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Update of OECD DART guidelines with endocrine disruptor relevant endpoints: Practical considerations

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ABSTRACT

In 1998, the OECD initiated a high-priority project aimed at revising existing test guidelines and developing new test guidelines for screening of potential endocrine disruptors. In 2011, OECD 443 was adopted, and in 2015 OECD 421 and OECD 422 were updated with endocrine disruptor relevant endpoints. A feasibility study for the enhancement of OECD 414 with endocrine disruptor relevant endpoints is currently ongoing. The addition of these endpoints is considered crucial for gaining more information on endocrine disruptor potency of tested chemicals, however it should be noted that these additions have a major impact on the study designs and give rise to several practical challenges. The aim of this review is to discuss important aspects of these challenging study designs and to share our knowledge on their implementation in our laboratory. Together, this review can be used as guidance for other laboratories, study monitors and registration officers.

1. Introduction

Almost 20 years ago, an important endeavor was started by the OECD to update existing test guidelines and to create new guidelines for the screening and testing of potential endocrine disruptors [1]. In this context, parameters suitable for the detection of endocrine disrupting activity of test chemicals were added to OECD 407 (Repeated Dose 28-Day Oral Toxicity Study in Rodents) in 2008 [2]. More recently, OECD 443 (Extended One-Generation Reproductive Toxicity Study; EOGRTS) was implemented as new guideline in 2011 to replace OECD 416 (Two generation reproduction toxicity study; [3]). In 2015, OECD 421 (Reproduction/Developmental Toxicity Screening Test) and OECD 422 (Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test) were revised to include endocrine disruptor relevant endpoints [4–6]. A feasibility study for the enhancement of OECD 414 (Prenatal Developmental Toxicity Study) with these parameters was launched in April 2015, and is now in progress.

Prior to the revision of OECD 407, there was an extensive international program to test for the relevance and practicability of the additional parameters, the performance of these parameters for chemicals with (anti-)oestrogenic, (anti-)androgenic, and (anti-)thyroid activity, the intra- and interlaboratory reproducibility, and the interference of the new parameters with those parameters already required by the previous version of the OECD 407 guideline. The large amount of data obtained has been compiled and evaluated in detail [7], therefore no further elaboration will be given on OECD 407, as the current manuscript will focus on Developmental and Reproductive Toxicity (DART) studies.

The selection of endocrine disruptor relevant endpoints included in the updated OECD 421 and 422 guidelines (and also in OECD 443) were based on a feasibility study addressing scientific and technical questions related to their inclusion, as well as possible adaptations of the test design needed for their inclusion [8]. Table 1 provides an overview of the endocrine disruptor relevant endpoints added to OECD 421, 422 and 443. One notable point is that the EPA guidelines for the Reproduction/Developmental Toxicity Screening Test and Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (OPPTS 870.3550 and 870.3650) have not yet been updated with these parameters [9,10].

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Table 1
Overview of endocrine disruptor relevant endpoints added to OECD 421, 422 and 443.

OECD 421 and 422	OECD 443
Vaginal smears are examined daily for a period of 2 weeks prior to the onset of dosing. Only animals displaying a 4- to 5-day cycle continue in the study.	---
Oestrous cycle determination two weeks pre-mating until evidence of mating.	
The anogenital distance (AGD) of each pup is measured on the same postnatal days (between PND 0 and PND 4).	
The number of nipples/areolae in male pups is counted on PND 12 or 13.	
Blood samples are taken from: • 2 pups (one pooled sample) per litter on PND 4 for possible future analysis • 1 pup/sex/litter on PND 13 for T4 • all adult males at termination for T4 • all dams at termination for possible future analysis Further assessment of blood samples from the dams and PND 4 pups as well as of other thyroid hormones (TSH) are done if considered relevant.	Blood samples are taken from: • 2 pups (one pooled sample) per litter on PND 4 (if considered necessary) for T4. • 1 pup/sex/litter on PND 22 (F_1-pups not selected for cohorts) for T4 and TSH. • 10 F_0-males and females per dose group at termination for T4 and TSH. • 10 Cohort 1A males and females per dose group at termination for T4 and TSH.
Thyroid weight and histopathological examination not required in OECD 421; this is required in OECD 422.	Thyroid weight and histopathological examination required.
---	3 additional endocrine disruptor relevant endpoints are included in the OECD 443, namely vaginal patency, balano-preputial separation, and day of first estrus.

The addition of these endpoints is considered crucial for gaining more information on the endocrine disruptor potencies of tested chemicals. However, it should also be noted that these additions have a major impact on the study designs and give rise to several practical challenges, especially as there are currently a growing number of OECD 421 and 422 studies being performed for REACh. These recent additions have a huge impact on laboratory capacities as the studies are more labor-intensive and now take approximately 11 weeks instead of 7 weeks for the in-life phase.

Implementing the new OECD 443 study and the changes in OECD 421 and 422 studies at our laboratory has revealed important practical aspects, knowledge of which can be invaluable for other laboratories. To be in accordance with the 3R's, the authors of this manuscript have therefore decided to share a review of the best practices, insights and guidance for the most important aspects of these complex study designs. Such information would be highly valuable for study monitors and registration officers.

This manuscript focuses on endocrine disruptor relevant endpoints, and discusses the following topics: legislation, practical aspects of the recently included endocrine disruptor relevant endpoints to OECD 421, 422 and 443, details on this for the OECD 414 guideline, and other important considerations. All other important practical considerations relevant to OECD 443 have recently been published [11].

2. Legislation

In 2012, the OECD 421 and 422 test guidelines were updated by the Joint Meeting of the OECD council, which agreed on the status of deleted and former versions of Test Guidelines with regards to the Mutual Acceptance of Data (MAD). An 18-month transitional period between the Council Decision and the effective date of deletion was agreed, after which no new test using the deleted or the former version of a Test Guideline can be initiated [12]. Thus if a test is initiated during the transition period (i.e. 28 July 2015 to 28 January 2017), the previous version of an OECD test guideline will be applied, and the test results would be acceptable under the principles of MAD. Evaluating authorities might however request additional data upon evaluation.

With regard to data evaluation, it is important to note that, to date, there is no globally accepted strategy to identify endocrine disruptors. As a consequence, the data requirements for addressing potential endocrine disruption in humans differs depending on the type of substance being registered and the region of interest. To illustrate this, the requirements for industrial chemicals, biocides and agrochemicals in the European Union (EU) vs. United States (U.S.) are highlighted below (a full overview of global requirements is outside the scope of this manuscript).

In the EU, under REACh law, every industrial chemical registration at 10 t/annum or more should include test data to address potential effects on development and reproduction. The minimum data requirement for Annex VIII (10–100 t/a) consists of a repro screening study (OECD 421/422), at higher tonnages the prenatal developmental toxicity study (OECD 414) and the EOGRTS (OECD 443) may also be required. The current dossier format does not include a separate endpoint to address potential endocrine disrupting activity of a substance, but this endpoint should be assessed within other sections (e.g. Toxicity to Reproduction). In the absence of well-defined criteria for endocrine disruption, a well-defined testing strategy is lacking, although the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters [13] can be used as guidance for testing. Upon evaluation by the European Chemicals Agency (ECHA), endocrine disrupting activity can however be a factor that determines if a substance is regarded as a Substance of Very High Concern (SVHC), which can trigger a ban from the market.

The U.S., unlike the EU, does not have fixed data requirements for industrial chemicals. New chemicals can be added to the Toxic Substances Control Act (TSCA) Chemical Substance Inventory through the submission of a Premanufacture Notice (PMN), in which all currently available data should be included. After submission, the Environmental Protection Agency (EPA) evaluates the data via the application of several tools including,but not limited to, structure activity relationships, data from analogues, predicted mechanisms of toxicity, and expert judgment. As a follow-up, the EPA can request further information, however it should be noted that almost 90 percent of Premanufacture Notices submitted to the EPA complete the review process without being restricted or regulated in any way [14]. As a result, for a number of substances used