

Critical Evaluation of Animal Alternative Tests for the Identification of Endocrine Active Substances

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In the past 20 years, considerable progress in animal alternatives accompanied by advances in the toxicological sciences and new emphases on aquatic vertebrates has appeared. A significant amount of current research is targeted to evaluate alternative test methods that may reduce, replace or refine (3Rs) the use of animals, while ensuring human and environmental health and safety. In 2009, the US EPA began implementation of the Endocrine Disruptor Screening Program which includes Tier 1 screening assays in fish and frog species which are closely aligned with the OECD test guideline series 229 and 231. However, these assays use a large number of animals and are quite long in duration relative to an ideal screening assay. As the Tier 1 assays screen and prioritize a large number of chemicals for possible endocrine activity shorter-term tests would be advantageous. In order to identify potential alternatives, a literature search was conducted and a database with alternatives to fish and frog testing methodologies assembled. Data from 1995 to present were collected related to the detection/testing of estrogen-, androgen-, and thyroid-active chemicals in the following test systems: cell lines, primary cells, fish/frog embryos, yeast, bacteria, cell free systems, and “omics” technologies. A critical analysis was performed to (1) determine the strengths and limitations of each alternative assay identified and (2) present conclusions regarding chemical specificity, sensitivity, and correlation with *in vivo* data. A summary of the most promising alternative assays will be presented.

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