PeToPLA

A meta-study on the persistence and toxicity of PLA, and the formation of microplastics in various environments

Technical Summary Report

prepared by

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The sponsors had no influence on the outcome of the meta-study and did not interfere with the interpretation of the scientific evidence.

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Technical Summary

This meta-study reflects the current knowledge in the publicly available scientific literature and in institutional and company reports on polylactide, or poly(lactic acid), with focus on the aspects of biodegradation in the open environment, environmental impact and toxicology, and the implications for standard testing, certification, and regulation.

PLA is a versatile polymer with properties that can change and can be modulated over wide ranges. On its way from the producer as pellets until it becomes available on the market as a plastic product, and later possibly ends up in the open environment, the neat PLA may experience all kinds of material modifications. During production, the polymer is made with only a few ingredients and with every further step the material may be treated mechanically and with heat, further chemicals may be added, or other materials are combined or come in contact with until the final article is available.

All these aspects are not further regarded in depth, and mainly studies on neat PLA as a base material, either self-made on laboratory scale or from industrial production are considered in this report. It must be emphasized that, because of the above-mentioned potential material modifications, a final article made from or with PLA may have different environmental performance and effects than the material that leaves the polymer producer’s factory as raw material pellets.

Regarding the fate of PLA in the open environment the lead questions of this meta-study were:
How fast and to what extent does PLA degrade in the open environment and are microplastics formed, which are persistent? What is known about the ecotoxicity of PLA? Additionally, the policy and regulation on microplastics on EU level were addressed and test methods, standards and certifications regarding biodegradability and biodegradation in the environment were briefly summarized.

Hydrolysis is the dominant process for PLA degradation

PLA is a polyester and thanks to its polar groups is slightly hydrophilic. Water as moisture or in liquid form is taken up by PLA by diffusion. The presence of water molecules leads to the hydrolytic cleavage of ester bonds. The hydrolysis rate of PLA depends on a number of external factors including moisture level or water availability, time, temperature, pH and internal polymer-related properties such as molecular weight, crystallinity, ratio of L- and D-lactic acid, and the possible presence of accelerators or additives. Hydrolysis is temperature-dependent, and the rate of ester hydrolysis follows Arrhenius behavior within the boundaries of a physical state, i.e., below the glass transition temperature T_g, between T_g and the melting temperature T_m, and above T_m. There are discontinuities in the reaction rate when these boundaries are crossed, but within the ranges the reaction rate is predictable for given conditions. The ester groups within the polymer molecules are cleaved randomly by hydrolysis which leads to a decrease in molecular chain length, thus molecular weight. Each ester group hydrolyzed in the PLA chain generates a corresponding carboxylic acid end group. The occurrence of free carboxylic acid end groups in the PLA polymer matrix accelerates the hydrolysis by autocatalysis and renders the polymer matrix more polar which results in higher water uptake. Once the cleavage products are small enough to become soluble, such as oligomers and lactic acid monomers, they can leave the polymer matrix by diffusion, resulting in mass loss. During hydrolysis of initially high-molecular weight PLA the molecular weight decreases with time, while the polydispersity, which is a measure of the distribution of molecules of different chain length in the polymer matrix, hardly changes. This also explains that, although the molecular weight decreases continuously, at the beginning no or only little mass loss is measurable, and microbial mineralization of the polymer carbon to carbon dioxide is small and may initially not be detectable. This also leads to a substantial lag phase in biodegradation tests where carbon dioxide can only be measured after the soluble hydrolysis products are assimilated by microbes and then ultimately mineralized to carbon dioxide. When carbon dioxide becomes detectable, molecular weight reduction of the PLA polymer chains via hydrolysis has been going on for quite some time but remained unnoticed by this method. The observation of significant molecular weight reduction before mineralization commences as a manifestation of the bulk hydrolysis mechanism for PLA.

The effect of autocatalysis by acid end groups within the polymer matrix indicates that the hydrolysis of PLA is also influenced by the pH of the environment. PLA hydrolysis is slowest at neutral pH of the surrounding water, faster at very low pH and fastest at very high pH. Natural freshwater and seawater systems usually have a pH between 6 to 9 so here this effect is low.

PLA can consist of amorphous and crystalline regions. Hydrolysis occurs preferentially in the free amorphous parts of the polymer matrix and the crystalline domains can only be hydrolyzed from the edges. The reduction of the molecular weight of polymer chains by hydrolysis lowers the inner order and can lead to relaxation within the PLA and a recrystallization of the amorphous parts neighboring the crystallites. This means that during the degradation process the remaining polymeric material is changing its conformation, making PLA microstructure a ‘moving target’.

PLA also takes up water from moist air and hydrolysis at high humidity can be even faster than if immersed in water. In air, the acidic degradation products cannot diffuse out of the polymer matrix and therefore accelerate the rate of hydrolysis. In water, those acid hydrolysis products of low enough molecular weight (estimated to be <1000 g/mole) can diffuse out of the bulk into the hydrolysis medium and the autocatalytic effect is thereby slowed down. The rate of hydrolysis for PLA immersed in water is slightly lower than in highly humid air.

Hydrolysis affects all ester groups in the whole polymer matrix. This leads to hydrolytic degradation throughout the whole PLA article, in a process called *bulk erosion*. In thicker PLA articles immersed in water, autocatalysis is more pronounced in the core, below an outer layer or ‘skin’ of about 200 µm (Li et al. 1990a). PLA objects with a thickness or diameter below a threshold estimated to 200-300 µm (Grizzi et al. 1995), thus also PLA films, fibers, and small particles, do not exhibit this accelerated inner erosion pattern.

Direct *bio*degradation of high-molecular PLA by microbes can happen by *enzymatic hydrolysis*, which has been shown in laboratory experiments with natural microbes, and also with solutions of isolated enzymes. Because enzymes are too big to diffuse into the polymer matrix, enzyme catalyzed hydrolysis of PLA is a *surface erosion* process. In order to be active in PLA hydrolysis, microbes need to come in contact with the polymer, physically attach (Kimkes and Heinemann 2020; Zheng et al. 2021), and chemically sense the substrate. When signaling molecules have reached a threshold concentration, they induce microbial production and secretion of the enzyme onto the polymer surface (Harapanahalli et al. 2015), such that the enzyme’s active site is in proximity to ester groups in PLA chains.

Most of the 27 PLA depolymerases described so far are esterases, lipases, cutinases, and proteases from the serine hydrolase group (EC 3) and are characterized by the presence of a signal peptide enabling their secretion onto an insoluble substrate. The activity of some PLA-degrading bacteria can be enhanced by adding traces of protein such as silk fibroin, gelatin, soybean, or keratin to the medium.

Microbes identified and confirmed to directly hydrolyze PLA in controlled laboratory experiments were isolated from soil, compost, sludge, garbage, or waste dump sites and comprise 78 bacteria, 27 fungi and two archaea. As some soils were wet soils from e.g., rice fields or dump sites their potential presence in freshwater systems is likely. Few PLA-degraders were isolated from marine samples. So far, no evidence for direct enzymatic hydrolysis of PLA in the open environment has been provided, maybe due to the lack of suitable methods.

However, even without direct depolymerization of PLA by microbial enzymatic hydrolysis, it can be assumed that lactic acid and short-chain PLA oligomers produced by chemical hydrolysis do not accumulate in the environment. These low-molecular hydrolysis products are bioassimilated, i.e. used by a variety of microbes as building blocks for biomass and as a source of energy, and eventually completely mineralized. For a comprehensive overview of PLA-degrading enzymes, see Tournier et al. (2023).

The occurrence of PLA microplastic in nature has been reported only a few times from environmental samples, with the findings of a single or a few fibers or particles in soil, freshwater, and the marine environments. In large sampling campaigns in the last years, biodegradable plastic materials in general were not in focus, and analytically not considered. As the market volume is increasing and PLA applications for outdoor use also increase, it is likely that findings will increase, at least occasionally.

From exposure experiments, PLA is reported to disintegrate in soil, freshwater, and marine settings over the period of several months to years at ambient temperature. Mass loss, along with changes in mechanical properties and molecular weight was used as measure for PLA degradation. However, several *short-term* field experiments and laboratory tests over several months with PLA articles placed in soil, water, and sediment have been published with the statement that PLA is not biodegradable in the open environment (e.g., Royer et al. 2023). Most of these studies seem to neglect or are not aware of the fact that the molecular degradation of PLA by hydrolysis is a bulk erosion process, which for a longer initial time is not measurable as mass loss but only as a decrease in molecular weight until the molecular fragments are small enough to become soluble and diffuse out of the polymer matrix. Long-term studies that take into account slow degradation rates are rare, and there is no systematic picture yet. Methods for long-term degradation studies and direct analytical methods for field tests are missing.

The formation of micro- and nanoparticles is part of the degradation process of natural polymers such as cellulose, chitin, or keratin and degradable synthetic plastic polymers alike. Even rapidly biodegrading plastics do not dissolve but physically fall into pieces and consequently, as a transition stage are present in the form of micro- and nanoplastics before they are completely assimilated and mineralized. Hydrolysis as the basic molecular mechanism of environmental PLA degradation continues as long as there is moisture or water available. Thus, the size of the fragments decreases, passing micrometer and nanometer range until the polymer chains are so short that the material becomes soluble in water and are not particles anymore. The dynamics of PLA micro- and nanoplastic formation and their lifetimes until complete mineralization in different environments are not systematically known and may range from several months to several decades or more.

In contrast, non-biodegradable, non-hydrolysable carbon-carbon backbone polymers, such as polyolefins, form micro- and nanoplastics only under the influence of UV light, abrasion, and other mechanical action, which suddenly stops acting on the material when the particles are covered with dirt, overgrown by algae, or trapped in soil or sediment. Micro- and nanoplastics from polyolefins and other conventional plastic polymers are likely to persist and permanently accumulate in the environment.

Environmental effects of PLA

In this section we refer to plastic in general and point out where PLA is specifically addressed. The physical effects of macro- and microplastics on organisms and ecosystems is widely documented. Many terrestrial, freshwater, and marine species all over the planet are killed, injured, or reduced in fitness by the physical interaction with plastic of various dimensions, with larger effects not only on an individual level but in some cases also on population and ecosystem level.

The presence of small micro- and nanoplastics (MNPs) within the respiratory and alimentary ducts of humans and animals has been proven and the uptake of a low percentage of ingested particles of ~5µm and smaller into other tissues across the gut, gill or lung epithelium seems possible. For a limited number of organisms (daphnids, mussels, crabs, fish), the uptake and translocation of MNPs has been demonstrated in the laboratory. However, it is not clear whether this also occurs in other species and whether it occurs in nature. Findings of large microplastic particles e.g., in liver or brain tissue seem unrealistic and are questionable. Currently, for the detection, identification, and quantification of environmental *nano*plastics in organisms, there are no validated analytical methods available.

The detrimental effects of micro- and nanoplastics (MNPs) in organisms are documented in some cases, but the overall consequences for plant, animal and human health are not yet clearly defined. The latest high-level reports on this topic (SAPEA 2019; WHO 2022; EC 2023a) are not conclusive but send clear warnings and call for high attention. If microplastic emissions to the environment remain the same, the ecological risks of microplastics may be widespread within a century. The review by Koelmans et al. (2022) is recommended for further reading.

Given its slow environmental degradation, the presence of PLA articles and MNPs may range from several months to several decades or more until full mineralization. During this time the effects of PLA in the open environment and on organisms are similar to conventional non-biodegradable plastic polymers in some respects. The pure *physical* effects of larger objects and articles (e.g., pellet, fork, cup, fibers) are expected to be basically the same.

Studies on the *chemical* effects of PLA rarely refer to the 'neat' (i.e., commercial raw material grade) polymer, but to processed and formulated grades or end products made from or with PLA such as cutlery, shampoo bottles, cups, or straws. In these plastic articles, PLA may be mixed with other polymers and additives, and may be contaminated by chemicals that have migrated from the material present in the packaged product (e.g., shampoo sold in a plastic bottle), for example, which likely blurs the picture of studies on the ecotoxicological effects of neat PLA. Several studies showed no adverse effects of PLA on algae, plants, and animals. In most studies where an effect on organisms was demonstrated, the measured effect could only be described for the totality of the tested material and could not be attributed to the neat polymer or other individual components.

The environmental behavior and impact of a final plastic product or its residues, e.g., as waste, on the environment can be drastically different from the original neat polymer. Throughout the life cycle of the product, from polymerization through plastic production, product manufacture and service life, to its entry into the environment, and then during its continued fate in or journey through various environmental compartments, all physical, chemical, and biological interactions influence the properties of the product and thus its degradation and impact.

Additives and foreign substances found in articles from the market, or in plastic samples collected from the environment attract high scientific and public interest which must be taken seriously. However, the mere presence of a certain substance detected by highly sophisticated analytical methods might be interesting but should not be specifically alarming at first sight. The actual concentration and thus potential doses should be considered to evaluate a potential risk. The background concentration (e.g., in food, organisms, environment) should be used for comparison and the presence of such substances in the plastic article may be due to migration from the contents of the article rather than a compound of the plastic *per se*.

Effects of plastic articles, also made from or with PLA, usually are assessed with unspecific tests and the effect mechanism remains unknown. This is of special importance for tests with high doses in confined vessels. One material-specific aspect of PLA is that ester hydrolysis increases the acid concentration and eventually those acids will become of low enough molecular weight that they become soluble and mobile to leave the item and enter the environment. A decrease of the water pH can be detrimental to sensitive organisms that cannot escape in a small test vessel in contrast to an open system. Interpretations must consider the environmental relevance of such artificial test conditions. Given the low hydrolysis rate of PLA at ambient temperatures, moderate pH changes are expected to be only effective in the immediate vicinity and quickly diluted in open aquatic environments.

The chemical effects of PLA-based macro-, micro- and nanoplastic beyond the formation of acidic degradation products comprise all substances that at a certain point of time are part of the particle, regardless of its origin. Plastic particles, being apolar and of low hydrophilicity, provide a space with high affinity and solubility for lipophilic substances in a biosphere which is mainly water-dominated, thus hydrophilic. This results in a steep concentration gradient from the medium to the surface and into the bulk of the particle with diffusion of chemicals with suitable properties into the bulk or adsorption to the polymer surface. Thus, plastic particles of any size can act as adsorbents that enrich certain chemicals to (much) higher concentrations than in the environment. With their spatial dispersal plastic particles are also vectors for all chemicals they contain. Generally, upon changes of the environmental conditions, solubilities may change, and previously adsorbed chemicals can desorb e.g., at the transition from the water phase to the sediment in freshwater and marine systems, or reversely, or upon internalization into organisms. In this sense they temporarily store certain chemicals taken from the environment rather than act as source for such chemicals. There must be a significant change in the surroundings for the plastic particle to later release the lipophilic chemicals. In the extreme, the total hydrolysis of the PLA particle releases all entrained chemicals. MNPs may also adsorb chemicals from highly contaminated organisms when ingested and via egestion clear the organism from toxins.

The constant background exposure to natural particles of mineral (e.g., dust, fly ash) and biological origins (e.g., pollen) and the long history of research on man-made nanoparticles in the air show that organisms have evolved countermeasures that are largely efficient. The presence of unwanted MNPs, particularly of PLA, in tissues has not yet been critically studied in detail. As bad as foreign particles may be in organisms' tissues, the intrinsic property of PLA of being rapidly hydrolyzed and subsequently metabolized in warm-blooded animals and humans, and its far-reaching approval as a suture, implant, and scaffold material, suggest a low risk posed by (a few) MNPs from PLA at temperatures prevailing in the body (~37 °C). Systematic information is lacking for cold-blooded animals and plants.

Summarized, the effects of PLA on organisms have been demonstrated both in lab and field tests but the explanations proposed for molecular effect mechanisms that could be PLA-specific are scarce and debatable.

Regarding the testing of hydrolysis, biodegradability, and biodegradation rates on the one hand and ecotoxicity tests on the other hand, the environmental relevance and the transferability from one scenario to another or from lab to environmental scale has to be evaluated for each case. Very strict criteria must be applied not to overinterpret or underestimate results and draw environmentally relevant conclusions not backed by the experimental or observational results.

The main difference when compared to non-biodegradable polymers is that PLA will eventually be fully hydrolyzed and biodegraded, and no persistent particles will remain and accumulate. The question of accumulation however is a balance between the rate at which MNPs are being produced and the rate at which they are degrading and leaving the environment. Less plastic waste littered should affect and eventually reduce the former rate. As soon as a PLA object is fully mineralized the environmental impact ends and a recovery of organisms, and the ecosystem can start. We are not aware of any studies that look at the impact and recovery in this sense.

Test methods and standards for environmental biodegradability

In laboratory tests, a direct measurement method such as quantifying the conversion of polymer carbon to carbon dioxide must be used to demonstrate biodegradability. The tests are usually done under optimized lab conditions. Subsequently, after inherent biodegradability has been proven, the rate of biodegradation will be estimated by indirect means using proxies such as disintegration, mass loss or surface erosion rate, in mesocosms and field tests under environmentally relevant conditions, while preventing physical deterioration. This 3-tier test scheme has proven to be effective and can be used for soil, freshwater, and marine scenarios. Ultimately the impact of the material on organisms shall be assessed with ecotoxicity tests. The tests should be done according to standards adopted on CEN level for comparability and reliability.

Certification serves as an external inspection level to check the accuracy of the measurements carried out. As a rule, the validity of the certificate and the use of the label is limited in time and requires renewal after its expiry, or if changes are made in the tested material.

Setting up a catalogue of criteria and an overarching testing scheme will be helpful for the further development and improvement of policy work, as well as certification schemes. To accelerate progress, an overarching Technical Committee (TC) on biodegradability at CEN level is proposed.

Although many standards have been introduced in recent years, there are still gaps that need to be closed in a timely manner. The European Commission is requesting a revision of the industrial composting standard EN 13432 (BS EN 13432 2007) to clarify the concepts of biodegradability and compostability. Examples of missing standards are for tests covering anoxic conditions in soil, freshwater, and marine environments, including a feasible way to integrate deep sea conditions. In addition, freshwater and soil tests for all tiers - laboratory, tank, field, and impact - as well as impact assessments for biodegradable plastics at the ecosystem level need to be completed at the standardization level.

The main difference when compared to non-biodegradable materials is that biodegradable materials will eventually be fully degraded, and the organisms and ecosystem can then recover. The duration of the impact and the rate of recovery are important aspects for an extended risk assessment, as they make it possible to evaluate the benefits in terms of reduced risk.

So far, there are only a few ecotoxicity tests for solid substances such as plastics. Therefore, tests for soluble substances have been applied so far. It must be examined whether the pass levels need to be adjusted to remain environmentally relevant.

Conceptually, it is not yet satisfactorily solved how the different environmental conditions and their variability can be taken into account. Furthermore, the integration of different timeframes of “weeks”, “months” and “years” (as proposed in the EU Policy framework) into test schemes is still lacking. This however is needed to do justice to those applications that have a longer use life and are thus intended to degrade more slowly (e.g., controlled release fertilizers), or for applications used in less favorable conditions for biodegradation. Therefore, new tests for longer biodegradation times, and also accelerated tests with new ideas and innovative approaches might be needed, potentially deviating from current tests and standards.

All these actions will support a consistent adaptation of testing and certification schemes for soil, freshwater, and marine, and so policy recommendations. Finally, the question remains as to what are acceptable timeframes and concentrations that should be allowed for biodegradable macroplastics and MNPs in the open environment.

The special character of PLA where *direct bio*degradation in the environment is assumed to play an insignificant role, and environmental degradation is dominated by chemical hydrolysis is not taken into account in the context of testing, certification and regulation. With the currently existing testing and evaluation scheme PLA remains non-biodegradable in the environment and is rather seen as a risk, and its potential as a beneficial material alternative for certain applications cannot unfold.

Accurate data on the environmental behavior of PLA, especially its hydrolysis rate under common environmental temperatures, are necessary to lead the discussion and the development of standards into this direction.

Policy and regulation concerning microplastic

On a policy level biodegradable plastic polymers and additives are seen as a possible solution for specific applications, and against persistent microplastic pollution. However, such materials are treated with caution for two reasons: First, it is considered essential that their introduction does not cause any lasting environmental pollution or other burden, and second, that biodegradation really does take place completely and that it is known how long it will take. For biodegradable polymers and additives, it must be demonstrated that they are biodegradable. The timeframe to biodegrade in the receiving environment must be estimated, and where they are likely to end up if there is the risk to be transferred between environmental compartments, e.g. from agricultural soil to freshwater. The impact on the environment should be minimal and claims must be based on existing standards and certification schemes (EC 2022a).

The adopted EU Action Plan: "Towards Zero Pollution of Air, Water and Soil" aims for 30% less microplastic release to the environment by 2030, showing that means for concrete measures need to be put in place as fast as possible. The Regulation 2019/1009 for safe and effective fertilizing products on the EU market is the first act that legally binds biodegradability to polymers in applications mentioned. For further processes, such as the Microplastic Initiative, negotiations are ongoing. The strategy is to look for measures which provide benefit and are not in conflict with other scopes of the EU Green Deal. As part of the ongoing work, the benefits of additional legislation on the unintentional release of (micro)plastics will be assessed. The focus is on understanding the sources of unintentional release of microplastics from selected product groups and then assessing possible measures and options for action. Data for such assessments is not available or available data is not useful for this purpose. Such assessments are time-consuming and should be carried out with care before proposing a policy measure that could have an impact on an entire industry. They should therefore be prioritized and presented to policy makers as soon as possible.

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