into IACUC review of proposed work with animals. This consideration may require requests for additional information from the investigator or consultation with an external reviewer. If the investigator is to be queried for additional information, the IACUC is advised to ensure these questions are specific enough to elicit an appropriate response.

1. Public Health Service. *Policy on Humane Care and Use of Laboratory Animals*. (US Department of Health and Human Services, Washington, DC, 1986; revised 2015).
2. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals* 8th edn. (National Academies Press, Washington, DC, 2011).
3. United States Department of Agriculture. *Animal Welfare Act regulations* (2013).
4. Mann, M.D. & Prentice, E.D. Should IACUCs review scientific merit of animal research projects? *Lab Anim* (NY). **33**, 26–31 (2004).
5. Bier, E. *Drosophila*, the golden bug, emerges as a tool for human genetics. *Nat. Rev. Genet.* **6**, 9–23 (2005).
6. Bejsovec, A. Wingless/Wnt signaling in *Drosophila*: the pattern and the pathway. *Mol. Reprod. Dev.* **80**, 882–894 (2013).

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RESPONSE

A need for more justification

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Tuckerton's response is not sufficient to justify the use of mice. The invertebrate model may not be appropriate, however the IACUC is required to assure that the researcher has considered alternatives, and his opinion alone is not enough of an assurance¹. According to the Guide a researcher should consider methods of relative animal model replacements, including "replacing animals such as vertebrates with animals that are lower on the phylogenetic scale"². The AWAR Policy 12 also states that alternative methods such as "methods that use non-animal systems or less sentient animal species to partially or fully replace animals (for example, the use of an *in vitro* or insect model to replace a mammalian model) "should be considered"¹. Tuckerton states that it is his opinion that the invertebrate model is

not appropriate, but does not provide data driven evidence. Tuckerton has not done an adequate job of justifying why he needs to do this work specifically in the mouse. The PHS Policy requires IACUC proposals to specifically indicate the appropriateness of the species and numbers used³. The fruit fly may be an appropriate model for studying the Wnt pathway, and it is Tuckerton's responsibility to clearly describe why the proposed species needs to be used in the selected study. A secondary concern arises from the fact that this is just a pilot study and will be followed up by a large-scale study that would use a high number of animals. There is no statistical justification provided as to why he would need to use that many animals after the pilot study. Perhaps he could use the mice to find the initial dose and then switch to fruit flies for the larger experiment. The reverse could also work, if he could use the flies to determine the dose.

Based on Tuckerton's initial response, the IACUC should consider asking additional questions. What type of data is he collecting from these animals? At what developmental stage will he be examining the embryos, or will he be bringing them to term? These questions will give greater detail into the level of pain and discomfort these animals will be feeling and give the committee more info about his model.

Does he have any physiological evidence that fruit flies and mice do not have similar responses to PGE2 with regards to embryonic development and the Wnt signaling pathway? There are differences in masculinization of sex behavior between mice and fruit flies and while PGE2 plays a role in masculinization in both species, steroidal sex hormones play a major role in mice, which is different from fruit flies⁴.

Drosophila is a common embryonic development model. Why then, in this case, do they not provide a good translational model? Can the development of the longitudinal (anteroposterior) axis of the body not be compared between vertebrate and invertebrate species? There is some research that concludes that while there are some differences between CNS evolution in vertebrates and insects, there are also many similarities in cells types⁵.

The information provided to the committee does not justify his use of species and animal numbers. Further information

is necessary for the committee to make an informed decision about his animal use.

1. United States Department of Agriculture. *Animal Welfare Act regulations* (2013).
2. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals* 8th edn. (National Academies Press, Washington, DC, 2011).
3. Public Health Service. *Policy on Humane Care and Use of Laboratory Animals*. (US Department of Health and Human Services, Washington, DC, 1986; revised 2015).
4. Amateau, S.K. & McCarthy, M.M. Induction of PGE2 by estradiol mediates developmental masculinization of sex behavior. *Nat. Neurosci.* **7**, 643–650 (2004).
5. Arendt, D. & Nubler-Jung, K. Comparison of early nerve cord development in insects and vertebrates. *Development* **126**, 2309–2325 (1999).

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RESPONSE

If it's only an opinion, it won't fly

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As part of protocol review, the IACUC has a responsibility to assure that Tuckerton has made a reasonable and good faith effort to determine if alternatives are available and consider whether any identified alternatives could be used to accomplish the objectives described in the animal care and use proposal¹. McCarthy's question, "Can this study be performed in fruit flies?" suggests that, based on the information provided in the proposal, the IACUC wouldn't be able to assure that this requirement had been met.

What are the expectations for this reasonable and good faith effort? The framework is defined in the law (ref. 2; §2.31), policy¹, the *Guide for the Care and Use of Laboratory Animals*³, and ideally, by each institution's animal care and use committee. A tenet of each is Russell and Burch's Three R's approach for assessing alternatives: replacement, reduction and refinement. Further, there is an expectation for a written narrative detailing the method of assessment, which is generally a