From:	Hunt, Piper (FDA/CFSAN)
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Subject:	[NTP Web] NTP Interagency Center for the Evaluation of Alternative Toxicological Methods Public Comments Submission [81FR42718-42719]
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Attachments:	2016 Hunt C. elegans in Tox Testing.pdf 2015 Boyd ToxCast C. elegans vs zebrafish rats rabbits.pdf 2016 Harlow C. elegans to predict mammalian mechanism of tox.pdf 2009 Sprando rank toxicity compounds in worms.pdf 2000 Olson Tox concordance humans and animals.pdf

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Attention NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
Federal Register Notice: (81FR42718-42719)
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Comments Submitted on Behalf of a Sponsoring Organization?: No
Organization Name:
Comments: Caenorhabditis elegans is a small nematode that can be maintained at low cost and handled using standard in vitro techniques. Unlike toxicity testing using cell cultures, C. elegans toxicity assays provide data from a whole animal with intact and metabolically active digestive, reproductive, endocrine, sensory, and neuromuscular systems. Toxicity ranking screens in C. elegans have repeatedly been shown to be as predictive of rat LD50 ranking as mouse LD50 ranking [1]. Additionally, many instances of conservation of mode of toxic action have been noted between C. elegans and mammals. These consistent correlations make the case for inclusion of C. elegans assays in early safety testing and as one component in tiered or integrated toxicity testing strategies.

In a recent study of hundreds of compounds from the ToxCastTM libraries, C. elegans larval growth identified rat or rabbit developmental toxins with a balanced accuracy of 52-53%, while the concordance for developmental toxicity between rat and rabbit was 58% [2]. These levels of predictivity are consistent with an earlier meta-study which found that a single-species rodent study alone predicted human toxicity less than 50% of the time [3]. Unlike a study in mammals however, a C. elegans larval growth assay that evaluates multiple compounds or mixtures at multiple concentrations can be conducted by a single technician in less than a week.

Interestingly, the sensitivity of the aforementioned larval growth assay for the detection mammalian developmental toxins was high, but the balanced accuracy was brought down by low specificity [2]. In contrast, in a recent study evaluating C. elegans egg viability using 72 compounds of known developmental activity in mammals, the specificity of the egg viability test was high while the sensitivity was low [4]. Thus, it is possible that the C. elegans larval growth and egg viability assays could be used together to improve the detection of mammalian developmental toxins. Additionally, both of these assays used E. coli as a feeder organism, and neither publication discusses efforts at Good C. elegans Culture Practice (GCeCP) which can substantially alter test results [1]. While early C. elegans axenic media formulations slowed growth, newer media produce identical growth rates to cultures fed the feeder organism, without the complication of xenobiotic metabolism [5]. It may be that the use of a combination of axenic media and GCeCP could improve the predictivity of both the C. elegans larval growth and egg viability assays, making them a useful addition to developmental toxicant testing strategies.

1. Hunt, P.R., The C. elegans model in toxicity testing. Journal of Applied Toxicology, 2016.
2. Boyd, W.A., et al., Developmental Effects of the ToxCast Phase I and II Chemicals in and Corresponding Responses in Zebrafish, Rats, and Rabbits. Environ Health Perspect, 2015. 124: p. 586-593.
3. Olson, H., et al., Concordance of the toxicity of pharmaceuticals in humans and in animals. Regul Toxicol Pharmacol, 2000. 32(1): p. 56-67.
4. Harlow, P.H., et al., The nematode Caenorhabditis elegans as a tool to predict chemical activity on mammalian development and identify mechanisms influencing toxicological outcome. Sci Rep, 2016. 6: p. 22965.
5. Sprando, R.L., et al., A method to rank order water soluble compounds according to their toxicity using Caenorhabditis elegans, a Complex Object Parametric Analyzer and Sorter, and axenic liquid media. Food Chem

Toxicol, 2009. 47(4): p. 722-8.
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