways for biological funneling of PS-, HDPE-, and PET-derived oxygenated intermediates to β-ketoadipate or polyhydroxyalkanoates. Engineered expression changes to genes encoding the corresponding proteins are depicted in bold font, where gene deletion is indicated by an "×" and gene overexpression is indicated by a circle. Subscripts indicate a protein of heterologous origin, where ADP1 indicates *A. baylyi* ADP1, RHA1 indicates *R. jostii* RHA1, and E6 indicates *Comamonas* sp. E6. AW162: *P. putida* ΔhsdMR::P<sub>tac</sub>:tphA2<sub>II</sub>A3<sub>II</sub>B<sub>II</sub>A1<sub>II-E6</sub>fpvA:P<sub>tac</sub>:tpaK<sub>RHA1</sub> ΔglcR P<sub>tac</sub>:glcDEFG: PP\_3749 ΔpaaX::P<sub>tac</sub>:dcaAKIJP<sub>ADP1</sub> ΔpsrA. AW307: AW162 ΔpcaIJ. Genotypes and

strain construction details are provided in tables S5 to S8. CoA, coenzyme-A; ATP, adenosine 5'-triphosphate; AMP, adenosine 5'-monophosphate; ADP, adenosine 5'-diphosphate; PPi, pyrophosphate; ETF $_{\rm cx}$ , an oxidized electron-transfer flavoprotein; ETF $_{\rm red}$ , a reduced electron-transfer flavoprotein; NAD $^+$ , oxidized nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; TCA cycle, tricarboxylic acid cycle; Cyt $_{\rm ox}$ , an electron-transfer quinone; Cyt $_{\rm red}$ , an electron-transfer quinol; DCD, 1,2-dihydroxy-3,5-cyclohexadiene-1,4-dicarboxylate. GcoT is also referred to as GcdG (PP\_0159); all enzyme names and corresponding genes can be found in the BioCyc Database.



**Fig. 4. Bioconversion of effluent from mixed plastics to target products.**(A) AW162 was engineered to use all intermediates as substrates for growth or polyhydroxyalkanoates production. (B to E) Utilization of intermediates in effluent from mixed PS beads and PE beads [(B) and (C)] or in effluent from mixed PS beads, HDPE beads, and PET powder by AW162 [(D) and (E)].
(F) Composition of polyhydroxyalkanoates produced from effluent from mixed PS beads and HDPE beads by AW162. (G) AW307 was engineered to use acetate

and dicarboxylates for growth and convert benzoate and terephthalate to

β-ketoadipate. (**H**) Utilization of intermediates in effluent from mixed PS beads and HDPE beads by AW307. (**I**) β-ketoadipate yields (percentage of theoretical maximum) from control mixtures, effluent from mixed PS beads and HDPE beads, or effluent from mixed postconsumer EPS cups and HDPE bottles by AW307. (**J** and **K**) Utilization of intermediates in effluent from mixed PS beads, HDPE beads, and PET powder by AW307 (**J**) and β-ketoadipate yields from the same or control mixtures (K). Data are means  $\pm$  SDs; N = 3 trials. Genotypes and strain construction details are provided in tables S5 to S8.

polymer resins, all dicarboxylates except C<sub>6</sub> and C<sub>7</sub> were completely used, and benzoate was converted to  $\beta$ -ketoadipate at an 81.0  $\pm$  7.2% molar yield (Fig. 4, H and I, and fig. S43B). AW307 cultivations in effluent from mixed postconsumer EPS cups and HDPE bottles displayed similar growth and substrate utilization, generating  $\beta$ -ketoadipate at 84.8  $\pm$  4.7% molar yield (Fig. 4I and fig. S43C). Incomplete utilization of C<sub>6</sub> and C<sub>7</sub> dicarboxylates was observed, and β-ketoadipate decreased at extended time points (fig. S43), which may indicate promiscuous activity of PcaLJ on C6 and C7 dicarboxylates and/or DcaIJ on β-ketoadipate. In effluent from mixed PS. HDPE, and PET commercial polymer resins. β-ketoadipate was produced at a 75.5  $\pm$  8.5% molar yield (Fig. 4, J and K, and figs. S44 and S45). Assuming that no losses occurred during the processing of samples between the chemical and biological processes, overall molar β-ketoadipate vields from the aromatic constituents of the original plastics were 64% for mixed PS and HDPE commercial polymer resins; 69% for mixed PS and HDPE postconsumer plastics; and 57% for mixed PS, HDPE, and PET commercial polymer resins (tables S13 and S14).

This work demonstrates a process concept for pairing chemical and biological catalysis to convert mixed plastics into valuable products. Metal-catalyzed autoxidation offers a feedstockagnostic approach for mixed-polymer deconstruction into oxygenated small molecules, with advantages for biological funneling owing to their solubility in water and known catabolic pathways. Extensions from the individual and mixed PS, HDPE, and PET materials demonstrated in this work to other polymers susceptible to autoxidation, including polypropylene and polyvinyl chloride (14), are readily envisioned. Continuous reactor systems, such as those used in the Amoco process, should support higher polymer loadings by improving oxygen delivery and continuous removal of products to limit in situ degradation. Improvements in process integration will increase recovery of acetic acid and catalysts between the chemical and biological steps. Separations could also enable the isolation of valuable autoxidation products before bioconversion. Further metabolic engineering will enable bioprocess improvements to access higher titers and rates and production of other products (31) beyond the two examples we have demonstrated here. Ideal targets include the synthesis of monomers for intrinsically circular polymers (10, 32). Techno-economic analysis and life cycle assessment will be necessary to guide future progress toward economical and sustainable processes (11).

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#### **ACKNOWLEDGMENTS**

We thank R. Allen, A. Cuthbertson, S. Hesse, K. Knauer, J. Kruger, C. Lahive, D. Moore, N. Rorrer, C. Tassone, N. Wierckx, Y. Ackermann, W. McNeary, H. Alt. and members of the BOTTLE Consortium for helpful discussions. We thank K. Beers and S. V. Orski at the National Institute of Standards and Technology for supplying a 1475A HDPE standard. Funding: Funding was provided by the US Department of Energy, Office of Energy Efficiency and Renewable Energy, Advanced Manufacturing Office (AMO), and Bioenergy Technologies Office (BETO). This work was performed as part of the BOTTLE Consortium and was supported by AMO and BETO under contract no. DE-AC36-08G028308 with the National Renewable Energy Laboratory (NREL), operated by the Alliance for Sustainable Energy, LLC. The BOTTLE Consortium includes members from MIT, funded under contract no. DE-AC36-08G028308 with NREL. Contributions by S.S.S. were supported by the US Department of Energy, Office of Basic Energy Sciences, under award no. DEFG02-05ER15690. Author contributions: Conceptualization: G.T.B., K.P.S., A.Z.W., K.J.R., L.D.E., and S.S.S. Investigation: K.P.S., A.Z.W., K.J.R., L.D.E., J.B., B.A.B., X.D., and I.P. Visualization: A.Z.W. and K.P.S. Funding acquisition: G.T.B., A.M.G., and Y.R.-L. Resources: D.G.B., F.B., B.L.B., S.J.H., M.A.I., M.O.K., W.E.M., J.M., and S.P.W. Supervision: G.T.B., S.S.S., and Y.R.-L. Writing - original draft: K.P.S., A.Z.W., and G.T.B. Writing – review & editing: All authors

have reviewed and approved of the manuscript. **Competing interests:** G.T.B., L.D.E., K.P.S., and A.Z.W. have filed a patent application on this concept, PCT/US2021/063725 "Upcycling Mixed Waste Plastic Through Chemical Depolymerization and Biological Funneling." The patent filer is the Alliance for Sustainable Energy, LLC. The authors declare no other competing interests. **Data and materials availability:** All data are available in the main text or the supplementary materials. Strains and plasmids are available upon request through a material transfer agreement with NREL. **License information:** Copyright © 2022 the authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original US government works. https://www.science.org/about/science-licenses-journal-article-reuse

#### SUPPLEMENTARY MATERIALS

science.org/doi/10.1126/science.abo4626 Materials and Methods Supplementary Text Figs. S1 to S45 Tables S1 to S14 References (33–56) Data S1

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Submitted 12 February 2022; resubmitted 7 July 2022 Accepted 19 August 2022 10.1126/science.abo4626



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Science, 378 (6616), • DOI: 10.1126/science.abo4626

### Funneling mixed waste with microbes

Current plastic recycling methods require sorting by chemical composition, a method that is expensive and results in products that are of lower quality and value than the starting plastic. If plastic waste could instead be converted to valuable chemical intermediates, then economical use of mixed waste as a feedstock might be feasible. Sullivan *et al.* developed a two-stage oxidation and biological funneling approach that can break down and reform mixtures of common consumer plastics (see the Perspective by Yan). The end products can be adjusted by metabolic engineering of the microbes in the second step, which should enable tailored conversion into various platform or specialty chemicals. —MAF

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