

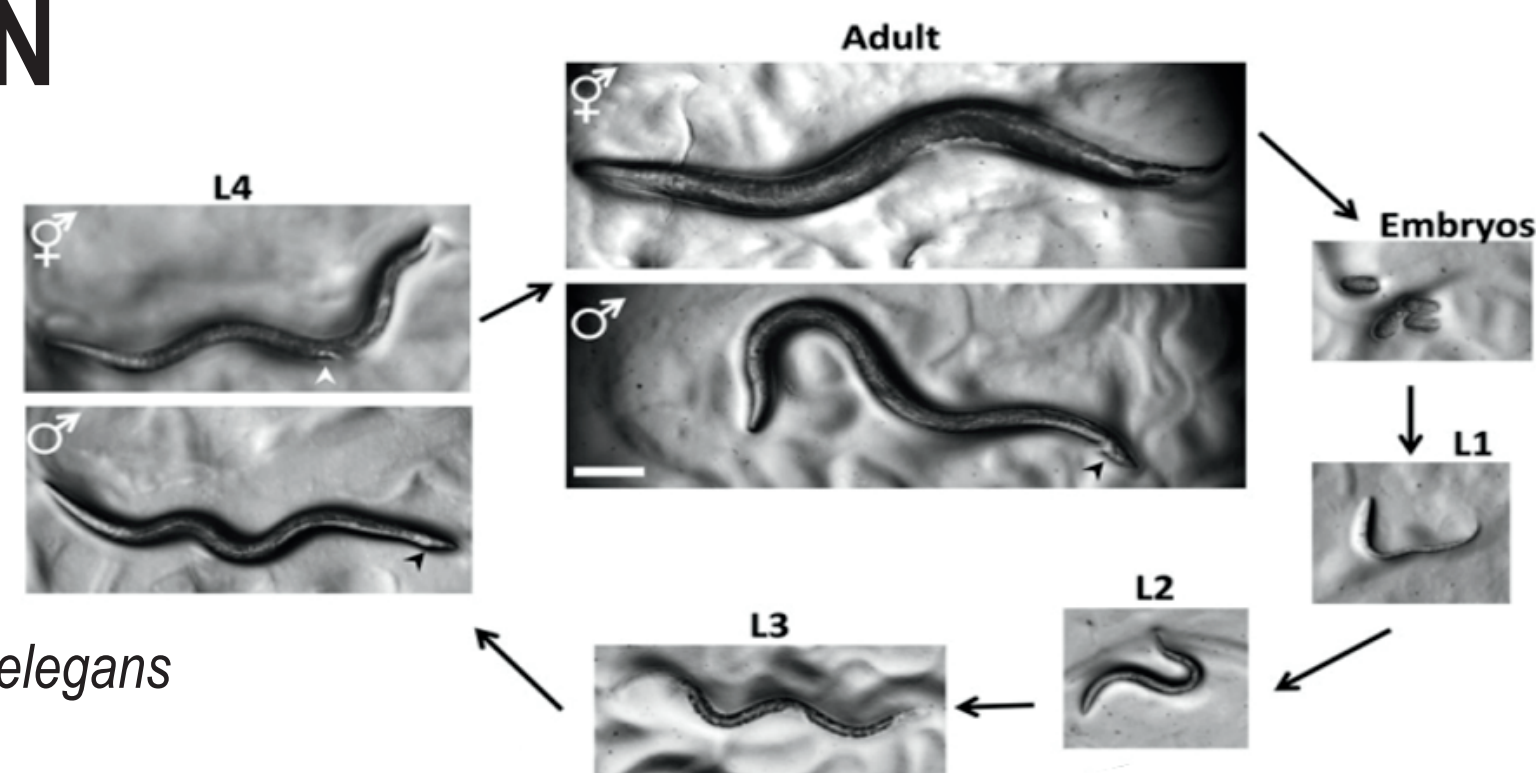
Optimization of the use of *Caenorhabditis elegans* in reproductive toxicology: in quest of the most optimal test conditions

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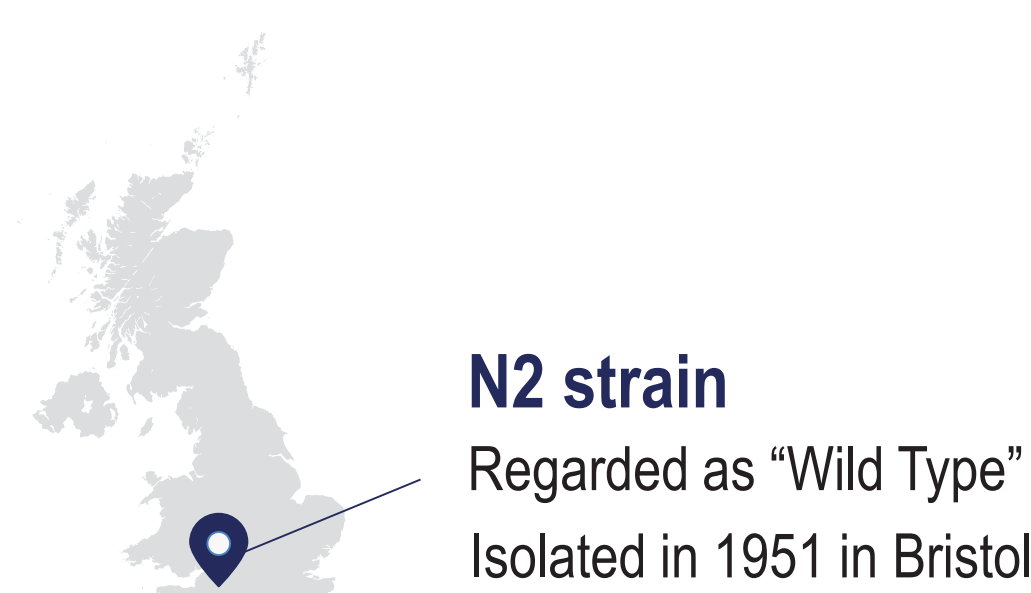
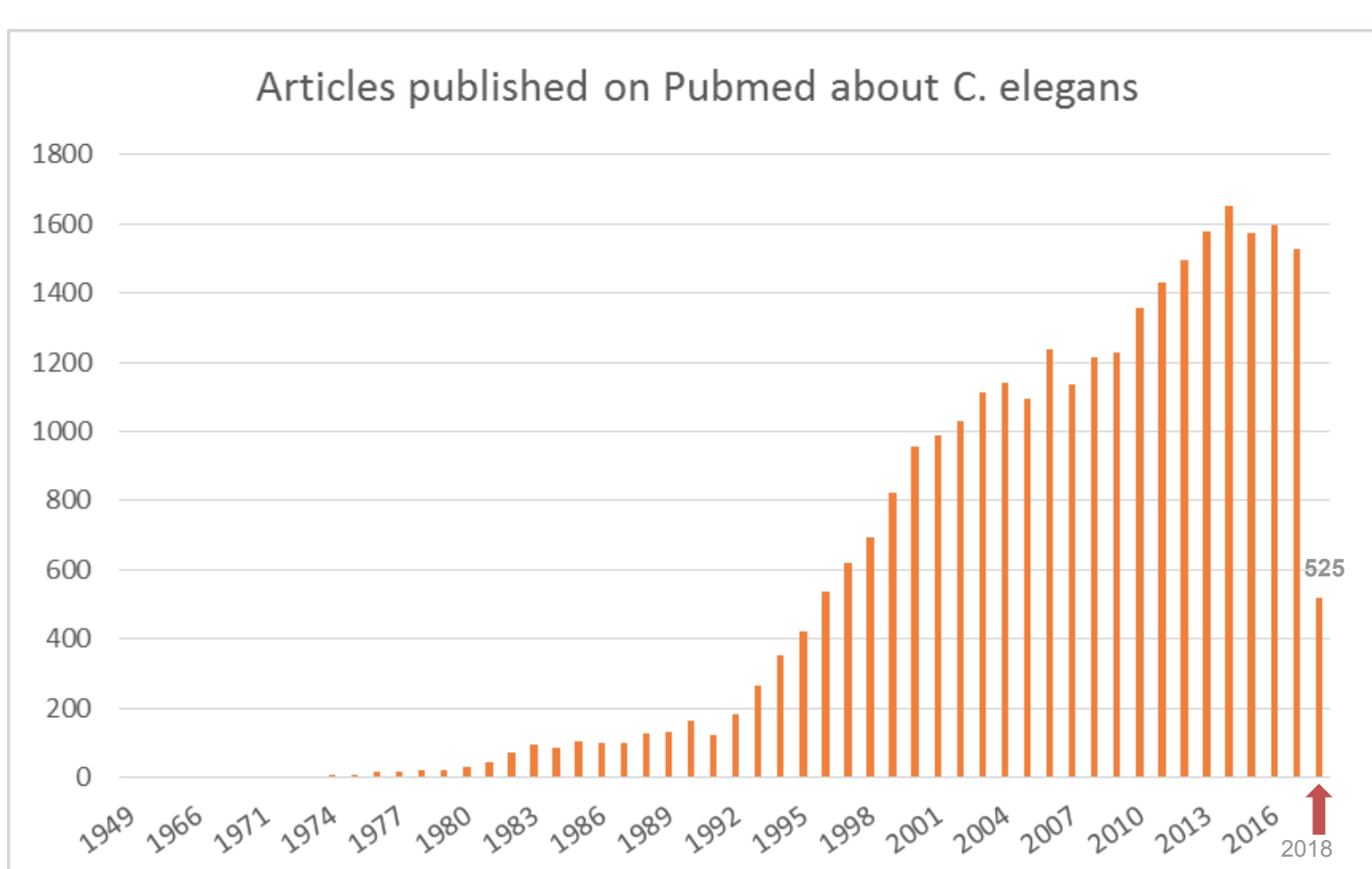
1 CAENORHABDITIS ELEGANS - INTRODUCTION

- Soil living organism
- Small in size (~ 1mm)
- Self-fertilizing hermaphrodite
- Fast reproduction cycle

- Increasing interest in the model organism *C. elegans*



Corsi et al., 2015

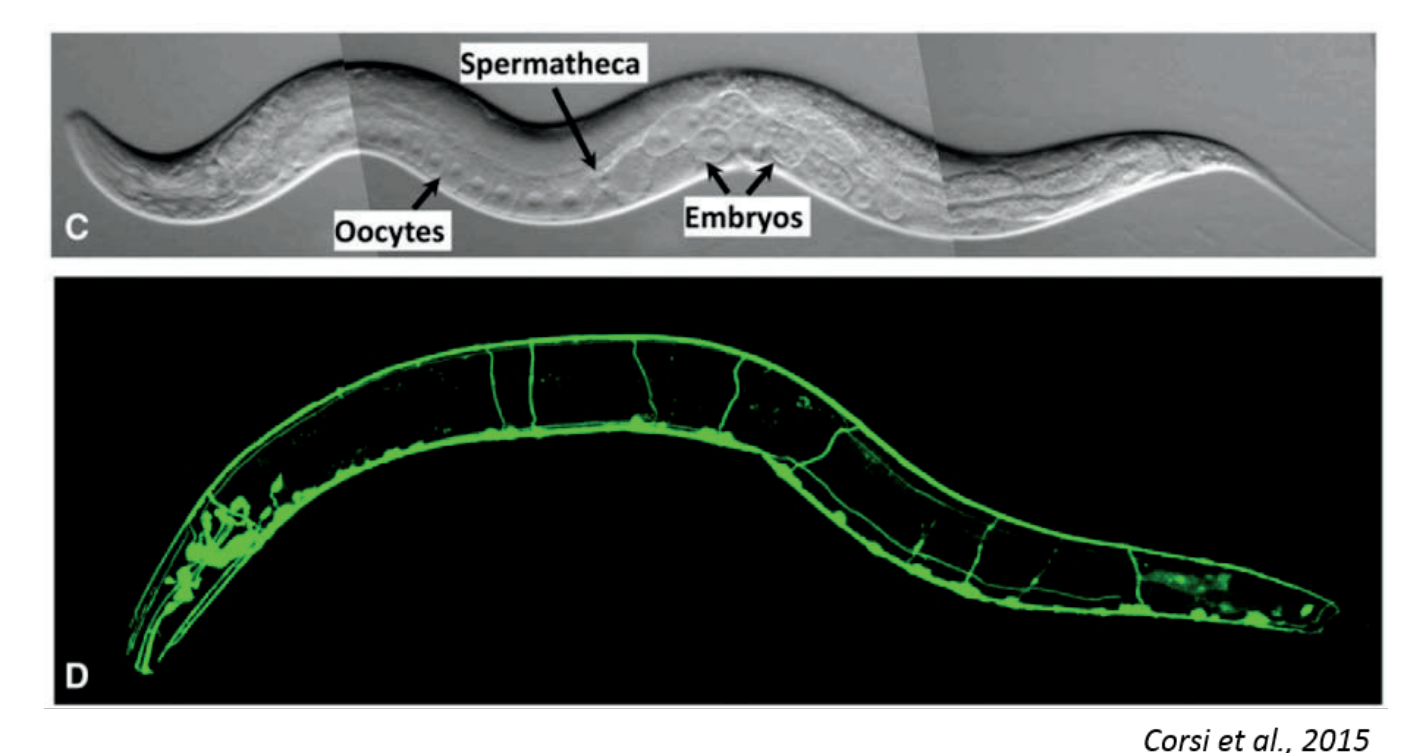


2 WHY IS THIS ORGANISM USEFUL AS AN ALTERNATIVE?

- Well studied**
Pattern of cell division is determined and fixed numbers of cells

- Fast and cheap**
Short life cycle of 2-5 days depending on temperature

- Transparent**
Ability to study cell division, cell migration, fluorescence etc.



Corsi et al., 2015

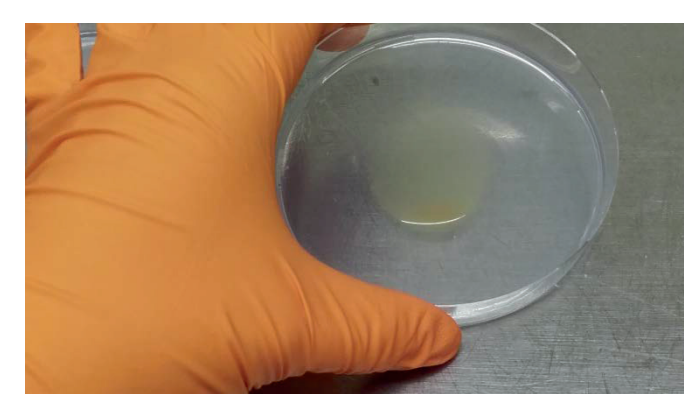
C. elegans reproductive toxicity test might be used for the screening and classification of compounds. Together with other established alternatives can form an *in vitro* testing battery.

3 TEST CONDITIONS FOR REPRODUCTIVE TOXICITY AND THEIR ADVANTAGES

A MAINTANCE: FAST, EASY AND LOW COSTS

Nematode Growth Medium (NGM) agar

- Easy to transfer
- Easy to synchronize
- Easy to store nematodes over time due to starvation

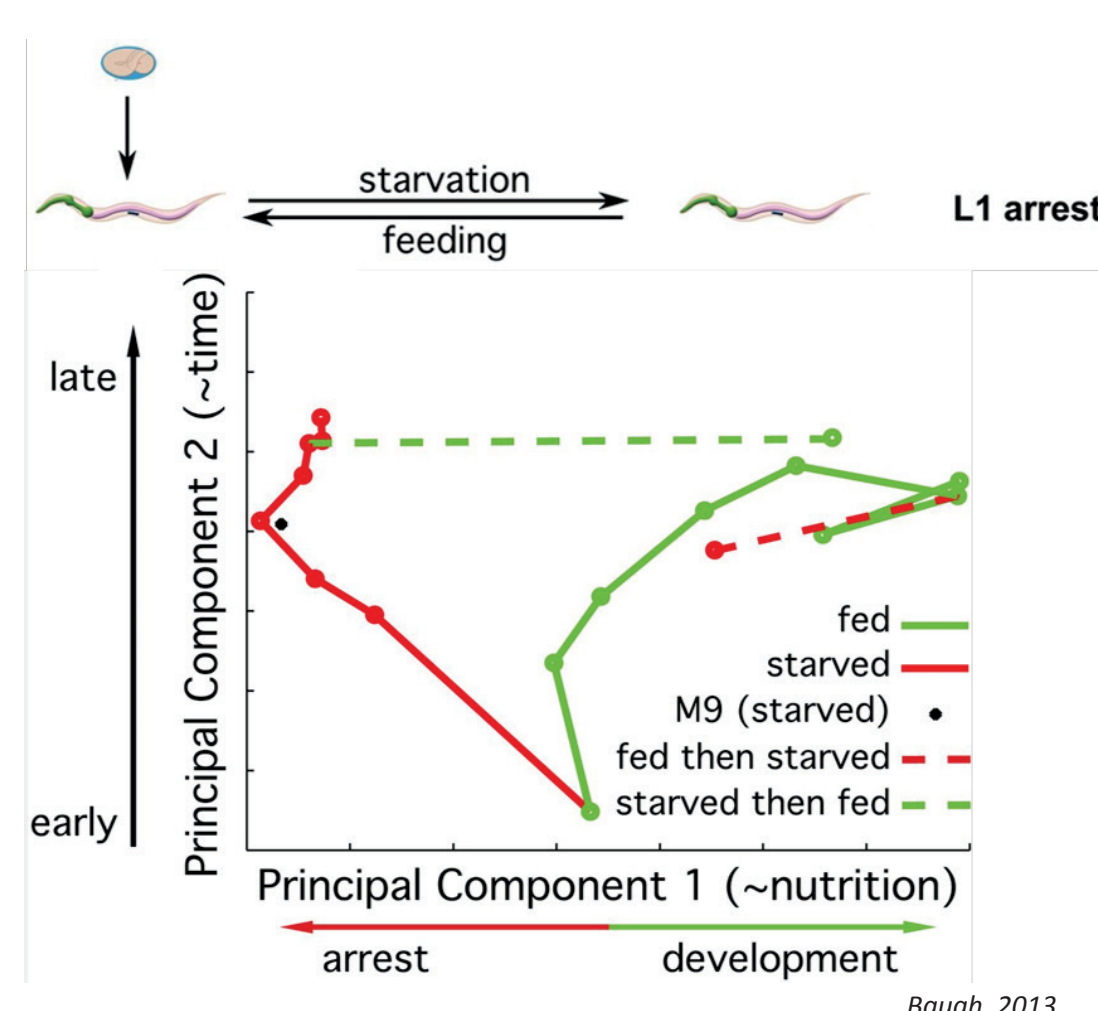


Bacterial food source → uracil-deficient *Escherichia coli* strain OP50

- In presence of food constant pace of nematode development
- OP50 allows visualization of nematodes due to reduced bacterial growth

B AGE-SYNCHRONISATION PRIOR TO REPRODUCTION TEST

- Age-synchronous nematodes needed to determine reproductive outcome.
- Alkaline hypochlorite treatment to obtain synchronised fertilized eggs from adult nematodes.
- Hatching of eggs in absence of food causes larval arrest.



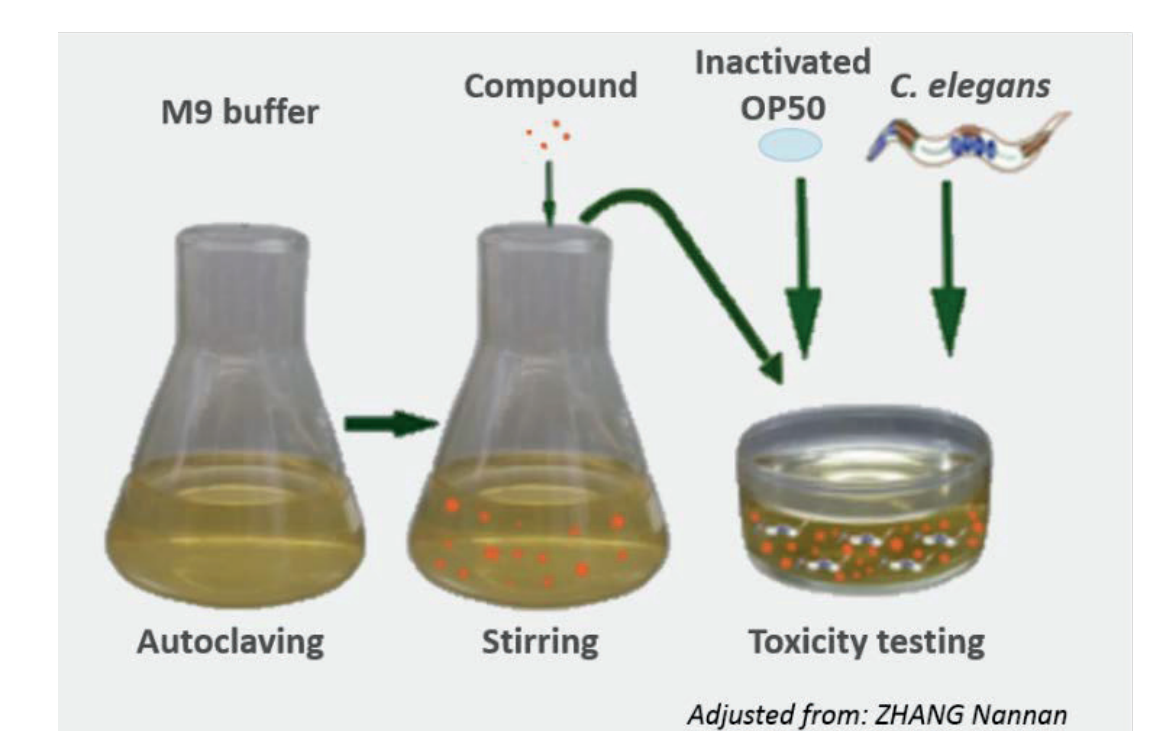
Baugh, 2013

C START EXPOSURE AT LARVAL STAGE L4

- Exposure during formation and maturation spermatocytes and oocytes
- No effect on development of the parental worms
- Easy to recognize by white spot

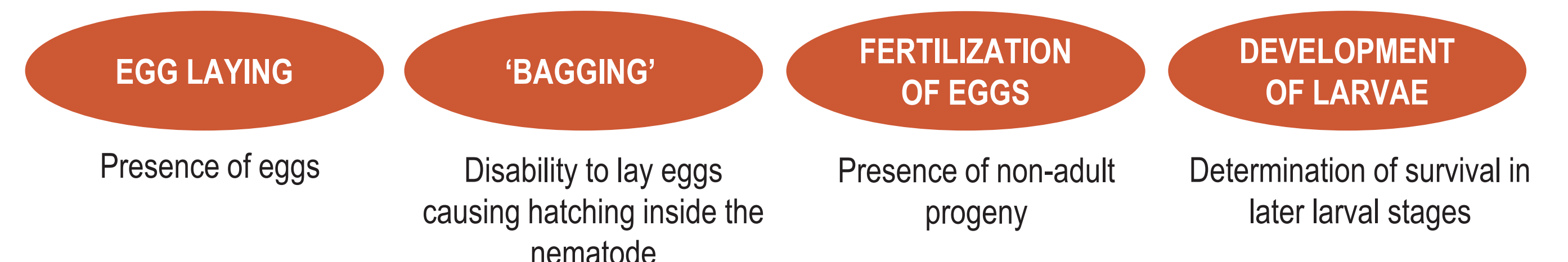
D EXPOSURE IN LIQUID MEDIUM

- Homogenous distribution of test compound within medium
- Exposure to known concentrations
- No avoidance behaviour from the nematodes
- Absorption through both cuticle and ingestion



Adjusted from: ZHANG Nannan

E COMBINING MULTIPLE OUTCOMES TO EVALUATE THE REPRODUCTIVE TOXICITY



4 FUTURE ROAD FOR DEVELOPMENT OF REPRODUCTIVE TOXICITY SCREENINGS TEST USING *C. ELEGANS*

