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The era of 3Rs implementation in developmental and reproductive toxicity (DART) testing: Current overview and future perspectives

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ABSTRACT

Since adoption of the first globally implemented guidelines for developmental and reproductive toxicity (DART) testing for pharmaceuticals, industrial chemicals and agrochemicals, many years passed without major updates. However in recent years, significant changes in these guidelines have been made or are being implemented. These changes have been guided by the ethical drive to reduce, refine and replace (3R) animal testing, as well as the addition of endocrine disruptor relevant endpoints. Recent applied improvements have focused on reduction and refinement. Ongoing scientific and technical innovations will provide the means for replacement of animal testing in the future and will improve predictivity in humans. The aim of this review is to provide an overview of ongoing global DART endeavors in respect to the 3Rs, with an outlook towards future advances in DART testing aspiring to reduce animal testing to a minimum and the supreme ambition towards animal-free hazard and risk assessment.

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1. Introduction

The guiding principles supporting humane and ethical use of animals in scientific research are called the three Rs (i.e. replace, reduce and refine) and were first described by Russell and Burch in 1959 [1]. Any researcher planning to use animals in their research must first show why there is no alternative and what will be done to minimize animal numbers and suffering, i.e.: 1) Replace the use of animals with alternative techniques, avoiding the use of animals altogether, 2) Reduce the number of animals used to a minimum, to obtain sufficient information from fewer animals, and 3) Refine the way experiments are carried out, to make sure animals suffer as little as possible. This includes better housing and improvements to procedures which minimize pain and suffering and/or improve animal welfare.

For toxic hazard assessment of chemicals in the regulatory domain, animal studies are being performed as default. For Developmental and Reproductive Toxicity (DART) testing, the first harmonized guidelines were published in the 1980's for industrial chemicals (OECD 414 in 1981, OECD 415 and 416 in 1983) [2–4] and in the 1990's for pharmaceuticals (ICH S5 in 1993) [5]. In addition to these definitive tests, a relatively quick screening method which can give initial clues about possible fertility and developmental effects of chemicals is the OECD 421 screening study adopted in 1995 (and the combination with 28-day repeated dose, OECD 422, adopted in 1996) [6,7]. All DART tests demand large numbers of animals, as parental and offspring generations are involved, and depending on circumstances, developmental toxicity studies can be required to be executed in two species (i.e. one rodent and one non-rodent). Since adoption of the first version of these guidelines, many years passed without major updates. However in the last couple of years, significant changes in these guidelines have been or are being implemented. These changes have in part been guided by the ethical drive to reduce, refine and replace (3R) animal testing, as well as the addition of endocrine disruptor relevant endpoints. Recent applied changes have focused on reduction and refinement. Scientific and technical innovations like assays based on human cells, non-mammalian models, high throughput testing, omics approaches, systems biology and computational modeling can provide the basis for replacement of animal testing in the future. Importantly, using these innovative models will greatly enhance the relevance and predictivity for humans.

Table 1 provides an overview of the most recent versions for DART guidelines with some details on the required numbers of animals and performance timing.

Already a lot of effort is made in the optimization of individual alternative methods for developmental toxicity assessment. Good examples are the rodent whole embryo culture, zebrafish embryo assay, and embryonic stem cell assay [8]. These alternative tests are currently mainly being used as screening tools for pharmaceuticals, but are of course also applicable for agrochemicals and industrial chemicals. In addition to these individual alternative methods, initiatives such as the US National Academy of Sciences report on Toxicity Testing in the 21st Century [9], the US EPA ToxCast high throughput screening test battery have provided fresh ideas in the 3R arena. The advent of computational systems toxicology has further stimulated development of a human-based animal-free hazard and risk assessment. These initiatives are providing innovative approaches and tools for an eventual transition to human focused, animal-free chemical and pharmaceutical hazard and risk assessment.

The current publication will provide an overview of other ongoing 3Rs activities worldwide in the field of DART (Chapter 2), with an outlook towards future 3Rs developments in DART testing (Chapter 3). Chapter 2 is divided into three topics: pharmaceuticals, industrial chemicals and agrochemicals. The aim is to provide a platform by which these three fields can learn from each other as similar DART discussions are ongoing in these areas. A general discussion and conclusion on future important steps follows in Chapter 4.

2. Hot topics in 3Rs for DART – current overview

2.1. Pharmaceuticals

This chapter describes the current hot topics in the field of 3Rs for DART testing of pharmaceuticals. It will cover 1) the upcoming revision of the ICH S5 guideline, 2) the use of the second species in embryo-fetal developmental (EFD) studies, 3) the use of microsampling for toxicokinetic (TK) assessment, and 4) the unnecessary use of satellite rats in EFD studies.

2.1.1. Update ICH S5

In March 2015 a final concept paper was endorsed by the ICH Steering Committee for revision of the ICH S5(R2) guideline [10]. It mentions "*Since implementation of ICH S5(R2), not only has experience been gained with the testing of pharmaceuticals using the current and novel testing paradigms; but scientific, technological and regulatory knowledge has also significantly evolved. Consequently there are now opportunities for modernizing testing paradigms to enhance human risk assessment, while also potentially reducing animal use*". It furthermore states that two of the major issues that need to be resolved are 1) Development of basic principles for possible regulatory acceptance of in vitro, ex vivo, and non-mammalian in vivo embryo-fetal development (EFD) assays, and 2) Design of optional integrated testing strategies involving an in vivo mammalian EFD assessment and in vitro, ex vivo and non-mammalian in vivo EFD assays, and the limited circumstances under which such a testing strategy would be considered. In this light there are currently several initiatives ongoing which focus on collaborative optimization of the zebrafish embryo assay, namely the ILSI (International Life Sciences Institute) HESI- (Health and Environmental Sciences Institute) DART DASTON workgroup [11] and a European Teratology Society (ETS) task force.

The anticipated milestone for the ICH step 4 document (i.e. adoption of the ICH harmonized guideline) is expected in Q3 of 2019 [12].

2.1.2. Second species in EFD studies

The reasoning for the need for a second species (usually the rabbit in addition to the rat) in developmental toxicity testing of pharmaceuticals goes back to the thalidomide tragedy that happened around 1960 [13]. Thalidomide, used for morning sickness during pregnancy, caused phocomelia in children exposed in utero. These defects could be reproduced in rabbits but not in rats, which prompted regulators to request developmental toxicity studies in two species. Important to note is that even though rat fetuses did