Extrapolating New Approaches into a Tiered Approach to Mixtures Risk Assessment

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Conflict of Interest Statement

- The speaker has no conflicts with the subject or sponsors of this symposium

- Based on risk assessment guidelines of the US Environmental Protection Agency, and US Agency for Toxic Substances and Disease Registry, and European Commission.

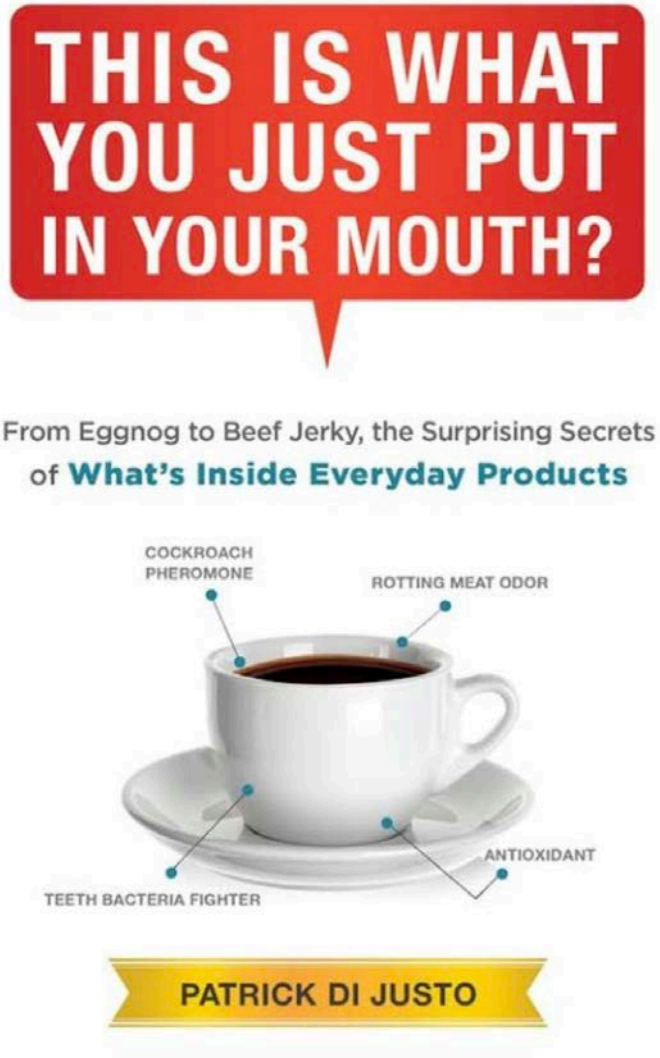
Mixtures in Food?

This is what you just put in your mug:

Caffeine
A plant toxin (like nicotine and cocaine) that plants use to kill bugs. It stimulates humans by blocking neuroreceptors for adenosine.

Water
Hot H_2O is a super solvent, leaching flavors and oils out of the coffee bean.
A good cup of joe is 98.75 percent water and 1.25 percent soluble plant matter.

And in that plant material are over 1000 other chemicals…including 20-30 mutagens and carcinogens



So Is Coffee Harmful?

- Carcinogenicity study of instant coffee in Swiss mice by Stalder et al., 1990
- Excerpted Abstract: Regular instant coffee was given in the diet to barrier-maintained, specified pathogen-free Swiss mice for 2 yr. Groups of 150 males and 150 females were fed 10, 25, or 50 g instant coffee powder/kg. The incidence of total neoplasms decreased from 70.6 and 56.8% in control males and females, respectively, to 34.8 and 36.2%, respectively, in the high-dose group. From this study it is concluded that instant coffee did not increase the incidence of malignant neoplasms.
- Results were confirmed in rats by Palm et al., 1984.

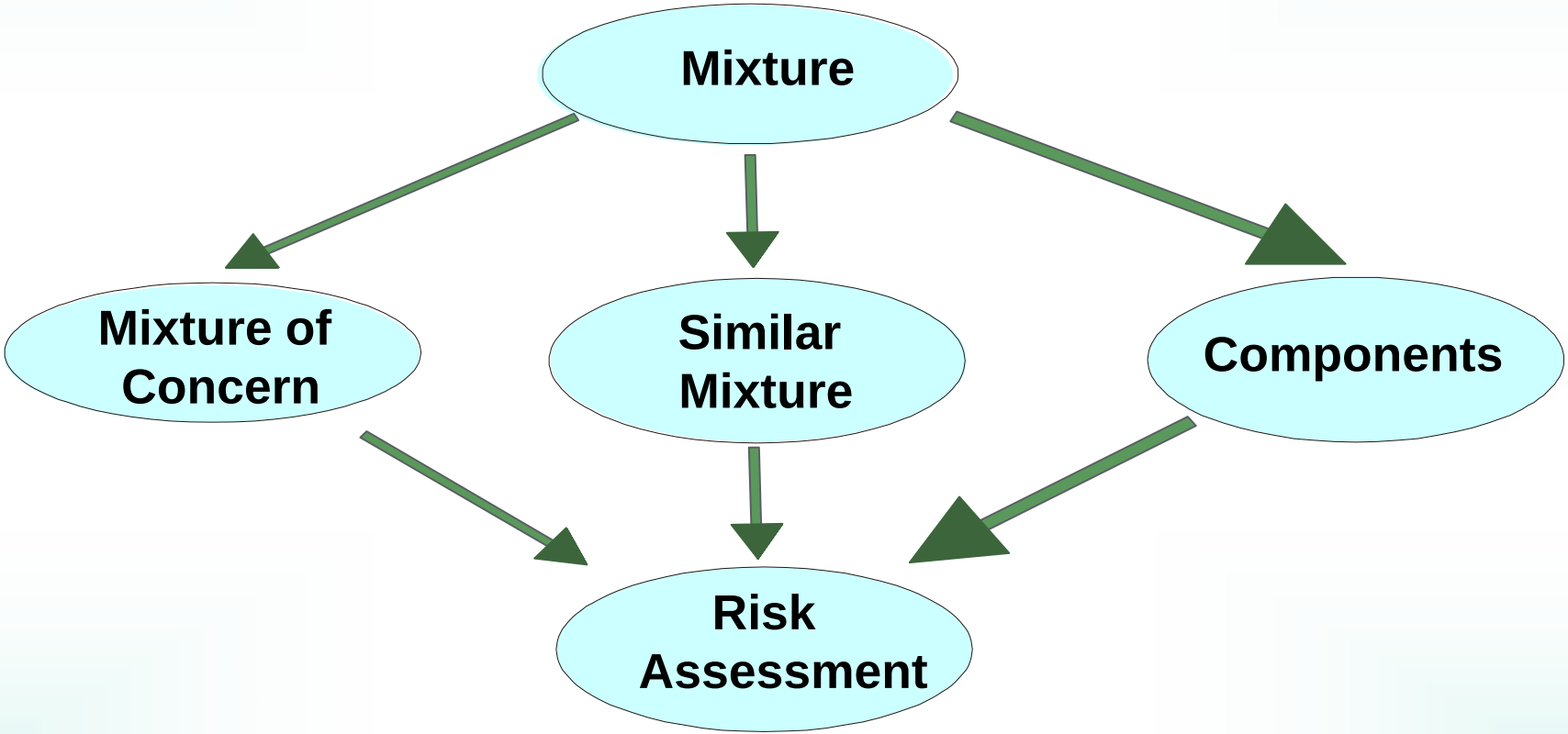
Key Definitions

- **Aggregate Risk** – involves consideration of exposures to a single compound from multiple pathways (food, drinking water, residential or occupational sources)
- **Cumulative Risk** - is an analysis, characterization, and possible quantification of combined risks to human health or environment from multiple agents or stressors. [EPA Framework For Cumulative Risk Assessment (2003)]
- **Multiple exposures**, to the same or different compound, may interact in a way that generates risk different from those when assessed individually
  - It is how real-life works
  - Characterization of significant sources of similar risk may be important for risk management
  - Still a question of how to use cumulative risk information

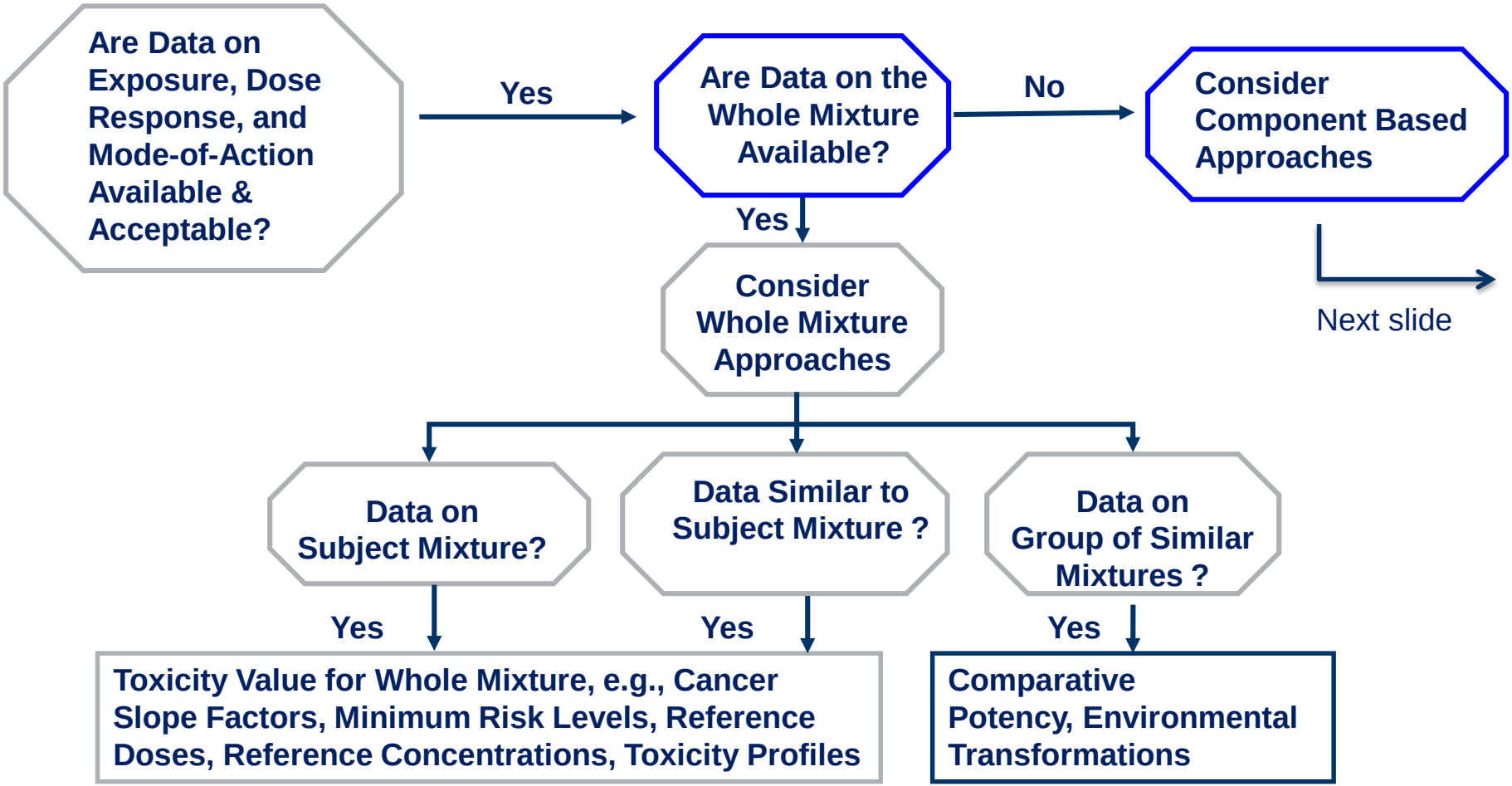
Chemical Mixture Interactions

- **Additive**: effect is equal to individual effects added together: 2 + 2 = 4
- **Synergistic**: combined effect of exposure to two or more chemicals is greater than the sum of their individual effects: 2 + 2 = 10
- **Antagonistic**: two chemicals when administered together interfere with each other's actions or one interferes with the actions of the other, e.g., Calcium blocking Tetracycline absorption: 2 + 2 = 1
- **Potentiation**: non-toxic chemical causes a toxic chemical to become more toxic or more active (adjuvants): 0 + 2 = 7
- **Coalitive**: several agents that have no known toxic effects interact to produce a toxic effect: 0 + 0 + 0 = 8

Principles of Mixtures Evaluation



Data Driven Approaches to Mixtures Assessment



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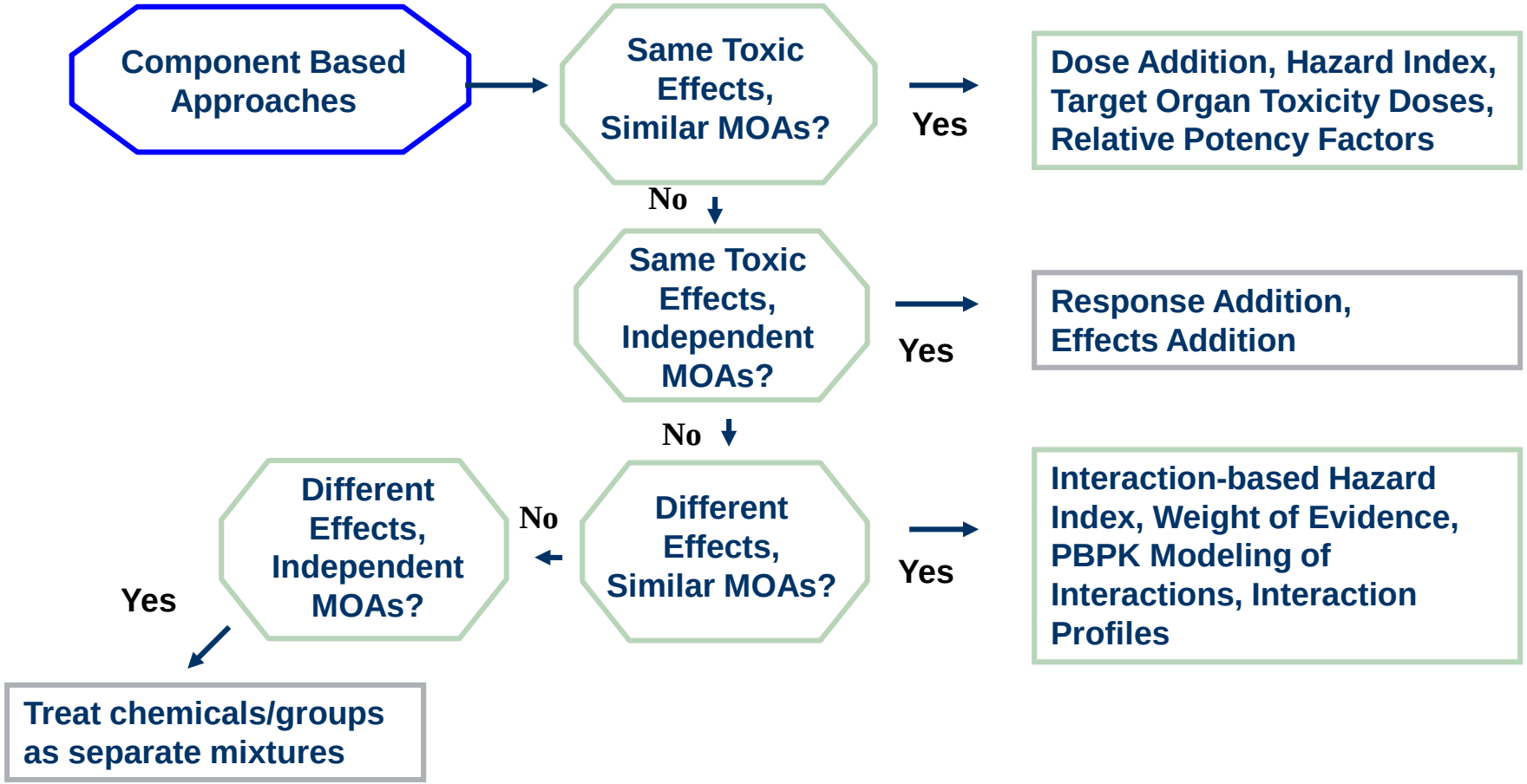
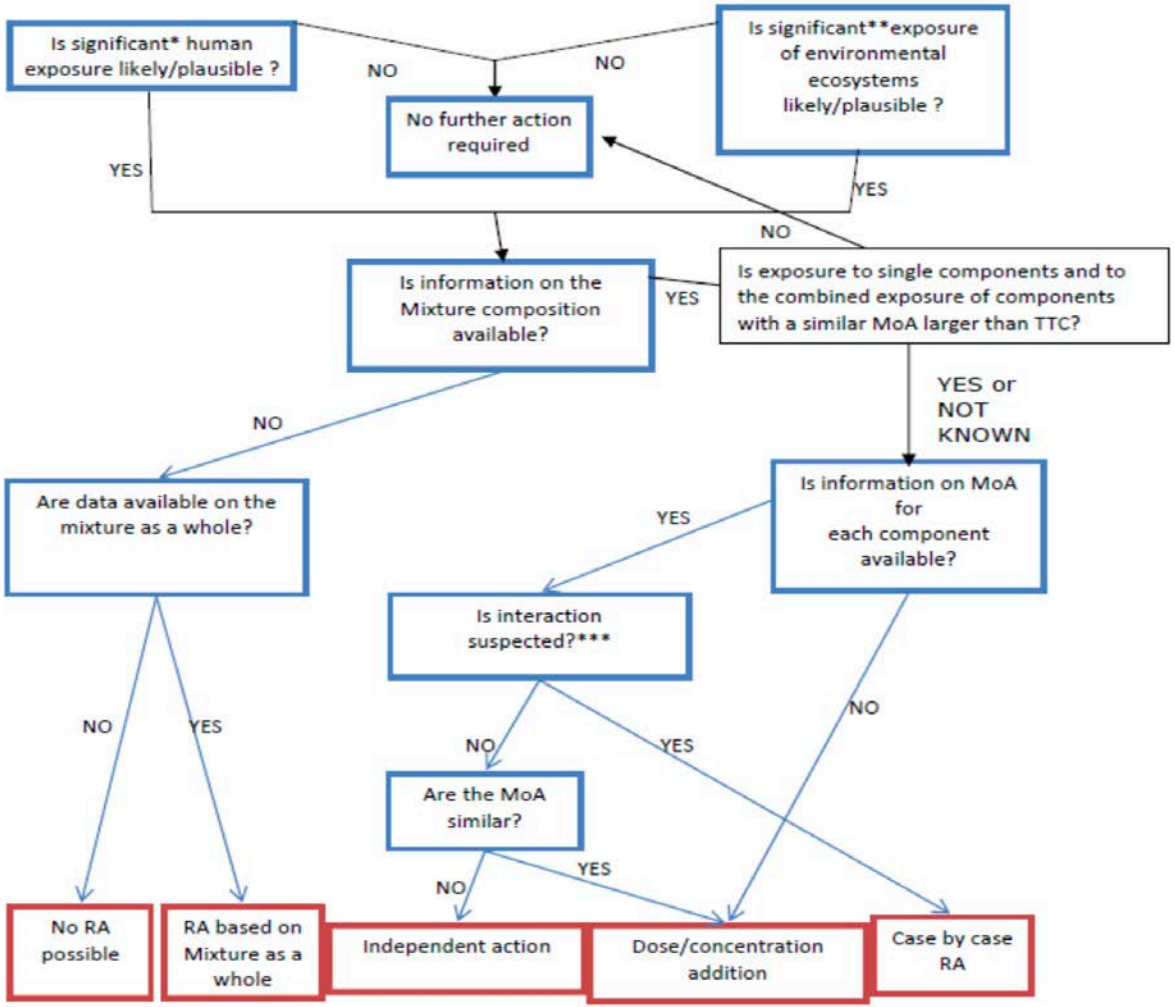


Decision tree for the risk assessment of mixtures (EC, 2011b) Source: European Commission



Summary and Conclusions of High-Throughput Screening: Michael DeVito

- HTS can provide screening level information on biological activity.
- Moderate throughput screening (libraries of 100 test articles or less) has advantages in that these efforts are more hypothesis based and can more easily be replicated in an iterative process.
- Combining chemical and biological data enhance our ability to implement sufficient similarity approaches.
- Sufficient similarity approaches all us to use prototype mixtures that have sufficient toxicological data and apply that data to untested mixtures that are deemed “sufficiently similar.”
- Alternative approaches can provide useful information for hazard assessment of complex mixtures in the context of sufficient similarity

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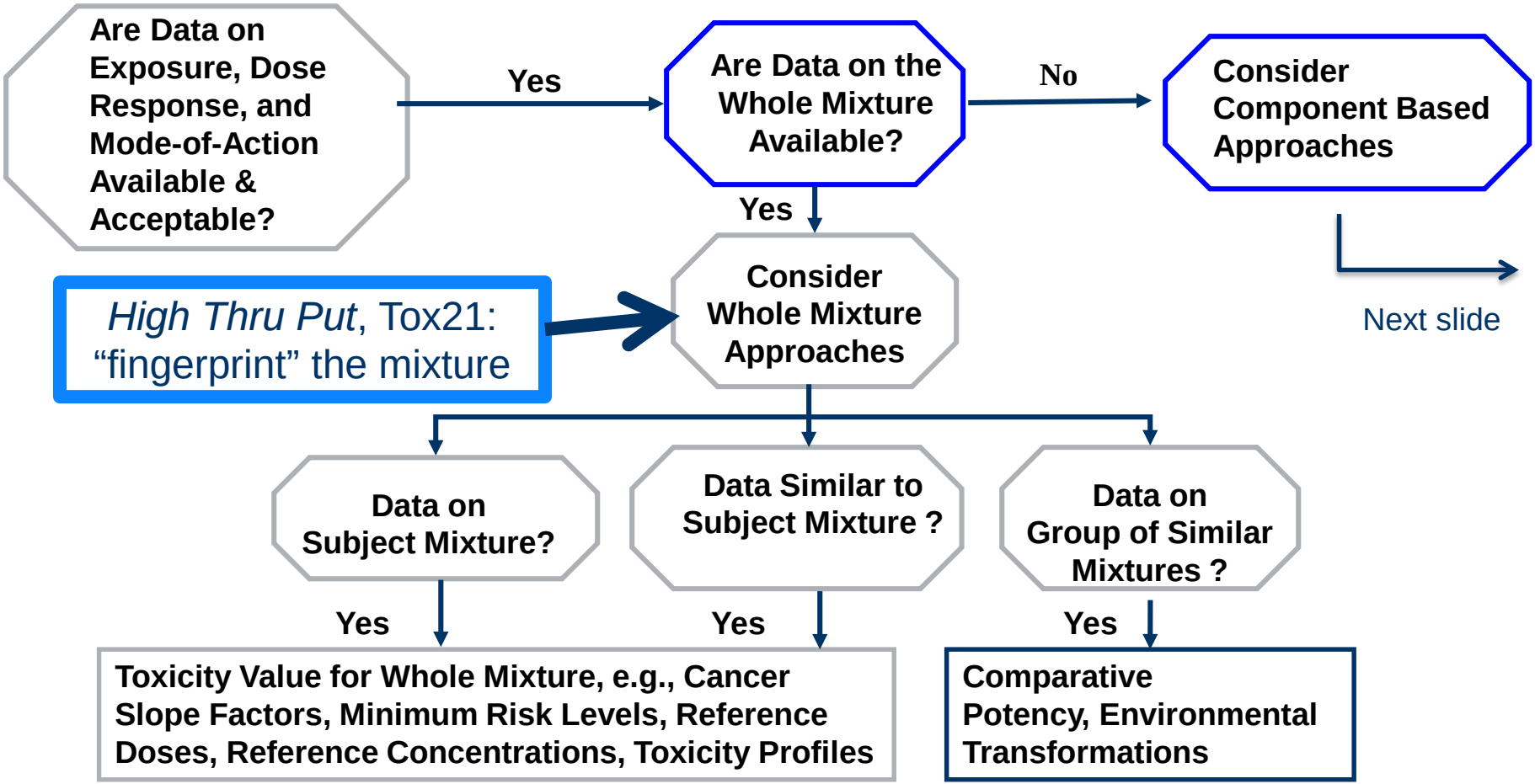

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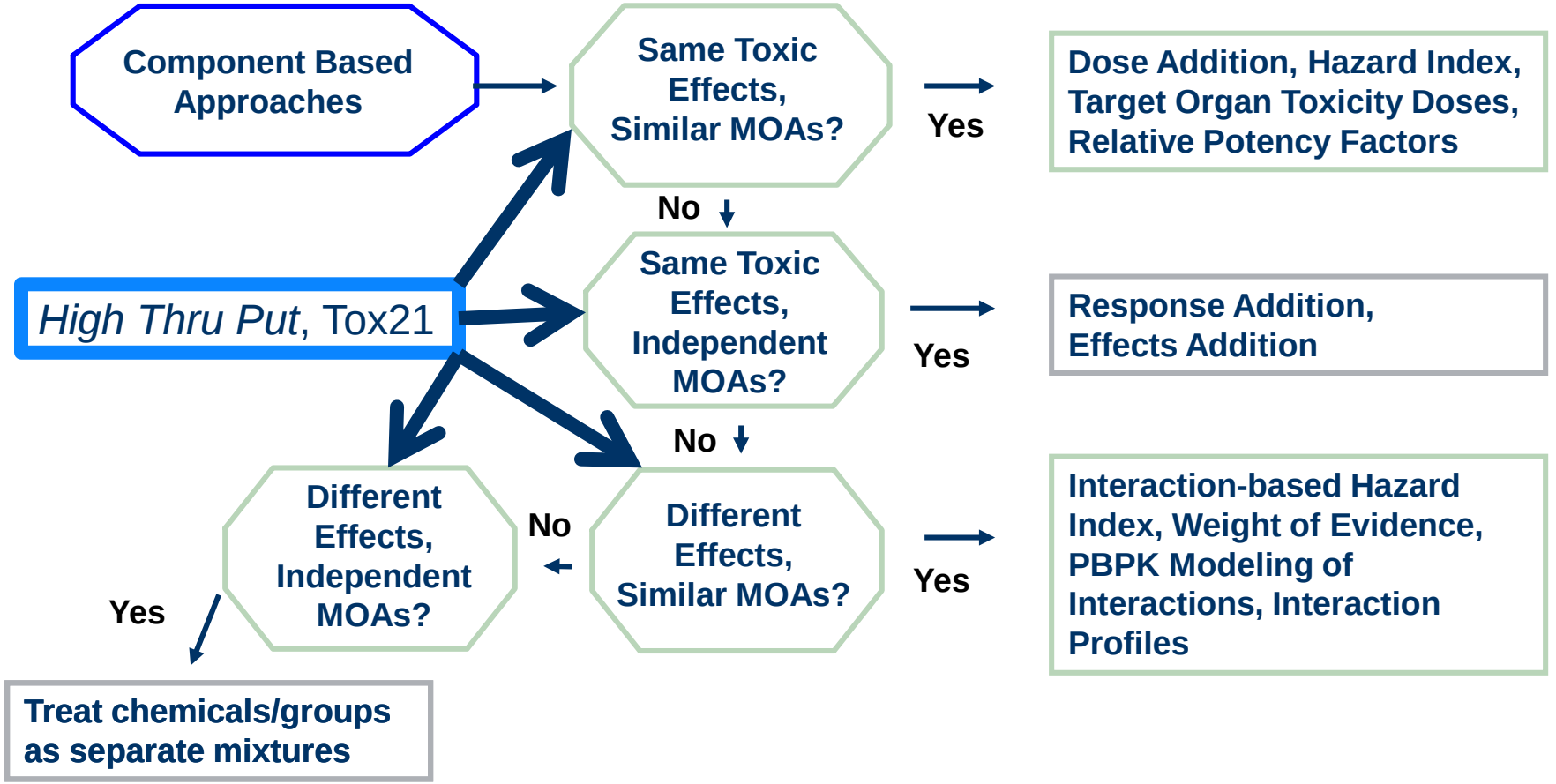

Summary of Constituent Characterization and Identification-*In Silico* Approach: Catherine Mahony

- Thoroughly vet the scientific literature-Define the question(s)!
- Advanced multi-detector analytical characterization technique to establish botanical constituent composition (simultaneous ID and quantitation)
- Each identified constituents is processed through a decision-tree to resolve questions;
    - *Use Thresholds for Toxicological Concern (TTCs)*, or otherwise close safety gaps, inform supportable exposure levels, or need for safety studies
- $\Rightarrow$ a focused approach for detecting possible bad actors in botanical extracts, the variables involved

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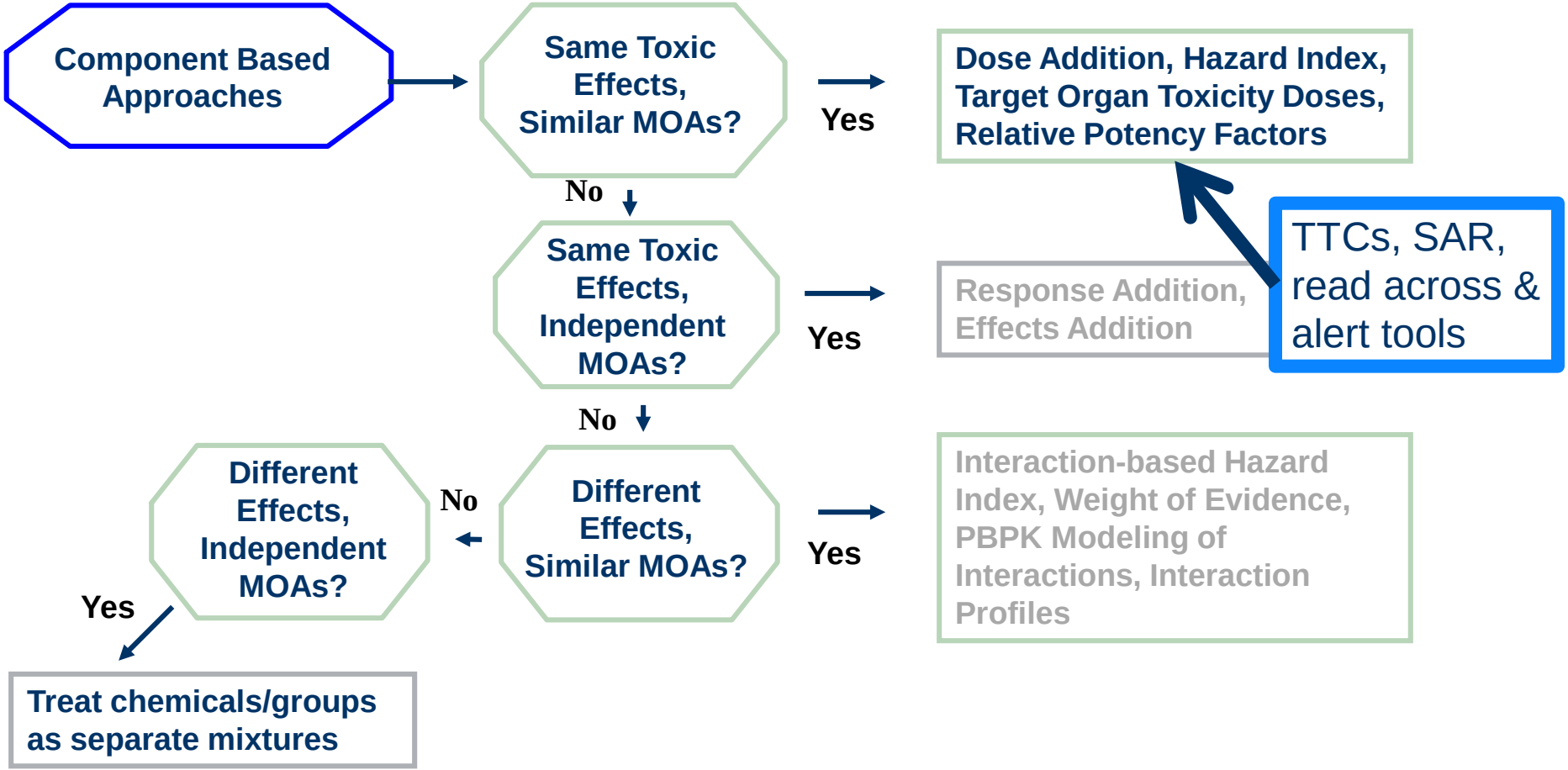

Summary of *In Vitro* MoA Approach: Catherine Mahony

- Biological activity of botanicals can be characterized through in vitro approaches

- Best approach will include both methods
  - CMAP to identify functional analogs (but more complex analysis)
  - Cerep to provide better focus (esp. to rule out false positives)

- Further work ongoing to utilize data for risk assessment purposes
  - Comparing concentrations to assess relative potency
    - CMAP effect/no-effect concentration ; Ki/IC50 for key receptors
  - Extending both panels for greater coverage of systemic toxicity MoA

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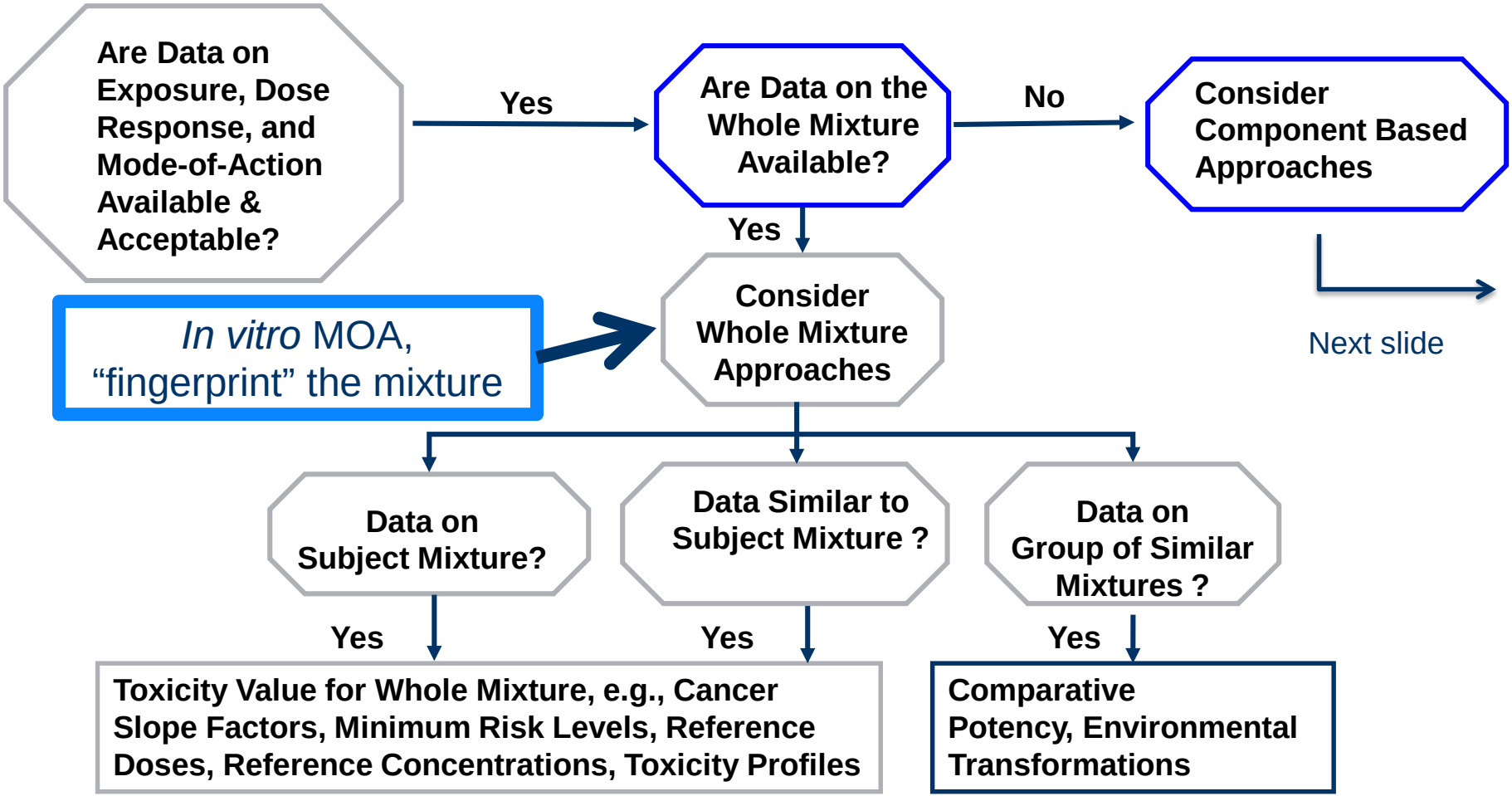

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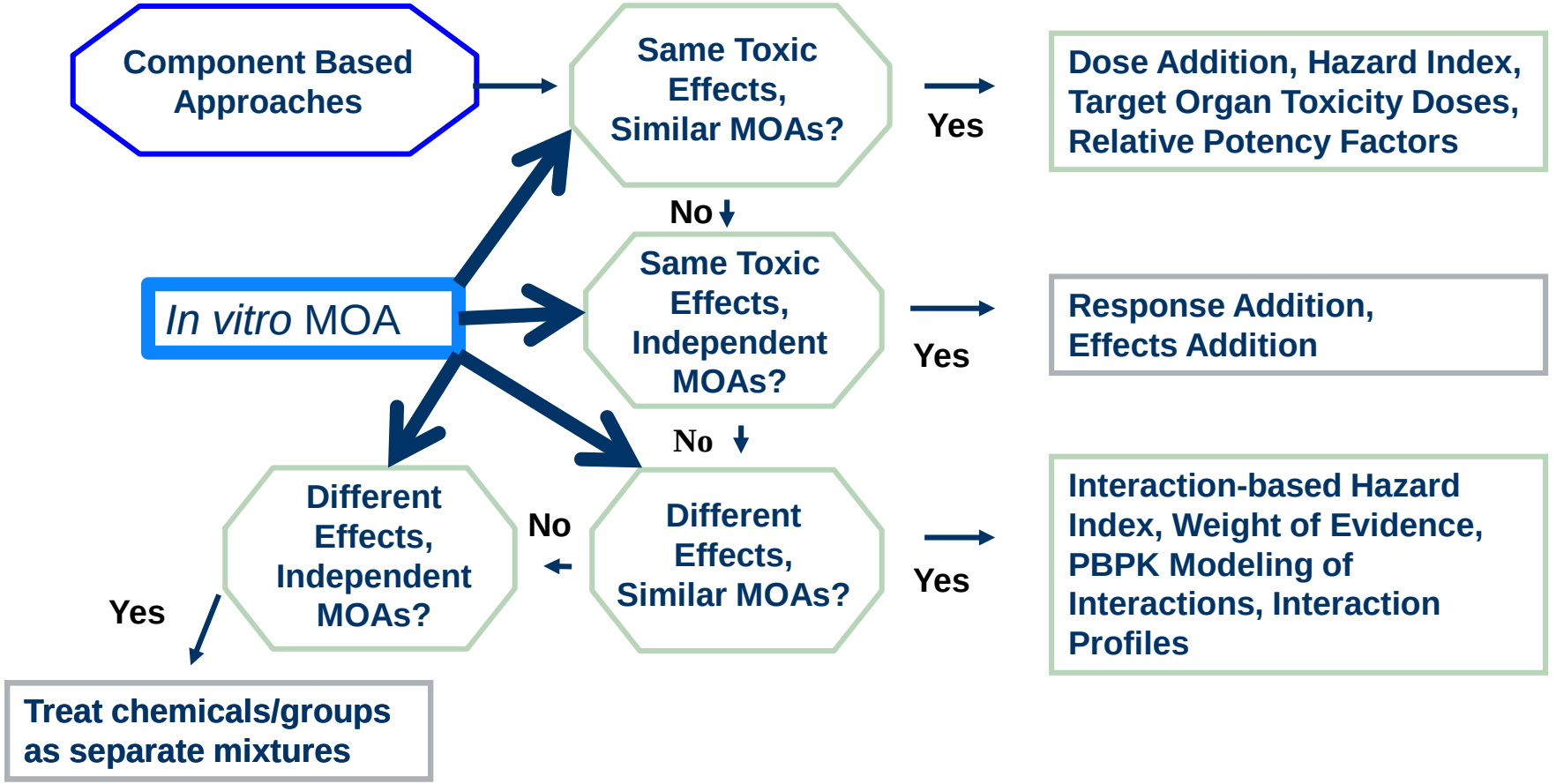

Summary of Piper Reid Hunt

- *C. elegans* assays are rapid and inexpensive, facilitating mixture assessment
- Concordance has been demonstrated between:
  - *C. elegans* LC50 ranking and rat LD50 ranking
  - *C. elegans* motility and mammalian neurotoxicity
  - *C. elegans* larval growth and mammalian developmental toxicity
  - *C. elegans* gene expression and mammalian mechanisms of toxicity
- **Validation studies are urgently needed**

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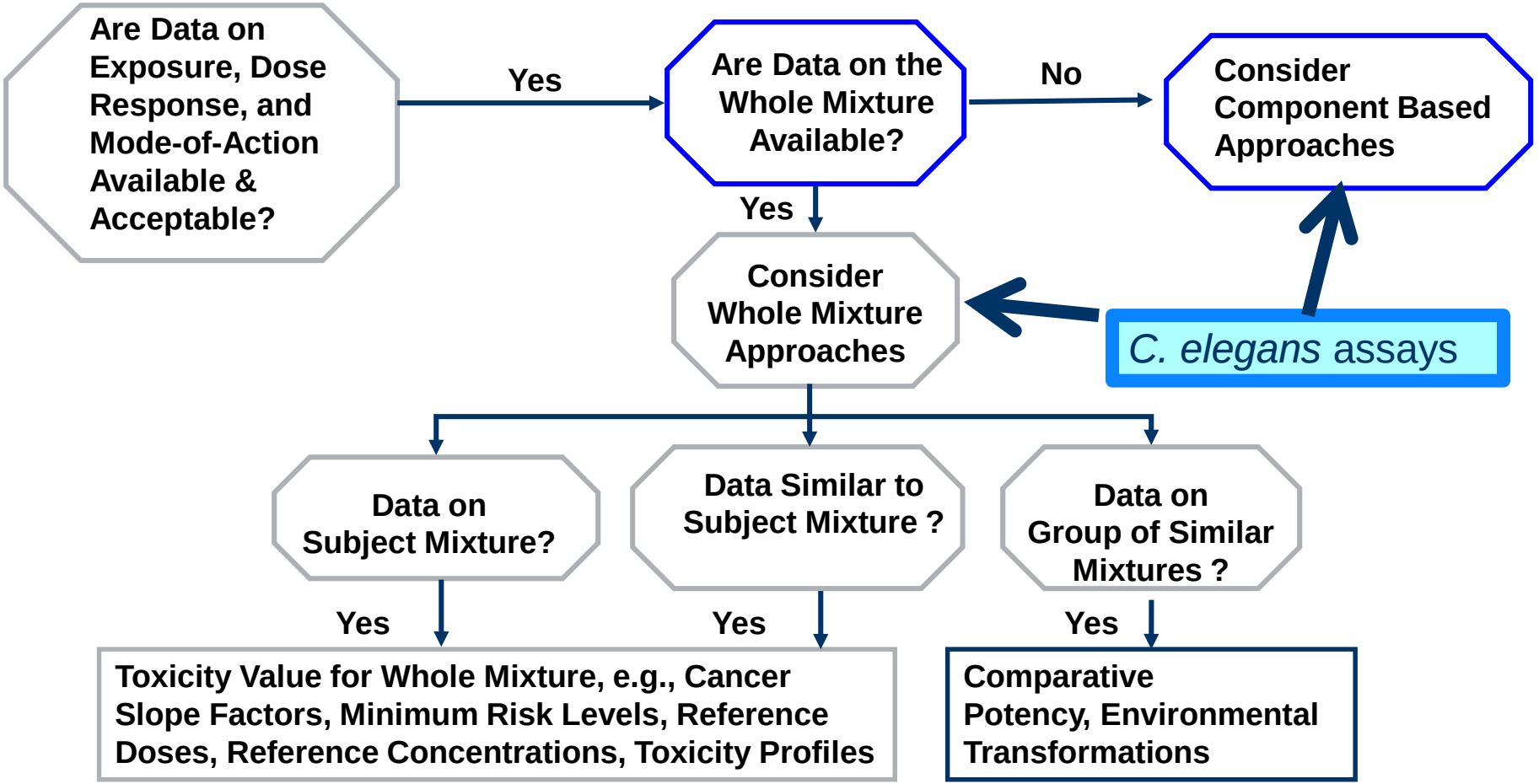

Summary

- Exposures to chemical mixtures is the rule, not the exception.
- Guidelines exist to sort our way though this sticky wicket, but improvements are always welcome.
- New methods will offer a significant improvement in the hazard assessment of chemical mixtures, and with appropriate exposure determination, should continue to promote the credible protection of public health.

Acknowledgements

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