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Regulatory acceptance and use of the Extended One Generation Reproductive Toxicity Study within Europe

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ABSTRACT

The two-generation study (OECD TG 416) is the standard requirement within REACH to test reproductive toxicity effects of chemicals with production volumes >100 tonnes. This test is criticized in terms of scientific relevance and animal welfare. The Extended One Generation Reproductive Toxicity Study (EOGRTS), incorporated into the OECD test guidelines in 2011 (OECD TG 443) has the potential to replace TG 416, while using only one generation of rats and being more informative. However, its regulatory acceptance proved challenging. This article reconstructs the process of regulatory acceptance and use of the EOGRTS and describes drivers and barriers influencing the process. The findings derive from literature research and expert interviews. A distinction is made between three sub-stages; The stage of Formal Incorporation of the EOGRTS into OECD test guidelines was stimulated by retrospective analyses on the value of the second generation (F2), strong EOGRTS advocates, animal welfare concern and changing US and EU chemicals legislation; the stage of Actual Regulatory Acceptance within REACH was challenged by legal factors and ongoing scientific disputes, while the stage of Use by Industry is influenced by uncertainty of registrants about regulatory acceptance, high costs, the risk of false positives and the manageability of the EOGRTS.

1. Introduction

Chemicals are subjected to a broad range of requirements to guarantee safety for humans, animals and the environment. The requirements describe the endpoints for which chemical substances have to be assessed and generally also the test procedures that need to be performed for a particular endpoint. Reproductive and developmental toxicity are two of the main endpoints in the assessment of industrial- and agrochemicals. These endpoints include the toxic effects of a substance on an organism's reproduction and development of its offspring. The reproduction cycle of mammals, being a highly complex process, is very difficult to investigate *in vitro*. For this reason regulatory reproductive and developmental toxicity tests are still conducted in laboratory animals with a prenatal developmental study in rodents and a non-rodent species and a one- or two generation reproduction toxicity study in rats (Janer et al., 2007b).

Since the 1980s the OECD 416 two-generation study has been the most comprehensive reproductive toxicity study (OECD, 2001). Up to 30% of the reproductive toxicity tests conducted are

Abbreviations: ACSA, Agricultural Chemical Safety Assessment of the ILSI HESI; ARA, Actual Regulatory Acceptance by regulatory authorities; CARACAL, Competent Authorities for REACH and GLP; CLP, classification and labelling process; CMR, Carcinogenic, Mutagenic and Reprotoxic; DIT, developmental immunotoxicity; DNT, developmental neurotoxicity; EC, European Commission; ECETOC, European Centre for Ecotoxicity and Toxicology of Chemicals; EURL-ECVAM, European Centre for the Validation of Alternative Methods; ECHA, European Chemicals Agency; EOGRTS, Extended One Generation Reproductive Toxicity Study; EOGRTS EG, Expert group on the EOGRTS established within CARACAL; F2, second generation of offspring; FI, Formal Incorporation into regulatory requirements; HESI, Health and Environmental Sciences Institute; ICAPO, International Council on Animal Protection in OECD programmes; ILSI, International Life Sciences Institute; MSC, Member States Committee of ECHA; MSCA, Member State Competent Authority of ECHA; OECD, Organisation for Economic Co-operation and Development; OPTS, EPA Office of Pesticides and Toxic Substances; RAC, Risk Assessment Committee of ECHA; REACH, Registration, Evaluation, Authorization and restriction of CHemicals; RIVM, Dutch National Institute for Public Health and the Environment; TG, test guideline; TG 416, test guideline two-generation reproductive toxicity study; TG 443, test guideline EOGRTS; TG 426, test guideline neuro developmental study; UI, Use by Industry for regulatory purposes; US EPA, United States Environment Protection Agency.

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two-generation studies (Spielmann and Vogel, 2006), requiring around 2600 animals per study (Lilienblum et al., 2008). The two generation test is estimated to use nearly 40% of the laboratory animals under REACH (Janer et al., 2007a) and thereby is one of the major users of rodents in safety test programs.

In anticipation of the introduction of the European Directive for the Registration, Evaluation, Authorization and restriction of CHemicals – REACH (EU, 2006) concern was expressed that reproductive toxicity testing would lead to a significant increase in numbers of animals needed. Reproductive and developmental toxicity were even estimated to become the largest animal user for safety testing within REACH (Pedersen et al., 2003; Van der Jagt et al., 2004) since approximately 10,000 chemicals with an annual volume of >100 tonnes would have to be tested on reproductive toxicity. The estimates ranged from 40% to 90% of the total number of animals to comply with REACH that would be needed for reproductive toxicity testing purposes (Van der Jagt et al., 2004; Spielmann and Vogel, 2006; Hartung and Rovida, 2009; Martin et al., 2011). At about the same time, several studies became available that questioned the added value of the second generation (Cooper et al., 2006; Janer et al., 2007a,b; Martin et al., 2009; Piersma et al., 2011) and criticized the limited predictive value of the OECD TG 416 for developmental immunotoxic and neurotoxic parameters (See Section 2.1.).

In 2006 the Agricultural Chemical Safety Assessment (ACSA) Technical Committee of the ILSI Health and Environment Sciences Institute (HESI) proposed a whole new testing paradigm, which constituted a tiered approach of toxicity testing. Part of this paradigm was a proposal for an alternative protocol for OECD TG 416 which required only one generation of animals while being more informative in data obtained (Cooper et al., 2006). This protocol became the basis for the Extended One Generation Reproductive Toxicity Study – EOGRTS – with a reduction of up to 40% in animal use – i.e. a total of 1200 animals per study – compared to the two-generation study. In addition the EOGRTS protocol includes parameters for developmental neurotoxicity-DNT – and developmental immunotoxicity-DIT –. The Cooper protocol was proposed to the OECD secretariat for incorporation into the OECD guidelines in 2007 and accepted in 2011 as OECD TG 443 after a process in which many amendments were made, as will be described in Section 2.1. of this manuscript.[1]

The EOGRTS matches with the ambition of the European Commission to diminish the use of laboratory animals and to stimulate the acceptance and use of models to replace, reduce and refine (3Rs) existing animal models (Russel and Burch, 1959). This ambition is laid down in Directive 2010/63/EU on the protection of animals used for scientific purposes and in REACH. Directive 2010/63/EU states in article 13.2 that in choosing between procedures, those which use the minimum number of animals shall be selected (EU, 2010). Furthermore, REACH states in article 25 (1) that in order "*…to avoid unnecessary animal testing, testing on vertebrate animals for the purpose of this Regulation shall be undertaken only as a last resort*". (EU, 2006) (See also Section 2.2.). Despite these legislative stimulants and the incorporation of the EOGRTS into the OECD test guidelines, the regulatory acceptance and use of the EOGRTS within Europe has been a point of strong disparity. This raises the following key questions which will be addressed in this paper:

- *Which factors influence the regulatory acceptance and use of the EOGRTS within Europe?*[2]
- *What is needed to augment the current process?*
- *Which lessons can be drawn from the case of the EOGRTS for future processes?*

To improve the use of the 3Rs-congruent with the EC's ambition – an exhaustive comprehension of the process of regulatory acceptance and use and its drivers and barriers is needed. In order to understand and examine the regulatory process, we made a distinction between the following three successive stages:

Sub stages of regulatory acceptance and use of a new test method
FI: Formal Incorporation into the OECD test guidelines
ARA: Actual Regulatory Acceptance by regulatory authorities
UI: Use for regulatory purposes by Industry

Full regulatory acceptance and use means that a 3R model has passed all three stages.

This manuscript builds on earlier work of the authors (Schiffelers et al., 2012, 2014) which examined the process of regulatory acceptance and use of 3R models from a technology acceptance perspective (see also Section 3). The reconstruction of the EOGRTS case offers additional in depth knowledge of this process.

The EOGRTS is currently in a critical phase. Although there is agreement on the inclusion of the EOGRTS in the fifth adaptation of the REACH test methods regulation, the discussion on the actual regulatory acceptance (ARA) and the use of the EOGRTS by industry (UI) for the release of chemicals is still taking place within Europe. Disentangling the process from a more general perspective of technology acceptance can offer relevant input for this discussion and lessons for future processes.

2. Results

This section reconstructs the process of the acceptance and use of the EOGRTS and gives an overview of the barriers and drivers on this process throughout the three sub stages of Formal Incorporation (FI) of the EOGRTS in the OECD Test Guidelines – Section 2.1.; the Actual Regulatory Acceptance (ARA) by European regulatory authorities for chemical registration and authorization purposes under REACH – Section 2.2.; and the Use by Industry (UI) for chemical registration and authorization purposes under REACH – Section 2.3. The findings derive from examination of available documents connected to the acceptance process (e.g. meeting- and workshop reports) and a series of interviews with experts involved in this process (see Appendix A for a description of the methodology). To elucidate the results, several quotes from respondents are inserted in the description of drivers and barriers.

[1] "*This Test Guideline is designed to provide an evaluation of reproductive and developmental effects that may occur as a result of pre- and postnatal chemical exposure as well as an evaluation of systemic toxicity in pregnant and lactating females and young and adult offspring. In the assay, sexually-mature males and females rodents (parental (P) generation) are exposed to graduated doses of the test substance starting 2 weeks before mating and continuously through mating, gestation and weaning of their pups (F1 generation). At weaning, pups are selected and assigned to cohorts of animals for reproductive/developmental toxicity testing (cohort 1), developmental neurotoxicity testing (cohort 2) and developmental immunotoxicity testing (cohort 3). The F1 offspring receive further treatment with the test substance from weaning to adulthood. Clinical observations and pathology examinations are performed on all animals for signs of toxicity, with special emphasis on the integrity and performance of the male and female reproductive systems and the health, growth, development and function of the offspring. Part of cohort 1 (cohort 1B) may be extended to include an F2 generation; in this case, procedures for F1 animals will be similar to those for the P animals*".; http://www.oecd-ilibrary.org/environment/test-no-443-extended-one-generation-reproductive-toxicity-study_9789264122550-en.

[2] Although this paper focusses on the European situation, major parts of the discussion in the US are also covered in this manuscript.