Disentangling the Web: The Impacts of Binary Chemical Mixtures on Binary Species Interactions:

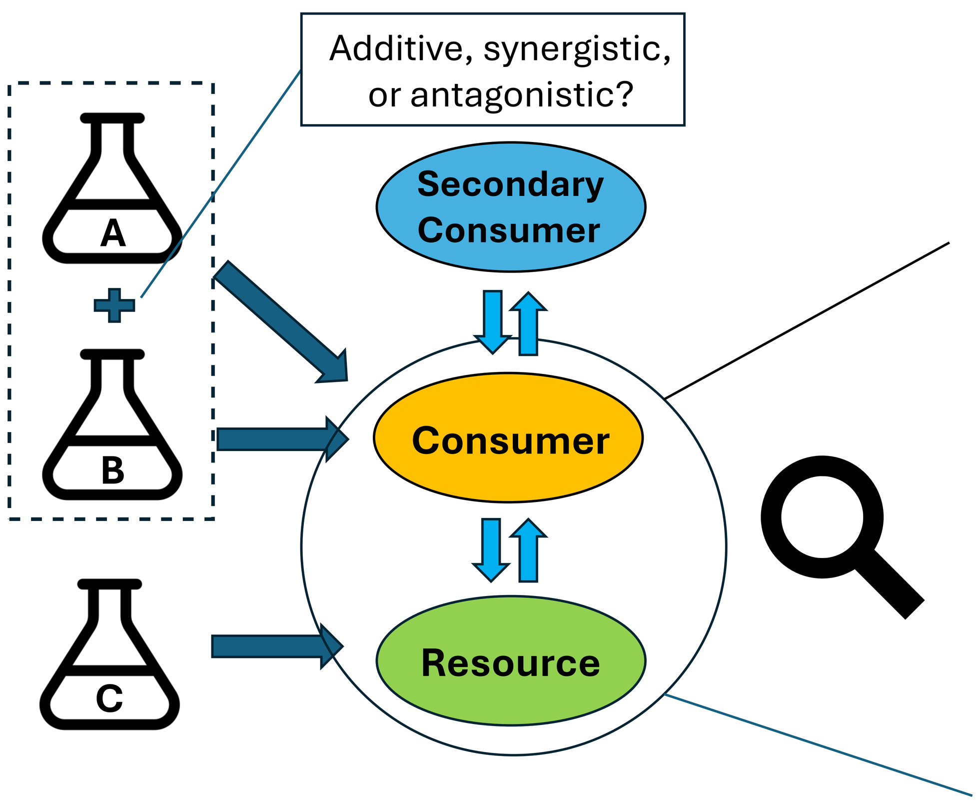
Dylan J Asbury[1], Amy Ockenden[1], Andrew P Beckerman[1], Alistair Boxall[2], Claudia Rivetti[3], Regianne Sanchez-Natumi[3] & Lorraine Maltby[1]

1. Department of Biosciences, University of Sheffield, UK; 2. Department of Environment and Geography, University of York, UK; 3. Science Environmental and Regulatory Science (SERS), Unilever®, Colworth Science Park, Sharnbrook, UK

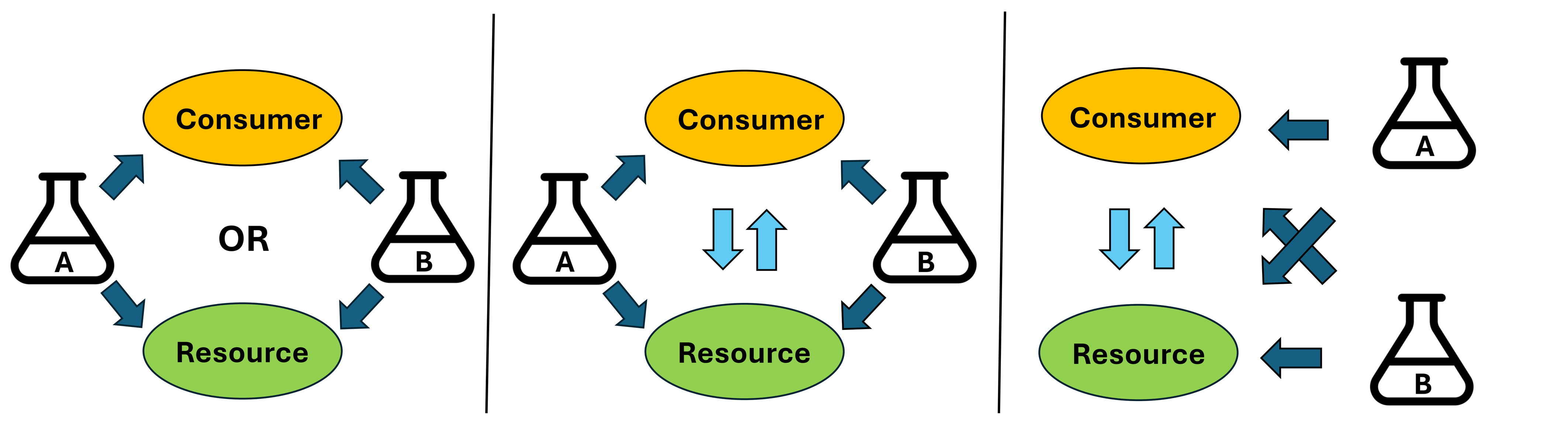
djasbury1@sheffield.ac.uk

Conceptualisation: The impacts of chemical mixtures on species interactions:

- Freshwater ecosystems support a rich diversity of species that are potentially exposed to complex chemical mixtures.
- Understanding the impact of multiple chemicals on multiple interacting species is a major challenge.



- The effects of mixtures can be additive, greater-than-additive or less-than-additive with respect to their components



Our current view:

- Current focus on the effects of chemicals and their mixtures is on individual species

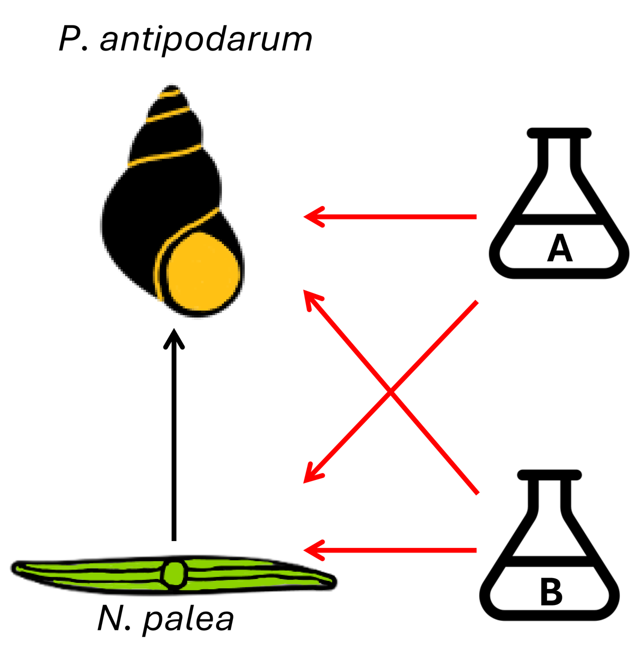
What we should consider:

- Species do not exist in isolation; they exist in complex communities where chemical effects on one species can have knock-on effects on others

How can we achieve this?

- Crossed-design experiments that focus on consumer-resource interactions allow mechanistic effects to be investigated

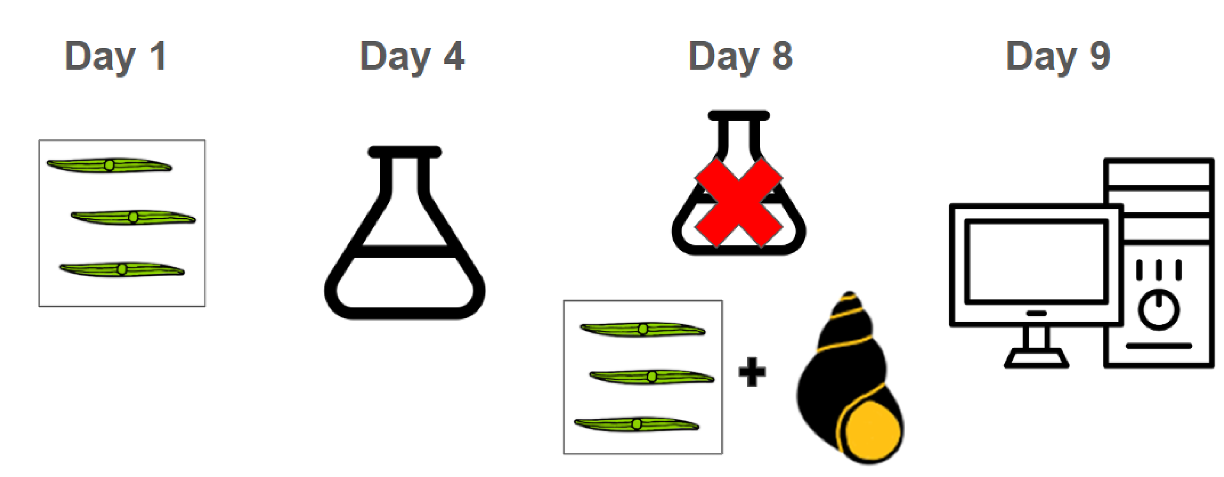
The System: Cross-design Post-exposure Experiments on a Grazer-Producer Interaction:



- Focus on a simple grazer-producer feeding interaction
- Key measure is feeding rate (i.e. cells consumed)

Chemical:	Concentration:	Sensitive Species:	Component in Mixture:
Copper	18 µg/L	Snail (consumer)	A
Azoxystrobin	180 µg/L	Snail (consumer)	A & B
Triallate	34 µg/L	Diatoms (resource)	B

- Two binary mixtures, one targeting the consumer (mixture A), one targeting both the consumer and the resource (mixture B)



- Day 1: Biofilm inoculation
- Day 4: Individual acute-exposure begins
- Day 8: Exposure ends; species interaction initiated
- Day 9: Interaction terminated; results analysed

Q1. Are the effects of binary chemical mixtures on feeding rate dependent on whether it is the consumer or resource exposed?

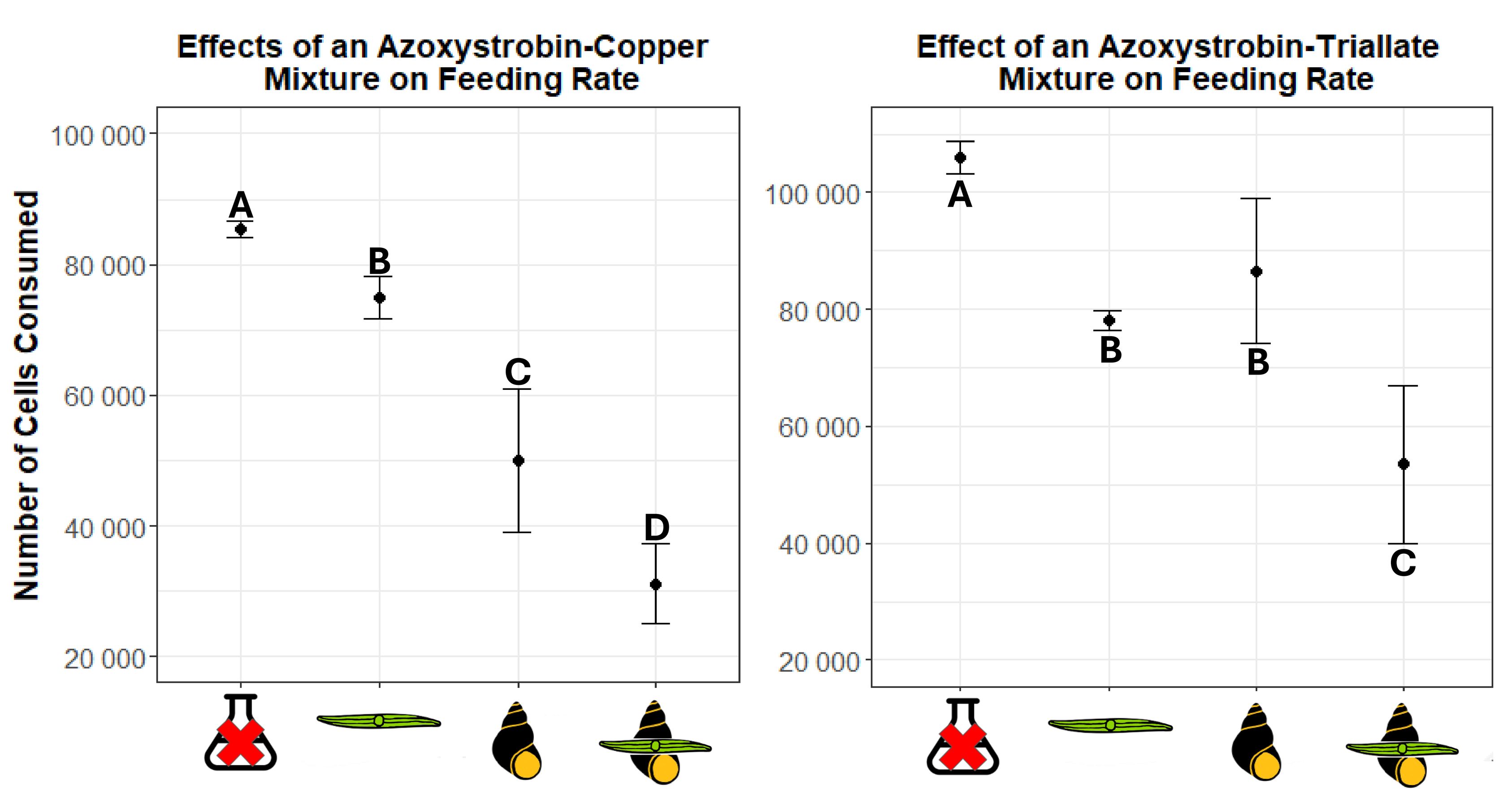
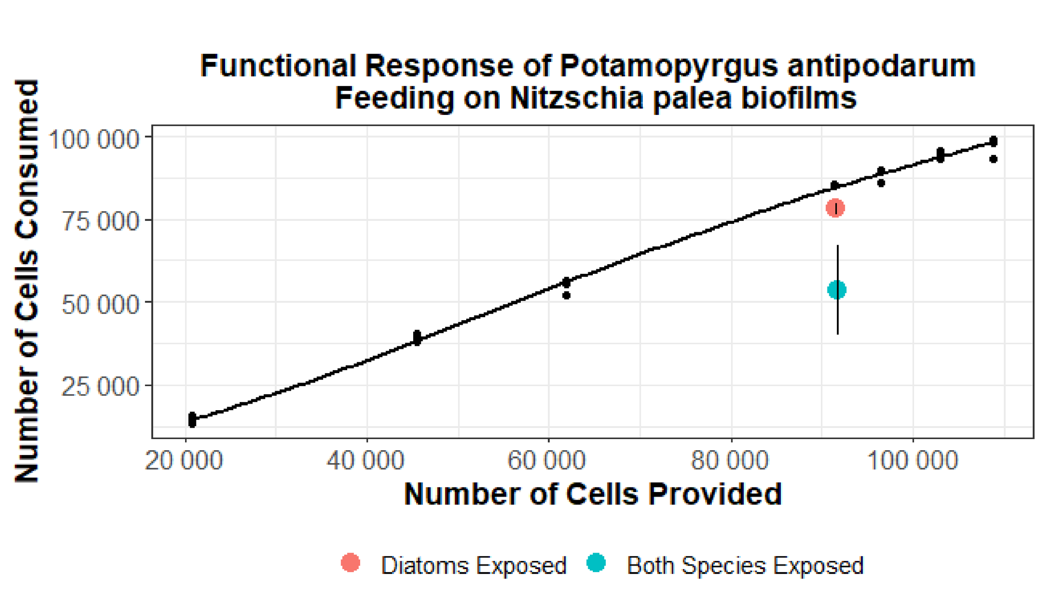


Figure 1. Target dependent effects of two chemical mixtures (azoxystrobin plus copper, azoxystrobin plus triallate) on snail post-exposure feeding rate. Letters denote significantly different treatments.

- The effects of binary mixtures on post-exposure feeding rate were dependent on species exposed and the mixture in question
- Binary mixture effects were greatest when both the consumer and the resource were exposed

A1. The effects of binary mixtures were dependent on species exposed, with the case where both species were exposed having the greatest impact under both mixtures



Are triallate effects only due to reduced diatom abundance?

- Triallate reduces the number of cells on biofilms
- However, the reduction in cells consumed when biofilms were exposed is greater than would be expected as shown by the functional feeding curve

Q2. Are the effects of binary chemical mixtures on feeding rate additive with respect to their components?

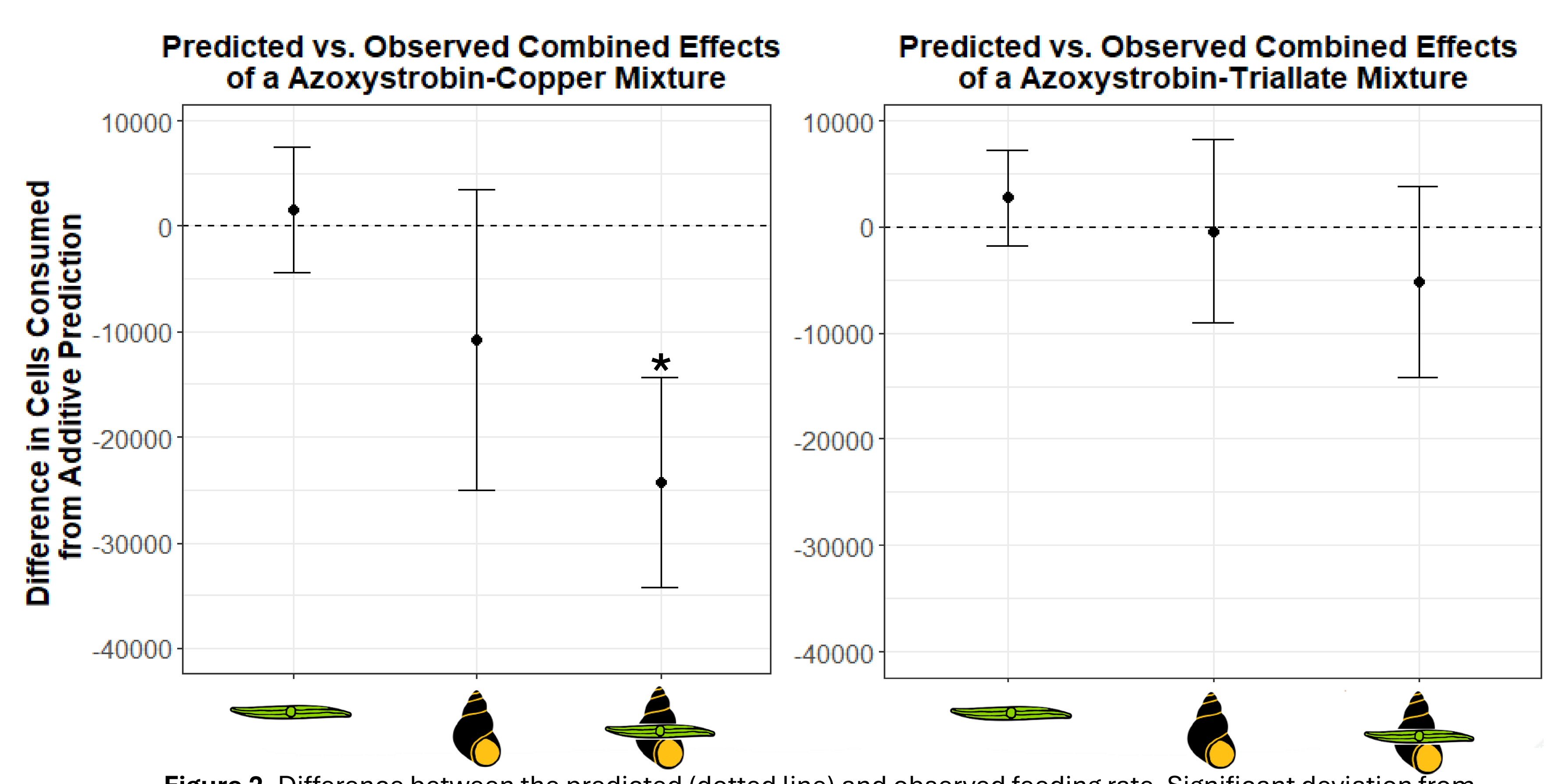


Figure 2. Difference between the predicted (dotted line) and observed feeding rate. Significant deviation from additive prediction is denoted as an asterisk. Data are mean ± 2 standard error

- Binary mixture effects were additive in almost all cases. Exception was the azoxystrobin-copper mixture when both species were targeted (where the consumer was sensitive to both chemicals)

A2. The effects of the binary mixtures were additive with respect to their components, except in the case where both species were exposed to azoxystrobin-copper

How are additive predictions tested?

- Contrasts that test whether the mean effect of the individual chemicals subtracted from the mean mixture effects are significantly different from zero

$$H_0: \mu AB = \mu A + \mu B - \mu C$$
$$=$$
$$H_0: (\mu AB - \mu C) - (\mu A - \mu C) - (\mu B - \mu C) = 0$$
$$=$$
$$H_0: \text{Effect of } AB - \text{Effect of } A - \text{Effect of } B = 0$$

(Where μA & μB are the individual chemical effect means, μAB is the mixture mean and μC is the control mean)

C(1, -1, -1, 1)

- Coefficient for Control (µC): 1
- Coefficient for Chemical A (µA): -1
- Coefficient for Chemical B (µB): -1
- Coefficient for Chemical A + B: 1

Disclaimer: This research was partially funded by Unilever®. This presentation reflects only the views of the author.

References:
- Klaminder *et al.* (2016) "Drug-induced behavioural changes: using laboratory observations to predict field observations"
- Microsoft Paint® V.11.2502.161.0 (2025): *Used for creating images for figures.*
- Rohr *et al.* (2006) "Community ecology as a framework for predicting contaminant effects"
- Saaristo *et al.* (2018): "Direct and indirect effects of chemical contaminants on the behaviour, ecology and evolution of wildlife"
- Scäfer *et al.* (2023): "Chemical mixtures and multiple stressors: Same but different?"

University of Sheffield

University of York

Unilever

ECORISC