

Development of a Reptile Egg Screening Assay for Endocrine Disrupting Chemicals

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This study is a continuation of research to evaluate fence lizards as reptile models for assessment of endocrine-mediated toxicity. Previously, a population of western fence lizards (WFLs) (*Sceloporus occidentalis*) from the San Joaquin Valley of California was identified as a good candidate for a laboratory reptile model. The purpose of this phase of the research was to continue studying WFLs, to develop a lizard-egg screening assay, and to determine if it could be used for detecting all three major groups of endocrine disrupting chemicals (i.e., estrogenic, androgenic, and thyroidogenic). Much of the research was directed toward obtaining baseline information including: the best breeding strategy for fence lizards, the optimal substrate moisture level for incubating eggs, the optimal incubation temperature, techniques to arrest development of embryos so they can be stored for up to a month, and the best methods for exposing eggs to endocrine disrupting chemicals.

Fence lizard embryos have two sets of secondary sex characteristics that are useful for developing a lizard-egg screening assay. First, both male and female embryos develop external hemipenal structures early in development. However, females reabsorb their hemipenal structures mid-way through development, whereas males do not. Males develop two enlarged post-anal scales prior to hatching, whereas females do not. Injecting eggs with an estrogenic chemical, 17 α -ethinylestradiol, feminized males and they hatched as phenotypic females without enlarged post-anal scales. In addition, their hemipenal structures were reduced in size or absent, depending on the dose of 17 α -ethinylestradiol injected. Therefore, fence lizard embryos have good potential as a screening assay for estrogenic chemicals. We also evaluated the effects of exposing eggs to androgenic chemicals.

Embryos tolerated both methods of exposure very well. Both DHT and testosterone affected sex ratio but exposure to either DHT or testosterone resulted in a higher percentage of phenotypic females, not males. Overall, painted eggs resulted in more phenotypic females than injected eggs. Apparently, embryos aromatized both DHT and testosterone into estrogen. We also evaluated the effects of a thyroid-inhibiting chemical on development of fence lizard embryos. Embryos were exposed to five dose levels of sodium perchlorate. The two highest dose levels reduced oxygen consumption of embryos, reduced thyroid hormone levels, and inhibited the piping response. Our original hypothesis was that embryonic fence lizards would be sensitive to all three major groups of endocrine disrupting chemicals, and our research supports this hypothesis.

Implications: WFLs from the San Joaquin Valley of California are good candidates for a laboratory reptile model to evaluate endocrine mediated toxicity of chemicals. WFLs survive well under laboratory conditions, mature when they are around four months old, lay multiple clutches of eggs per year, and produce embryos that are sensitive to estrogenic, androgenic, and thyroidogenic chemicals. WFL eggs are tolerant of injections of many solvents used as vehicles for endocrine disrupting chemicals, and embryonic mortality due to solvents is low. Therefore, the WFLs are a good candidate for use in a reptile egg assay for regulatory screening assays.

Start and end date: April 2002 – March 2007

Presentations:

Cooper, N.L., and Talent, L.G. (2004). Effect and type and amount of carrier injected into lizard eggs on hatching success. Presentation at Annual Meeting of the Society for Environmental Toxicology and Chemistry. Portland, OR, November 14, 2004.

Redick, M.S., Janz, D.M., and Talent, L.G. (2004). Effects of long term *in ovo* exposure to sodium perchlorate on incubation and growth of sceloporine lizards. Presentation at Annual Meeting of the Society for Environmental Toxicology and Chemistry. Portland, OR, November 16, 2004.

Salice, C.J., Suski, J.G., and Talent, L.G. (2004). Effects of lead on the western fence lizard, *Sceloporus occidentalis*. Presentation at Annual Meeting of the Society for Environmental Toxicology and Chemistry. Portland, OR, November 14, 2004.

Talent, L.G., Cooper, N.L., Redick, M.S., and Janz, D.M. (2004). Window of sensitivity when the phenotypic sex of male fence lizards can be altered. Presentation at Annual Meeting of the Society for Environmental Toxicology and Chemistry. Portland, OR, November 14, 2004.

Talent, L.G. (2004). Fence lizards have potential for use in environmental risk assessments. Presentation at Annual Meeting of the Society for Risk Analyses. Palm Springs, CA, December 7, 2004.

Melson, D.C., Burnham, D.K., Jarrell, V., and Talent, L.G. (2005). Reptile macrophages as indicators of the consequences of estradiol exposure on innate immune responses. Presentation at the Annual Meeting of the Society of Environmental Toxicology and Chemistry. Baltimore, MD, November 17, 2005.

Melson, D., Talent, L.G., and Burnham, D.K. (2005). The effects of hibernation recovery on total peripheral blood leukocytes and phagocytic activity of peritoneal macrophages in *Sceloporus occidentalis*. Presentation at the Federation of American Societies for Experimental Biology. San Diego, CA, April 6, 2005.

Redick-Harris, M.S., Talent, L.G., and Janz, D.M. (2005). Effects of *in-ovo* exposure to sodium perchlorate on growth, breeding success, and hormone levels of eastern fence lizards. Presentation at the Annual Meeting of the Society of Environmental Toxicology and Chemistry. Baltimore, MD, November 14, 2005.

Redick-Harris, M.S., Talent, L.G., and Janz, D.M. (2005). Effects of *in ovo* exposure to sodium perchlorate on histology and hormone levels of hatchling and mature male western fence lizards. Presentation at the Annual Meeting of the Society of Environmental Toxicology and Chemistry. Baltimore, MD, November 15, 2005.

DuRant, S.E., Hopkins, W.A., Talent, L.G., and Romero, L.M. (2006). Effect of exogenous corticosterone on respiration in a reptile. Presentation at the Annual Meeting of the Society for Integrative and Comparative Biology. Orlando, FL, January 5, 2006.

DuRant, S.E., Hopkins, W.A., Talent, L.G., and Romero, L.M. (2006). Effect of exogenous corticosterone on respiration in a reptile. Presentation at the Annual Meeting of the Virginia Academy of Science. Blacksburg, VA, May 25, 2006.

Talent, L.G. (2006). Evaluation of fence lizards for use as reptile laboratory models for ecotoxicological studies. Presentation at the Annual Meeting of the Ozark-Prairie Chapter of the Society of Environmental Toxicology and Chemistry. Columbia, MO, May 23, 2006.

Peer-reviewed publications:

Brasfield, S.M., Weber, L.P., Talent, L.G., and Janz, D.M. (2002). Dose-response and time course relationships for vitellogenin induction in male western fence lizards (*Sceloporus occidentalis*) exposed to ethinylestradiol. *Environmental Toxicology and Chemistry* 21(7): 1410-1416. (Received the SETAC/AstraZeneca Best Student Publication in Environmental Research Award - accepted in Vienna, Austria at SETAC Europe meeting).

Talent, L.G., Dumont, J.N., Bantle, J.A., Janz, D.M., and Talent, S.G. (2002). Evaluation of western fence lizards (*Sceloporus occidentalis*) and eastern fence lizards (*Sceloporus undulatus*) as laboratory reptile models for toxicological investigations. *Environmental Toxicology and Chemistry* 21(5): 899-905.

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Hopkins, W.A., Staub, B.P., Baionno, J.A., Jackson, B.P., and Talent, L.G. (2005). Transfer of selenium from prey to predators in a simulated terrestrial food chain. *Environmental Pollution* 134(3): 447-456.

Brasfield, S. M., Talent, L.G., and Janz, D.M. (2008). Reproductive and thyroid hormone profiles in captive western fence lizards (*Sceloporus occidentalis*) following a period of brumation. *Zoo Biology* 27(1): 36-48.

DuRant, S.E., Romero, L.M., Talent, L.G., and Hopkins, W.A. (2008). Effect of exogenous corticosterone on respiration in a reptile. *General and Comparative Endocrinology* 156(2008): 126-133.

Rich, C.N., and Talent, L.G. (2008). The effects of prey species on food conversion efficiency and growth of an insectivorous lizard. *Zoo Biology* 27(3): 181-187.

Talent, L.G., and Talent, S.G. Effects of social interaction on reproductive parameters of captive female western fence lizards, *Sceloporus occidentalis*. *Zoo Biology*. (Submitted).

Talent, L.G., Cooper, N.L., and Talent, S.G.. Effects of injecting western fence lizard eggs with solvents used as vehicles on embryonic survival and growth. *Environmental Toxicology and Chemistry*. (Submitted).

Redick-Harris, M.S., Talent, L.G., and Janz, D.M. Perinatal Corticosterone and Thyroid Hormone Levels in Western Fence Lizards (*Sceloporus occidentalis*). (In preparation).

Redick-Harris, M.S., Talent, L.G., and Janz, D.M. Effects of *in ovo* perchlorate exposure on growth and reproduction of eastern fence lizards (*Sceloporus undulatus*). (In preparation).

Redick-Harris, M.S., Talent, L.G., and Janz, D.M. Effects of *in ovo* perchlorate exposure on hormones, hatch rate, and oxygen consumption in western fence lizards (*Sceloporus occidentalis*). (In preparation).

Talent, L.G. and Janz, D.M. Effects of EE2 on post-anal scale development, survival, and development of western fence lizards (*Sceloporus occidentalis*). (In preparation).

Talent, L.G. and Janz, D.M. The developmental window when embryos of western fence lizards (*Sceloporus occidentalis*) are sensitive to EE2. (In preparation).

Other publications:

Brasfield, L.M. (2002). Development of a laboratory reptile model for assessment of endocrine-mediated toxicity. Oklahoma State University, M.Sc. Thesis.

Redick, M.S. (2006). Effects of *in ovo* exposure to perchlorate on life history parameters of the western fence lizard (*Sceloporus occidentalis*). Oklahoma State University, Ph.D. Dissertation.

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