Summary for the final report
Development of a test strategy for the comprehensive safety assessment of plastic recyclates (PolyCycle)

The Polycycle project intended to develop a testing strategy for the comprehensive safety assessment of plastic recyclates. The European Circular Economy Strategy calls for recyclable packaging, but there are major challenges regarding its safety, especially as food contact materials. The collection and recycling process can introduce unknown substances into the material that, although undetectable by current methods, have a DNA-reactive effect and could pose a health risk. To detect this DNA-reactive effect, in vitro bioassays are particularly suitable, but these are optimized for the analysis of pure substances and not of polymer extracts. Therefore, the following objectives should be achieved during the project period:

1. development and validation of an assay strategy for DNA-reactive carcinogens in recycled plastics (in particular the calculation of detection limits; optimization of extraction methods, sample preparation and concentration techniques for in vitro bioassays; chemical analysis for substance groups not covered by in vitro bioassays).
2. analysis of selected packaging-relevant recycled materials (PE, PP, PET, PS) with in vitro bioassays and chemical methods (GC, HPLC, MS)
3. enabling the use of recycled packaging materials without compromising safety.

In WP2, the required detection limits for DNA-reactive carcinogens in recycled materials were calculated based on migration models and exposure estimates of four polymers at 11 different application scenarios. For high diffusivity polymers such as HDPE and PP, total transfer of the substances was expected, with detection limits ranging from 0.0021 mg/kg (infant milk bottle) to 0.536 mg/kg (instant coffee lid). For low-diffusive polymers such as PET and PS, an activation energy-based diffusion model served as the basis for calculation. The realistic detection limits ranged from 0.0011 mg/kg (PET bottle for baby water based on low molecular weights) to 2030 mg/kg (PS yogurt cup for infants based on high molecular weights). Based on the diffusion coefficients and activation energies, higher molecular weight substances migrate only to a very small extent, therefore higher concentrations in the recycle-containing packaging can be tolerated.

In WP 3, a sample processing method was developed and optimized for the representative extraction and concentration of DNA-reactive genotoxic substances from post-consumer materials. In WP 5, a method was developed and optimized for performing Ames assays on polymer extracts. The ideal combination of experimental parameters was performed on 208 samples using two test strains (TA98 and TA100) with and without the addition of rat liver S9 and with and without the addition of a spike control. A majority of the samples resulted in toxic effects with TA98 without S9, which were not reversed by adjustment of the control substance. In studies with strain TA100, the majority of results were unremarkable with and without the addition of S9. An unexpectedly large proportion of samples (approximately 35%) showed clear and reproducible positive results in the Ames-MPFTM assay with TA98 with the addition of S9.

In WP 4, the 208 samples were analogously analyzed for volatile compounds and additives and identified substances were toxicologically classified. Unidentified substances were classified as potentially genotoxic. Differences were detectable between polymers in the number of substances found and the number of substances evaluated as toxicologically conspicuous (PET less "non-intentionally added substances" NIAS, polyolefins more NIAS), but beside diffusivity other influencing factor were responsible. Post-consumer substances were more frequently evaluated as toxicologically suspicious compared to compounds from virgin materials, but post-consumer substances were less frequently present in the plastics and less frequently correlated with a mutagenic classification from the Ames tests. For polyolefins and polystyrene, positive results were obtained in the Ames tests; for PET, all samples measured were clearly negative in the Ames test. Correlations between contained substances and mutagenic classification for polyolefins and polystyrene were partially present, but these could also be coincidental. Causalities with volatiles or additives were unlikely, but a common causal factor could be oxidative stress. Samples classified as mutagenic were not clearly distinguishable from the non-mutagen classified samples in the score. Clear conclusions are not possible based on volatiles.

As there were delays due to the restrictions caused by the COVID19 situation and the project duration was extended on the Austrian side, the results of WP6 and WP7 are not yet fully available. The chemical analysis was validated and verificated at IVV.

Since volatile compounds could be excluded as a direct cause of the mutagenic effect in the Ames tests, preliminary tests were carried out to find causes within other substance classes. Previous results discussed a contamination with MOAH, mycotoxins or printing ink reaction products. For mineral oil components, a correlation between mutagenic classification of the sample and the MOAH content was suggested, however, the examination of the MOAH fraction resulted in negative Ames tests. Since the mutagenic effect occurred only when liver enzymes were added, contamination with mycotoxins, especially aflatoxin, was suspected, but aflatoxin could not be detected in the polymers. It was noticeable that the mutagenic effect occurred only in re-extruded granules and not in flakes. This suggested an activation of the DNA-reactive effect under the influence of temperature, which could be confirmed on a laboratory scale. Investigation of various printing inks revealed an increase in mutagenic effect when nitrocellulose was used. The analytical characterization of the inks provided a reduction of all possible substances to small organic non-volatile molecules, which have polar functional groups, such as nitroso compounds. These substances can possibly cause the DNA-reactive effect and explain the lack of correlation with volatile compounds. In the follow-up project "Safecycle", these compounds are to be analyzed and the cause of the DNA-reactive effect found so that their occurrence in plastics can be reduced or avoided in the future and recycled packaging materials can be used without compromising safety.

Overall, a test strategy for DNA-reactive substances from plastics was developed and validated, and chemical and in vitro bioassay analysis was applied to selected samples. However, no clear correlation could be found between samples classified as mutagenic and detected substances, so that in the case of polyolefins and polystyrene it is not possible to use recycled plastics without compromising safety. However, the "SafeCycle" research project offers great potential for achieving this goal in the future.

IVLV members can download the complete final German project report from our homepage. All you need is to register in the section “My IVLV”. Non-members can request the final report from the IVLV office at office@ivlv.org.

Supported by:

Federal Ministry
for Economic Affairs
and Climate Action

on the basis of a decision
by the German Bundestag

Forschungsnetzwerk
Mittelstand

The IGF project no. 258 EN presented here by the Research Association of the Industrial Association for Food Technology and Packaging (IVLV e.V.) is funded by the Federal Ministry for Economic Affairs and Climate Action via the AiF as part of the program for the promotion of industrial community research (IGF) based on a decision of the German Bundestag.