23.0 OTHER APPLICATIONS FOR *XENOPUS*

23.1 *Xenopus* Tail Resorption Assay

The *Xenopus* Tail Resorption Assay is an endocrine (thyroid) disruption assay using advanced *Xenopus* larvae to screen materials that may disrupt thyroid function (Fort et al., 2000b). In this assay, tadpoles are exposed for approximately 14 days from Developmental Stages between 58 and 60 through Developmental Stage 66. Ten tadpoles at the "just bud" stage (average tail length between four to five centimeters) are exposed to varying concentrations of the test material. Test organisms are fed twice daily and dead organisms removed and mortality counted. At a minimum, all solutions are renewed and exposure containers cleaned on Days 4, 7, and 10. Photographic images of the test organisms are taken on Days 0, 4, 7, 10, and 14 and tail length determined. At the completion of the 14-day exposure period, any malformed organisms are maintained for further observation. All other test organisms are euthanized. Data on gross effects on tail resorption are collected. The test period may be extended if tail resorption in the control organisms is not complete at the end of the 14 days. Also, the renewal and photographic days may be modified as necessary to meet differing test requirements.

In a recent evaluation of this assay (Fort et al., 2000b), short-term static-renewal studies were performed on *X. laevis* embryos with 16 selected test materials from day 50 (Developmental Stage 60) to day 64 (Developmental Stage 66) (14-day test). The test materials were 6-AN, acetyl hydrazide, cadmium, copper, endosulfan, iodine, lindane, methimazole, methoprene, nonylphenol, pentachlorophenol, perchlorate, propylthiouracil, semicarbizide, thyroxin, and triiodothyronine (T_3). Of these 16 test materials, ten (acetyl hydrazide, cadmium, endosulfan, lindane, methimazole, methoprene, pentachlorophenol, perchlorate, propylthiouracil, and semicarbizide) were found to significantly inhibit the rate of tail resorption. Four test materials (iodine, nonylphenol, thyroxin, and T_3) were found to stimulate metamorphosis. Two test materials (6-AN and copper) had no appreciable effect on the rate of metamorphosis. In an effort to determine if the morphological effects observed were related to an alteration in thyroid activity, measurement of T_3 in the treated embryos, and co-administration studies using thyroxine (agonist) or propylthiouracil (antagonist) were performed based on the morphological

response noted during tail resorption. Of the ten compounds found to inhibit the rate of tail resorption, eight were found to reduce the levels of T_3. In each case, the inhibitory response could be at least partially alleviated by the co-administration of thyroxine. Larvae exposed to the four stimulatory agents had somewhat elevated levels of T_3 and were responsive to propylthiouracil antagonism. Twelve of the 14 compounds tested in this study that altered the rate of tail resorption did so via the thyroid axis. The investigators concluded that *Xenopus* might be a suitable system for evaluating the impact of environmental agents and chemical products on thyroid function.

This methodology has been used to evaluate the effects of two sulfonylurea herbicides, sulfometuron methyl and nicosulfan, on tail resorption (Fort et al., 1999c). The analytically impure, but not the pure, sulfonylurea herbicides slowed tail resorption rates significantly. Also, Fort et al. (1999a) demonstrated that pond water, sediment, and sediment extracts from ponds inhabited by mal-developed frogs was capable of inhibiting tail resorption in *Xenopus*.

23.2 *Xenopus* Vitellogenin Assay

Another endocrine disruption assay involving *Xenopus* is based on the detection of vitellogenin in the blood of treated males (Palmer and Palmer, 1995). One of the most important and sensitive responses to estrogen is the upregulation of protein production. A particularly well known estrogenic response in all oviparous and ovoviviparous vertebrates is the induction of the lipoprotein vitellogenin in liver cells. In females, vitellogenin is transported in the blood to the ovaries, where it is incorporated into the developing ovarian follicles as yolk. Due to their normally low levels of endogenous estrogens, male *X. laevis* have no detectable levels of vitellogenin in blood. However, their liver is capable of synthesizing and secreting vitellogenin into the blood in response to exogenous estrogen stimulation. In the *Xenopus* Vitellogenin assay, adult males are given intraperitoneal injections of a test substance daily for 7 days, and plasma is collected on day 14 (Palmer and Palmer, 1995). The estrogenic activity of the test substance is determined by measuring the induction of plasma vitellogenin. Vitellogenin is identified by precipitation, electrophoresis, Western blot, and enzyme-linked immunosorbant assay (ELISA).

Vitellogenin may prove useful as a biomarker in *Xenopus* for identifying xenobiotics with estrogenic activity. The test is relatively noninvasive, requiring only small (microliter) quantities of plasma or serum. It can be used in the laboratory to identify substances with *in vivo* estrogenic activity and *in situ* to indicate the presence of environmental pollutants with estrogenic activity. The expression of vitellogenin is through known physiological and biochemical pathways. The induction of vitellogenin is sensitive to any estrogenic contaminant, and the response is quantifiable. Finally, the assay for vitellogenin can be performed relatively easily and inexpensively. A major limitation of this assay is that it provides no direct information regarding the female or developing embryo. However, if estrogen receptors are being stimulated in the liver of males, receptors in other organs such as the testes and prostate gland of males and reproductive tissues of females and embryos may likewise be affected. Also, in ecotoxicological studies, vitellogenin production does not indicate what substance(s) may be causing the effect. However, the assay may be used as a rapid, sensitive, and economical initial screen, followed (as indicated by positive vitellogenic responses) by more costly screens to identify the specific contaminating substances.

23.3 Evaluation of Reproductive Toxicity using *Xenopus*

Fort et al. (1999d) has evaluated the utility of *X. laevis* for assessing reproductive toxicity. Cadmium, boric acid, and ethylene glycol monomethyl ether (EGME) were evaluated for reproductive and developmental toxicity in *X. laevis.* Eight reproductively mature adult male and eight superovulated female *X. laevis* were exposed to at least five separate sublethal concentrations of each material via the culture water for 30 days. Four respective pairs were mated and the offspring evaluated for developmental effects; an evaluation of reproductive status was performed on the remaining four specimens. Ovary health, oocyte count, oocyte maturity and maturation capacity, and necrosis were evaluated in the female, while testis health, sperm count, dysmorphology, and motility were studied in the male. Based on this assessment, each test material exerted reproductive toxicity in *X. laevis*, but with varying potencies. The investigators concluded that this model appears to be a useful tool in the initial assessment and prioritization of potential reproductive toxicants for further testing. In a related series of studies,

low boron levels in the diet were associated with adverse reproductive performance (Fort et al. 1999e, f, g).

23.4 *Xenopus* Limb Bud Assay

The *Xenopus* Limb Bud Assay is a test method for exploring limb mal-development, including possible mechanisms of action (D. Fort, personal communication). This assay is proposed as a model for screening materials that may cause limb deformities in the workplace or the environment. The assay uses blastula stage *Xenopus* embryos raised to about Developmental. Stage 58 to 59. The first four days of the test are similar to the standard FETAX test. However, at the end of the 96-hour exposure period, the developing embryos are transferred to larger containers. Chemical solution renewal and tub cleaning are reduced to a minimum of two times per week. However, the frequency of renewals may be increased if the test chemical in solution is easily degraded or volatilized. The pH is maintained between 7.8-8.0, and each container is aerated throughout the remainder of test. Test organisms are fed twice daily; dead organisms are removed and mortality counted. The length of time to complete the assay will vary and is dependent on the rate of hind limb development in the control sets (generally 45 to 60 days). The assay can be stopped when greater than 80% of the control organisms reach Developmental Stage 58 to 59 with developed hind limbs (femur, tibia, fibula, and foot with digits or toes and the beginning formation of claws). At the end of the exposure period, the incidence of malformations, survival, and total organism counts are determined. This methodology has been used to identify agents associated with limb mal-development in pond water and sediment collected in Minnesota, U.S. (Fort et al., 1999a), to evaluate the developmental toxicity of thalidomide (Fort et al., 2000c), and to evaluate the effects of two sulfonylurea herbicides, sulfometuron methyl and nicosulfan, on limb development (Fort et al., 1999c). The analytically impure, but not the pure, sulfonylurea herbicides induced abnormal limb development.

23.5 Section 23 Conclusions

Other tests using *Xenopus* are being evaluated for their ability to identify substances or environmental samples that may disrupt endocrine function (the *Xenopus* Tail Resorption Assay, Vitellogenin Assay), for assessing reproductive toxicity, and for exploring limb mal-development, including possible mechanisms of action (*Xenopus* Limb Bud Assay). These developing test methods require appropriate validation.