iTAP Workshop report – analytical challenges with environmental testing of polymers and other difficult chemistries

A workshop held at the SETAC-North America Meeting (Toronto, Ontario, Canada)

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(iTAP, Cefic LRI ECO46)

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Introduction

The growing regulatory attention and increased use of water-soluble polymers in consumer cleaning products is leading to a need for industry and the scientific community at large to ensure the use of polymers can be supported by robust environmental safety assessment methodology. One important aspect in the ecological risk assessment process is the availability of high-quality data from safety studies where the test material is well-characterized and concentrations in exposure solutions are quantified by analytical measurement. With advances in mass spectrometric instrumentation, reliable and sensitive approaches are possible with many low molecular weight species or simple organic chemistries. Quantitation of polymers in environmental studies have historically been challenged by their structural complexity with varying repeat subunits (polydispersity). Well-established approaches to measure and quantify polymers in environmental effects studies include wet chemistry and non- and semi-specific methodology.

Polymers can be analyzed by a multitude of techniques for identification/characterization purposes and to evaluate performance in the intended application. A variety of chemical properties are routinely examined including: molecular weight and structure, number of repeat subunits, rheology, crystallinity and thermal mechanical properties (for solid polymers used in packaging). However, it is not likely these methods are relevant or appropriate for quantitation at low concentrations from environmental matrices or safety studies. With the anticipated needs for polymer registration in chemical management programs, there is a need to consider the availability of methods for low level quantitation and to evaluate the relevance of analytical methods for quantifying polymer in exposure solutions in effects testing.

Workshop Description

To begin to understand the state of the science with polymer analytical methodology and the limitations and potential solutions, a workshop was held at the 40th SETAC NA meeting in November 2019 with the purpose to:

1) To provide a 'prospective review' of published and yet unpublished analytical approaches for polymers and other similar and difficult to analyze materials in the context of environmental safety testing

2) Discuss general challenges with water-soluble polymers – limitations of current methods

3) Understand feasibility of an accessible universal approach

This workshop was a companion to the session on "*Environmental Considerations for the Risk Assessment of Polymers, Rubber and Macroplastics: State of the Science*", and representatives from industry, academia and government were invited to attend to share their perspectives and experiences from working with polymers in a safety context (see workshop invitation in Figure 1 below).

Figure 1: Workshop Invitation

The workshop was well attended with 23 external participants from industry; regulators and academia from North America; Europe; and Africa (see Figures 2 and 3). The workshop employed a World Café style methodology with four sub-topics. Participants rotated between each sub-topic providing increasing and collective input to the subject matter discussed. The slides for the workshop are included in the Appendix. In brief, one sub-topic focused on watch-outs and guidance for extraction approaches for polymers from test matrices. The other three sub-topics were centered on wet chemistry, non- and semi-specific approaches and mass spectrometry as potential analytical methods to measure polymers in environmental safety studies. The following considerations were used to guide the discussions within each sub-group:

- What question is being asked? Characterization, detection or quantitation?
- How can I apply this tool?
- What factors are important in tool selection?
- Who needs the data?
- How will the data be used?
- What environmental safety studies (fate, effects, field, etc.)
- What is the matrix/type of study?

- How low do we need to go?
- How specific does the method need to be?
- Is this instrumentation available/accessible?

Each group brainstormed and converged on key findings using flipcharts. Group rotation enabled the addition of builds and contribution of new ideas to the discussion (Fig. 2). Upon completion of the rotations, ideas were synthesized with a focus on guidance and watch-outs, advantages and disadvantages, and recommendations for scenarios where lower tiered methods could be envisioned.

Fig 2: Snapshots from the workshop

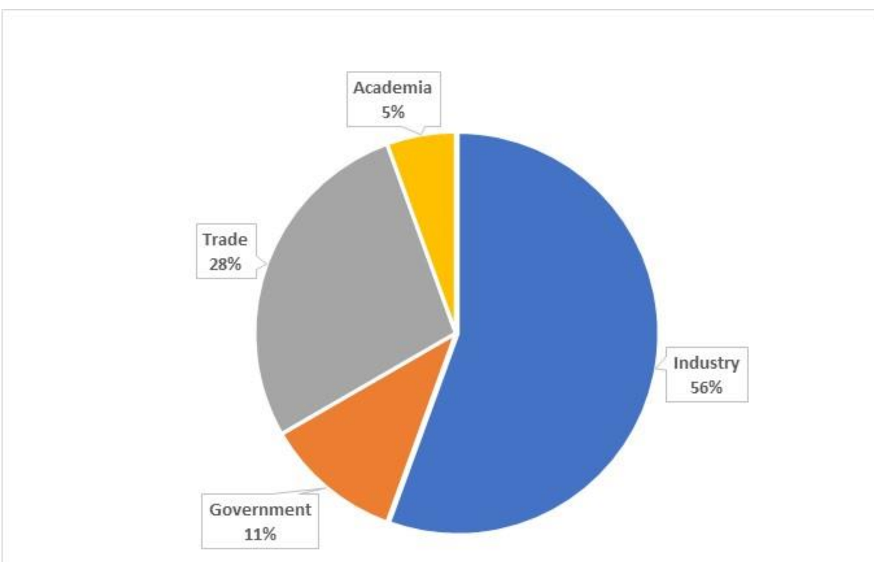

Figure 3. Distribution of Workshop Participants

The findings from each sub-group are summarized in the next several sections.

Extraction

(H. Sanderson, sub-group discussion leader)

When extracting polymers from aquatic effects samples for analytical measurements, one may need to consider if the matrix is capable of causing issues with specificity, sensitivity or signal interference. This sub-group focused on methods to improve polymer extraction methods and the following guidance was shared:

Guidance:

a) It is critical to understand relevant physical-chemical properties of the polymer. A key property to determine is water solubility.
b) Polymers could be separated from the matrix using size exclusion filtering in a pre-extraction clean-up step.
c) Selected solvents for polymer extraction/isolation should not alter the polymer structure or distribution.
d) The selection of extraction strategy and method for analytical detection are not mutually exclusive. The extraction approach must be appropriate and suited to the analytical method of detection.
e) Consider an *in-situ* derivatization provided the polymer chemical properties are not significantly altered. Possible derivatization methods include metal addition.

Watch-outs:

a) Caution may be needed with some polymer radiolabeling methods that involve heating. This may alter polymer composition and distribution.
b) Extraction of polymers from sediments and soils is an analytical challenge and significant effort is needed to advance the science.
c) Analytical extraction efficiency or recovery of polymer from the matrix may need to be considered when interpreting data from safety studies.

Wet Chemistry

(S. Belanger, sub-group discussion leader)

This sub-group was tasked to provide input on the potential for wet chemistry methods to inform aspects of understanding polymers for testing, characterization or for environmental measurements. These include methods such as selective ion electrodes (SIE) and FTIR, but certainly is not limited to such. The following captures discussions of the sub-group. Feedback was often in the form of open, unresolved questions, reflecting the uncertainties in applications of existing methods.

Selection Ion Electrodes (SIE)

These are generally older methods and if applied would come with a number of assumptions. To be applicable, a SIE would need to cover the full molecular weight (MW) distribution of the polymer to avoid bias. Thus, it might be suitable only for certain ranges of MW and charge densities. Interference with environmental modifiers (organic carbon, anions, cations) would likely require upfront application of a separation method to isolate the polymer except in the most clean of matrices. This would then also require some additional assumptions. The general conclusion was that SIE may be most useful for "clean" matrices such as stock solutions or relatively high exposures. It is not clear if SIE would be sensitive enough to be useful for low level quantifications; although this was not an area that workshop participants had direct experience with.

Fourier Transform Infra-Red Spectroscopy (FTIR)

While FTIR would not be useful for quantitation of exposures or field measurements, it may be highly useful for determination of chemical identity. Improved sensitivity of FTIR could be gained using derivatization with a compound providing a better analytical handle and could be explored. However, at this point investment in development of a suitable derivatized material would likely be so onerous that simply moving to more selective and sensitive methods would be easily justified. Derivatization would also require some new assumptions regarding efficiency across all components of the polymer distribution. FTIR could also be useful/applicable in identifying functional groups present on polymer molecules. Unexplored uses still include a potential application in verifying dose with some assumptions (for example, needing to compare traces before and after placement of polymers into dose solutions). Similar to SIE, limits of detection may be an issue overall, especially for polymers that carry a significant toxicity signature (e.g., cationic polymers).

As a side note, FTIR coupled with Raman spectroscopy remains as a mainstay of quantifying solid microplastic particles at environmental levels (also with known assumptions regarding size limits of field or biological samples).

Additional wet chemistry ideas

An example of successful application of using metal complexation in a product context was shared. By measuring total metal (e.g., copper) in a solution where the metal was also truly free, and then placing the polymer into the solution, detection of the remaining free metal would correlate with the levels of polymer in the system. However, this approach would have limited application in environmental testing, given metals are ubiquitous in environmental matrices.

Another option could be measurement of a counter ion known to exist in a particular molar ratio with the polymer. Thus, by measuring the presence of the counter ion, the total polymer present may be estimated. This would require the use of a counter ion which is not prevalent in the environment.

Lastly, conductivity as a surrogate was also identified as a possible wet chemistry method. Polymers delivered within a chloride solution may be detectable as an inflection in conductance.

It is worth noting that other than the product/complexation example, participants did not have any direct experience with any option discussed in this breakout.

Conclusions:

1. Wet chemistry methods are highly dependent on a high level of characterization so assumptions of the measurements can be clear. This would be common to any of the methods discussed in this session (FTIR, SIE, complexation, derivatization, counter ion measurements and conductivity).
2. Wet methods will have a limited scope of utility with respect to testing applications. Potential issues would include limits of detection/quantitation, specificity, clear articulation and knowledge of assumptions, and knowledge of the testing matrix.
3. Interesting alternative options which have not been explored include complexation, measurement of counter ions and conductivity.
4. FTIR will have utility for determination of chemical identity or functionalizations of the polymer.

Non- and Semi-specific Methods (e.g. TOC, Atomic methods)

(M. Lam, sub-group discussion leader)

This sub-group focused on the use of non- and semi-specific analytical methods to provide input on the potential use of these approached for quantitative measurements in environmental fate and effects studies. Methods included in this discussion were Total Organic Carbon (TOC), and atomic spectroscopy methods such as Inductively Coupled Plasma (ICP), Atomic Emission Spectroscopy (AES), Atomic Absorption Spectroscopy (AAS). The following are highlights from the discussions on the advantages and disadvantages of using these techniques, and considerations that should be addressed when using methods that are non- or semi-specific. The method that most had direct experience of quantifying experimental polymer concentrations with Total Organic Carbon (TOC). This approach is commonly used to verify exposure solutions and is considered well-established and highly accessible.

The general advantages of using non- or semi-specific approaches to provide a quantitative measure of polymers in environmental safety studies are these methods are generally quick, easy, well-established and accessible. These methods could serve as a cost-effective proxy for more sophisticated techniques, provided there is adequate method validation to support the use of the less specific approach. Non- and semi-specific methods could have utility in screening studies to guide resource and timing needs for more definitive or comprehensive studies and evaluations.

The disadvantages of non- or semi-specific analytical methods are the lack of specificity, low sensitivity (LOD ~1 mg/L for TOC), background or matrix interferences, and overall a limitation to drawing robust conclusions from the study. Additional limitations are these methods inform on the presence of an atom, but it is often challenging to distinguish the between the presence of the atom as associated with the analyte of interest or freely available in the matrix. Along this line of thought, there are potential limitations with non- and semi-specific methods to support acute studies only. Using TOC as an example, feeding is typically needed with chronic studies, however it may be difficult to use total organic carbon as a measure of the polymer when carbon is incorporated into the biomass. However, these concerns could be addressed if TOC analyses is used when expected exposure levels are relatively high. Scenarios of when these techniques could be possible are discussed in the next paragraph.

Despite the limitations raised above, the use of a non- or semi-specific analytical method is possible in certain circumstances. Using TOC as an example, scenarios where this method alone may be possible include:

- Polymer is stable to degradation;
- No losses of polymer due to sorption;
- Polymer exhibits moderate to low toxicity;
- Polymer level is significantly above background;
- Well-characterized polymer, e.g. low residuals etc.

Conclusions:

1. Non- and semi-specific methods have limited utility with differentiating between components in a sample (monomer vs polymer vs residuals). Therefore, it is critical to have a well analytically characterized material if one chooses to use a non/semi-specific method.

2. TOC could be useful for screening studies, or even in definitive studies if there is sufficient robust data available to document and to support the use of a non-specific method like TOC.
3. It is highly recommended to pair TOC with another analytical technique to improve the specificity. It could be envisioned to use higher tiered methods initially to validate and support the use of TOC in definitive studies.

Mass Spectrometry-Based Methods

(Y. Sun, sub-group discussion leader)

This sub-group was tasked to provide some input on the potential of the mass spectrometry-based methods for polymer environmental safety testing and assessment. Mass spectrometry has been demonstrated as a powerful tool for high sensitivity detection, identification and quantitation of various chemical materials. It has been widely used in supporting environmental toxicity and chemistry research, albeit predominantly for the small molecules or impurities. The key findings from this sub-group discussion on using mass spectrometry for polymer quantitation in environmental studies are summarized below:

Mass spectrometry has the potential to be a sensitive and specific tool for polymer characterization and quantitation, but it is expensive and may not be easily accessible. It is considered high-end and sophisticated instrumentation and requires highly skilled and trained analytical chemists to operate; expertise and experience are needed in developing an analysis strategy and data interpretation.

Currently, mass spectrometry is predominantly used for the analysis and quantitation of low MW monomers and oligomers in polymeric materials to gauge synthesis and processing efficiency and this information can also be leveraged for the conductance of a material safety assessment. There has been less focus on the analysis and quantitation of intact polymers using mass spectrometry, largely due to the difficulties and challenges from their high MW and polydispersity, sample complexity, variable solubility and ionization efficiency.

Current common mass spectrometry methods used for the quantitative analysis of polymers include Electrospray Ionization Mass Spectrometry (ESI-MS), Matrix-assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF-MS), Pyrolysis Gas Chromatography Mass Spectrometry (pyGC-MS), and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). On-line and off-line coupling of Gel Permeation Chromatography (GPC) and Liquid Chromatography (LC) to mass spectrometry could be useful approaches for addressing the complexity of polymer mixtures, the separation of polymers from matrices, and even quantification of polymers in media from aquatic effects studies. Other approaches such as dia-filtration, MW cut-off filtration, solvent precipitations were also proposed as potential solutions for polymer isolation to enable high sensitivity detection and quantitation by mass spectrometry. The sub-group leader provided anecdotal and perspectives with mass spectrometric approaches based on hands-on experiences within industry. Significant development effort is needed for both instrumentation and methodology to provide sufficient sensitivity and robust quantitation of sub-ppm concentrations accurate for environmental toxicity and safety studies.

Due to the diversity of polymer scaffolds, structures and polydispersity used across the range of applications, it does not seem likely universal one-size-fits-all method is possible with polymers. Mass spectrometry could play an important role in providing a more accurate, sensitive and specific method for the quantitation of polymers, but method development will need to be case-by-case as specific methods are envisioned for polymer from different classes, or with different functionalities or chemical properties.

Conclusions:

Mass spectrometry provides high potential for delivering the need for a high sensitivity, robust approach for polymer identification, structure characterization and quantitation of polymers at low levels in environmental effects testing. However, further efforts in the environmental safety and analytical communities are needed to meet the technical challenges in gaining a deep understanding of polymer materials for testing, characterization and environmental measurements.

Overall Conclusions and Next Steps:

Perspective on the latest advancement and practical considerations for analytical quantitation of polymers in environmental safety testing was shared by representatives from industry, academia and government at this workshop. Based on the discussions, some general conclusions may be drawn in addition to some recommendations for future actions to further advance the discussions on analytical methods for cationic polymers, and polymers in general.

The key conclusion is that the strategy for analytical method is influenced by the problem statement and an understanding of what the test material is. It is important to have a comprehensive characterization of the polymer, including relevant chemical properties and residuals, as these will be critical in guiding method development and will ultimately help with data interpretation. Another important consideration is how the data will be used and for what purpose. Overall, the process in developing an appropriate analytical method must include a clear picture of what the end goal is. Practical considerations include accessibility, ease of analysis and resources.

The overarching guidance and considerations that are highlighted as the following points below:

1. It is critical to have a well analytically characterized polymer to guide the selection of an appropriate analytical method and the design of environmental safety studies.
2. Analytical method selection will depend on the specific need and also the polymer of interest being studied. Additional factors such as polymer fate and effects need to be considered.
3. Consider technical Influencing of regulators to accept waivers due to technical feasibility or irrelevance of data to assessment.
4. Industry representatives agreed that much knowledge on polymers is privately held data, and a mechanism to openly share and access this knowledge base would be useful.
5. Since SETAC does not have very deep representation of scientists interested in polymer chemistry, another venue to consider outreach are the American Chemical Society (ACS) and attending and presenting at meetings that are more frequented by polymer experts.

In addition to the analytical methods discussed in this workshop, other approaches were suggested but it should be clear that feasibility will be a case-by-case decision based on the structure of the polymer and ability to leverage these approaches. Considerations for alternative approaches includes; Derivatization post sampling – fluorescent-labelling, metal addition; and the use of Electrophoresis.

Appendix 1: Workshop slides

Polymer Analytical
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