Special Event Publication

CO-ORGANISERS

knoell worldwide registration

ENVIGO

TSGE Consulting

ORGANISED BY

TSGE FORUM

THE EUROPEAN REACH CONGRESS 2015

24-25 NOVEMBER | DÜSSELDORF

MEDIA PARTNERS

CHEManager INTERNATIONAL

Speciality Chemicals Magazine

HEADLINE SPONSORS

BioReliance by SAFC

EBRC Consulting GmbH

History of Developmental and Reproduction Tests (DART) and their significance for safety compliance in REACH

How did the harmonized guidelines come into existence?

Samir Pandya | Manager - Business Development
Jai Research Foundation, N H No 8, Near Daman Ganga Bridge,
Valvada, Vapi, Gujarat, India, 396191

The need of inclusion of developmental and reproduction screening in the safety assessment of chemicals became a matter of concern after several hazardous incidences came to light in the early twentieth century. No one can forget the thalidomide tragedy of the late 1950s. Heavy metal ions and exposure to endocrine disrupting chemicals are also known to affect normal human reproductive cycles and cause birth defects in infants.

In January 1990, an ad hoc Meeting of Experts was held by the Organization for Economic Co-operation and Development (OECD) to discuss the Screening Methods for Reproductive Toxicity. The committee discussed and agreed to establish a protocol for a "Preliminary Reproduction Toxicity Screening Test". This protocol was drafted in such a way that it could be effectively utilized in the initial evaluation of existing chemicals. The draft was then adopted by the council during a meeting of Nominated Experts on Reproductive Toxicity Screening Methods, held in Tokyo, October 1992. Finally, it was converted to OECD guidelines - 421 and 422, which, in turn, became the reference for several safety assessment programs worldwide including REACH.

In June 1995, an OECD Working Group on Reproduction and Developmental Toxicity held a meeting in Copenhagen. The working group discussed the need to update the OECD Test Guidelines for reproduction and developmental toxicity as well as the development of new Guidelines for endpoints not yet covered. The Working Group recommended a revision to the Guideline for Developmental Toxicity. Thus OECD 414 guideline came into existence. In 2006, OECD 443 was adopted after a publication of proposal by a Joint Technical committee comprising International Life Science Institute (ILSI)-Health and Environmental Sciences Institute (HESI), and Agricultural Chemical Safety Assessment (ACSA).

What is expected in REACH?

Study	Annex VII	Annex VIII	Annex IX	Annex X
OECD 421 or 422	Not Required	Required	Not Required	Not Required
OECD 414	Not Required	May be Required if severe concern for prenatal developmental toxicity is observed.	Required in one species; second species may be triggered	Required in two species
OECD 416 or 443	Not Required	May be Recommended instead of 421/422 if severe concern for fertility is observed	Required if triggered	Required

What kind of developmental and reproductive testing is expected in REACH?

The European REACH regulation established the prerequisite to evaluate reproductive toxicity using the reproduction screening tests according to OECD 421 and 422, for all substances above the tonnage level of 10 tons/year. OECD 414 and 443 are conditionally required for substances having tonnage level above 100 tons/year, whereas both OECD 414 and 443 are mandatory for substances above the tonnage level of 1000 tons/year.

What is the significance of performing these tests?
OECD 421 is the Reproduction/Developmental Toxicity Screening Test. It is intended to produce information pertaining to the effects of a test substance on male and female reproductive performance such as gonadal function, mating behavior, conception, development of the conceptus, parturition and histopathological data on reproductive organs.

OECD 422 is a Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test. This test involves assessment of basic parameters to be considered in repeated dose toxicity with similar endpoints as OECD 421. This test is preferred over OECD 421 when there is no sufficient data from a 90 day repeated dose toxicity study.

OECD 414 is the Prenatal Developmental Toxicity Study that provides a focused assessment of potential effects subsequent to prenatal exposure, although it includes only effects that are manifested before birth. In particular, this study is intended to provide information on substance-induced effects on growth and survival of the foetuses, and increased incidences in external, skeletal and soft tissue malformations and variations in foetuses.

OECD 443 is the Extended One-Generation Reproductive Toxicity Study (EOGRTS). It involves the effects of the substance on the integrity and functioning of the adult male and female reproductive system, offspring viability, physical and development of the functions until adulthood. The focus of the study in REACH is on fertility. Therefore, it requires 10-week pre-mating exposure duration and the highest dose showing systemic or reproductive toxicity for all variant study designs of EOGRTS. The F study design focuses on assessment of the fertility of parental animals as well as of some parameters on postnatal development until adulthood including sexual maturity and histopathology of gonads.

The extension of the Cohort 1B (mating of the Cohort 1B animals to produce the F2 generation) also provides information on the fertility of the offspring, i.e. the F1 generation, which has been exposed to the test compound during germ cell formation, preimplantation, in utero and postnatal periods. Cohorts 2A and 2B provide information on developmental neurotoxicity and Cohort 3 on developmental immunotoxicity.

Earlier, REACH requirements required data from OECD 416, which is a two generation study. However, under the amendment of REACH (EC REG 2015/282), the less animal intensive OECD 443 has replaced this test.

How the end-points of these tests are compared?

Test method	End points	
	Developmental toxicity	Impairment of fertiliy
OECD 421/422	Screening	Screening
OECD 407	Not Relevant	Screening
OECD 416/443	Limited relevance	Definitive test
OECD 414	Definitive test	Not Relevant

What should be expected from a CRO while outsourcing these tests?
These tests are being offered by several Contract Research Laboratories worldwide. However, it is pertinent to choose carefully in outsourcing such critical and sensitive studies. The following are some considerations in the selection process of a CRO.

1. OECD GLP certification: History of continued GLP accreditation is a basic prerequisite. Look for a CRO with an unblemished history of GLP accreditation. Moreover, one should also check whether the CRO is fully accredited or partially accredited; in other words, whether the CRO is authorized to run all the tests under GLP within full premises or a few tests within a confined location.

2. SOPs:

a. Ensure that the CRO has robust SOPs for handling the critical aspects of the studies.

b. The CRO must have SOPs which mandate periodical validation using the recommended positive controls.

3. Animal handling expertise: The animals must be handled during the live phases as well as termination in such a way that the environment/ handling does not cause hormonal imbalances. Experience and expertise is required in handling the animals under pregnant / juvenile stage. This requires scientists and technicians dedicated to handling animals expertly and humanely.

4. Other expertise: Other skills of importance include specialized staining and evaluation techniques such as sperm/follicular counts and assessing congenital abnormalities, if any, at birth.

5. Strong Historical / Background control data: This is possibly the most important factor. It not only shows the experience of the CRO, but also ensures existence of reliable data from high quality performances of tests.

6. State-of-the-art facility:

a. The experimental environment for conducting these tests must be carefully maintained.

b. CROs should have barrier-maintained (BMR) facilities to run these tests and use specific pathogen free (SPF) animals. Ideally, facilities should be designed to have animal rooms within the clean and return corridor and perfect maintenance of barriers ensuring pressure gradient and unidirectional flow of air in such a way that accidental contamination is prevented.

c. The facility must have 100% fresh HEPA/ULPA filtered air and 15+ air changes per hour.

d. The BMR system must ensure digital logging and control of the environmental parameters, such room temperature, humidity, air pressure as well as performance of the utilities in real time.

e. Entry in an animal room, where such studies are conducted, must be strictly pre-authorized and recorded.

f. Provisions with dedicated areas for species isolation must be a norm.

g. Augmented and sophisticated necropsy and histopathology, use of certified and tested diet from renowned sources, accreditation from AAALAC etc. give confidence on the professionalism.

7. Other factors: There may be some other factors to consider such as transparent and realistic pricing, timeline compliance, a professional approach and attitude, communication skills and scientific understanding. However, these factors are objective and based on individual perceptions and expectations. Publications and surveys are helpful, but should be approached with caution as they may be misleading due to unseen biases, cherry-picking of data and other such issues. The historical approach to geography, size or prices of CROs is less relevant in today's global environment. One must always evaluate the cost vs. benefit ratio for CRO selection personally.

To know more about JRF Global's capabilities in developmental and reproductive toxicity (DART) testing, and other REACH requirements, please visit www.jrfglobal.com or write to us at bd@jrfonline.com. Our highly trained scientists and regulatory staff will always be on hand for all your REACH related queries!

References: OECD TG 421, 422, 414, 416 and 443, Draft Guidance on information requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance