Guidance on Dose Level Selection for Developmental and Reproductive Toxicity (DART) Studies

Background and Purpose:

In 2022, the European Chemicals Agency (ECHA) issued advice on the selection of high dose levels for DART studies, indicating 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) that would compromise the interpretation of co-occurring reproductive effects."* In addition, a recent publication advocated that a 10% decrease in body weight gain should be removed from test guidelines as a criterion for maximum tolerated dose and replaced with a 10% decrease in body weight. Thus, the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) gathered a group of experts to evaluate this advice on dose selection and its potential impact on study outcomes and interpretation.

Methods:

Recommendations on dose level selection in existing OECD test guidelines and guidance documents were consulted for compatibility with the new dose selection proposals. Data on representative DART guideline studies were analyzed to determine the impact of a 10% decrease in maternal body weight during pregnancy and to examine the contribution of fetal growth to maternal body weight gain during gestation. In addition, scientific literature was reviewed to identify other factors (not related to body weight) that should be considered when selecting high dose levels for DART studies.

Results:

It was concluded that the dose selection advice was not in line with guidance given in OECD test guidelines, including humane endpoints guidance. Furthermore, analysis of representative data indicated that a 10% decrease in maternal body weight during gestation would equate to an approximately 25% decrease in body weight gain, a level that exceeds a consensus recommendation of DART experts at a 2010 ILSI/HESI workshop. Previously published DART studies indicate that high dose selection may be based on other factors such as maternal clinical signs of toxicity, food consumption/nutritional intake, clinical chemistry parameters, circulatory/cardiovascular changes, target organ toxicity, maternal stress and toxicokinetics.

Conclusions:

Excessive dose levels that cause frank toxicity and overwhelm homeostasis in pregnant animals should be avoided to limit the potential for secondary effects on reproduction that are not relevant for real-word hazard characterization or human health risk assessment. Dose level selection should use a biological approach considering all available data and the complexity of the maternal-placental-fetal model. Current advice on dose level selection for DART studies should consider a more holistic approach.

Figure 1. Implications of removing 10% Body Weight Change as MTD for Pregnant Rats

- 10% ↓ in maternal BWt in DevTox study (Fig. 1A) = an ~25% ↓ in BWt gain in pregnant rats (Fig. 1B)
- ≥ 20% ↓ in gestation BWt gain not supported by DART experts (Beyer *et al.*, 2011)

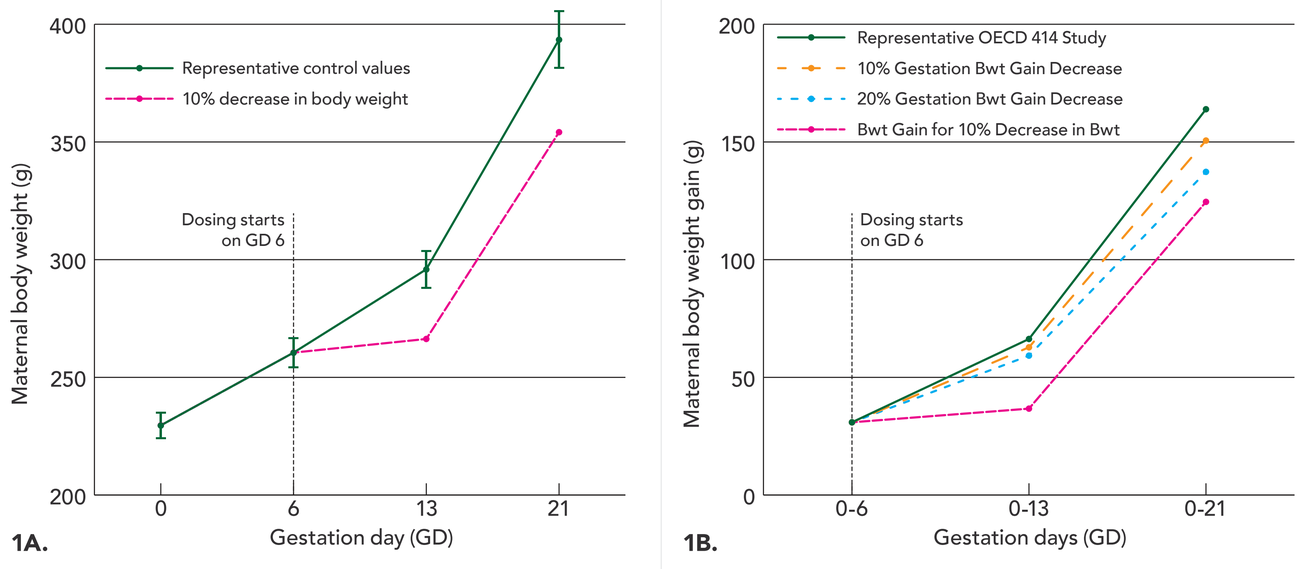

Table 1. Top Dose Guidance: Recent Advice from ECHA Compared with OECD TGs and GDs

- ECHA advice is not aligned with OECD TGs or humane endpoints GD (allows more severe effects)

ECHA recent top dose selection advice 2022	• 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. • ...the top-dose selection 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 (>10%) as signs of severe suffering)** that would compromise the interpretation of co-occurring reproductive effects.
OECD Guidance Documents: 19 (Humane endpoints), 43 (Reproductive toxicity testing), 150 (Evaluating chemicals for endocrine disruption)	• GD 43 on Repro Tox: Ideally, unless limited by the physico-chemical nature or biological effects of the test substance, the highest dose level should induce toxicity **but not mortality in the parental animals.** • GD 19 on Humane Endpoints: A humane endpoint can be defined as the **earliest indicator in an animal experiment of severe pain, severe distress, suffering,** or impending death (note severe suffering is not indicated). • GD 19 on Humane Endpoints:Studies must be designed to **minimise any pain, distress or suffering** experienced by the animals, consistent with the scientific objective of the study • GD 19 on Humane Endpoints: Annex 3 – Clinical signs indicating the need for closer observation or humane killing (This annex includes many clinical signs such as **dehydration, difficulty breathing, jaundice, altered motor activity, abnormal vocalization, abnormal posture, decreased grooming, abortion, agalactia, etc.)... Body weight loss or emaciation**: Particularly when **bodyweight has decreased by more than 20%** compared with control animals, or bodyweight has decreased by more than 25% over a period of 7 days or more.
OECD TG 414 developmental toxicity study	• The highest dose should be chosen with the aim to induce some developmental and/or maternal toxicity **(clinical signs or a decrease in body weight) but not death** or severe suffering.
OECD TG 443 EOGRTS, GD 151 on EOGRTS	• OECD TG 443: If dose levels are based on toxicity, the highest dose should be chosen with the aim to induce some systemic toxicity, **but not death or severe suffering of the animals**.
OECD TG 421/422 Repro screening studies	• The highest dose level should be chosen with the aim of inducing toxic effects **but not death** or severe suffering.

Background

ECHA reviewed Extended One-Generation Reproductive Toxicity Studies (EOGRTS, TG 443) submitted to REACH:

- Concluded 20% did not have adequate high-dose levels for EU regulatory purposes

In 2022, ECHA's Issued Advice on Dose Selection for DART Studies:

- *"Setting the dose level by toxicokinetic considerations only 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".*
- *"…the highest dose tested should aim to induce clear evidence of reproductive toxicity without excessive toxicity and severe suffering in parental animals."*

This top dose selection advice is supported by a series of papers, including Van Berlo *et al.* (2022):

- There is no universal definition of MTD (Maximum Tolerated Dose)
- Different body weight criteria appear across different OECD test guidelines (TGs) and guidance documents (GDs)
- MTD as a 10% change in body weight or body weight gain should be removed from virtually all TGs and GDs

Other Points to Consider During DART Top Dose Selection

- Dose selection should consider the complexity of the maternal-placental-fetal model
- Maternal health impacts embryo-fetal development
- Excessive maternal toxicity can produce secondary (non-specific) effects in embryo/fetus
- Overdosing can compromise the ability to correctly interpret DART/endocrine potential of chemicals

ECHA Dose Selection Advice is too focused on P1 parental fertility assessments

- DART studies also evaluate survival, development and reproductive capacity in offspring treated during critical stages of development (gestation, lactation, maturation)
- ECHA does not permit reducing dose levels to ensure sufficient offspring for DevTox assessments
- Requiring effects on parental fertility increases the likelihood of insufficient pups in next generation
  - Limits study sensitivity to detect treatment-related changes (smaller n)
  - If top dose level is removed due to excessive toxicity, only the control and two dose levels for dose-response modeling

Conclusions

- Good dose selection approaches allow for both accurate risk assessment and identification of relevant hazards for hazard-based classification
- Toxicokinetics should be an option for dose setting to avoid nonlinearity and ensure relevant findings
- Conducting studies at dose levels that cause frank toxicity and overwhelm homeostasis leads to:
  - Unwarranted animal suffering
  - Hazard misclassification of chemicals for DART leading to unnecessary market restrictions
- Recommend: Holistic approaches to dose setting that consider maternal-placental-fetal complexity

Information from ECETOC manuscript:

Lewis RW, Andrus AK, Arroyo J, Brescia S, Botham PA, Corvaro M, Daston GP, Hofmann T, Rodriguez C, Sewell F, van Ravenzwaay B, Wiench K, Marty S. (2024). Considerations for the Development of Guidance on Dose Level Selection for Developmental and Reproductive Toxicity Studies. *Regul. Toxicol. Pharmacol.* https://doi.org/10.1016/j.yrtph.2024.105585.

ecetoc
We are the Centre for chemical safety assessment

AUTHORS: Fiona Sewell[1], Richard W. Lewis[2], M. Sue Marty[3], and Philip A. Botham[4]

[1]UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), London, UK; [2]Regulatory Science Associates, Inverkip, Glasgow, Scotland; [3]The Dow Chemical Company, Midland, MI, USA; [4]Syngenta, Jealott's Hill, UK

REFERENCES

Beyer *et al.* (2011). ILSI/HESI maternal toxicity workshop summary: maternal toxicity and its impact on study design and data interpretation. Birth Defects Res B Dev Reprod Toxicol. 2011 Feb;92(1):36-51.

ECHA. (2022). Advice on dose-level selection for the conduct of reproductive toxicity studies (OECD TGs 414, 421/422 and 443) under REACH. Available at: https://www.flashpointsrl.com/app/uploads/2022/01/211221_echa_advice_dose__repro_en.pdf

van Berlo *et al.* (2022). 10% Body weight (gain) change as criterion for the maximum tolerated dose: A critical analysis. *Regul. Toxicol. Pharmacol.* 134, 10523S