Prolonged test duration for testing the biodegradability of polymers in OECD 301 improves reproducibility of test results

Marlies Bergheim[a], Christian Kastner[a], Julia Wilmers[a], Kathleen McDonough[b], Glauco Battagliarin[c], Jennifer Menzies[b], Jared Bozich[d], Bjorn Hidding[c], Bahar Koyuncu[e], Georg Kreutzer[f], Hans Leijs[d], Yash Parulekar[g], Meera Raghuram[h], Nathalie Valloton[i], Jan Robinson[e], Johannes Tolls[a]

[a]Henkel AG & Co. KGaA, Düsseldorf, Germany
[b]Procter and Gamble Company, Mason, USA
[c]BASF, Ludwigshafen, Germany
[d]IFF, Hazlet, USA
[e]AISE, Brussels, Belgium
[f]Givaudan, Vernier, Switzerland
[g]MonoSol, a Kuraray company, Merrillville, USA
[h]Lubrizol Advanced Materials, Cleveland, USA
[i]DOW Europe GmbH, Horgen, Switzerland

THE BIODEGRADABILITY OF POLYMERS – TESTING IS A CHALLENGE

Why is the information of biodegradability needed for polymers?

- **Persistence (P):** Element of new EU CLP hazard classes PMT/vPvM & PBT/vPvB: biodegradability assessments are of greatest importance for persistency (P) assessments.
- When polymers are included in the scope of REACH (**REACH for polymers**), the determination of degradability will be key.
- Use in broad number of applications including products with **wide-dispersive use** emissions.

Why do not use the current OECD screening tests?

- Screening tests acc. OECD are **standardized** procedures and widely accepted[3]. They are **proven to be successful** for low molecular weight and easy-to-test molecules[2]. Nevertheless, polymeric substances were not considered in the validation of the test guidelines.

Why is it so difficult to determine the biodegradability of polymers?

- Polymers are often **multi-constituent substances** with **varying chemistry** and environmental behavior (e.g. adsorption and/or low water solubility). This can result in **limited bioavailability** in screening tests[2].
- Polymers often consist of **large molecules** (typically > 1000 Da)[1], making them inaccessible to internal enzymes of bacteria without prior fragmentation[2].

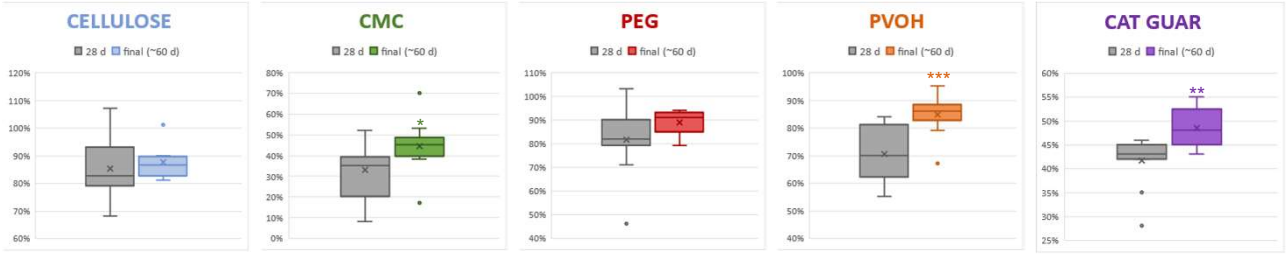
RESEARCH QUESTIONS

- How can screening tests be adapted to enhance their suitability for polymeric substances?
- How does a prolonged test duration of OECD 301 influence the test outcome for polymers?

EXPERIMENTAL SETUP

- 5 polymeric substances with different structural components, molecular weight, water solubility, substitution type and charge were tested.
- 11 OECD 301 studies (8 OECD 301 F & 3 OECD 301 B) were conducted at 8 different laboratories in 4 different countries by members of the A.I.S.E task force for polymeric biodegradation.
- 6 studies followed the test guidelines while 5 studies had modified test conditions (altered test substance concentration or inoculum levels).
- 9 studies had an extended test duration (~60 days).

BIODEGRADATION DATA (MCDONOUGH ET AL. 2023[1]) FOR SOLUBLE POLYMERS



- The cumulated results of all OECD 301 test results performed by the task force are displayed as box plots. The grey boxes on the left represent the results after 28 days, respectively. Colored boxes show results at the end of the test (60.1 ± 6.6 days). The boxes are delimited by the 25% quartiles, with the median in between. The calculated mean values are represented as crosses. The vertical lines represent the highest and lowest individual test results and outliers are shown as dots. The asterisks indicate significance (Student's *t*-test): * p <0.05, ** p <0.01, and *** p <0.001.
- *Test materials:*
  Microcrystalline cellulose / Carboxymethyl cellulose (CMC), MW= 200,000, DS=0.6 / Polyethylene glycol (PEG), MW=35,000 / Polyvinyl alcohol (PVOH), MW=130,000, DH=0.88 / Cationically Modified Guar, MW=1,500,000

RESULT 1: VARIABILITY OF BIODEGRADATION RESULTS

- **Decreased variability** of biodegradation results due to extended test duration could be observed.
- The **variance of results was** significantly **lower** when prolonging the test to ~60 days.
- The **mean quartile distance was almost halved** (12.2% vs. 6.2%) by extension of test duration from 28 to ~60 days.

Prolonged test duration increases the reproducibility of biodegradation results.

RESULT 2: MEAN DEGREE OF BIODEGRADATION

- The average biodegradation levels were **4 – 15% higher at test end (~60 days)** than after 28 days.
- The extension of the test duration allows for a more precise **assessment of final mineralization.**

Prolonged test duration significantly increases the degree of biodegradation.

CONCLUSIONS AND OUTLOOK

- For the tested polymers, the mean **degree of biodegradation** increased with increasing test duration, thus illustrates that the assessment of ultimate degradation requires longer study durations.
- The **variability** of the test results (variance) was significantly lower when comparing the test data after ~60 days with respective data after 28 days.
- The **accurate evaluation and reproducibility of the test results** is therefore improved by extending the test duration.
- The criteria (e.g. timeframe for degradation) of current screening tests may need to be changed to conclude on polymer biodegradability due to their differences compared with low-MW substances. The **fragmentation** via biotic and/or abiotic processes is the first essential step for large complex polymers prior to degradation. Thus, extending the test duration for polymers can be of help to reach the plateau phase of mineralization.

REFERENCES

1) McDonough et al. 2023: Multi-laboratory evaluation of the reproducibility of polymer biodegradation assessments applying standardized and modified respirometry methods. Sci Total Env 901.
2) ECETOC 2020. Technical Report No. 133-2: Applicability of Analytical Tools, Test Methods and Models for Polymer Risk Assessment.
3) Kowalczyk et al. 2015: Refinement of biodegradation tests methodologies and the proposed utility of new microbial ecology techniques. Ecotoxicol Environ Saf 111.