Progress in understanding the ecotoxicology of cationic polymers: evidence with Polyquat 10's

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Background

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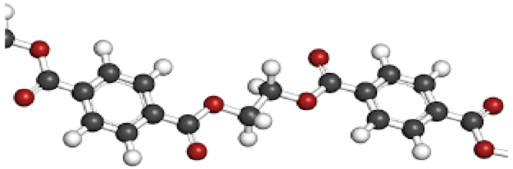
Improved aquatic Testing and Assessment of cationic Polymers (iTAP)

- To support the development of methods to improve the accuracy and precision of the aquatic environmental risk assessment of cationic polymers

- Builds on the recognition that:

  - Microplastics debate re-shaped the thinking on polymers in the environment
  - New approaches are needed to address this (very large and diverse) group of chemicals
  - Polymers are *very* understudied relative to other industrial chemicals
  - Polymers have moved into the regulatory scope
  - Very importantly, that cationic polymers are already recognized as a class that will require registration and assessment due to known toxicity profile/lack of biodegradability

P&G



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iTAP Objectives

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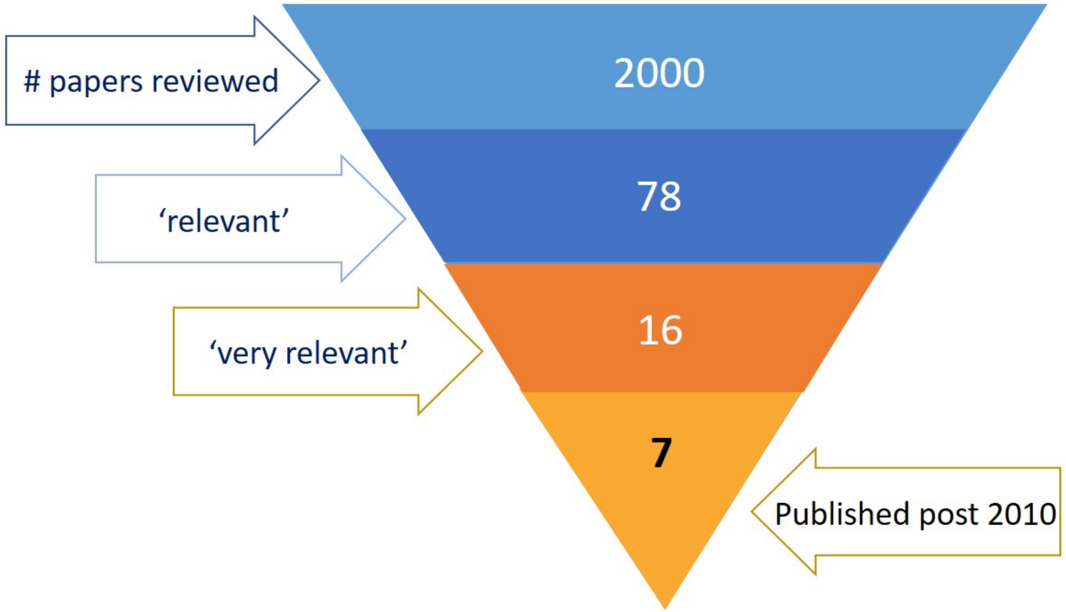
- Develop new aquatic toxicity approaches (methods and test designs) that will be useful to inform regulators on aquatic hazards
- Develop QSAR approaches (if possible) for cationic polymers
- Devise recommendations for means to improve aquatic risk assessments of polymers
- Review the literature with an eye towards testing that would be acceptable in a regulatory environment
- Lay the foundation for regulatory acceptance



Polymers

- Polymers are large macromolecules consisting of repeating monomer units
  - Polymers are an exceptionally diverse group of compounds (with varying properties and uses)
  - Polymers may be described as linear, branched or cross-linked
  - They may exist as homopolymers and have one repeated monomer, or they may be copolymers and contain two or more monomers combined in random or ordered approaches
  - They can both be synthetic, such as plastics and rubbers (e.g. polystyrene; PET; PE; etc.) or natural, (e.g. DNA; starch; amino acids, proteins, cellulose, polyamides; polysaccharides)
  - Hence, while *all plastics are polymers - not all polymers are plastic*

- Polymers are currently exempt in REACH and generally not reviewed in most regulatory programs.
  - This exemption is due to the assumption that there would be little toxicological concern due to their high molecular weight, low water solubility and reduced reactivity of polymers in environmental compartments

- "Truths" about polymers being challenged
  - Can't find 'em
  - Can't measure 'em
  - They aren't' toxic
  - They don't biodegrade

- Polymers will no longer receive a "free pass"

Challenges

- Polymers are typically large and not expected to pass biological membranes
  - Making toxicity testing problematic as they still exert demonstrable effects
- Expected to interact with the outer membranes of aquatic organisms and thereby affect their functionalities
  - This behavior makes it hard to describe the dose-response relationship as responses likely confound external physical effects and internal uptake
- Cationic polymers combine two contrasting elements – a positive charge and a long hydrophobic chain
- Low water solubility due to hydrophobicity of the polymer chains
- Polymer charge (neutral, anionic, cationic, amphoteric) can also modulate toxicity
- Not toxicologically available (non-toxic by definition)?
- Mechanical/physical effects are possible

P&G Internal Work

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Polymers tested: high molecular weight, low charge densities

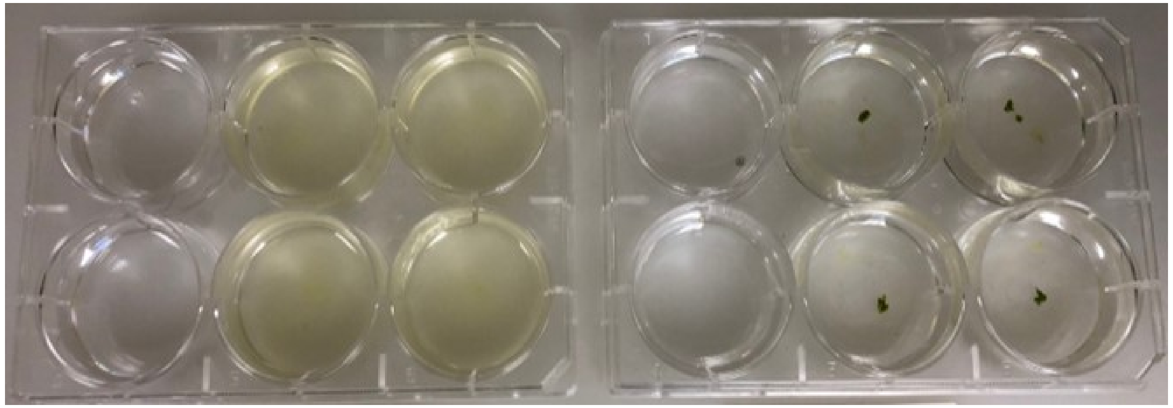
Algae	Daphnia

Algae

- Algae tend to be most sensitive
  - Expected as algae are generally the most sensitive species for cationic materials
  - Algae tended to ‘clump’ together
  - Not seeing an exposure response over an order of magnitude
    - Suggesting this is being driven by a physical phenomenon and not inherent toxicity

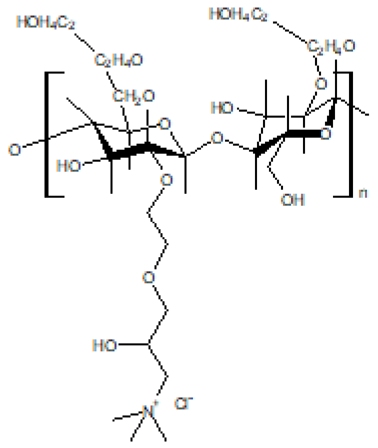
Daphnia

- Carapace adhering to *Daphnia magna* / organisms sticking together
  - Toxicity likely due to physical impairment
- Mitigation due to humic acid can be substantial (25-fold)
  - Increasing concentrations of humic acid did not mitigate further



Materials: Polyquaternium 10's

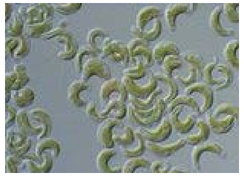
Tradename	MW	Viscosity (mPa/s) (2% aq soln)	%-N	Charge density
UCARE JR125	435 (low)	75-125	1.5-2.2	1.02 (high)
UCARE JR30M	2202 (high)	30000	1.5-2.2	1.04 (high)
UCARE JR400	723 (low)	300-500	0.4-0.6	1.16 (high)
UCARE LR30M	1363 (high)	300-500	0.8-1.1	0.58 (low)
UCARE LR400	432 (low)	75-125	1.5-2.2	0.59 (low)



- Polyquaternium-10 (PQ10) is a cationic cellulose polymer with quaternary ammonium functionality, varying in charge density and molecular weight
- Due to widespread usage and lack of biodegradability the potential for environmental exposure is high

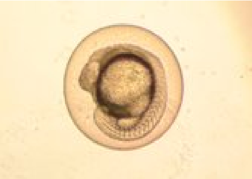
Methods

Employed standard environmental toxicity assays to determine hazard of select polymeric materials



Algal OECD 201 growth inhibition test (acute and chronic)
Additional observations: Cell membrane surface

Daphnia magna OECD 202 and 211 tests (acute and chronic)
Additional observations: Gill function, feeding, reproduction



Zebrafish embryo OECD 236 test (acute)
Additional observations: Gill function, hatching rate, interactions with chorion

- Analytical methods were developed and used to measure freely dissolved concentrations of cationic polymer
- Mitigation potentials were investigated
    - Will yield information on the nature of the charge interaction
- Strategically tested compounds in order to determine the relationship between physical descriptors/functionality and observed organism responses
    - Non-traditional endpoints assessed
        - Light microscopy and investigations of bioavailability are being used to gain insight into toxicological mechanisms

What part does humic acid play in this?

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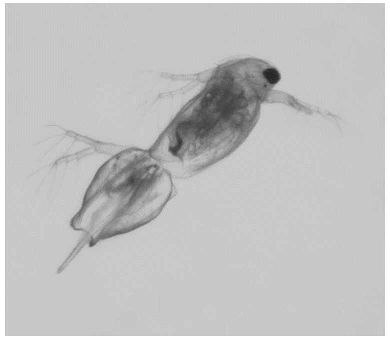
- Humic acid is a known modifier of toxicity by altering bioavailability of cationic compounds
- A USEPA ecotox Test Guideline and testing strategy is dedicated to assessing HA-ecotox interaction
- OECD test guidelines limit organic carbon levels present in tests at 2 mg/L
  - Standard test media used for aquatic hazard testing usually has low TOC content compared to surface waters, which may results in artificially high toxicity of some cationic test chemicals
- Humic acids are complex, differ from place to place, and may form and react differently
- Impactful for high charge density cationics as cationic polymers are thought to bind to dissolved organics in surface waters resulting in a decrease in the availability of the charged polymer
  - However, personal care and consumer products usually have much lower charge densities so interactive effects are less

Effects of Humic Acid_Daphnia

- **Observations:**
  - Daphnid survival improves in the presence of humic acid (clean water)
    - Some level of HA ingestion
  - Addition of humic acid did not seem to heavily mitigate toxicity to *D. magna* but did ameliorate toxicity to *Ceriodaphnia*
  - Interaction of polymer and humic acid causing excessive stickiness / physical effects in *D. magna*
    - At times, humic acid is increasing the toxicity of the chemical
      - The higher the concentration of polymer in addition to the higher concentration of humic acid the more drastic the sticking effect is

control

Attached carapace (polymer, no HA)



Humic acid + polymer attached to organism

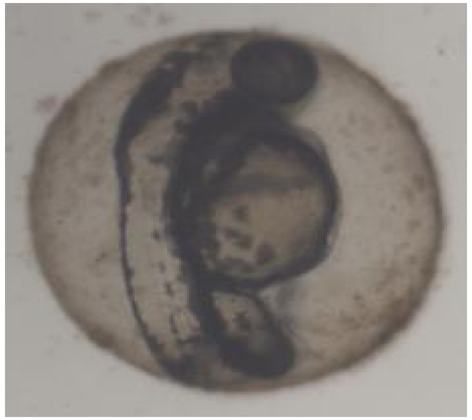
Organisms sticking together (humic acid + polymer)

Effects of Humic Acid_FET

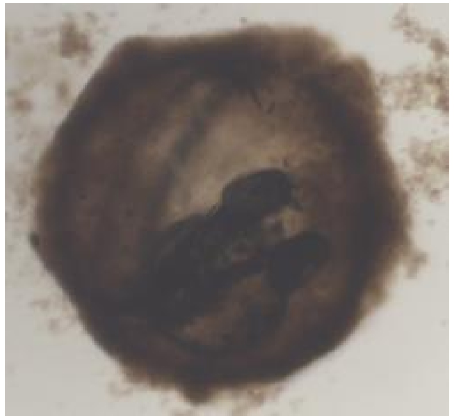
- Humic acid interacts very strongly with the chorion in a dose-dependent manner (in clean water)
- Presence of cationic polymers with HA reduces the amount of humic acid interacting with the embryo: bioavailability impact
- For a given level of humic acid, Suwannee River humic acid ameliorates toxicity more than Sigma-Aldrich humic acid in preliminary investigations
- The observations correspond with toxicity outcomes and analytical measurements

48-h, No HA

48-h, 2 mg HA/L



48-h, 20 mg HA/L



48-h
10 mg JR30/L
20 mg HA/L

48-h
100 mg JR30/L
20 mg HA/L

Conclusions

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- Most of our findings thus far represent “firsts” for environmental risk assessments of polymers
- Organic carbon can play an important role in the biological availability of cationic polymers
- Experimental data to date suggests unique toxicity patterns with issues concerning bioavailability, mitigation and physical impairment versus conventional organic compounds
  - Humic acid reacting strongly with the chorion
  - Interaction of polymer and humic acid causing (in some cases) an increase in toxicity with *Daphnia magna*
  - Different humic acids are reacting differently within toxicity assays
- Seeing exposure without uptake: external surface interactions, clogging of gill/gut structures are environmentally relevant
- These research outcomes are highly significant for the scientific development and justifications anticipated for registration of cationic polymers, particularly in Europe
- The need for development of more advanced methods and uniformity in using these methods for polymers by regulators has been made apparent

Future Needs

- Further explore differences in humic acid supply
  - Use same humic acid for all assays in future?
  - Explore mitigating effects in terms of TOC rather than humic acid concentration
    - Difference in binding capacity of different humic acid sources
- PQ10 exposure verification
- Improved microscopy, quantify carapace shedding rate and completeness; levels of ingestion (is this food?)
- Temporal aspects regarding bioavailability
- Is HA a potential secondary toxicity source when tested alone
- Role of different media types
- Role for dechorionation and does this result in differential interaction with different tissues

Questions?