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Dose level selection for Developmental and Reproductive Toxicology (DART) studies

Workshop report

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Introduction

1. This report summarises the outcomes from the 'Dose level selection for Developmental and Reproductive Toxicology (DART) studies' workshop held as a satellite event to EUROTOX 2024 in Copenhagen on 11 September 2024. The meeting was co-organised by the NC3Rs[1], ECETOC[2], Charles River Laboratories and European Chemicals Agency (ECHA). The meeting brought together stakeholders globally from a range of sectors to understand different perspectives on dose selection for reproductive toxicity studies (OECD TGs 414, 421/422 and 443) conducted under EU legislation for the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), following advice issued by ECHA in 2022.

Background

2. In 2022, ECHA issued advice on the selection of high dose levels for DART studies (OECD TGs 414, 421/422 and 443) conducted under REACH indicating that the highest dose tested should aim to clarify whether or not a substance is a reproductive toxicant without excessive toxicity and severe suffering in parental animals [1]. The ECHA advice followed an evaluation of existing Extended One-Generation Reproductive Toxicity (EOGRT) studies with respect to design, conduct and toxicological findings, and concerns that potential hazardous effects could be missed due to inadequate dosing. The resulting advice specified that the highest dose tested should "*demonstrate an aim to induce clear evidence of reproductive toxicity without excessive other toxicity and severe suffering in parental animals (e.g. prostration, severe inappetence (lack of appetite), excessive mortality as signs of severe suffering)*".

3. The advice received some criticism from contract research organisations (CROs) and industry with uncertainty around the implications of the new advice on animal welfare, and the impact on study outcomes and interpretation for different chemicals and sectors [2, 3, 4]. The main issues raised included concern that this could lead to testing at unnecessarily high doses in animals and how the recommendations align with advice in existing Organisation for Economic Cooperation and Development (OECD) test guidelines (TG) and guidance documents.

[1] The NC3Rs is the UK's national organisation that provides scientific leadership to the development and implementation of new models and tools that minimise the use of animals in research and testing and/or improve animal welfare (the 3Rs).

[2] The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) is a scientific, non-profit, non-commercial and non-governmental association.

Workshop aims and agenda

4. The workshop was organised to understand the concerns relating to the ECHA advice, with a focus on ensuring a high level of human health protection, whilst balancing scientific considerations and high standards of animal welfare.

5. The workshop therefore aimed to bring together stakeholders from a range of sectors globally to better understand the advice issued by the ECHA on dose selection for reproductive toxicity studies, and how it is being interpreted by those conducting the studies, to identify areas where further clarification may be needed and provide input into future updates on the advice.

6. The session included introductory presentations and background as outlined below, ending with an interactive discussion and Q&A session. Slides are available on request.

    - Introduction. *Fiona Sewell, Head of Toxicology, NC3Rs*.
    - Dose level selection for REACH purposes. *Ingo Bichlmaier, Scientific Area Leader Reproductive Toxicology, ECHA*.
    - Case study: Confounding of reprotox studies by under-nutrition and maternal toxicity. *Mike McMahon, Principal Toxicologist, Penman Consulting*.
    - Review of the ECHA guidance: Dose level selection for DART studies – examples and questions. *Interactive discussion led by Daphne Peperkamp-van den Oetelaar, Charles River Laboratories*.

7. Fiona Sewell introduced the session, explaining that the workshop was originally put together in response to the ECHA advice and the differing viewpoints around dose-level selection. However, whilst working together with ECHA to prepare for the workshop and hearing the rationale behind the advice, there was a realisation that there was greater alignment than expected. The aim of the workshop was therefore to provide others with the background ECHA had at the time of writing the advice to alleviate the concerns and bring the different perspectives together. The interactive survey tool, Mentimeter, was also introduced and tested. Over 120 delegates attended the session from the UK, mainland Europe, USA, and Asia, representing a range of sectors, including industry (49%), regulatory body (16%), academia (10%), testing laboratory (7%) and other (17%). Approximately 85% had experience of commissioning, conducting or interpreting reproductive toxicity studies. However, only 54% of responders indicated they had read the ECHA advice.

8. Ingo Bichlmaier provided the background to the ECHA advice, explaining that this was based on a review of EOGRTS [5]. ECHA's EOGRTS database shows that 20% of studies were underdosed and 2% overdosed. The hazard-based approach followed by ECHA was introduced and an overview of the different DART studies used to characterise reproductive hazards was given, along with the

considerations for appropriate dose level selection in these studies. Examples from the EOGRTS review were presented, and dose-limiting factors such as reduced bodyweight gain discussed. Data presented suggested a 10% reduction in bodyweight gain during gestation corresponds to a 2% to 4% reduction in absolute bodyweight compared with controls, which ECHA would not consider to be dose-limiting as it is within accepted background levels. However, it was noted that this requires further analysis. It was recommended the top dose should be 'as high as possible' within the given limits/context, avoiding death, severe suffering or corrosivity, and that dose-level spacing in studies (e.g. TG 421, TG 422 and TG 443 studies) should be two- to four-fold. Inclusion of all data in the submission, including dose range finding (DRF) studies, will help the regulator understand the justifications supporting decisions on dose selection. Another example included studies where the dose levels were too low based on concern that insufficient pups would be available at higher doses, resulting in no findings (i.e. the reproductive concern was not clarified by the study), showing no clear effects on sexual function or fertility and no effects on development. It was concluded that concern about fertility should be prioritised, even if it results in an insufficient number of pups for allocation. Furthermore, dose level selection should be considered in both sexes to ensure reproductive toxicology effects are not overlooked in either sex. In practice, it was recommended that the less sensitive sex should be tested at higher dose levels than the more sensitive sex. Setting the dose level on the basis of only toxicokinetic considerations is not allowed under REACH because dose-level selection should be based on toxicity to ensure that the data generated are adequate for hazard identification.

9. Mike McMahon gave a presentation on the potential for under-nutrition and maternal toxicity to confound the interpretation endpoints relevant to reprotoxicity. Data from 15 TG 422 and nine TG 414 studies were presented, with animals treated by dietary administration. Generally, there was a mild and consistent effect with good correlation, but not fully conclusive on causation. A pair-feeding study is planned in future to support this theory.

10. Daphne Peperkamp-van den Oetelaar led an interactive session where key parts of the advice were selected and discussed. The purpose of this session was to understand how specific aspects of the advice had been interpreted and the impact this had on decision making for dose selection, with a view to informing where clarifications and updates to the advice would be useful. Extracts of the advice discussed are presented in boxes below, followed by a summary of the key points and discussion outcomes. The voting system Mentimeter was used to capture specific feedback on how aspects of the advice have been interpreted. The Mentimeter results are included below where relevant (see slides for the full results).

Interactive workshop session: Working through the ECHA advice

Alignment with OECD TGs

Alignment of the advice with OECD TGs was one of the main criticisms against the ECHA advice, particularly with regards to the wording in the extract below.

> How to comply with provisions about dose selection: Reproductive toxicity studies.
>
> "*…Irrespective of the specifications in the OECD TGs regarding the selection of the highest dose, for classification and labelling, it is critical that the tested doses are sufficiently high to also be able to conclude on a lack of clear evidence on reproductive toxic properties warranting a classification as Repr. 1B for the tested parameters…*"

This was presented as an area which would benefit from further clarification, particularly the implications of the wording "*irrespective of the specifications in the OECD test guideline*" and "*sufficiently high*" (see further discussion on this below). The majority (88%) of responding audience members agreed that clarification is needed.

Outcome: There is no intention to disregard what is set out in the OECD TGs. However, further clarification on this point would be appreciated.

Highest dose level selection

There was extensive discussion around the selection of the highest dose level, what is intended by the "*highest possible dose level without severe suffering*" (see extract below), and the impact the current wording has had on dose selection since the advice was issued.

> How to comply with provisions about dose selection: Specifications for highest dose level.
>
> "*…For the highest dose level, it should be demonstrated that the aim is that it is the highest possible dose level without severe suffering or death, or the limit dose concept shall be used…*"

Concern was raised that the identification of severe suffering is subjective and could therefore lead to differences between laboratories and interpretation by ECHA and it was unclear how to prove the dose was high enough. There was concern that the dose spacing outlined in the OECD TGs (i.e. two- to four-fold) may not be accepted if the No Observed Adverse Effect Level (NOAEL) was unclear (e.g. if the high dose was selected as two- to four-fold below levels that cause death or severe suffering). It was questioned whether, if mortality was seen at 1000 mg/kg/day but no severe signs at 500 mg/kg/day, would another DRF study be required?

The audience were asked via Mentimeter how the "*highest dose level possible*" wording was interpreted. Over half (58%) understood this to mean a requirement to demonstrate severe signs/death in a DRF study, while a smaller proportion (11%) responded to say they understood this to mean demonstration of a lower maternal body weight gain of 10% to 15%, with almost a third (31%) answering 'other'.

Outcome: ECHA will consider providing further clarification, particularly with regards to what is outlined in the OECD TGs.

Dose selection based on differences in sex

Male and female reproductive toxicity.

"*…If existing information, including results from a dose-range finding study, show that the sensitivity between male and female animals differs significantly, the dose setting should take these differences into account. The less sensitive sex should be tested at higher doses than the more sensitive sex…*"

The ambiguity around "*differs significantly*" was highlighted and discussed, with Mentimeter collecting comments on how "*significantly*" was interpreted here. Responses included statistically significant differences, two- to- three-fold differences and toxicologically significant differences.

Outcome: It was noted that differences in sensitivities did not necessarily mean differences in dose sensitivity (i.e. not a fold change difference) and would also include differences in clinical observations seen in males versus females. If existing information, including results from a DRF study, show that the sensitivity between male and female animals differs significantly, then the less sensitive sex should be tested at higher doses than the more sensitive sex. This should be a case-by-case evaluation, where the Study Director should interpret the data and justify decisions.

Specific aspects for dose-level selection for OECD TG 414 in rabbits

"*…Sometimes, the dose-response can be steep in rabbits and further preliminary studies may be needed to understand the dose-response relationship…*"

Mentimeter captured suggestions for what further preliminary studies could be considered in this scenario. Responses included additional dose group(s), a new DRF study, use of data from general toxicity studies, palatability studies, and running a maximum tolerated dose study in non-pregnant animals prior to the DRF study. Toxicokinetic studies were also suggested, but it was noted that these are rarely performed on TG 414.

Outcome: It was noted that this scenario (i.e. steep dose response) is extremely rare, and it was suggested to start with a non-pregnant tolerability study before the DRF study. Again, the Study Director is expected to interpret these findings and justify decisions. ECHA will consider providing further clarification.

Example cases for dose selection in the OECD TG 414 study in rabbits

The conclusions from the two examples for TG 414 included in the ECHA advice were presented and discussed:

Example 1: DRF study in pregnant rabbits shows no maternal or developmental effects.

"*…A DRF study conducted in pregnant rabbits exposed during gestational days 6-28, at 0, 50, 150 & 500 mg/kg bw/day. The study provides relevant information for an OECD TG 414 study in rabbits with respect to test species and exposure duration. The results show no maternal or developmental effects up to 500 mg/kg bw/day.*

Conclusion: Testing is mandated up to the level of toxicity or the limit dose of 1000 mg/kg bw/day and hence a new DRF study with higher doses should be conducted, to establish a dose-level selection with the aim to induce some developmental and/or maternal toxicity but not death or severe suffering…"

The conclusion to conduct a new DRF study was questioned, as the need to conduct a new DRF study seems excessive. The audience were asked what they would suggest doing in this scenario. Only 12% answered that they would start a new DRF study, with the rest instead suggesting the addition of one (67%) or two (20%) dose groups. The use of additional dose group(s) would use fewer animals than a new DRF, and it was noted that, in addition, there would also likely be information already available in a tolerability study at 1000 mg/kg/day.

The audience were also asked what they would recommend doing if severe toxicity was observed at 1,000 mg/kg. Almost half (45%) responders suggested testing another intermediate dose group (i.e. between 500 and 1000 mg/kg), approximately one-third (38%) suggested going straight to the main study using 500 mg/kg as the high dose and 18% suggested going straight to the main study using a high dose in between 500 and 1000 mg/kg.

Outcome: There was agreement that additional dose group(s) would suffice in this scenario, and that it is important to present all of the information used to select the dose levels (i.e. information from a tolerability study). However, 500 mg/kg/day would be accepted based on the two-to-four dose-fold spacing outlined in the OECD TGs. The data for 1000 mg/kg/day must be included in the submission so the dose selection justification is clear.

Example 2: DRF study in pregnant rabbits shows some evidence of maternal toxicity but no severe suffering or death.

"*…A DRF study is conducted in pregnant rabbits exposed during gestational days 6-28, at dose levels of 0, 150 and 300 mg/kg bw/day. The study provides relevant information for an OECD TG 414 study in rabbits with respect to test species and exposure duration.*

The dose-range finding study shows no effects at 150 mg/kg bw/day. There is some evidence of maternal toxicity (such as clinical signs and lower food consumption compared to control animals) but no severe suffering/death at 300 mg/kg bw/day. In addition, the gravid uterus weight was 15 % lower due to embryotoxicity (resorptions and/or lower foetal weights).

Conclusion: The highest dose of the main OECD TG 414 study should be 300 mg/kg bw/day because clear developmental effects are expected at that dose level without severe suffering or deaths of dams…"

This example was highlighted as in need of expansion to help understand the conclusions drawn. It was noted that data prior to the DRF would be useful here, as well as the individual animal data from the DRF to help to assess animal variation due to the low number of animals in this group (only six animals). The 15% lower uterus weight was not considered to be a clear effect and there was a discussion around the lower fetal weight, as this may not be direct indication of embryotoxicity.

Outcome: More information on the example and the individual animal data would be appreciated.

Specific aspects for dose-level selection for OECD TG 421/422

Prolongation until weaning if used as a DRF for TG 443

"*…It [OECD TG 421 or 422] can be used also as a dose-range finder for OECD TG 443, where prolongation until weaning is recommended to cover the sensitive life stages of pups from parturition to weaning during lactation…*"

This area was highlighted as in need of attention, as the advice to prolong dosing (where the TG 421/422 is used as a DRF for TG 443), is in potential contradiction with the advice not to adjust dose levels in order to obtain sufficient offspring. The audience were asked when they would suggest prolonging the study until weaning. Approximately half (48%) said they would prolong in all cases when the 421/422 is used for dose setting of a 443 study, and 50% in specific cases based on anticipated toxicity.

Outcome: Either option is acceptable, with appropriate justification from the Study Director. It is important to ensure all data used in the justification is provided.

Systemic versus reproductive toxicity in OECD TG 421/422

> "*…Where there is a need to provide information both on specific target organ toxicity after repeated exposure (for which doses causing effects are relevant for classification) and adverse effects on sexual function and fertility/developmental toxicity (for which there is no specific threshold dose above which classification is not warranted), and the dose-setting for these objectives would lead to conflict between the requirements of classification for these two, then registrants should ensure that there are additional dose levels so that there is information provided for both objectives. Such dose level setting should be specially justified. Due to its screening nature for both sexual function and fertility, and development, and potential lack or limitations of previous information on reproduction, the selection of the top dose for OECD TG 422 should be as high as possible without causing deaths or severe suffering…*"

This topic was raised to understand how often reproductive toxicity drives dose level selection over systemic toxicity. The audience were asked if they were aware of any examples where this scenario has occurred. There were no comments on this, however four out of 33 respondents to this question said they did have examples of this, but no further details were provided.

Outcome: It was noted that this is an extremely rare case. More information on the example where reproductive toxicity has driven dose selection over systemic toxicity would be appreciated.

Special aspects for dose-level selection for OECD TG 443

There was discussion around valid scientific justifications used to select lower doses for longer-term studies.

> "*…Evidence-based justification. Dose-level selection should be based on existing information, not theoretical considerations. For example, a longer premating exposure duration in the OECD TG 443 study compared to that of reproductive screening tests according to OECD TG 421/422 (10 vs 2 weeks, respectively) alone is generally not enough to justify a reduction of dose levels in the OECD TG 443 study. There should be a case-specific and science-based expectation that the longer premating exposure duration could result in severe suffering or death of the test animals to justify the reduction of the highest dose in the OECD TG 443 study…*"

Discussion and comments collected by Mentimeter highlighted that some would consider a longer dose period justification to lower the dose. For example, a longer premating exposure duration in the OECD TG 443 study compared to that of reproductive screening tests according to OECD TG 421/422 (10 vs. two weeks, respectively). Examples would be useful to explain what would be considered sufficient information to lower the dose levels (and what is insufficient).

Outcome: Dose-level selection should be based on existing information, not theoretical considerations and, alone, a longer dosing period is generally not enough to justify a reduction of dose levels in the OECD TG 443 study. In most cases, speculation about high-dose toxicity causing non-specific secondary toxicity is not a valid reason for limiting the dose level. The guidance should be clarified for this point.

Example cases for dose-level selection in OECD TG 443

The conclusions from one of the TG 443 examples included in the ECHA advice was presented and discussed:

Example 4: Existing information shows no severe suffering or death, but severe and clear effects on development result in classification for Repr. 1B H360D.

"*…The results of the repeat-dose toxicity studies (e.g. OECD TG 407 and 408) do not show severe suffering/death up to a limit dose of 1 000 mg/kg bw/day.*

The results of the reproductive screening test according to OECD TG 422 shows clear evidence of post-implantation loss (30 % at 100 mg/kg bw/day, 60 % at 300 mg/kg bw/day and 100 % at 1000 mg/kg bw/day) in pregnant females and associated decreased litter sizes/number of pups after parturition in the absence of severe co-occurring maternal toxicity. Post-implantation loss was observed also in a pre-natal developmental toxicity study in rats.

Conclusion: The substance should be self-classified as Repr. 1B H360D based on the clear and severe specific effects on post-implantation loss. However, this self classification for developmental toxicity is not a valid adaptation for the OECD TG 443 study. As explained above, the priority of the OECD TG 443 study under REACH is to investigate sexual function and fertility. Therefore, and despite the developmental effects observed, the highest dose in OECD TG 443 should be 1000 mg/kg bw/day to properly investigate potential effects on sexual function and fertility in parental males and females. If this dosing results in an insufficient number of pups, allocation to Cohorts 1A and 1B take precedence according to OECD TG 443…"

In this example there was a developmental effect (high post-implantation loss) and the advice was to self-classify as Repr. 1B. Audience members were asked if they would still proceed with a TG 443. The majority (~67%) thought that an TG 443 study would not be required if a substance was already self-classified as Repro 1B, whereas 28% were unsure and wanted clarification, and only ~5% would run a TG 443. However, there was discussion around the fact that legally a TG 443 would still be required, as this is designed to assess both sexual function and fertility, so for this case fertility still needs to be characterised.

Outcome: It was agreed that, whilst it may not seem logical to proceed with a TG 443 study when there is a clear Repro 1B decision based on insufficient offspring, the current legal text is clear: Repro 1B/D is not sufficient to waive the EOGRTS. This would require a change to the legislation.

Conclusions

The workshop discussions were positive and constructive, resulting in both a general acceptance, and a better understanding, of the different perspectives on dose-level selection for DART studies.

There are some areas of the advice that would clearly benefit from further clarification and examples to ensure the selection of appropriate dose levels to ensure relevant scientific information is collected in order to protect human reproductive health, whilst maintaining high standards of animal welfare.

It is important to clearly justify dose level selection so that the evaluators can understand all the considerations that have led to the decision-making process, including limitations stemming from national law.

ECHA will consider providing further clarification, with a planned update to the advice.

Next steps

We will continue to engage on this topic to work towards a better understanding of the requirements for dose-level selection in DART studies, and an update of the ECHA advice.

This workshop report will feed into discussions due to take place at the ECHA workshop in Helsinki, November 2024 “Contract research organisation days – bridging *in vivo* laboratories and regulatory scientists”.

This topic and the outputs of the workshop will be discussed at sessions accepted at the Society of Toxicology annual meeting in March 2025 (Orlando, US) “*Global Implications of the changing EU expectations on dose-level setting in reproductive and developmental studies*” and at EUROTOX 2025 in September 2025 (Athens, Greece): “*Dose level selection for Developmental and Reproductive Toxicology studies under REACH*”.

We plan to write the full results of the workshop and resulting discussions as a peer-review publication.

Further information

Workshop slides are available on request, including the full Mentimeter results. Please email Fiona Sewell (fiona.sewell@nc3rs.org.uk) for a copy.

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