By JRF GLOBAL

JRF SPOTLIGHT: DART

2016

JRF receives approval at USEPA

As part of a recent regulatory submission (June 2015) a number of JRF studies were reviewed and classified as 'Acceptable: - Guideline' by USEPA.

The registration submission included a number of complicated studies from JRF's non-clinical Developmental And Reproductive Toxicity (DART), Immunotoxicity and Neurotoxicity divisions. The regulatory submission timelines required a strong combination of scientific excellence and project management skills to deliver study data within tight time and quality expectations. This adds further experience with USEPA regulatory submissions to JRF's non-clinical portfolio building on a number of previous USEPA submissions over the past three years.

"We are delighted with the approval and acceptance of our client's study data. We are proud to offer our scientific contributions to non-clinical services globally. We strongly believe these positive achievements position us as a global CRO with strong and reliable scientific, quality and project management capabilities for our global clients.

Dr. Abhay Deshpande,
Director Global - JRF

About JRF Global

JRF Global is a multinational Contract Research Organization, with an expansive portfolio of non-clinical regulatory research services. We serve our global client base by offering high quality GLP studies and ensuring rapid turnaround time, on-time delivery and constant communication and updates.

HISTORY OF DEVELOPMENTAL AND REPRODUCTION TESTS (DART) AND THEIR SIGNIFICANCE FOR SAFETY COMPLIANCE IN REACH

How did the harmonized guidelines come into existence?[1]

The need of inclusion of developmental and reproduction screening in the safety assessment of chemicals has become 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)[2] to discuss the "Screening Methods for establishing potential of a chemical to cause 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. This draft, was then adopted by the council during a meeting of Nominated Experts on Reproductive Toxicity Screening Methods, held in Tokyo, October 1992[3]. 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[4]. 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 TG 414 guideline came into existence. In 2006, OECD TG 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)[5].

What kind of developmental and reproductive testing is expected in REACH?[7]

Study	Annex VII	Annex VIII	Annex IX	Annex X
OECD TG 421 or 422	Not Required	Required	Not Required	Not Required
OECD TG 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 TG 416 or 443	Not Required	May be Recommended instead of 421/422 if severe concern for fertility is observed	Required if triggered	Required

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

Significance of performing the Reproductive Toxicity testing[1]

OECD TG 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 behaviour, conception, development of the conceptus, parturition and histopathological data on reproductive organs.

OECD TG 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 coupled with endpoints as expected of the test using OECD TG 421. This test is preferred over OECD TG 421, when there data from a 90 day repeated dose toxicity study is deemed insufficient.

OECD TG 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. This study is intended to establish substance-induced effects on growth and survival of the fetuses, adverse effects leading to external, skeletal and soft tissue malformations and variations in fetuses.

OECD TG 443 is the Extended One-Generation Reproductive Toxicity Study (EOGRTS). This is a comprehensive study, which helps to assess 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 F1 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 substance 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.

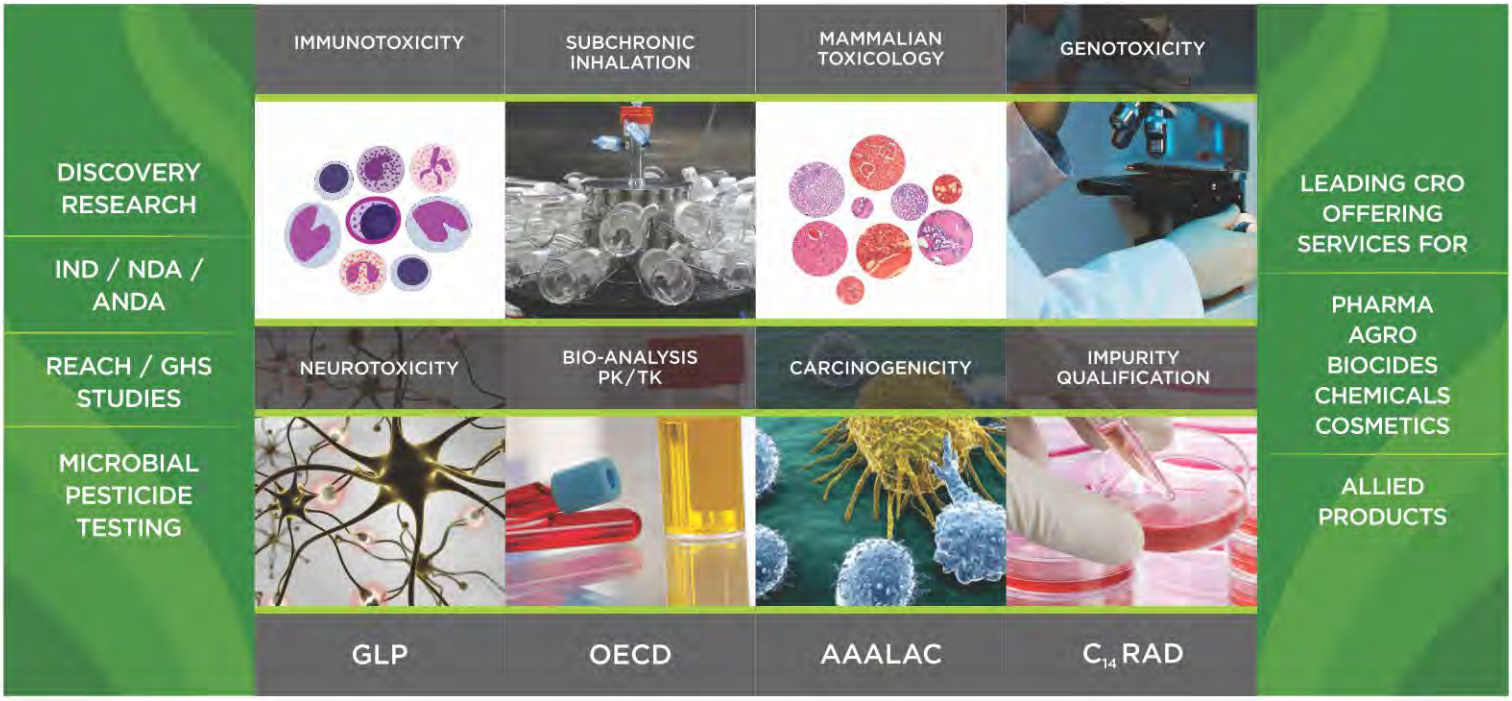
Earlier, REACH requirements required data from OECD TG 416, which is a two generation study. However, under the amendment of REACH (EC REG 2015/282), the less animal intensive OECD TG 443 has replaced this test.

The commission regulation (EU) 2015/282 of 20 February 2015[6] states;

EOGRTS is considered to offer a number of advantages in comparison to the two-generation reproductive toxicity study. It assesses a greater number of animals of the first filial (F1) generation and addresses additional parameters, thus improving the sensitivity and level of information that can be obtained from the test. Furthermore, as breeding of the F2 generation is not part of the basic test design, a significant reduction in the number of animals used is achieved if this design is used.

How the end-points of these tests are compared?[7]

Test Method	End points	
	Developmental toxicity	Impairment of fertility
OECD TG 421/422	Screening	Screening
OECD TG 407	Not Relevant	Screening
OECD TG 416/433	Limited Relevance	Definitive Test
OECD TG 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.

01. OECD GLP Certification: History of continued GLP accreditation is a basic prerequisite. Sponsor should select 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 Reproductive Toxicity tests under GLP within full premises or a few tests within a confined location.

02. SOPs:

a. Ensure that the CRO has very 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.

03. Expertise in handling animals during the live phase under the Reproductive Toxicity testing:
The animals must be handled during the live phases as well as termination in 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.

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

05. 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.

06. 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. A dedicated holding area and sacrifice area to minimize environment/movement related distress, which could adversely disturb the hormonal balance augmented and sophisticated necropsy and histopathology, use of certified and tested diet from renowned sources, accreditation from AAALAC etc. help the sponsor to gain a confidence upon the CRO, where they are investing faith, time and money![8]

07. Professionalism: 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. 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.

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 our expert Dr. Manish Patel patelmv@jrfonline.com. Our highly trained scientists and regulatory staff are happy to help you in rationally and economically designing the Testing paradigm for all your Reproductive Toxicity needs!

References:

1. OECD Test Guidelines 421, 422, 414, 416 and 443
2. OECD, Paris (1990). Room Document No. 1 for the 14th Joint Meeting of the Chemicals Group and Management Committee.
3. OECD, Paris (1992). Chairman's Report of the ad hoc Expert Meeting on Reproductive Toxicity Screening Methods, Tokyo, 27th-29th October, 1992.
4. OECD (1995) Draft Report of the OECD Ad Hoc Working Group on Reproduction and Developmental Toxicity. Copenhagen, Denmark, 13-14 June 1995.
5. Cooper, R.L., J.C. Lamb, S.M. Barlow, K. Bentley, A.M. Brady, N. Doerr, D.L. Eisenbrandt, P.A. Fenner-Crisp, R.N. Hines, L.F.H. Irvine, C.A. Kimmel, H. Koeter, A.A. Li, S.L. Makris, L.P. Sheets, G.J.A. Speijers and K.E. Whitby (2006), "A Tiered Approach to Life Stages Testing for Agricultural Chemical Safety Assessment", Critical Reviews in Toxicology, 36, 69-98.
6. Official Journal of the European Union, 21.2.2015, L 50/1, COMMISSION REGULATION (EU) 2015/282 of 20 February 2015
7. Draft Guidance on information requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance
8. J. Charles Eldridge James T. Stevens "Endocrine Toxicology" 3rd Edition, pg 53, CRC Press.

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Presented at SOT Baltimore, MD, 2009

ABSTRACT

There are innumerable hazardous contaminants in our environment. Some of these agents are known to be neurotoxic in animals and humans. Foetuses and neonates are known to be a high-risk groups. In several studies it has been shown that exposure to environmental toxic agents such as PCB, DDT, nicotine, organophosphorous compounds can be neurotoxic as well as lead to disruption of adult brain function. The present study was carried out in order to observe the enhanced developmental neurotoxic effects in pregnant female rats using acrylamide. Pregnant Wistar rats (9-11 weeks old, weighing 190-205 gms) were dosed with acrylamide at 0, 8, 12 and 16/18 mg /kg b. wt. from day 6 of gestation to day 21 of lactation; No maternal death or toxic signs were noted during gestation however, two dams from the high dose group were found dead during lactation. The maternal body weight and feed consumption were significantly decreased in the mid and high dose groups during gestation and lactation.The mean body weight and feed consumption of pups were significantly decreased in all treatment groups. The survival index of the pups was decreased in the high dose group on postnatal day 4, 21 and 28. During neurobehavioral observation of the pups, circling behaviour (PND 11), abnormal gait (PND 21) and impaired mobility (PND 21) were observed in male and female pups from the high dose group only. The pups from the high dose group were also observed to fall immediately during the wire manoeuvre observation, whereas pups form the mid and low dose could complete the manouvere. The mean ambulatory activity on postnatal days 13 and 17 was decreased in the male and female pups from the mid and high dose groups. On PND 23, abnormal air righting reflex of the male and female pups was observed in the mid and high dose groups and mean foot splay (mm) of male and female pups was significantly decreased at the high dose. Based on results of present study, It can be concluded that acrylamide did not show selective developmental neurobehavioral toxicity, because neurotoxic effects in the pups were only observed as doses associated with maternal toxicity. It was evident that the test substance was found to possess maternal toxicity but failed to reveal potential developmental toxicity.

OBJECTIVE

This experiment was designed to provide data, including dose-response characterizations, on the potential functional effects on the developing nervous system of the pups following exposure in utero and during early life to a known neurotoxic substance.

RESULTS

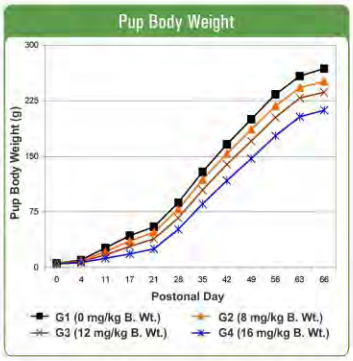
PARENTAL FEMALES

TOXIC SIGNS: No toxic signs were observed during gestation period upto the dose level of 18 mg/kg body weight. Body weight and food consumption were reduced in all treated groups, compared to the controls. However a pedalling gait was observed in the rats from the 12 and 16 mg/kg bwt/day group during the lactation period.

NEUROBEHAVIORAL OBSERVATION: During the neurobehavioral observations of the dams, rear count was decreased at 12 and 18/16 mg/kg b wt/day during gestation and lactation. Abnormal gait, impaired mobility and low arousal were observed at 16 mg/kg b wt/day during lactation.

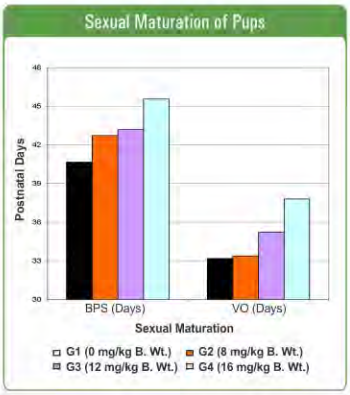
PUPS

TOXIC SIGNS: Treatment related mortality was observed in the 16 mg/kg bwt/day dose group. Body weight and food consumption were reduced in all treated groups, compared to the controls. Neurobehavioral Observations: During neurobehavioral observations of the pups, circling behaviour (PND 11), abnormal gait (PND 21) and impaired mobility (PND 21) were observed in male and female pups from the high dose group.



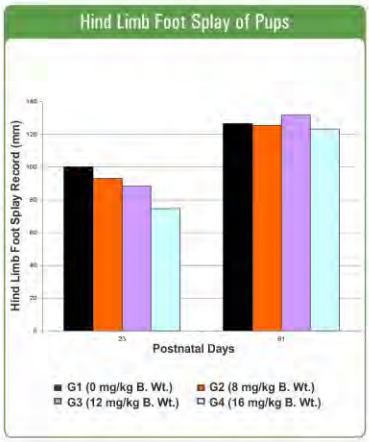
SURVIVAL INDEX: The survival index of the pups was decreased at 16 mg/kg bwt/day on postnatal days 4, 21 and 28. On day 28 onwards, mortality of pups falls up due to the treatment of acrylamide to the lactating dam was discontinued after day 21.

PHYSICAL DEVELOPMENT LANDMARKS: Due to acrylamide treatment, delayed in the normal appearance days were observed in the unfolding of pinna, ear and eye opening, incisor eruption and hair growth at 16 mg/kg bwt. While, incisor eruption and hair growth at 12 mg/kg bwt and only incisor eruption at 8 mg/kg bwt. when compared with the concurrent control group. The sexual maturation was hastened in all treatment groups as compared to the control group.



BEHAVIOURAL ONTOGENY: The pups from the 16 mg/kg bwt/day dose group fall immediately during the wire manoeuvre observation and were unable to complete the task.

MOTOR ACTIVITY: The mean ambulatory activity of male and female pups on postnatal Motor and Sensory Function: An abnormal air righting reflex was observed in the male and female pups on postnatal day 23 at 12 and 16 mg/kg bwt/day.



CONCLUSION

It can be concluded that acrylamide did not showed selective developmental neurobehavioral toxicity. It was evident that the test substance was found to possess maternal toxicity but failed to reveal potential developmental toxicity.