charles river

Summary

The EOGRTS (OECD 443) is an improvement on the traditional two-generation study (OECD 416) since, despite having more endpoints and therefore an increase in the data generated, fewer animals are required.

SAFETY ASSESSMENT

Design Considerations in Extended One-Generation Reproductive Toxicity Studies

Background

The Extended One-Generation Reproductive Toxicity Study (EOGRTS) evaluates specific life stages not previously assessed by other types of toxicity studies including any effects resulting from pre- and postnatal exposure to chemicals.

Prior to study initiation, it is useful to have some background data on reproductive endpoints such as spermatogenesis (testicular histopathology) for males and ovarian integrity (histopathology) for females. Data from repeat-dose studies or short term endocrine disrupter screening assays can also be used to evaluate effects on male and female reproductive organs. An EOGRTS includes functional endpoints and serves as a test for reproductive endpoints that require the interaction of males with females, females with conceptus, and females with offspring and the F_1 generation until after sexual maturity[1].

Points of Consideration in the Study Design

Review existing data on the test substance

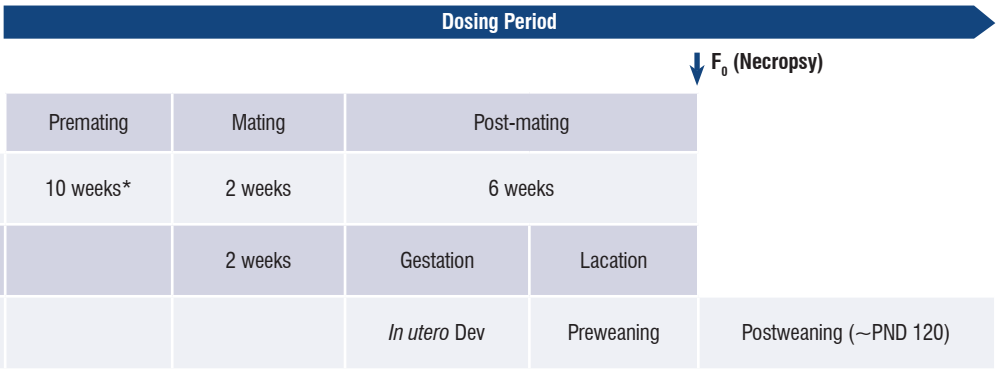
Aside from the typical considerations of dose route, levels and frequency, vehicle, and species, a thorough review of existing data is beneficial. This could include physicochemical data, toxicokinetics, toxicodynamic properties, structure-activity relationships, *in vitro* metabolic processes, results of previous toxicity studies, and structural analogs. Other information that is useful to have includes:

- Data that estimates internal dosimetry
- Data that confirms exposure in the fetuses/pups
- Data that evaluates potential dose-dependent saturation of kinetic processes
- Metabolite profiles
- Concentration-time courses

Study Duration

The duration of the basic study design is typically 21 weeks commencing with a 2 week premating period followed by mating, post-coitum, and lactation for the P generation. The end of the study is defined by an evaluation if a second generation is required using cohort 1B animals. Adding the developmental neurotoxicity or developmental immunotoxicity cohorts will not lengthen the in-life period of the study. The various elements of the study can be seen in Fig. 1.

Fig. 1, Schematic of EOGRT study schedule



*Although OECD 443 suggests a minimum 2 week premating period, the rationale behind increasing the premating exposure period to 10 weeks (as recommended by ECHA) is based on the fact that the focus of the study under REACH is the assessment of fertility. The standard design, with its 2 week premating treatment period, focuses only on the fertility of the parental animals, which are only exposed as adults.

During the F_1 dosing phase, the pups will receive test substance (TS) through the milk until weaning at postnatal day (PND) 21, although there will be additional exposure to the TS during the last week of lactation in a diet or water exposure study, when the pups begin to eat and drink on their own. Direct dosing of the F_1 pups starting at PND 10 may be considered if there is poor or inconsistent exposure during the lactation phase.

Cohort Assignment

A range of assessments are conducted on the F_1 offspring during the pre-weaning period and prior to weaning on PND 21 pups are randomly selected to constitute the F_1 generation and assigned to each of the three main cohorts (Fig. 2). The subgroups in each cohort may be assigned as shown in Fig. 3. The F_1 pups that are not selected are identified for blood collection for thyroid hormone assessments on PND 21 and also subjected to a gross macroscopic examination following termination.

Fig. 2, F_1 generation pups are assigned to each of the three main cohorts and cohort subgroups

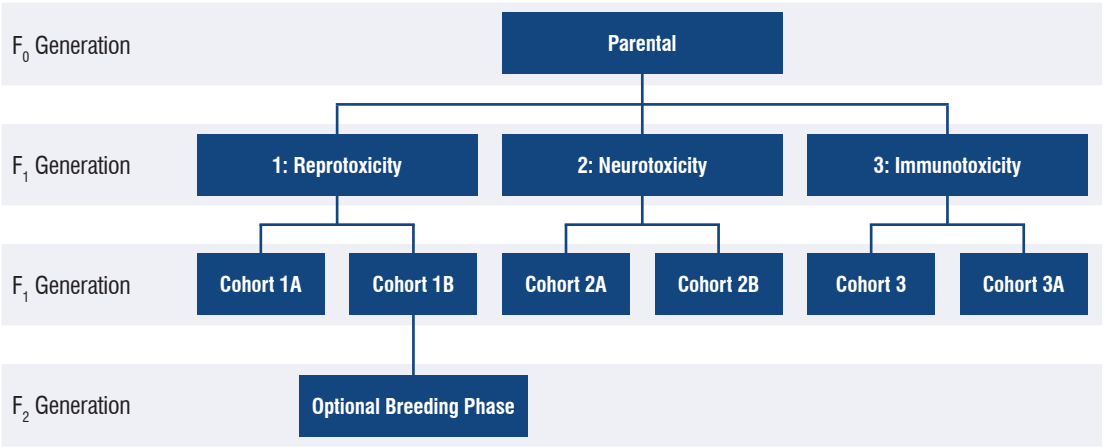


Fig. 3, Cohort sub-group assignments for F_1 endpoints

Cohort	Number of Animals	Primary Assessments
1A	1 pup/sex/litter/group (20/sex/group)	Primary reproductive/developmental toxicity testing
1B	1 pup/sex/litter/group (20/sex/group)	Follow-up reproductive assessment, if required
2A	1 pup/litter (10/sex/group)**	Neurobehavioral testing and neuropathology
2B	1 pup/litter (10/sex/group)**	PND 21 neuropathology
3	1 pup/litter (10/sex/group)**	Primary antibody response using sRBC challenge (PND 56 ± 3 days)
3A	1 pup/litter (10/sex – positive control)	Positive control group for sRBC challenge (PND 56 ± 3 days)

**One pup per litter selected such that selected pups represent as many litters as possible

One important aspect of cohort selections is the inclusion of a positive control subgroup in Cohort 3, identified as Cohort 3A in Figs 2 & 3, which should be considered if immunotoxicity is being assessed on the study. In order to conduct the T-dependent antigen response (TDAR), additional animals will be required from the F_0 control females to ensure there is sufficient F_1 control pups for assignment to Cohort 3A, which serves as the cyclophosphamide-positive control group.

It is also important to remember that in the event that insufficient pups are available for assignment to study, the guideline does provide direction on assignment priorities, with the maximum priority given to the primary assessment of general toxicity and of effects upon reproductive systems i.e., Cohorts 1A and 1B. If litters are standardized, the preference is for five per sex/group to allow distribution of pups across all litters, however it should also be noted that culling reduces the number of pups available for cohorts should there be any mortality.

Clinical observations, as well as detailed examinations, are then carried out according to protocolled cohort requirements and PND age. Further mating of the F_1 generation may also be undertaken to follow up any potential reproductive toxicity concerns. At the termination of the study, gross necropsy and, where indicated, further histopathology/neurohistopathology is undertaken.

Summary

An EOGRTS will provide data on the effects of repeated exposure to a TS during all phases of the reproductive cycle. In particular, the study will provide information on potential effects on the reproductive system as well as on development, growth, survival, and functional endpoints of subsequent generations.

Although large and complex, the benefit of this revised study design is that it provides information that addresses endpoints that historically required several separate studies. Cooper et al.[2] estimated that the EOGRTS requires approximately 1,400 animals compared with 3,880 animals in the multi-generation reproduction study and developmental neurotoxicity studies, thereby representing a large saving in animal resources.

References:

1. OECD. Test No. 443: Extended One-Generation Reproductive Toxicity Study [Internet]. OECD Publishing; 2012 [cited 2017 Mar 31]. Available from: http://www.oecd-ilibrary.org/environment/test-no-443-extended-one-generation-reproductive-toxicity-study_9789264185371-en
2. Cooper RL, Lamb JC, Barlow SM, Bentley K, Brady AM, Doerrer NG, Eisenbrandt DL, Fenner-Crisp PA, Hines RN, Irvine LFH, Kimmel CA, Koeter H, Li AA, Makris SL, Sheets LP, Speijers G, Whitby KE. A tiered approach to life stages testing for agricultural chemical safety assessment. Crit Rev Toxicol. 2006 Jan;36(1):69–98. PMID: 16708695