

Rapid screening in *Dugesia japonica* planarians

Planarian HTS: Danielle Ireland¹, Christina Rabeler¹, and Eva-Maria S. Collins^{1,2,3}

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Planarians: non-vertebrate organismal model

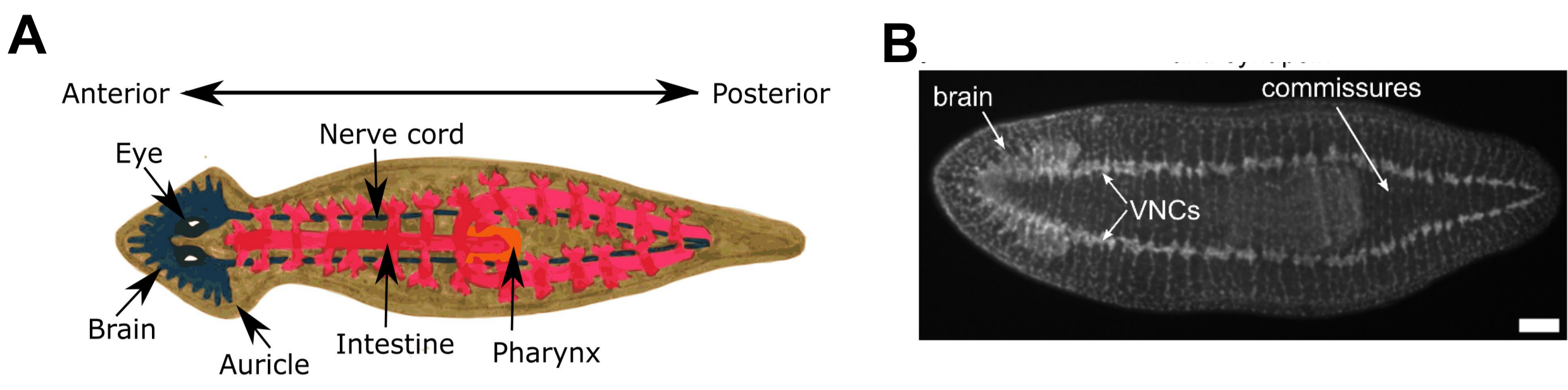
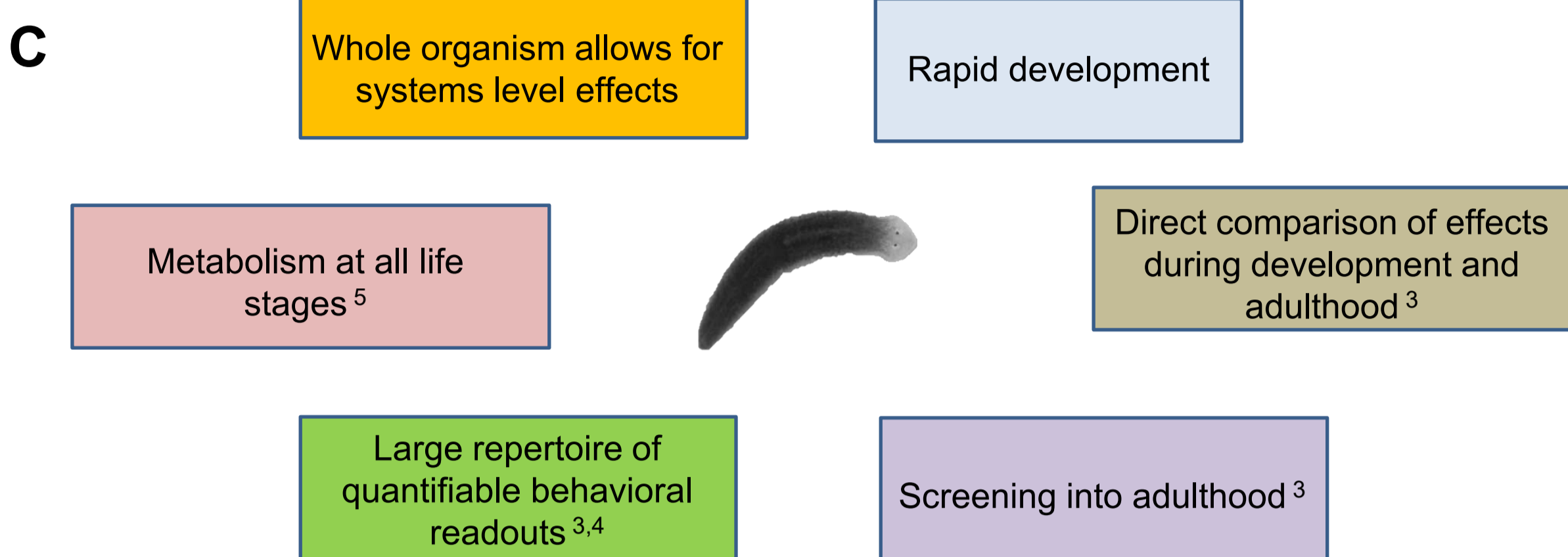


Figure 1. (A) Planarian anatomy, from¹. (B) Visualization of the planarian central nervous system using immunohistochemistry. VNC: ventral nerve cords. (C) Planarians provide unique advantages over other models.



Assays: Lethality and Behavior

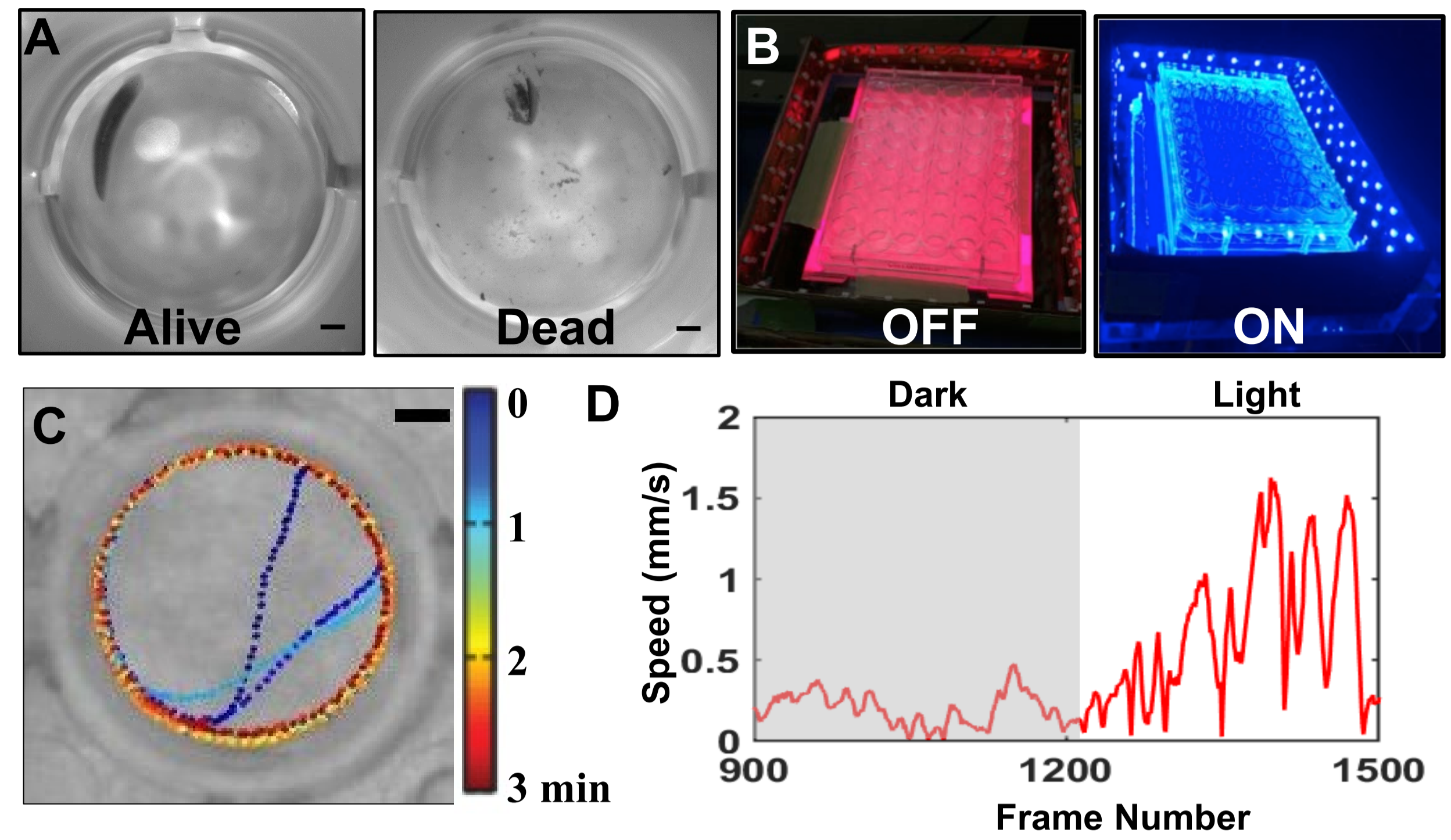


Figure 2. (A) Lethality can be determined by the presence or absence of a planarian since dead planarians disintegrate. (B) Set up of phototaxis assay showing the light OFF and ON. (C) Representative center of mass (COM) tracking of planarian locomotion, color coded by time, showing a gliding planarian. COM tracking is performed in both the dark and light periods to calculate speed and time spent resting. (D) In a normal phototaxis response, planarians increase their speed in response to the light pulse.

Applications of planarian HTS

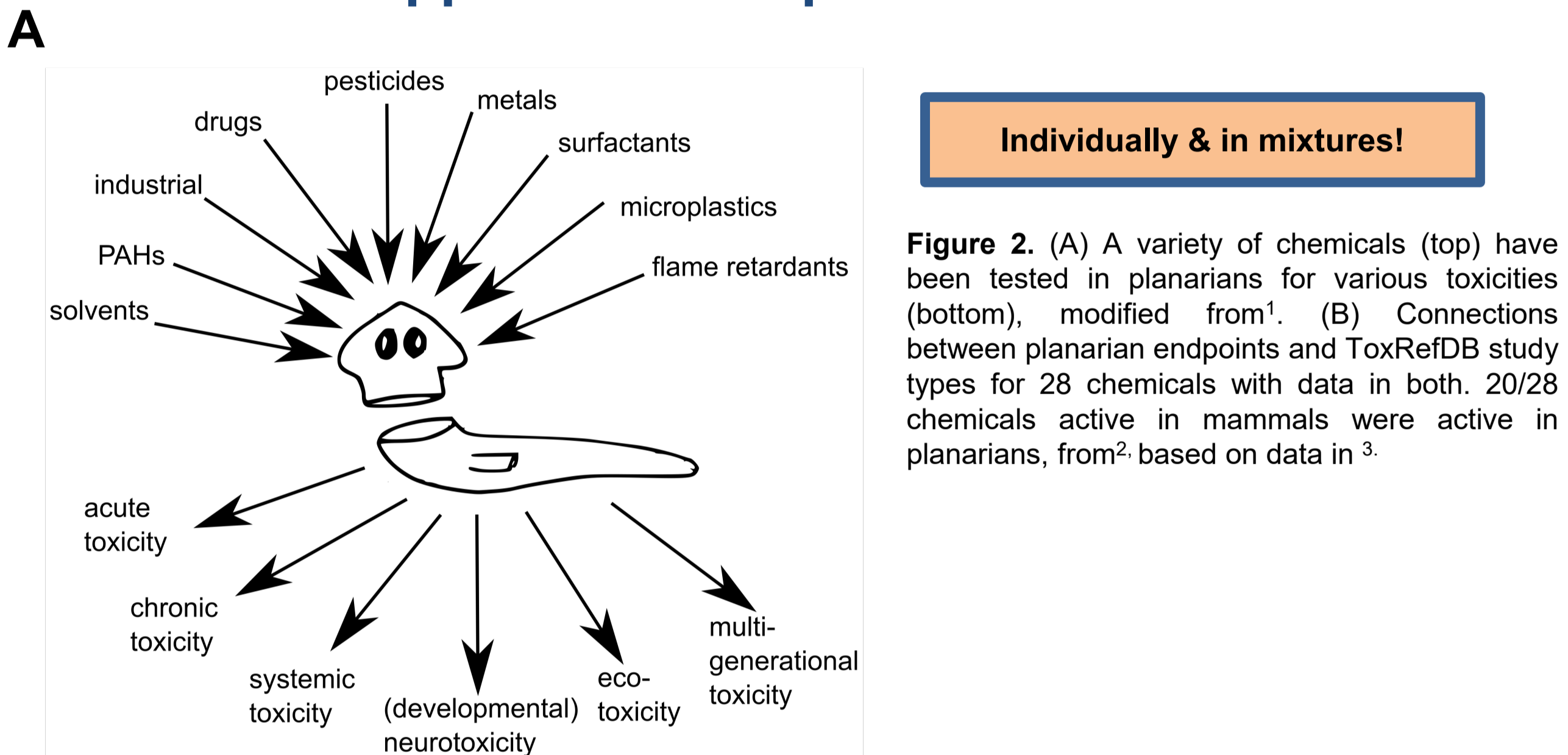


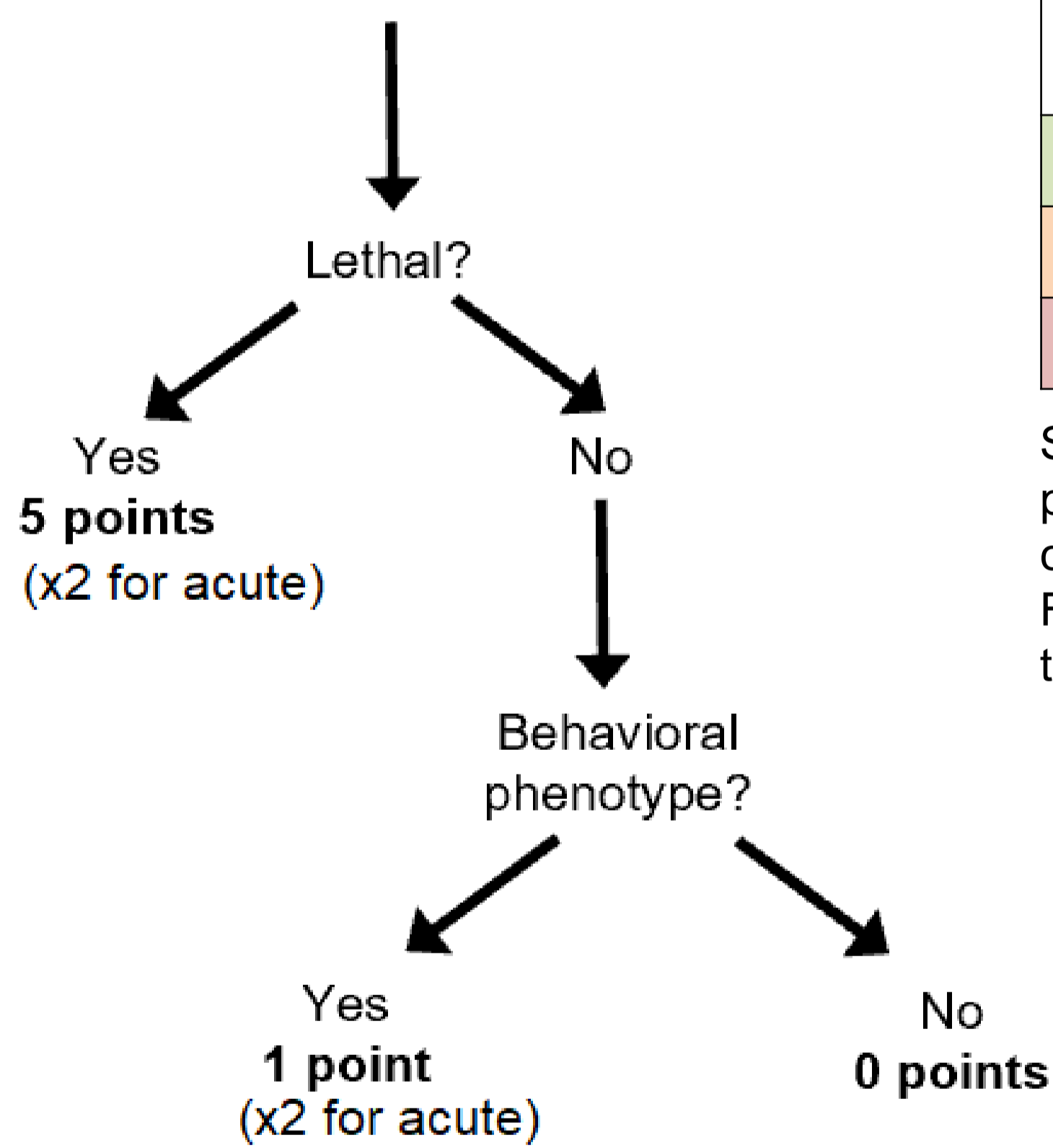
Figure 2. (A) A variety of chemicals (top) have been tested in planarians for various toxicities (bottom), modified from¹. (B) Connections between planarian endpoints and ToxRefDB study types for 28 chemicals with data in both. 20/28 chemicals active in mammals were active in planarians, from²; based on data in³.

Endpoint	Raw analysis	Statistical test
Lethality	Automated object detection	Fisher's exact test with BH correction
Behavior	COM tracking to calculate speed and resting in the dark and light periods.	Speed: Welch's ANOVA, followed by Tamhane-Dunn pairwise Resting: Kruskal Wallis, followed by Dunn pairwise test (BH correction)
	Binary classification of phototaxis based on changes in speed.	Phototaxis: Fisher's exact test with BH correction
		If any specific behavioral endpoint is significantly affected, a behavior effect will be counted.

BH: Benjamini-Hochberg. All statistics are performed in R.

Phenotype scoring and classification scheme

Per chemical concentration

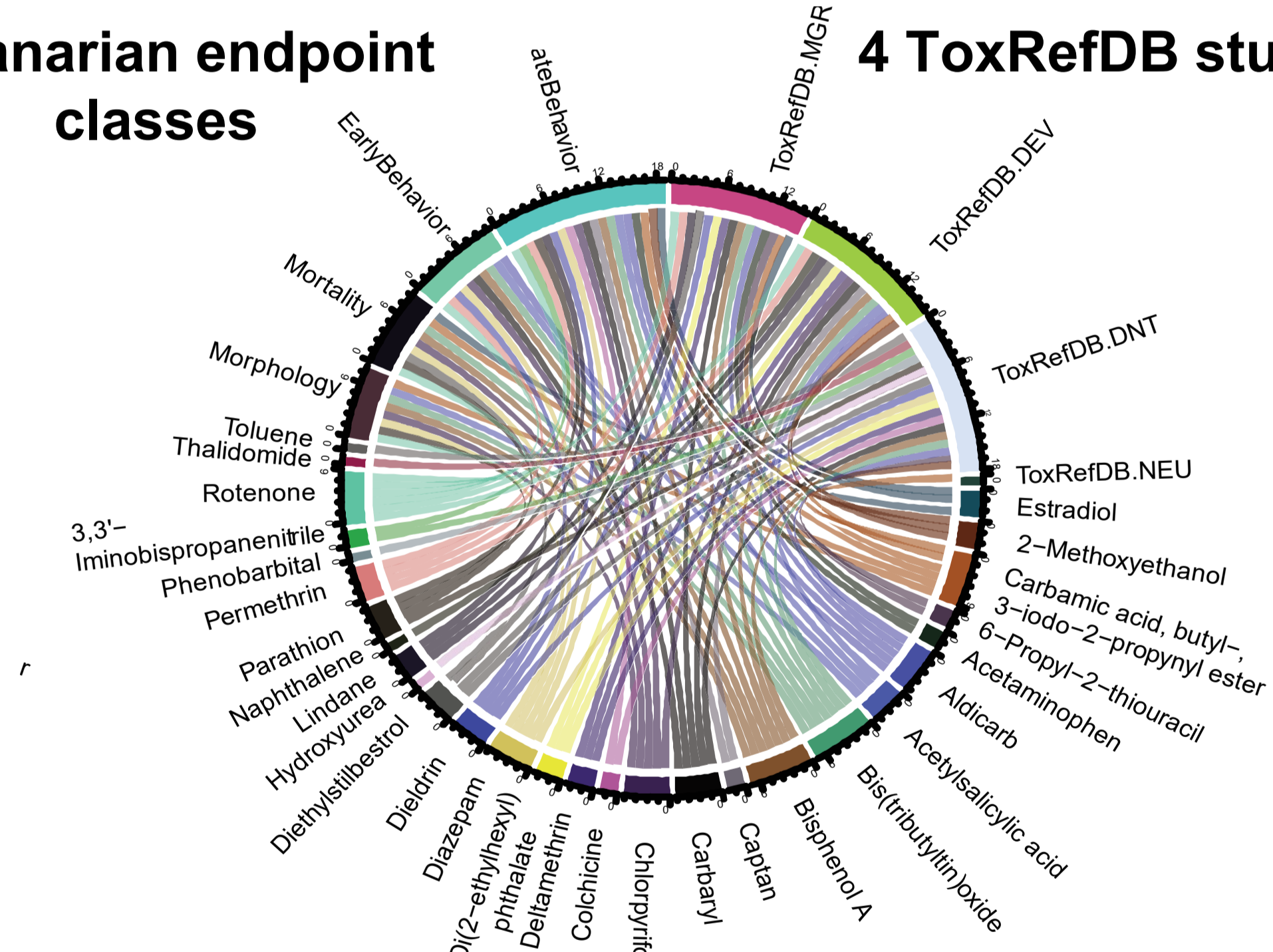


Concern category	Total score
Low	0-4
Medium	5-9
High	>9

Scores for adult and regenerating planarians could be summed or calculated separately. Final chemical score is sum of all time points and concentrations.

4 Planarian endpoint classes

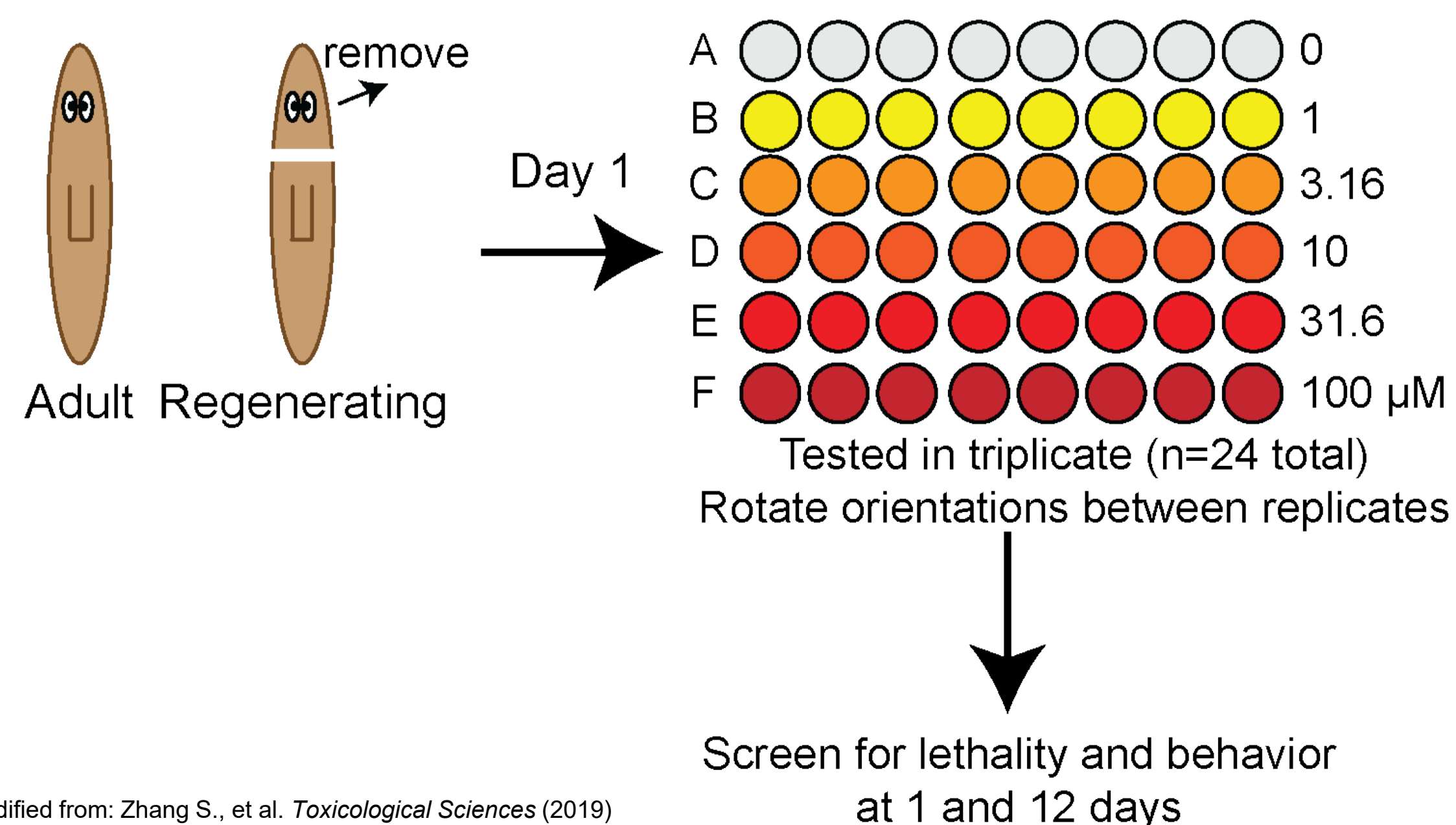
4 ToxRefDB study types



28 chemicals with animal studies in ToxRefDB

Testing for systemic toxicity with planarian HTS

- Automated screening in 48-well plates (patent pending)
- 0.5% DMSO control included in every plate
- Assays run in triplicate (total n=24)
- Analysis is blinded.
- Adult and regenerating planarians are assayed in parallel to determine developmental-specific effects.



Modified from: Zhang S., et al. *Toxicological Sciences* (2019)

Strengths and limitations of planarian HTS

- As an invertebrate organismal model, planarians provide systems-level insight into systemic toxicity at all life stages.
- Scoring is based on timing and severity of phenotypes.
- Allows for direct comparison of adult and developing organisms to determine effects on different life stages.

Current limitations :

- Very few of the reference compounds have been tested in planarians so far, thus uncertainty whether the proposed classification scheme works
- Chemicals must allow for aqueous exposure
- Little is known about ADME for most chemicals in planarians
- Planarians lack certain anatomical structures / molecular targets

References

- Ireland, D., et al. *Current Protocols* (2022)
- Hagstrom D., et al., *Toxicological Sciences* (2019)
- Zhang S., et al. *Toxicological Sciences* (2019)
- Ireland, D. et al., *Frontiers in Toxicology* (2022)
- Ireland, D. et al., *Archives of Toxicology* (2022)

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Conflict of Interest: EMSC is the founder of Inveritek, LLC, which offers planarian screening commercially.