FETAX FOR ECOTOXICOLOGICAL HAZARD ASSESSMENT USING WATER/SOIL/SEDIMENT SAMPLES

Much of the information provided in the sections dealing with FETAX for human developmental hazard identification (**Sections 1** through **11**) are applicable to the use of FETAX for ecotoxicological hazard assessment using water/soil/sediment samples. However, for ease of comparison, this BRD will continue to follow the structure described in the evaluation criteria guidelines found in the *Evaluation of the Validation Status of Toxicological Methods: General Guidelines for Submissions to the Interagency Coordinating Committee on the Validation of Alternative Methods* (**Appendix 15**).

12.0 INTRODUCTION AND RATIONALE FOR THE USE OF FETAX IN ASSESSING DEVELOPMENTAL HAZARDS IN WATER/ SOIL/SEDIMENT SAMPLES

12.1 Scientific Basis for the Use of FETAX

In developing alternative testing methods for ecotoxicology, there is a need to clearly define strategies and goals when undertaking testing procedures (Walker et al., 1998). Reduction, replacement, or refinement in animal use will be served by:

- developments and improvements in assays incorporating new techniques from biochemical/molecular biology that relate to mechanisms;

- further development of nondestructive assays for vertebrates, and assays for invertebrates;

- selection of the most appropriate species, strains and developmental stages in the light of new knowledge (but no additional vertebrate species for basic testing); and

- better integrated approaches incorporating biomarker assays, ecophysiological concepts, and ecological end points.

Maximum success depends on a flexible approach and expert judgment in interpretation. Testing protocols need to be realistic, taking into account particular problems with mixtures and volatile or insoluble chemicals (Walker et al., 1998).

The purpose of FETAX for ecotoxicological assessment is to identify and prioritize aquatic environments, soils, or sediments that contain naturally occurring or anthropogenic substances, which pose a developmental hazard to living organisms. Ecotoxicological testing is usually performed using multiple test species. For each species, it is a combination of toxicants, water quality, and the susceptibility of the organism itself that defines the hazard for a specific concentration of a toxicant within defined water quality conditions. Ecotoxicological standards are generally based on the susceptibility of the adult animal, which may not provide adequate protection for embryonic development and reproduction in many species. Early embryonic and juvenile stages are often the most susceptible periods for the toxic effects of many environmental contaminants. Furthermore, it is inherently impossible to evaluate developmental toxicity without exposing animals throughout development and assessing for adverse effects at multiple life stages. Due to the sensitivity of embryonic development in amphibians to water quality, FETAX is thought to be relevant as a conservative 'sentinel' estimator of ecotoxicologic hazard (ASTM, 1991; 1998).

12.2 Intended Uses of FETAX

12.2.1 Intended Regulatory Uses and Rationale

FETAX, without metabolic activation, has been used to identify and prioritize the potential developmental hazards of contaminated surface waters, sediments, waste site soils, and industrial wastewater (Fort et al., 1995; 1996b; Fort and Stover, 1997). The rationale for use is based on the sensitivity of amphibian embryonic development to water quality. Based on this sensitivity, FETAX might be useful in estimating the chronic toxicity of a test substance to aquatic

organisms (ASTM, 1998). FETAX also has potential for deriving water quality criteria for aquatic organisms, for studying bioavailability (ASTM, 1998), or for evaluating the efficacy of wastewater treatment procedures (Vismara et al., 1993).

12.2.2 Currently Accepted Water/Soil/Sediment Developmental Toxicity Testing Methods

FETAX is not currently accepted by U.S. Federal agencies. U.S. Federal and international regulations pertinent to the potential use of FETAX include the following:

Under the Clean Water Act, the EPA has developed guidance (40 CFR 132) that sets minimum water quality standards, policies, and implementation procedures for the Great Lakes System to protect aquatic life and wildlife. The methodology for collecting the required data requires acceptable acute or chronic tests with at least one species of freshwater animal in at least eight different families, including a family in the phylum Chordata (e.g., fish, amphibian). However, data from species that do not reproduce in the wild in North America are not acceptable. Although *X. laevis* is not native to North America, recent reports have indicated the occurrence of naturally reproducing populations in some portions of the United States (J. Burkhart, personal communication).

The EPA guidelines for evaluating whole effluent toxicity are provided in **Appendix 14**. This final rule amends the *Guidelines Establishing Test Procedures for the Analysis of Pollutants,* 40 CFR part 136, by adding methods for measuring the acute and short-term chronic toxicity of effluents and receiving waters.

EPA's Significant New Alternatives Policy Program (40 CFR 82.170) identifies acceptable substitutes (compounds believed to present lower overall risks to human health and the environment) for ozone-depleting compounds. Under this program, ecotoxicological studies of a substitute and its components can include data from tests for effects on invertebrates, fish, or other animals.

12.2.3 Ability of FETAX to Assess Potential Developmental Hazards in Water/Soil/Sediment Samples

FETAX has been used to evaluate the potential developmental hazards of contaminated surface waters, sediments, waste site soils, and industrial wastewater (Fort et al., 1995; 1996b; Fort and Stover, 1997), to demonstrate the efficacy of wastewater treatment processes (Vismara et al., 1993), and to identify possible causes of malformations in natural frog populations in the United States (Burkhart et al., 1998; Fort et al., 1999a, b). However, very few comparative studies have been conducted to evaluate the relative ability of FETAX versus that of other similar bioassays to prioritize the hazard associated with contaminated water/soil/sediment samples (see **Section 16**).

12.2.4 Intended Range of Water/Soil/Sediment Samples Amenable to Test and Limits According to Physico—Chemical Factors

FETAX is considered to be applicable, with appropriate modifications, to aqueous effluents; surface and ground waters; leachates; aqueous extracts of water-insoluble materials; and solid-phase samples, such as soils and sediments, particulate matter, sediment, and whole bulk soils and sediments (ASTM, 1991; 1998). The test method is incompatible with materials (or concentrations of materials) that alter the pH, hardness, alkalinity, and conductivity of the FETAX Solution beyond the acceptable ranges specified by the ASTM FETAX Guideline (1991, 1998). Testing of solids is generally limited by the water solubility of the constituents. The effects of other physical/chemical properties (e.g., nitrate levels) on *Xenopus* embryonic development needs to be fully evaluated.

12.3 Section 12 Conclusions

The scientific basis for FETAX and its intended use(s) in ecotoxicology are adequately described. Test limits are defined but only limited information is available on the complete range of environmental samples amenable to test. The test method is incompatible with environmental samples that alter the pH, hardness, alkalinity, and conductivity of the FETAX

Solution beyond the acceptable ranges. Testing of solid environmental samples is generally limited by the water solubility of the constituents. The effects of other physico-chemical properties (e.g., nitrate levels) on *Xenopus* embryonic development needs to be evaluated.