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# Advice on dose-level selection for the conduct of reproductive toxicity studies (OECD TGs 414, 421/422 and 443) under REACH

## Background

The revised legal text now includes provisions about dose selection in the introductory part of each annex from VII to X[1]:

*'Where a test method offers flexibility in the study design, for example in relation to the choice of dose levels, the chosen study design shall ensure that the data generated are adequate for hazard identification and risk assessment.* ***To this end, testing shall be performed at appropriately high dose levels.*** *If dose (concentration) selection is limited by the physicochemical properties or biological effects of the test substance, justification shall be provided.'*

Examples of such physicochemical properties are explosivity and flammability, but also viscosity, solubility, vapour pressure and particle size may be limiting factors for certain routes of administration. Examples for limiting biological effects are lethality, (respiratory tract) irritation/corrosivity or any other manifestation of toxicity which leads to death or severe suffering. The lack of biological effects of a substance would normally lead to a limit test.

## How to comply with the provisions about dose selection in the annex preamble

*General criteria*. The dose-level selection should ensure conclusive data generation for classification and labelling, risk assessment, and whether the substance meets the criteria for a substance of very high concern regarding endocrine disruption according to Article 57(f) of REACH.[2,3,4] This means that the highest dose level should be sufficiently high to be able to conclude on hazardous properties for the tested parameters, and the lowest dose level should show no toxicity to allow a no-observed-adverse-effect level (NOAEL) to be set. The dose levels should be spaced to produce a gradation of toxic effects. Generally, at least three dose levels and a concurrent control should be used, except where a limit test (1 000 mg/kg bw/day) does not produce observable toxicity. Expected human response may indicate the need to use a dose level above 1 000 mg/kg bw/day. The conditions for applicability of a limit test are provided in the individual test methods for reproductive toxicity. For inhalation exposure, *OECD guidance document 39*[5] should be considered.

*Reproductive toxicity studies*. There are aspects to be considered in the dose level setting of OECD TGs 414, 421/422 and 443. Common to these TGs is that the lowest dose should not produce any evidence of toxicity (to allow the NOAEL to be set). Dose-level selection should also demonstrate any dose response, meaning that the mid-dose level should produce observable toxic effects. However, there are some differences in the specifications

[1] COMMISSION REGULATION (EU) 2021/979 of 17 June 2021 amending Annexes VII to XI to Regulation (EC) No 1907/2006
[2] Paragraph 22 of OECD TG 443
[3] Section 1.0.1 of Annex I and Section 11.1.1 of Annex II to the REACH Regulation
[4] Recital 7 to Regulation No 2015/282
[5] OECD Guidance Document on Inhalation Toxicity Studies (Second Edition). ENV/JM/MONO(2009)28/REV1

for the setting of the high-dose level (see below). 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.

In OECD TGs 414, 421/422 and 443, the specifications for the highest dose level refer to observation of toxicity and guides to avoid death or severe suffering:

"***the highest dose level should be chosen with the aim***

- (OECD TG 414) *to induce some developmental and/or maternal toxicity (clinical signs of a decrease in body weight)*
- (OECD TG 421/422) *of inducing toxic effects*
- (OECD TG 443) *to induce some systemic toxicity*

***but not death or severe suffering***".

In this respect, it is highly important to consider the CLP criteria for different categories for reproductive toxicity, which is one of the objectives of these studies. The classification of a substance in **Category 1B** is largely based on **clear evidence** of an adverse effect on sexual function and fertility or on development, whereas classification in **Category 2** is based on **some evidence** of an adverse effect on sexual function and fertility, or on development (see Section I Table 3.7.1 (a) of the CLP Regulation). Consequently, the dose selection should set the highest dose so that it aims at "some developmental toxicity" or "some systemic toxicity" that provides either clear evidence of adverse effects on reproduction or evidence that allows exclusion of clear evidence on reproduction for the tested parameters.

The top dose selection should indeed also take regulatory requirements in the EU into account (see, for example paragraph 22 of OECD TG 443), i.e. its applicability for being able to achieve conclusive decisions on classification and labelling. **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.**

*Toxicokinetics.* In selecting dose levels, information should be considered from existing studies, as well as from any dose-range finding studies that may need to be conducted. 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. For substances under REACH, the available toxicokinetic data is typically insufficient to conclude on toxic dose levels and, therefore, guide on dose-level selection. However, toxicokinetic information may provide reasons to adjust, for example, the dosing route and regime. Toxicokinetic analysis is very useful to investigate lactational transfer to conclude on the need of direct dosing of pups for the extended one-generation reproductive toxicity study according to OECD TG 443.

*Pregnant animals*. Furthermore, toxicity, toxicokinetics and sensitivity in pregnant animals may differ from those in non-pregnant animals. The selection of the highest dose level for the study based solely on studies on non-pregnant animals may lead to excessive toxicity in one or more groups of pregnant animals, with the result that the study in pregnant animals is not fit for purpose. Registrants should carefully evaluate the need to conduct a dose-range finding study in pregnant animals. ECHA recommends that a dose-range finding study in pregnant animals should be conducted where relevant.

*Classification and labelling*. It is important to get information about the reproductive toxicity profile of a substance including the spectrum of reproductive toxicity effects related to different dose levels (e.g. nature of effects, consistency of results, number of parameters/aspects affected) as well as information to allow the severity of reproductive toxicity of a substance to be evaluated. For classification and labelling, reproductive toxicity is divided into three main differentiations, which relate to:

1. adverse effects on sexual function and fertility (Annex I, Section 3.7.1.3. of the CLP Regulation);

2. adverse effects on development of the offspring (Annex I, Section 3.7.1.4 of the CLP Regulation); and

3. adverse effects on or via lactation (Annex I, Section 3.7.1.5 of the CLP Regulation).

For classification and labelling, it is important that the tested doses are sufficiently high to also conclude on a lack of reproductive toxic properties on the tested parameters for sexual function and fertility and/or development warranting a classification for reproductive toxicity, and that it is also possible to exclude category 1B if the effects are sufficient only to category 2 classification. Therefore, 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[6] as signs of severe suffering) that would compromise the interpretation of co-occurring reproductive effects. Severe suffering in animal studies should be avoided; identification of severe suffering is described in *OECD guidance document 19*[7], and reference should be made to national legislation on animal experiments and associated guidance on severe suffering.

*Male and female reproductive toxicity*. As explained above, it is also important that clear evidence of reproductive toxicity or sufficient severity of other toxicity in both female and male parental animals is seen in OECD TGs 421/422 and 443, (and in pregnant dams in OECD TG 414) to ensure that reproductive toxicity in either gender is not overlooked. 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. For all the TGs 414, 421/422 and 443, the aim to have an appropriate dose level setting should be demonstrated. Dose-level selection should be justified and documented to allow independent evaluation of the choice made.

*Reporting of preliminary and dose-range finding studies*. All preliminary (e.g. tolerability studies) and dose-range finding studies should be reported in separate endpoint study records of the IUCLID dossier. The methods and results of these preparatory studies should be described in sufficient detail to facilitate evaluation of the results and to understand the rationale for the design of the main studies. The endpoint study records of the main studies (e.g. OECD TGs 414 and 443) must contain a scientific rationale for dose-level selection, which is based on the results of the provided preliminary and dose-range finding studies. Detailed reporting of the results of these preparatory studies might also be very useful to interpret dose-response relationships.

In the following sections, specific aspects for dose-level selection in different reproductive toxicity studies are explained, with selected examples for studies/common cases where

[6] More than 10% mortality (Section 3.7.2.4.4 of Annex I to the CLP Regulation).
[7] OECD Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Human Endpoints for Experimental Animals Used in Safety Evaluation. ENV/JM/MONO(2000)7

dose-level selection tends to have issues (OECD TG 414 in rabbits, and OECD TG 443).

**Specific aspects for dose-level selection for OECD TG 414**

The study investigates developmental toxicity but can also inform on the ability of dams to maintain pregnancy. The highest dose should be set with the aim to reach as high a dose level as possible without death or severe suffering of the pregnant animals.

*Rats.* Dose-level selection for an OECD TG 414 study in rats should be based on existing information from the same species, i.e. from studies performed with rats and preferably the same strain. Typically, the technical dossier at Annexes IX and X to REACH already contains information from repeat-dose studies and reproductive toxicity screening tests with rats as test animals. This existing information is often sufficient to adequately design the prenatal developmental toxicity study in rats with respect to dose levels. However, a dose-range finding study should be performed when necessary.

*Rabbits.* Dose-level selection for an OECD TG 414 study in rabbits should be based on existing information on the same species, i.e. from studies performed in rabbits. However, typically the existing information for REACH requirements is limited to rodent data (mostly rats and sometimes mouse studies) and no prior information is usually available on rabbits. Therefore, to design the prenatal developmental toxicity study in rabbits, adequate information to inform on dose-level selection usually needs to be generated by conducting preliminary studies including a palatability/tolerance study in non-pregnant rabbits and a preliminary study investigating exposure to a small number of pregnant rabbits. Some developmental parameters should also be included in preliminary studies. Sometimes, the dose-response can be steep in rabbits and further preliminary studies may be needed to understand the dose-response relationship.

*Inter-species differences.* The rabbit and rat studies are complementary so as to detect effects only seen in the rat or the rabbit. Due to the significant inter-species differences between rat and rabbit, information gathered for the rat cannot be used to inform on dose-level selection for the rabbit and vice versa.

*Classification.* Clear effects on reproduction shown in rats and/or rabbits may warrant classification as Repr. 1B H360D.

*Adaptation.* If available information from existing studies including dose-range finding studies show clear evidence of an adverse effect on development, which meet the CLP criteria for Repr. 1B H360D, then the substance should be self-classified accordingly and is normally subject to harmonised classification and labelling under Article 36 of the CLP Regulation. The self-classification or harmonised classification in Category 1A or 1B for developmental toxicity can then to be used to adapt the OECD TG 414 study information requirement according to Column 2 of Section 8.7, Annex IX/X to the REACH Regulation if the available data are adequate to support a robust risk assessment.[8]

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

The following examples are illustrative to reflect dose-level selection rationales under REACH for two different scenarios.

*Example 1: Dose-range finding study in pregnant rabbits shows no maternal or developmental effects*

- A dose-range finding study is conducted in pregnant rabbits exposed during gestational days 6-28, at dose levels of 0, 50, 150 and 500 mg/kg bw/day. The

[8] ECHA Guidance R.7a, R.7.6.2.3.2

study provides relevant information for an OECD TG 414 study in rabbits with respect to test species and exposure duration.

- The results of this study 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 1 000 mg/kg bw/day, and hence a new dose-range finding 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.

Example 2: Dose-range finding study in pregnant rabbits shows some evidence of maternal toxicity but no severe suffering or death

- A dose-range finding 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.

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

*OECD TG 421*, the Reproduction/Developmental Toxicity Screening test, is designed to generate limited information concerning the effects of a test substance on male and female sexual function and fertility (such as gonadal function, mating behaviour, conception and parturition) and on development of the conceptus. It 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. 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 should be as high as possible without causing deaths or severe suffering.

*OECD TG 422*, the Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test, is also designed to generate limited information concerning the effects of a test substance on male and female sexual function and fertility (such as gonadal function, mating behaviour, conception, and parturition) and on development of the conceptus. Furthermore, the method comprises the basic repeated dose toxicity study. Similarly to OECD TG 421, it 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 from parturition to weaning during lactation.

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, see Annex I, Section 3.9.2.9.5 of the CLP Regulation) and adverse effects on sexual function and fertility/developmental toxicity (for which there is no specific threshold dose above which classification is not warranted, see Annex I, Sections 3.7.2.5.7-9 of the CLP Regulation), 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.

See also ECHA advice for dose-level selection for repeated dose toxicity studies published separately.

*Classification.* Clear effects on reproduction shown in these studies may warrant classification as Repr. 1B H360F and/or H360D.

*Adaptation.* If existing information, including results from a dose-range finding study, show clear evidence of an adverse effect on sexual function and fertility, which meet the CLP criteria for Repr. 1B H360F, the substance should be self-classified accordingly and is normally subject to harmonised classification and labelling under Article 36 of the CLP Regulation. The self-classification or harmonised classification in Category 1A or 1B for sexual function and fertility can then to be used to adapt the OECD TG 421/422 study information requirement according to Column 2 of Section 8.7, Annex VIII to the REACH Regulation if the available data are adequate to support a robust risk assessment.[9]

If available information from existing studies including dose-range finding studies show clear evidence of an adverse effect on development, which meet the CLP criteria for Repr. 1B H360D, then the substance should be self-classified accordingly and is normally subject to harmonised classification and labelling under Article 36 of the CLP Regulation. The self-classification can then to be used to adapt the OECD TG 421/422 study information requirement according to Column 2 of Section 8.7, Annex VIII to the REACH Regulation if the available data are adequate to support a robust risk assessment.[10]

**Special aspects for dose-level selection for OECD TG 443**

*Focus on sexual function and fertility*. The focus of the OECD TG 443 study in the REACH annexes is on sexual function and fertility[11], which should be prioritised in the study design of the OECD TG 443 study. Regarding the highest dose level, it is important to ensure that sufficient severity of toxicity in both female and male animals is achieved to ensure that potential effects on sexual function and fertility in either gender is not overlooked.[12] The highest dose should be as high as possible without causing death or severe suffering in parental P0 generation.

As the study should be designed to ensure adequate assessment of the effects on sexual function and fertility, the dose levels should not be reduced to get enough offspring for the assessment of developmental toxicity. Even if the number of offspring would be reduced due to effects on sexual function and fertility, any offspring available at that dose level should be investigated for adverse effects on development as well as those at lower dose levels.

*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

[9] ECHA Guidance R.7a, R.7.6.2.3.2
[10] ECHA Guidance R.7a, R.7.6.2.3.2
[11] Recital 7 to Regulation No 2015/282
[12] ECHA Guidance R.7a, R.7.6.2.2.3

severe suffering or death of the test animals to justify the reduction of the highest dose in the OECD TG 443 study.

*Classification.* Clear effects on reproduction shown in OECD TG 443 may warrant classification as Repr. 1B H360F and/or H360D.

*Adaptation.* If existing information, including results from a dose-range finding study, show clear evidence of an adverse effect on sexual function and fertility, which meet the CLP criteria for Repr. 1B H360F, the substance should be self-classified accordingly and is normally subject to harmonised classification and labelling under Article 36 of the CLP Regulation. The self-classification or harmonised classification in Category 1A or 1B for sexual function and fertility can then to be used to adapt the OECD TG 443 study information requirement according to Column 2 of Section 8.7, Annex IX/X to the REACH Regulation if the available data are adequate to support a robust risk assessment.[13]

If available information from existing studies including dose-range finding studies show clear evidence of an adverse effect on development, which meet the CLP criteria for Repr. 1B H360D, then the substance should be self-classified accordingly and is normally subject to harmonised classification and labelling under Article 36 of the CLP Regulation. However, this classification for developmental toxicity is not a valid adaptation justification for the OECD TG 443 study information requirement. In this case, the OECD TG 443 study should be performed with the main objective to clarify effects on sexual function and fertility as outlined above.[14]

***Example cases for dose-selection in the OECD TG 443 study***

The following examples are illustrative to reflect dose-level selection rationales under REACH for different scenarios.

*Example 1: Existing information shows no severe suffering or death, and no effects on sexual function/fertility.*

- The results of repeat-dose toxicity studies (e.g. OECD TG 407 and 408) show some adverse effects on organs/tissues but do not show severe suffering or death up to a limit dose of 1 000 mg/kg bw/day.

- The results of a pre-natal developmental toxicity study in rats and the results of a reproductive toxicity screening test (e.g. OECD TG 421 or 422) do not show effects on developmental toxicity or sexual function and fertility up to the limit dose of 1 000 mg/kg bw/day.

- Conclusion: The highest dose of the OECD TG 443 study should be the limit dose of 1 000 mg/kg bw/day (or even above if scientifically justifiable as outlined above) because the limit dose level does not show signs of severe suffering/death nor sexual function and fertility.

*Example 2: Existing information shows no severe suffering or death, but clear and severe effects on sexual function/fertility resulting in self-classification for Repr. 1B H360F.*

- The results of repeat-dose toxicity studies (e.g. OECD TG 407 and 408) do not show severe suffering/death at a limit dose of 1 000 mg/kg bw/day, although adverse effects on sperm parameters and male reproductive organs are observed at all dose levels.

[13] ECHA Guidance R.7a, R.7.6.2.3.2
[14] ECHA Guidance R.7a, R.7.6.2.3.2

- The results of the reproductive screening test according to OECD TG 421 shows male infertility in all P0 males at dose levels ≥200 mg/kg bw/day.

- Conclusion: The substance should be self-classified as Repr. 1B H360F based on clear evidence of adverse effects on male sexual function and fertility. Repr. 1B classification with the statement of H360F is a valid basis to adapt (i.e. no need to conduct) the OECD TG 443 study if the available data are adequate to support a robust risk assessment. Note that a classification of Repr. 2 is not a valid adaptation for the OECD TG 443 study.

*Example 3: Existing information shows no severe suffering or death, but moderate effects on sexual function/fertility.*

- The results of an OECD TG 408 study do not show severe suffering or death at a limit dose of 1 000 mg/kg bw/day. However, effects on sperm parameters (reduced sperm count of -5 %, -7 % and -10 % at low-, mid- and high-doses, respectively) were observed.

- The results of an OECD TG 422 study show similar effects on the sperm counts in parental males; however, no functional impairment is observed (no changes in mating and fertility parameters).

- The severity of effects is considered not sufficient to justify Repr. 1B, H360F classification (however, please note that effects on sperm even without impairment of functional fertility may in some cases be sufficient for classification in Category 1B for sexual function and fertility).

- Conclusion: The OECD TG 443 study should be conducted with the highest dose of 1 000 mg/kg bw/day to adequately investigate male sexual function and fertility and potential effects on sexual function and fertility in females. The reduction in sperm counts without smaller litter sizes after an exposure duration of 13 weeks in the OECD TG 408 study would not be a valid justification to reduce the highest dose in the OECD TG 443 study (to avoid smaller litter sizes) because the investigation of potential effects on sexual function fertility should be prioritised in the OECD TG 443 study.

- To justify a reduction in dose levels based on the consideration to produce enough offspring is not considered valid as already explained above. In this case, such justification would also not be considered substantiated because it has been shown that "*chemical induced reductions of up to 90 % of sperm production can still result in normal fertility rates"*[15] and that "*in rodents you must get down to a 20-fold reduction of sperm, or about 5 % of normal counts, to begin to see an increase in infertility.*"[16] Even if it would be likely that the number of offspring is reduced, the dose should not be lowered.

*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

[15] David Jacobson-Kram and Kit A. Keller (editors). *Toxicological Testing Handbook*. CRC Press (Boc Raton, London, New York), 2nd edition (2006), p. 328 of the 2019 paperback edition.

[16] Meistrich, M.L. *Evaluation of Reproductive Toxicity by Testicular Sperm Head Counts*. Journal of the American College of Toxicology; Volume 8(3), 1989, pp. 551-567.

clear evidence of post-implantation loss (30 % at 100 mg/kg bw/day, 60 % at 300 mg/kg bw/day and 100 % at 1 000 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 1 000 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.

*Example 5: Existing information shows severe suffering or death, and no effects on sexual function/fertility.*

- The results of the repeated dose toxicity study according to OECD TG 408 show treatment-related mortality of 0 % at the low- and mid-dose of 100 and 300 mg/kg bw/day, and 25 % in males and females at the limit dose of 1 000 mg/kg bw/day. No other effects qualifying as severe suffering were observed in this study.

- The results of the reproductive screening test according to OECD TG 422 with dose levels of 50, 200 and 750 mg/kg bw/day do not show any treatment-related mortality and no effects on sexual function/fertility and development are observed.

- Conclusion: The mortality observed at the highest dose in the OECD TG 408 study is dose limiting, i.e. a dose of 1 000 mg/kg bw/day cannot be chosen for the OECD TG 443 study. The OECD TG 443 study should, however, be dosed as high as possible to investigate sexual function and fertility in both genders in this definitive study. Therefore, a highest dose of around 750 mg/kg bw/day should be chosen because this did not result in mortality in males and non-pregnant and pregnant females in OECD TG 422. In cases where the relationship of dose and exposure duration with severe suffering/mortality is not clear, it might be necessary to investigate this aspect in a dedicated dose-range finding study to be able to determine an appropriate highest possible dose.

*Example 6: Asymmetric dosing regimen for males and females.*

- The results of a repeated dose toxicity study according to OECD TG 408 show prostration in all female animals at the highest dose of 1 000 mg/kg bw/day. No severe suffering/death were observed in low- and mid-dose females, and for male animals in any dose group up to the limit dose.

- The results of a pre-natal developmental toxicity study (OECD TG 414) in rats and the results of a reproductive toxicity screening test in rats according to OECD TG 422 with the highest dose of 600 mg/kg bw/day do not show prostration or any other signs of severe suffering/death in non-pregnant and pregnant females. Also, no effects on developmental toxicity or sexual function and fertility up to the highest dose were observed.

- Conclusion: The prostration in high-dose females in the OECD TG 408 study limits doses for female animals, i.e. a dose of 1 000 mg/kg bw/day cannot be chosen for females in the OECD TG 443 study. The OECD TG 443 study should, however, be

dosed as high as possible for female animals to investigate sexual function and fertility in this sex. Therefore, the highest dose of 600 mg/kg bw/day should be chosen as the highest dose for females because the pre-natal developmental toxicity study and reproductive screening test in rats show neither prostration nor other signs of severe suffering/death. Males, however, should be dosed at a limit dose of 1 000 mg/kg bw/day because no severe suffering/death was observed for this sex in any study up to the limit dose.