

# Use of Virtual Control Group for future assessments of effects of pesticides on avian reproduction

**Gunther du Hoffmann<sup>1\*</sup>, Diana Temple<sup>1</sup>, Thomas George Bean<sup>2</sup>, Carsten Lange<sup>3</sup>, Ryan Davis<sup>1</sup>**

<sup>1</sup> Eurofins EAG Agrosience Services, Easton, MD, USA, <sup>2</sup> BASF Corporation, Ecotoxicology, Research Triangle Park, NC, USA, <sup>3</sup> BASF Agricultural Solutions, Ecotoxicology, Limburgerhof, Germany; \*(presenting author): [Gunther.duHoffmann@AS.EurofinsUS.com](mailto:Gunther.duHoffmann@AS.EurofinsUS.com)

## Background

- Protocols for conducting avian reproduction studies based on the OECD 206 [1] and OCSPP 850.2300 [2] guidelines typically use 144 adult birds and ~2000 offspring during each test.
- There is a growing trend in mammalian toxicity testing towards 'virtual control groups' (VCG) as a way of reducing vertebrate testing, but to our knowledge this has yet to be explored for birds.
- This poster presents a preliminary investigation of whether virtual control groups that are constructed from avian reproduction study historical control data (HCD) be used to reduce bird numbers needed for testing.

## Material & Methods

### Construction of the VCG from Eurofins' HCD

- Two anonymized mallard reproduction studies were provided for the preliminary assessment of use of VCG in avian repro studies.
- 27 mallard repro studies from the Eurofins HCD database were used to construct the VCG:
  - 1 study with 10 replicates
  - 3 studies with 15 replicates
  - 10 studies with 16 replicates
  - 13 studies with 18 replicates→ total of 447 controls

### Endpoints evaluated

- 12 of the 15 endpoints required by US EPA were evaluated:
  1. Cracked eggs of eggs laid
  2. 14-d survivors of hatchlings
  3. 14-d survivor weight
  4. Eggs laid/hen/d
  5. Eggshell thickness
  6. Adult food consumption
  7. Hatchlings of egg set
  8. Hatchling weight
  9. Terminal female bodyweight
  10. Terminal male bodyweight
  11. Viable embryos of eggs set
  12. 3-wk live embryos of viable embryos

### NOEC as reported in the final report (Comparison with concurrent control)

- Study 1: NO NOEC determined based on statistical significance at all treatment levels for **(Endpoint #2) 14-d survivors of hatchlings** and **(#5) Eggshell thickness**.
- Study 2: The NOEC was determined to be the mid treatment concentration based on effects on **(#2) 14-d survivors of hatchlings** and **(#4) Eggs laid/hen/d** at the highest treatment concentration.

### Comparison against VCG

- 1000 studies were recreated for Study 1 and Study 2, each time replacing the control data with a VCG through systematic random selection of 18 replicates from the HCD which were resampled with replacement.
- Four statistical tests were run on each of the 12 endpoints for the 1000 studies comparing against the VCG:
  - a) Williams's Multiple Comparisons Test
  - b) Jonckheere-Terpstra Step-Down Test
  - c) Dunnett's Multiple Comparisons Test
  - d) Wilcoxon Test with Bonferroni-Holm Correction

Quantal and conditionally quantal data were arcsine square root transformed to make the data pseudo-continuous.

## Results



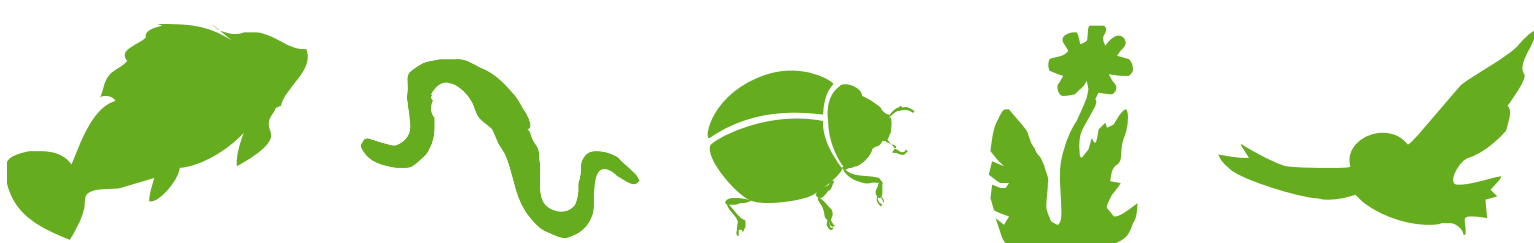
## Conclusion

- Over 92% of simulated VCGs generate same conclusion for study 1.
- Over 96% of simulated VCGs generate same conclusion for study 2 for eggs laid and a lower endpoint by one dose group for 14-d survivors of hatchlings, but outcomes varied by statistical test from 0% to 94% of simulated studies.
- Encouragingly, statistical reanalysis using a VCG in place of the concurrent control showed consistent and reliable results, providing robust statistical power and often providing a more conservative finding than the concurrent study control alone.
- Use of VCG in place of concurrent control could save up to 36 animals per test (i.e. 18 pairs) and tens of offspring for reproductive endpoints.
- That said, while the natural biological variability can be assessed using the HCDS, a smaller concurrent control may still be needed given the importance to assess validity criteria to ensure guideline compliance.
- 3-4 control pairs (6-8 birds) may be sufficient to compare against the HCDS to validate the use of the VCG.

## Next steps

- Larger scale industry collaboration to conduct a more comprehensive assessment of use of VCG.
- Assessment for both bobwhite quail and mallard.
- Evaluation of how the VCG could be used for BMD<sub>10</sub> derivation.
- Determine suitability of smaller number of controls to evaluate biological variability among batches of birds and for demonstrating validity criteria have been met.

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References  
[1] OECD (1984) Test Guideline 206: Avian Reproduction Test.  
[2] U.S. EPA (2012) OCSPP 850.2300: Avian Reproduction Test.