FETAX, a versatile tool in toxicology, can be conveniently integrated with molecular biology techniques

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Abstract. The Frog Embryo Teratogenesis Assay-Xenopus (FETAX) is a powerful and flexible bioassay for developmental toxicants that makes use of the embryos of the anuran *Xenopus laevis*. FETAX, thanks to its three endpoints (i.e., mortality, malformation, and growth inhibition), can also detect the xenobiotics that affect embryonic development, a weak link in the life cycle of an organism. The FETAX protocol, however, is amenable to modification in several ways and to being integrated with molecular biology techniques that considerably increase the capability of the test. As exposure to xenobiotics may alter gene expression and therefore mRNA and protein patterns, transcriptome and proteome modifications can be studied with the aim to obtain new insights on the mechanisms of embryotoxicity and teratogenesis or simply to obtain molecular markers of exposure useful in the early diagnosis of environmental stress.

Key words: Bioassay; hormesis; molecular markers; teratogenesis; *Xenopus*.

Introduction

The Frog Embryo Teratogenesis Assay-Xenopus (FETAX) (Dumont et al., 1983, 2003) is a powerful and flexible bioassay for developmental toxicants that makes use of the embryos of the anuran *Xenopus laevis*. It is considered an in vitro test as it uses cells, parts of embryos, or entire embryos. FETAX, thanks to its three endpoints (i.e., mortality, malformation and growth inhibition), can also detect the xenobiotics that affect embryonic development, a weak link in the life cycle of an organism. During embryogenesis, in fact, several processes must operate harmoniously to complete the developmental plan and such cooperation can be easily perturbed by numerous chemicals.

FETAX has not only been used for evaluating single compounds or their joint action, but also to test environmental mixtures, soils (Prati et al., 2000), sludges (Chenon et al., 2003) or the ecotoxicological efficiency of a water processing plant.
FETAX has also been used to challenge the issue of the decline of amphibian populations (Garber et al., 2004). The test has numerous advantages: a) it can be carried out in a short time and at low cost; b) it guarantees a good repeatability of results and provides a large amount of data for optimal statistical analysis; c) the use of a non-mammalian species better fulfills bioethical issues; and d) *Xenopus* are easy to maintain and can be bred throughout the year.

The FETAX protocol has been described in detail in several papers and in ASTM publications (ASTM, 1998). The FETAX protocol, however, is amenable to modification in several ways and to being integrated with molecular biology techniques that considerably increase the capability of the test. Here we stress these characteristics of versatility rather than discussing the validation issues necessary for regulatory purposes (Fort et al., 1998; Bantle et al., 1999).

**Embryo Production**

First of all, healthy embryos at the early gastrula stage are needed. They can be obtained either by inducing mating or by in vitro fertilization. To obtain them by mating, gonadotropins should be injected in the dorsal lymph sac of both males and females and the couple then left undisturbed. The day after, if everything proceeds normally, embryos can be collected at different developmental stages at the bottom of the breeding tank and placed in Petri dishes containing FETAX solution (Dawson and Bantle, 1987). This method is quite simple and, therefore, the one used most, but it yields a population of embryos with a wide age distribution.

With in vitro fertilization (Bernardini et al., 1996) synchronous embryo populations are obtained and, consequently, a simultaneous initiation of the treatment is made possible. In addition, the environment in which early development takes place can be more readily controlled, facilitating a highly accurate preselection of the embryos that will undergo the test. Moreover, in vitro fertilization makes the very early phases of development and even the fertilization process available for the assay. This initial period, from fertilization to the gastrula stage, when FETAX is started, is characterized by rapid and synchronous cleavage; the importance of this should not be underestimated. Unfortunately, to obtain a sperm suspension for in vitro fertilization, the male has to be sacrificed.

In both cases, before proceeding to the assay, the ‘bad’ eggs and all embryos with any abnormalities should be discarded through a screening procedure.

**Experimental Design**

A proper experimental design is needed to implement a biological test and help interpret its results; in our opinion, however, this issue has never been sufficiently addressed for FETAX. The number of embryos per Petri dish, the number of replicates, and the choice of the concentrations have never been optimized with