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Current developments in reproductive toxicity testing of pesticides☆

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ABSTRACT

A protocol to evaluate the potential developmental and reproductive effects of test chemicals has been developed by the Life Stages Task Force of the International Life Sciences Institute (ILSI)/Health and Environmental Sciences Institute (HESI) Agricultural Chemical Safety Assessment (ACSA) Technical Committee. Since the original publication, several international groups have provided public comment on conducting the test. The extended one-generation reproductive toxicity test is now under consideration as a potential test guideline. The protocol uses a flexible approach that is markedly different from the current multigenerational guidelines. It encourages the use of toxicokinetics when setting the doses, evaluates more than one rat per sex per litter in the F1 offspring and does not necessarily require mating of the F1 to produce an F2 (F1 mating may be triggered by the presence of effects in the P0 and developing F1 rats). A number of additional reproductive endpoints, and the neurotoxicity and immunotoxicity cohorts are included. The ACSA protocol was developed with the goal of assuring that the methods are scientifically appropriate and the toxicological endpoints and exposure durations are relevant for risk assessment. Compared to existing testing strategies, the proposed approach uses substantially fewer animals, provides additional information on the neonate, juvenile and pubertal animal, and includes an estimation of human exposure potential for making decisions about the extent of testing required. In this paper, the evolution of the protocol since the 2006 publication is discussed. These changes reflect the collective input of a U.S. expert panel of government and industrial scientist convened in 2007 and discussions of an OECD expert group held in Paris, France (October, 2008).

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1. Introduction

The process of reproductive and developmental toxicity testing for regulatory purposes is governed by a framework of guideline studies depicted in Table 1 [1–7]. The multigenerational tests listed in Table 1 are typically the cornerstone protocols of the overall testing requirements implemented under the various regulatory authorities and, as such, represent a critical part of human health risk assessment. They are designed to identify developmental and reproductive effects by examining parental animals and offspring dosed pre- and post-natally to establish a no-observed-adverse-effect level (NOAEL) for the most sensitive effects, thus providing the basis for quantitative assessments. Furthermore, these guideline studies are developed and issued through the combined efforts of the international community of researchers and regulators, a process that was formalized and introduced for the risk assessment process approximately 50 years ago in an effort to set acceptable exposure or tolerance levels. A basic underlying premise is that these studies are predictive of potential adverse human health impacts, and provide the necessary information for identifying potentially sensitive target organ systems, maternal toxicity, embryonic and fetal lethality, morphological anomalies, specific types of malformations, and altered birth weight and growth retardation. While guideline studies have served their intended purpose, there is a continuing need to revisit their design and endpoint requirements periodically to assure that they reflect the current state of the science and that they make maximum use of the available resources. The most recent of such efforts was a series of articles published by the HESI ACSA Technical Committee addressing the need for an improved approach to assessing the safety of crop protection chemicals.

2. The ILSI/HESI ACSA Committee

The extended one-generation reproductive toxicity test was developed within a broader effort that reconsidered risk assessment approaches for evaluating the safety and potential health risks

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Table 1
Current reproductive and developmental guideline studies required for chemical registration.

OECD guideline number	U.S. EPA guideline number	Other	Description
OECD 414 [2]	EPA 870-3700 [4]		Prenatal Developmental Toxicity Study
OECD 415 [14]			One-Generation Reproduction Toxicity Study
OECD 416 [3]	EPA 870-3800 [5]		Two Generation Reproduction Toxicity Study
OECD 421 [31]	EPA 870-3550 [32]		Reproduction/Developmental Toxicity Screening Test
OECD 422 [33]	EPA 870-3650 [34]		Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test
OECD 426 [22]	EPA 870-6300 [21]		Developmental Neurotoxicity Study
		US FDA [6,7]	Multigeneration Reproductive Toxicity; Developmental Toxicity
		ICH [1]	Multigeneration Reproductive Toxicity

of crop protection chemicals. The large and diverse group of international experts that carried out this task was convened by the ILSI Health and Environmental Sciences Institute (HESI) in 2000. The task of this group was to develop a credible and viable testing paradigm using scientifically appropriate studies that are necessary without being redundant, and that emphasize toxicological endpoints and exposure durations that are relevant for risk assessment. The final strategy proposed by ACSA utilizes fewer animals and provides an improved basis for the risk assessment of agricultural chemicals [8–11]. Although developed for pesticides, this scheme is applicable to other types of environmental chemicals. The ACSA tiered approach begins with consideration of existing data/knowledge of the chemical and its class, use pattern and estimated exposures (by route, duration, and magnitude). These factors aid in refining or modifying the animal testing. The key principle of this approach is to identify areas of potential concern early in testing and thus concentrate on a more detailed evaluation of those areas. Further enhancements of the proposed approach include the use of a tiered-testing system based on existing knowledge of the chemistry of the compound, human exposure scenarios, absorption, disposition, metabolism and elimination (ADME) information and improvements in data collection for risk assessment. This, in turn, assures a greater efficiency in testing, use of fewer animals, and thus, a better use of resources.

To accomplish these aims, ACSA formed three international task forces charged with designing the specific tests and approaches to evaluate what information would be needed to determine [1] the test compound's absorption, distribution metabolism and excretion (ADME) [2], systemic toxicity and [3] potential adverse effects on various life stages. The results of these efforts were published in a single volume of Critical Reviews in Toxicology in 2006 [8–11]. The *extended one-generation reproductive toxicity test* was developed by the Life Stages Task Force [11]. Although the broader ACSA tiered-testing proposal departed from the current standardized list of hazard studies used by many national authorities, it represented the first comprehensive effort of its kind in decades to scientifically redesign the risk assessment framework for agricultural chemicals. To date, the extended one-generation reproductive toxicity test is the only protocol to be proposed as a new test guideline. Although this life stage study incorporates many features from current EPA and OECD two-generation reproductive test guideline, it represents several critical improvements, including the:

- use of toxicokinetic/ADME information from pregnant animals and their offspring, as well as data from the young adult systemic toxicity testing information, when setting the doses to be used in the extended one-generation study,
- evaluation of systemic toxicity in young adults as a consequence of pre- and early postnatal exposure,
- assessment of multiple outcomes from the same population of animals including the incorporation of sensitive endpoints for endocrine effects, developmental neurotoxicity, and developmental immunotoxicity,
- mating of F1 offspring to produce an F2 only when data indicate the need to do so (i.e., triggered F2),
- Increased efficiency in animal use and subsequent overall reduction in the total number of animals rquired for testing by thoroughly evaluating structural and functional outcomes across multiple developing organ systems in the same litter/study.

3. Features of the extended one-generation reproductive toxicity test

The ACSA Life Stages Task Force proposed a tiered approach to toxicity testing that assessed a compound's potential to cause adverse effects on reproduction and development throughout the lifespan of the organism. The core of this approach includes conducting two key protocols, Rabbit Developmental Toxicity Study and the Extended One-Generation Reproductive Toxicity Study [11]. In this section, the extended one-generation protocol is outlined.

The reproduction study proposed by the ACSA Life Stages Task Force is an *extended* one-generation study (Fig. 1). This study uses a comprehensive range of endpoints to detect abnormalities of reproductive function and sexual development. In addition, it specifies that a larger number of F1 offspring (three per sex per litter) be maintained and dosed post-weaning. This allows an assessment of offspring maturation (including the timing of sexual development), along with limited evaluations of delayed or latent manifestations of some toxicities, and of additive effects that may be related to the amount of time over which exposure has occurred.

There are a number of endocrine-sensitive measurements that are added as required endpoints in this protocol. These include specific, endocrine-sensitive endpoints in the F1 offspring (anogenital distance = AGD in all pups, vaginal opening and preputial separation in at least three pups per litter, nipple retention in all F1 males, and ovarian function as measured by vaginal smears in a minimum of three F1 females per litter). Specific measurements of thyroid hormone (thyroxin and thyroid stimulating hormone) are specified in the dam, early postnatal animals, and adult F1 animals. Although some of these endpoints are optional in the post-1998 EPA 870.3800 [5] and OECD 416 [3] Reproductive Toxicity Guidelines, they are required in the ACSA Extended F1 Study [11].

The Extended F1 Study also emphasizes the value of using all relevant, available information on the test substance, i.e., physicochemical, TK (including species-specific metabolism), toxicodynamic properties, structure-activity relationships (SARs), in vitro metabolic processes, results of previous toxicity studies, and relevant information on structural analogues. Preliminary information on ADME and bioaccumulation may be derived from chemical structure, physico-chemical data, extent of plasma protein binding or TK studies, while results from toxicity studies give additional information (e.g., NOAEL, metabolism or induction of metabolism, target organ effects). However, if this information is not available, the protocol also specifies the need to conduct an ADME evaluation in both the dams and pups during gestation and lactation.