ANNEX

Questionnaire template used for evaluating the EOGRT studies including the overview table of investigations according to OECD TG 443 and OECD GD 151

EOGRTS REVIEW PROJECT

QUESTIONNAIRE FOR REPORTING

This questionnaire is tailored to the needs and objectives of the EOGRTS review project, as a standardised reporting tool for the analysis of the selected EOGRT studies. This assessment is scientific, and the conclusions drawn do not represent a regulatory outcome. The reporting in this questionnaire can be considered for other processes such as follow-up to dossier evaluation.

EOGRTS request in

- ☐Testing proposal decision
- ☐Compliance check decision
- ☐Substance evaluation decision
- ☐Commission decision

PUBLIC SUBSTANCE NAME: Click or tap here to enter text.

EC NUMBER: Click or tap here to enter text.

REGISTRATION NUMBER: Click or tap here to enter text.

IUCLID UUID: Click or tap here to enter text.

EVALUATING EXPERT/AFFILIATION: Click or tap here to enter text.

ECHA SUPPORTER: Click or tap here to enter text.

LEAD REGISTRANT: Click or tap here to enter text.

TEST LAB THAT CONDUCTED THE EOGRTS: Click or tap here to enter text.

PROBLEM WITH DOSE LEVEL SETTING: ☐YES ☐NO

Please indicate if the top dose was clearly set too low. Please complete the evaluation anyway because there might be parameters that can still be evaluated for this project. In case that a conclusion is not possible for specific parameter(s), please record as "no conclusion possible due to low dose-level setting", for example.

Contents

1. General information and adequacy of the study

1.1. Relevant dates

Specify the date of the decision (DD/MM/YYYY).
Click or tap here to enter text.

Specify the date of the full study report (DD/MM/YYYY).
Click or tap here to enter text.

Specify the date of initiation dosing of P0 in the EOGRTS (DD/MM/YYYY).
Click or tap here to enter text.

1.2. Study design

REQUESTS AND REASONING IN THE DECISION

Specify the EOGRT study design as requested in the decision.
Tick the appropriate checkboxes.
☐Testing with registered substance
☐Testing with analogue substance

☐Oral route: unspecified
☐Oral route: gavage
☐Oral route: feed
☐Oral route: drinking water
☐Inhalation route: unspecified
☐Inhalation route: whole body
☐Inhalation route: nose only

☐Testing in rats: strain not specified
☐Testing in rats: Sprague-Dawley
☐Testing in rats: Wistar Han
☐Testing in rats: other

☐At least 2 weeks premating exposure
☐10 weeks premating exposure

☐Extension of Cohort 1B to produce the F2 generation
☐DNT cohorts 2A and 2B
☐DNT cohorts 2A and 2B with inclusion of additional parameters
☐DIT cohort 3
☐DIT cohort 3 with inclusion of additional parameters

☐Other …

If the decision requested testing with a structurally analogue substance, specify this analogous substance and briefly summarise the decision's justification for requesting testing with this analogue.
Use identifiers such as substance name, EC and/or CAS numbers.
Click or tap here to enter text.

Did the decision specify the termination time of F2 pups?
☐Yes

☐No

If YES, specify the requested time of termination.
Typically, the decision asks to keep the F2 pups until weaning.
Click or tap here to enter text.

Does the decision contain specific instructions for dose level selection?
☐Yes
☐No

If YES, summarise these specific instructions for dose level selection.
Click or tap here to enter text.

If the decision requested testing in another rat strain, specify the requested strain.
Click or tap here to enter text.

If the decision requested testing in a specific rat strain such as Sprague-Dawley, Wistar, etc., briefly summarise the reason for this request.
Click or tap here to enter text.

If the decision requested the extension of Cohort 1B to produce the F2 generation, briefly summarise the reasons for this request.
Click or tap here to enter text.

If the decision requested the DNT Cohorts 2A and 2B, briefly summarise the reasons for this request.
Click or tap here to enter text.

If the decision requested the DIT Cohort 3, briefly summarise the reasons for this request.
Click or tap here to enter text.

If the decision requested inclusion of additional parameters in DNT Cohorts 2A and 2B, briefly specify these parameters.
For example, additional testing relating to learning and memory could be requested.
Click or tap here to enter text.

If the decision requested inclusion of additional parameters in DNT Cohorts 2A and 2B, briefly summarise the reasons for this request.
Click or tap here to enter text.

If the decision requested inclusion of additional parameters in DIT Cohort 3, briefly specify these parameters.
For example, the requested TDAR might include measuring IgG.
Click or tap here to enter text.

If the decision requested inclusion of additional parameters in DIT Cohort 3, briefly summarise the reasons for this request.
Click or tap here to enter text.

If you ticked "Other …", briefly specify these additional requested parameters.
Click or tap here to enter text.

If the decision requested inclusion of "Other…", briefly summarise the reasons for this request.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

CONDUCTED STUDY AND REASONING

Specify the EOGRT study design as conducted by the test house.
Tick the appropriate checkboxes.
☐Testing with registered substance
☐Testing with analogue substance

☐Oral route: gavage
☐Oral route: feed
☐Oral route: drinking water
☐Inhalation route: unspecified
☐Inhalation route: whole body
☐Inhalation route: nose only

☐Testing in rats: strain not specified
☐Testing in rats: Sprague-Dawley
☐Testing in rats: Wistar Han
☐Testing in rats: other

☐At least 2 weeks premating exposure
☐10 weeks premating exposure

☐Extension of Cohort 1B to produce the F2 generation
☐Developmental neurotoxicity cohorts 2A and 2B
☐Developmental neurotoxicity cohorts 2A and 2B with inclusion of additional parameters
☐Developmental immunotoxicity cohort 3
☐Developmental immunotoxicity cohort 3 with inclusion of additional parameters

☐Other …

If the test lab conducted the study with another rat strain, specify the strain used:
Click or tap here to enter text.

Does the conducted study comply with the request in the decision?
☐Yes, exactly as requested in the decision
☐Yes, however the test house added investigations (e.g. Cohort 1B was extended without being requested)
☐No because the test house did not conduct all requested investigations/ follow all specifications, for example.

If the test house added investigations/animal groups (e.g. additional cohorts), briefly summarise these (follow-up questions below).
Click or tap here to enter text.

If not all requests were considered by the test house, briefly explain these.
Click or tap here to enter text.

Did the conducted EOGRT study include cross-fostering?
☐Yes
☐No

Briefly summarise the registrant's / test lab's justification for performing cross-fostering.
Cross-fostering is typically performed to investigate if effects observed in pups are an indirect result of changed maternal care (e.g. negligence) or a direct treatment related effect.
Click or tap here to enter text.

The justification for design deviations is based on
☐ECHA Guidance Chapter R.7a, R.6
☐OECD GD 117 (internal triggering)
☐Other regulatory frameworks (e.g. conducting the study also for other regulatory regions)
☐Don't know/ not clear/ other

Briefly summarise the registrant's / test lab's justification for the deviation in study design.
Click or tap here to enter text.

EVALUATION

Do you agree with the justification given for the deviation(s) in study design?
☐Yes
☐No
☐No justification given

Explain why you think that the deviations in study design are / are not justified.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

1.3. Test animals

CONDUCTED STUDY

Specify the age of the male P0 test animals at start of mating in weeks.
According to ECHA Guidance R.7a, R.7.6, Appendix R.7.6-2 "*The exposure can be started when the animals are around 5 weeks old and mate them around 15 weeks of age.*"
According to paragraph 11 of OECD TG 443 the P0 animals should be of "*similar age (approximately 90 days) at mating*".
Click or tap here to enter text.

Based on the age of the male P0 animals at start of mating, how old were these animals at start of premating exposure.
If animals are younger than 5 weeks at start of premating exposure, consider this while evaluating sperm parameters as the exposure in animals with spermatogenesis is shortened.
☐Younger than 5 weeks
☐5 weeks and older

Specify the age of the female P0 test animals at start of mating in weeks.
Click or tap here to enter text.

Based on the age of the female P0 animals at start of mating, how old were these animals at start of premating exposure.
☐Younger than 5 weeks
☐5 weeks and older

If extension of Cohort 1B to produce the F2 generation is requested, specify the age of the male P1 test animals at start of mating in weeks.
According to OECD TG 443, cohabitation of P1 animals should begin "on or after PND 90, but not exceeding PND 120."
Click or tap here to enter text.

If extension of Cohort 1B to produce the F2, specify the age of the female P1 test animals at start of mating in weeks.
According to OECD TG 443, cohabitation of P1 animals should begin "on or after PND 90, but not exceeding PND 120."
Click or tap here to enter text.

How many P0 animals per sex per dose group were used?
Click or tap here to enter text.

Is the number of animals in the P0 generation enough to aim producing at least 20 pregnant animals in each of dose group in P0 generation?
Typically, 24 or 25 animals/sex are used.
☐Yes
☐No

Are there 20 animals per sex per dose group in Cohort 1A?
See also paragraph 34 of OECD TG 443 on selection of pups for post-weaning studies.
☐Yes
☐No, less than 20
☐No, more than 20

If NO, how many animals per sex per dose group are in Cohort 1A?
Click or tap here to enter text.

Are there 20 animals per sex per dose group in Cohort 1B?
See also paragraph 34 of OECD TG 443 on selection of pups for post-weaning studies.
☐Yes
☐No, less than 20
☐No, more than 20

If NO, how many animals per sex per dose group are in Cohort 1B?
Click or tap here to enter text.

Are there 10 animals per sex per dose group in Cohort 2A?
See also paragraph 34 of OECD TG 443 on selection of pups for post-weaning studies.
☐Yes
☐No, less than 10
☐No, more than 10

If NO, how many animals per sex per dose group are in Cohort 2A?
Click or tap here to enter text.

Are there 10 animals per sex per dose group in Cohort 2B?
See also paragraph 34 of OECD TG 443 on selection of pups for post-weaning studies.
☐Yes
☐No, less than 10
☐No, more than 10

If NO, how many animals per sex per dose group are in Cohort 2B?
Click or tap here to enter text.

Are there 10 animals per sex per dose group in Cohort 3?
See also paragraph 34 of OECD TG 443 on selection of pups for post-weaning studies.
☐Yes
☐No, less than 10
☐No, more than 10

If NO, how many animals per sex per dose group are in Cohort 3?
Click or tap here to enter text.

How many animals are in the control group of Cohort 3?
Click or tap here to enter text.

Does Cohort 3 contain a positive control group?
☐Yes
☐No

How many animals serve as positive control animals for the TDAR test in Cohort 3 and what positive control substance was used?
Click or tap here to enter text.

If there are any additional intentional cohorts (e.g. Cohort 1C) with planned purpose and size, specify the purpose, generation (e.g. P0, F1, P1) and number of animals allocated to it.
Click or tap here to enter text.

If DNT and/or DIT Cohorts are not triggered, are 3 pups/sex/litter evaluated for sexual maturation landmarks (VO, BPS)?

Paragraph 12 of EOCD GD 151 specifies that a total of 3 pups/sex/litter (i.e. a total of 60 animals/sex) should be evaluated.
☐Yes
☐No

If NO, how many animals are investigated for sexual maturation?
Some labs only investigate the animals of Cohorts 1A and 1B for sexual maturation, i.e. 2 pups/sex/litter.
Click or tap here to enter text.

Is sexual maturation statistically analysed by combining results of all F1 pups?
VO and BPS should be statistically analysed by combining results of all F1 pups of the same dose group to achieve higher statistical power.
☐Yes, the results of all F1 pup were combined
☐No, the results were analysed separately

EVALUATION
Deviations with respect to age and number of animals can influence the evaluation of the results addressed later in this questionnaire. Please take into consideration that testing of less animals can result in decreased statistical power and deviations in age can alter observed effects.

If there are deviations in animal numbers, briefly explain.
Please also consider paragraph 35 of OECD TG 443 that states that "if there is an insufficient number of pups in a litter to serve all cohorts, the cohort 1 takes precedence. Additional pups may be assigned to any of the cohorts in case of specific concern, e.g. if a chemical is suspected to be a neurotoxicant, immunotoxicant or reproductive toxicant. These pups may be used for examinations at different timepoints or for the evaluation of supplementary endpoints."
Click or tap here to enter text.

Is there a justification for deviation(s) in animal numbers?
☐Yes
☐No

If YES, briefly summarise this justification.
Click or tap here to enter text.

Do you agree with this justification?
☐Yes
☐No

Briefly explain why you agree or disagree.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

1.4. Mating

CONDUCTED STUDY

Is the mating procedure 1m+1f?
☐Yes
☐No

If YES, go to next section.

If NO, briefly summarise the deviation(s)?
Click or tap here to enter text.

Is there a justification for the deviation(s) provided?
☐Yes
☐No

Briefly summarise the provided justification.
Click or tap here to enter text.

EVALUATION

If a justification is provided, do you agree with it?
☐Yes
☐No

Briefly explain why you agree/disagree with the provided justification.
Click or tap here to enter text.

If no justification is provided, do you think that the deviation in mating procedure is acceptable?
☐Yes
☐No

Briefly explain why the deviation in mating procedure is acceptable/ not acceptable.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

1.5. Litter size adjustment

CONDUCTED STUDY

Did the adjustment of the litter size on day 4 after birth comply with paragraph 33 of OECD TG 443?
On day 4 after birth, was the size of each litter adjusted by eliminating extra pups by random selection to yield, as nearly as possible, 4 + 4 or 5 + 5 per litter. Note: Whenever the number of male or female pups prevents having five of each sex per litter, partial adjustment (for example, six males and four females) is acceptable.
☐Yes
☐No

If YES, go to next section.

If NO, briefly summarise the deviation(s).
Click or tap here to enter text.

Is there a justification for the deviation(s) provided?
☐Yes
☐No

Briefly summarise the provided justification.
Click or tap here to enter text.

EVALUATION

If a justification is provided, do you agree with it?
☐Yes
☐No

Briefly explain why you agree / disagree with the provided justification.
Click or tap here to enter text.

If no justification is provided, do you think that the deviation is acceptable?
☐Yes
☐No

Briefly explain why the deviation is acceptable / not acceptable.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

1.6. Dose selection & dosing

1.6.1. Basis for dose selection

CONDUCTED STUDY

Briefly summarise the test lab's dose selection rationale.
Click or tap here to enter text.

Did the Registrant conduct a new dose-range finder before the EOGRTS?
Tick the appropriate checkbox.
☐Yes
☐No
☐Not clear

EVALUATION

If NOT CLEAR, briefly explain why it is not clear that a new dose range finder was conducted?
For example, the registrant might not have reported the results of the dose range finder in the IUCLID dossier in a dedicated study record, so that bibliographic data is missing such as time period when the study was conducted.
Click or tap here to enter text.

If YES, what type of new dose-range finder was conducted before the EOGRTS?
Briefly summarise the study type. Also explain if the dose range finder was tailored to address specific issues of the EOGRTS. For example, OECD TG 421 or 422 was extended to weaning to address double exposure to test item of pups through lactation and feed. Briefly address deviations in parameters between the dose-range finder and the EOGRT study, which could explain possible inconsistencies between the studies: Route of administration, rat strain, vehicle, etc.
Click or tap here to enter text.

The dose selection was based on the results of the following information.
Briefly summarise the studies on which the dose-selection rationale is based (repeat-dose toxicity study/ies (e.g. OECD TG 407, 408, 413), prenatal developmental toxicity study/ies (e.g. OECD TG 414), reproductive toxicity screening test/s (e.g. OECD TG 421, 422), one-generation reproductive toxicity study/ies (e.g. OECD TG 415), two- or multigeneration reproductive toxicity study/ies (e.g. OECD TG 416), non-guideline studies, etc.). Briefly discuss differences in basic study parameters between these studies and the EOGRT study (route of administration, rat strain, vehicle, …).
Click or tap here to enter text.

Are all the studies, on which the dose selection is based, reported in IUCLID?
☐Yes, all
☐Only partially
☐No

Any additional comments:
Click or tap here to enter text.

1.6.2. Vehicle and control animals / groups

CONDUCTED EOGRT STUDY

Was the size of the control group the same as for the treated groups?

☐Yes
☐No

If NO, how many animals were used in the control group?
If the control group sizes deviated for specific investigations/cohorts, please specify.
Click or tap here to enter text.

Specify the vehicle used.
Click or tap here to enter text.

If an oily vehicle was used, was a maximum of 4 ml/kg oily vehicle applied (see paragraph 31 of OECD TG 443)?
☐Yes
☐No

Was the vehicle lipophilic (such as corn oil) despite the substance being very water soluble?
Using an oily vehicle while the substance is water soluble may decrease bioavailability.
☐Yes
☐No

Did the EOGRT study include an additional sham control group?
For example, if the test-item solution contains additionally an adjuvant, a sham control might be included in addition to the vehicle control to investigate if the adjuvant results in any effects.
☐Yes
☐No

Briefly summarise the registrant's/ test lab's justification for including a sham control group.
Click or tap here to enter text.

EVALUATION

Was the number of control group animals adequate?
Click or tap here to enter text.

Was the selection of vehicle adequate?
Click or tap here to enter text.

Is the sham control group used in evaluation?
Click or tap here to enter text.

Is the use of the sham control group justified in your opinion?
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

1.6.3. Actual doses

In this section, please specify doses used, additionally always as mg/kg bw/day. Usually, the registrant/ test lab should provide that information in the full study report and/or IUCLID dossier, in particular for oral feed and drinking water studies. For inhalation studies, often the actual dose received is not given as mg/kg bw/day. In these cases, calculate these values by applying the following formula as defined in ECHA Guidance

R.8, Example B. 4: The conversion factor from rat inhalation [mg/m^3/day] to rat oral is 0.29 m^3/kg bw followed by a correction for differences in absorption between routes (if the case): Effect level oral = Effect level inhalation [mg/m3/day] x 0.29 m3/kg bw x absorption inhalation / absorption oral.

CONDUCTED STUDY

Specify the doses applied in the EOGRTS as stated in the "Doses / concentrations" table in the IUCLID EOGRT study record.
Enter the dose with the appropriate unit (e.g. 100 mg/kg bw/day). Report with original descriptor and mg/kg bw/day. If the test item was administered through feed or drinking water, please specify additionally the mean and range for different generations, sex and study phase (such as pregnancy and lactation) in the study. If this information is not given in the IUCLID study record and/or full study report, please state this. Adjustment of dose level during lactation is specifically addressed also later.

- **Low dose:**

Click or tap here to enter text.

- **Mid dose:**

Click or tap here to enter text.

- **Top dose:**

Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

EVALUATION

Is there a significant deviation (> ±10 %) of the actual dose from the target dose (*i.e.* from the mean value in mg/kg bw/day, ppm, mg/L or mg/m^3)?
☐Yes
☐No

If YES, briefly explain whether there was over- and/or under-dosing of animals in the study.
Also indicate the study period (days), generation and sex.
Please note: Usually there should not be any deviations in gavage and inhalation studies. However, deviations can occur in feeding and drinking water studies depending on the consumption by animals, which can be influenced by the properties of the test item (palatability, smell, etc.) and its toxicity.
Click or tap here to enter text.

Please explain whether this over- or under-dosing of animals is acceptable or not in your opinion.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

1.6.4. Keeping of dose levels/groups

CONDUCTED STUDY

Were all the dose levels kept until the end of the EOGRT study?
☐Yes
☐No

If YES, go to next section.

If NO, briefly explain which dose level(s) for which groups were stopped at what time.
Click or tap here to enter text.

Did the test lab provide a justification for stopping dose level(s)?
☐Yes
☐No

EVALUATION

If YES, do you agree with the justification to stop dose levels?
☐Yes
☐No

Briefly explain why you agree/ disagree.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

1.6.5. Reduction(s) of dose levels due to excessive toxicity

CONDUCTED STUDY

Were one or more dose levels reduced due to excessive toxicity during other periods than lactation?
☐Yes
☐No

If NO, go to the next section.

If YES, list the reduced dose level, indicate to which dose level they were changed and during which study period (days), generation and for which sex.
Click or tap here to enter text.

If YES, briefly summarise the observed excessive toxicity per dose level and study phase (study days), generations and sex (magnitude, incidence, severity, and type).
Click or tap here to enter text.

Briefly summarise the provided justification for reduced dose level(s).
Click or tap here to enter text.

Did the reduction of the dose level(s) result in the recovery of test animals from excessive toxicity?
☐Yes
☐No
☐Partly

EVALUATION

Do you agree with the provided justification for reduced dose level(s)?
☐Yes

☐No

Explain why you agree or disagree with the provided justification.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

1.6.6. Dosing of dams during pregnancy and/or lactation

CONDUCTED STUDY

Was the exposure of dams discontinued at parturition/ early lactation?
Exposure is typically discontinued in gavage and inhalation around parturition to not disturb dams during this critical time.
☐Yes
☐No

If YES, specify the time period when exposure was discontinued.
Click or tap here to enter text.

Were the doses for dams reduced during lactation and/or pregnancy at any time?
☐Yes, during pregnancy and lactation
☐Yes, during pregnancy only
☐Yes, during lactation only
☐No

What was the reduced low dose?
If the low dose was not reduced, leave empty. Enter the dose with the appropriate unit (e.g. 20 mg/kg bw/day). If a stepwise reduction was performed, please specify all doses with timing. Enter the time as postnatal days (e.g. PND 1 to PND 12 or PND 1 to weaning).
Click or tap here to enter text.

What was the reduced mid dose?
If mid dose was not reduced, leave empty. Enter the dose with the appropriate unit (e.g. 60 mg/kg bw/day). If a stepwise reduction was performed, please specify all doses with timing. Enter the time as postnatal days (e.g. PND 1 to PND 12 or PND 1 to weaning).
Click or tap here to enter text.

What was the reduced top dose?
If mid dose was not reduced, leave empty. Enter the dose with the appropriate unit (e.g. 200 mg/kg bw/day). If a stepwise reduction was performed, please specify all doses with timing. Enter the time as postnatal days (e.g. PND 1 to PND 12 or PND 1 to weaning).
Click or tap here to enter text.

Briefly summarise the registrant's/ test lab's justification for reduced dosing of dams during pregnancy and/or lactation.
If there is no justification, fill in "No justification".
Click or tap here to enter text.

EVALUATION

Do you agree with the provided justification?
☐Yes

☐No

Briefly explain if you agree / disagree with the provided justification.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

1.6.7. Presence of the test item in milk

CONDUCTED STUDY

Did the test lab or a third party investigate if the test item is present in milk in a dedicated study before conducting the EOGRTS?
☐Yes
☐No

If YES, briefly summarise the methodology used.
Click or tap here to enter text.

If NO, is there an explanation provided why presence in milk was not investigated?
☐Yes
☐No

If NO, go to the next section

If YES, briefly summarise the provided explanation.
Click or tap here to enter text.

Briefly explain the used method to investigate the presence of the test item in milk.
Please include details such as use of time-mated animals, use of oxytocin and on which day the sampling was conducted.
Click or tap here to enter text.

If the test lab or third party conducted the investigation, was the test item present in milk?
☐Yes
☐No

What was the concentration of the test item in milk?
Click or tap here to enter text.

EVALUATION

Do you agree with the provided justification to investigate the presence of the test item in milk?
☐Yes
☐No

Briefly explain why you agree / disagree with the provided explanation to investigate the presence of the test item in milk.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

1.6.8. Direct dosing of pups

CONDUCTED STUDY

Were pups directly dosed during lactation?
☐Yes
☐No

If YES, was the decision to directly dose pups based on the finding that the test item was not present in milk?
☐Yes
☐No

Briefly summarise the provided justification for directly dosing pups during lactation.
Click or tap here to enter text.

Specify the time period when the pups were directly dosed during lactation.
Enter the time period as postnatal days (e.g. PND 1 to PND 12 or PND 1 to weaning)
Click or tap here to enter text.

Did the direct dosing of pups during lactation result in excessive toxicity (e.g. mortality, severe suffering of pups)?
Please consider test-item related mortality as well as gavage errors.
☐Yes
☐No

If YES, briefly explain the reason for the observed excessive toxicity (e.g. due to test-item related toxicity or gavage errors).
Click or tap here to enter text.

EVALUATION

Do you agree with the provided justification for directly dosing the pups?
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

1.7. Adequacy of dose selection

Please respond to this section 1.7 after finalising the toxicological assessment because the conclusions on the toxicological assessment determine if dose selection is adequate or not.

CONDUCTED STUDY

Is there toxicity (adverse effects) at top dose at least in one generation, sex or cohort?
☐Yes
☐No

If NO, is a justification provided, which explains that dose-selection is adequate despite not achieving such toxicity at top dose?
For example, the top dose was selected to achieve limit dose in the absence of toxicity.
☐Yes
☐No

If YES, briefly summarise the justification.
Click or tap here to enter text.

EVALUATION

Consider that the dose-selection must be adequate for classification and labelling: Are the doses sufficiently high for a conclusive conclusion on classification and labelling for the tested parameters on reproductive toxicity in accordance with CLP e.g. when there are no effects warranting classification for Repr. 1B, was the parental toxicity sufficiently high so that higher doses could not have been tested without severe suffering or mortality?

Do you agree with the justification?
☐Yes
☐No

Briefly explain why you agree / disagree with the justification.
For example, the substance might not induce any toxicity even at the limit dose, therefore, the requirement of toxicity cannot be achieved.
Click or tap here to enter text.

If no justification is provided, do you consider that the study was conducted with the aim to achieve toxicity at the top dose?
Consider the dose-selection rationale and based on what available information the dose-selection was done.
Click or tap here to enter text.

Overall, do you consider the dose-selection adequate?
Click or tap here to enter text.

Briefly explain why you consider dose-selection adequate or not.
Also consider proportionality here. For example, the top-dose might be 800 mg/kg bw/day with no clear toxicity observed. Is it then proportionate to re-run a EOGRT study to increase the top-dose to 1000 mg/kg bw/day?
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

1.8. Not conducted investigations and investigations with deviations

Annex I includes overview tables of all required investigations in the EOGRTS and male and female reproduction indices. To facilitate their use, these investigations are grouped into

- *male reproduction (sexual function and fertility),*
- *female reproduction (sexual function and fertility),*
- *litter observations,*
- *(developmental) neurotoxicity,*
- *(developmental) immunotoxicity,*
- *general & organ toxicity (other toxicity),*
- *adrenals, pituitary and thyroid,*
- *male reproduction indices, and*
- *female reproduction indices.*

These overview tables are meant to support the evaluator in his/her assessment as a quick reference informing on all required investigations and when and for which animals they are conducted.

1.8.1. Not conducted investigations

This section deals with required investigations, which have not been done at all. Therefore, limit your responses to missing investigations. The next section thereafter deals with required investigations, which have been conducted with deviations.

CONDUCTED STUDY

Is any of the required investigations missing?
☐Yes
☐No
☐Not clear

If NO, go to next section.

If YES, briefly summarise all missing investigations.
Click or tap here to enter text.

For each missing investigation, briefly summarise the registrant's justification for not conducting it.
If a justification is missing, please indicate.
Click or tap here to enter text.

EVALUATION

Do you agree with the provided justification(s) for not conducting the required investigation(s)?
☐Yes
☐No

Explain why you agree/ disagree with the provided justification(s).
Click or tap here to enter text.

If NOT CLEAR, briefly explain for which investigations it is not clear if they were conducted and why.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

1.8.2. Investigations with deviations

If there are more investigations with deviation(s), please copy-paste section 1.8.2.1 as often as needed so that for each investigation with deviation(s) one set of questions is a as answered.

1.8.2.1. [*Specify the investigation with deviation*]

CONDUCTED STUDY

Please specify the investigation for which you identified a deviation.
Click or tap here to enter text.

The deviation relates to
☐methodology used
☐animal groups/ numbers
☐timing/ frequency
☐Other …

Briefly summarise the deviation.
Click or tap here to enter text.

Is there a justification for the deviation(s) provided?
☐Yes
☐No

If YES, briefly summarise the justification(s).
Click or tap here to enter text.

EVALUATION

If a justification is provided, do you agree with it?
☐Yes
☐No

Briefly explain why you agree/ disagree with the justification.
Click or tap here to enter text.

If no justification is provided, do you think that the deviation is acceptable?
☐Yes
☐No
☐Not clear

If YES, briefly explain why you think that the deviations are acceptable.
Click or tap here to enter text.

If NO, briefly explain why you think that the deviations are not acceptable.
Click or tap here to enter text.

If NOT CLEAR, briefly explain why you think that it is not clear if the deviations are acceptable or not.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

1.8.3. Additional investigations

CONDUCTED STUDY

Has the test house added investigations, which were not requested in the decision?
E.g. test labs might add investigations on clotting times for F1A animals to have a comparison to P0 although only required in P0 according to OECD TG 443.
☐Yes
☐No

Briefly summarise the additional investigations.
Click or tap here to enter text.

Is there a justification provided explaining why the additional investigations have been conducted?
☐Yes
☐No

Briefly summarise the justification.
Click or tap here to enter text.

EVALUATION

The justification is relying on
☐ECHA Guidance Chapter R.7a, R.6
☐OECD GD 117 (internal triggering)
☐Other regulatory frameworks (e.g. conducting the study also for other regions)
☐Don't know

Do you agree with the provided justification?
☐Yes
☐No

Briefly explain why you agree / disagree with the justification.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

2. Toxicological assessment

Annex I includes overview tables of all required investigations in the EOGRTS and male and female reproduction indices. To facilitate their use, these investigations are grouped into

- *male reproduction (sexual function and fertility),*
- *female reproduction (sexual function and fertility),*
- *litter observations,*
- *(developmental) neurotoxicity,*
- *(developmental) immunotoxicity,*
- *general & organ toxicity (other toxicity),*
- *adrenals, pituitary and thyroid,*
- *male reproduction indices, and*
- *female reproduction indices.*

These overview tables are meant to support the evaluator in his/her assessment as a quick reference informing on all required investigations and when and for which animals they are conducted.

Please note that for certain investigations, besides group data, also individual data needs to be considered to investigate if an observed effect might stem from treatment, e.g. malformations (e.g. malformations of genital organs) and other rare events.

2.1. Body weight changes

Changes in parental and offspring body weight may be important findings to put other observed effects on reproduction into perspective (e.g. severe body weight loss in dams may correlate with reduced body weights in pups). Lower parental body weight development is also a useful indicator for unspecific general/systemic toxicity. That's why changes in body weights are considered already here before assessment of effects on reproduction. A more detailed assessment of severity of general toxicity, including also other signs of general toxicity, is done under the chapter of general toxicity (see 2.2).
Please note: If there are severe effects on parental body weight at a group level, a comparison of parental body weights and effects on reproduction (sexual function and fertility and development to be assessed separately) at the individual animal level is needed to assess if there is temporal and causal correlation between these effects.

Remember to include any relevant changes in body weights in assessment of general toxicity. If no changes, report that.

Summarise relevant body weight changes for relevant time points, in particular

- **P0 males at start premating, start mating, and termination**
  - Click or tap here to enter text.

- **F1 males (without ext. Cohort 1B) at start dosing (weaning), and termination**
  - Click or tap here to enter text.

- **P1 males (with ext. Cohort 1B): start dosing (weaning=start premating), start mating, and termination**
  - Click or tap here to enter text.

- **P0 females at start premating, start mating, start gestation, end gestation[1] or start lactation, end lactation, termination**
    - Click or tap here to enter text.

- **F1 females (without ext. Cohort 1B): start dosing (weaning), termination**
    - Click or tap here to enter text.

- **P1 females (with ext. Cohort 1B): start dosing (= weaning = start premating), start mating, start gestation, end gestation, start lactation, end lactation, termination**
    - Click or tap here to enter text.

- **During weaning (both sexes separately): birth weight, PND 4 (after culling), PND 21**
    - Click or tap here to enter text.

- **Other:**
    - Click or tap here to enter text.

EVALUATION

Is the largest difference in body weights over 20 % in body weights between treated animals and controls in respective sex and generations at times specified above?
☐Yes
☐No

If YES, briefly explain in which animals and if there is dose response.
This requires a careful assessment of the relationship between reproductive toxicity and unspecific general (systemic) toxicity in these animals.
Click or tap here to enter text.

Is the largest difference in body weights over 10 % (and below 20 %) between treated animals and controls in respective sex and generations at times specified above?
☐Yes
☐No

If YES, briefly explain in which animals and if there is dose response.
This may require considerations on potential relationship between reproductive toxicity and unspecific general (systemic) toxicity in these.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

[1] If possible as approximation of “corrected body weight”, i.e. maternal body weight at the end of pregnancy minus the sum of pup weights at birth

2.2. Observed effects

Only relevant (critical) test-item related effects should be reported, i.e. typically those effects that follow a dose-response relationship. Effects that are not considered related to treatment and do not follow a dose-response relationship should only be reported if a scientific justification can be given why these are considered treatment related (e.g. rare developmental events). As a rule, for this project, if the change is over 10% at the top dose it should be reported irrespective whether the change is statistically significant or not. Generally, patterns of effects in different parameters pointing to same direction should be given more weight than single findings. For haematological and clinical chemistry data (which are physiologically well controlled), the spectrum of the findings is more important than the magnitude.

Respond to the questions in section 2.2.1 ***for each relevant effect****. If you want to discuss more than one effect, copy this section as often as needed. Group and report in the following order: sexual function and fertility (adult males and females); offspring toxicity including sexual maturation; developmental neurotoxicity; developmental immunotoxicity; signs of endocrine activity (e.g. thyroid, pituitary and adrenal glands); general and organ toxicity (to other organs).*

2.2.1. [*Specify the observed effect*]

Please group effects in the order

- *male sexual function and fertility*
- *female sexual function and fertility*
- *litter observations*
- *(developmental) neurotoxicity*
- *(developmental) immunotoxicity*
- *organ toxicity (including adrenals, pituitary and thyroid)/general toxicity (other toxicity)*[2]

Briefly explain the observed effect considering

- *If observed effect results from comparison to concurrent and/or historical control*
- *In which animal groups/ generations/ sex the effect was observed or not (e.g. observed in Cohort 1A and (not) confirmed in 1B; please consider only those groups in which the affected parameter was investigated)*
- *The magnitude/ incidence/ severity/ type (MIST) of the observed effect*
- *Changes in magnitude/ incidence/ severity/ type between sexes/ generations*
- *A dose-response relationship (including effects only seen at top-dose)*
- *Statistical significance and biological relevance*[3]
- *Human (ir)relevance*[4]
- *Transiency/ reversibility*
- *Occurrence of the same/ similar effect in other studies (e.g. OECD TG 407, 408, 413, 415, 416, 421, 422 etc.)*
- *If the observed effect is a specific effect by the test item or solely secondary to non-specific consequence of other toxicity*

Click or tap here to enter text.

Is the magnitude/ incidence/ severity/ type of this effect similar across generations and/or sexes?

☐Yes

[2] Other toxicity refers to effects not covered by the other headers

[3] Please consider the EFSA papers on statistical significance and biological relevance:

- https://www.efsa.europa.eu/en/efsajournal/pub/4970
- https://www.efsa.europa.eu/en/efsajournal/pub/2372

[4] An effect is considered human relevant unless the opposite is demonstrated

☐No

If NO, briefly explain the differences magnitude/ incidence/ severity/ type of this effect between the generations and/or sexes.
Click or tap here to enter text.

If relevant, briefly explain the methodology, which was used to measure the observed effect.
Only discuss methodologies if they add an interesting aspect such as the TG or DG refer to different options or do not specify the methodology. Methodologies relating to thyroid hormone measurements, FOB, DNT and DIT are of special interest.
Click or tap here to enter text.

The applied methodology to measure the observed effect is
☐appropriate
☐appropriate, however, a "better" methodology is available
☐inappropriate

If INAPROPRIATE, briefly explain why.
Click or tap here to enter text.

If A BETTER METHODOLOGY IS AVAILABLE, briefly explain why.
Example 1: an open-field methodology for measuring motor activity might be appropriate to investigate motor activity per se. However, the employed method might not be able to analyse where in the open field the animals are. Therefore, no additional information on anxiety of the test animals could be drawn from such methodology because it does not localise the animals in the open field.
Example 2: Different methodologies to investigate auditory startle (e.g. pre-pulse inhibition).
Example 3: The method used might be known to have lower sensitivity/ specificity compared to other methods.
Click or tap here to enter text.

CONCLUDING ON OBSERVED EFFECT

Taking all the information into account, do you conclude that the observed effect is adverse (and not adaptive or incidental)?
In particular, consider your replies to the question above relating to consistency of effects in different animal groups/ generations within the EOGRTS, identical/similar effects in other relevant studies, dose-response relationship, transiency/ reversibility, statistical/ biological/ human relevance, specificity vs non-specificity of effects (as used in CLP)/ primary/ secondary effect, stress, etc.
☐Yes
☐No
☐Borderline

Briefly explain why you consider the effect adverse or not.
Click or tap here to enter text.

Is there a NOAEL for the observed effect identified above in your opinion?
☐Yes
☐No

Please specify the NOAEL for the observed effect, also indicating the generation and sex?
For example: NOAEL (systemic toxicity P0 males) = 300 mg/kg bw/day based on

nephropathy and NOAEL (systemic toxicity P1 males) = 300 mg/kg bw/day based on nephropathy.
Click or tap here to enter text.

Does this effect contribute to or indicate a concern according to Art 57(f) in your opinion?
☐Yes
☐No

Could this effect contribute to or meet the criteria for hazard classification (Repr., STOT RE/SE) in your opinion?
☐Yes
☐No

Is this effect observed at generally toxic dose level complicating the assessment of its relevance?
☐Yes
☐No

If YES, briefly explain the issue/difficulty.
Click or tap here to enter text.

Is this effect the most sensitive observation in its effect group? (observable at lower dose level than others)
Consider sensitivity of an effect within its effect groups:

- *male sexual function and fertility*
- *female sexual function and fertility*
- *litter observations*
- *(developmental) neurotoxicity*
- *(developmental) immunotoxicity*
- *organ toxicity (including adrenals, pituitary and thyroid)/general toxicity (other toxicity)*

E.g. if an effect is the most sensitive of litter observations or not.
☐Yes
☐No

Is this effect the most relevant in its group?
Consider both from regulatory and toxicological points of view. E.g. if an effect in spermatogenesis may be considered as the most relevant finding of all male sexual function and fertility findings. Thyroid hormone results may be toxicologically most relevant endocrine finding in the study but does not reach regulatory relevance (no regulatory action).
☐Yes
☐No

Any additional comments:
Click or tap here to enter text.

2.3. Use of Cohort 1B to clarify findings

According to paragraph 67 of OECD TG 443, "Cohort 1B animals should have the following organs weighed and corresponding tissues processed to the block stage:, Vagina (not weighed), uterus with cervix, ovaries, testes (at least one), epididymides, seminal vesicles and coagulating glands, prostate, pituitary, and identified target organs. Histopathology in cohort 1B would be conducted ***if results from cohort 1A are equivocal or in cases of suspected reproductive or endocrine toxicants.****"*

Note that the OECD TG 443 is not clear with respect to required investigations in Cohort 1B in case it is extended to produce the F2 generation. According to paragraph 41 of the OECD GD 151, "… Cohort 1B, is included for termination at approximately 14 weeks (if not mated) or 20-25 weeks (if mated) of age and should be subject to gross necropsy with organ weights and tissues processed to block for future analysis, if required." Therefore, it seems that independently of whether Cohort 1B is extended, the same procedure should be followed as stated above (paragraph 67 of OECD TG 443).

The EOGRT study at Annex IX can be triggered by concern for reproductive toxicity. The extension of Cohort 1B can be triggered by exposure plus indications of endocrine activity, mutagenicity and/or delayed steady state kinetics, which all indicate a concern for reproductive toxicity. Therefore, if these triggers are identified in the ECHA decision, histopathology in the extended Cohort 1B seems to be needed.

CONDUCTED STUDY

Has the test lab conducted histopathology of organs / tissues in Cohort 1B (in the extended or non-extended Cohort 1B)?
☐Yes
☐No

If YES, which of the organs / tissues were investigated?
☐Vagina
☐Uterus with cervix
☐Ovaries
☐Testes
☐Epididymides
☐Seminal vesicles
☐Coagulating glands
☐Prostate
☐Pituitary
☐Other identified target organs (specify): Click or tap here to enter text.

What was the justification for conducting histopathology of organs / tissues in Cohort 1B?
☐There are equivocal results in Cohort 1A
☐There is suspected reproductive/ endocrine activity
☐Other (specify): Click or tap here to enter text.

Briefly summarise the justification for conducting histopathology on organs / tissues in Cohort 1B.
Click or tap here to enter text.

EVALUATION

Do you agree with the conclusions in the full study report, whether or not histopathology needs to be performed in Cohort 1B?
☐Yes
☐No

If NO, briefly explain why you disagree.
Click or tap here to enter text.

If you disagree with the selection of organs investigated in Cohort 1B, which of the following organs should have been investigated in your opinion?
☐Vagina
☐Uterus with cervix
☐Ovaries
☐Testes
☐Epididymides
☐Seminal vesicles
☐Coagulating glands
☐Prostate
☐Pituitary
☐Other identified target organs (specify): Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

2.4. NOAEL values and concerns according to Art. 57(f)

For allowing an independent and detailed assessment of the results, and reducing misunderstandings, the NOAEL values should be reported in a detailed manner by the evaluator. This would also help in comparing the evaluators NOAEL values with those presented in the full study report and IUCLID dossier. We recognise that CROs may have different practises on how they present the NOAEL values in the full study report, and it may not match with the IUCLID template terminology.

Below in evaluator's assessment, reproduction means sexual function and fertility, if in the full study report or IUCLID dossier CRO/registrant has included also other parameters (such as postimplantation loss), please indicate that. Developmental toxicity belongs to offspring toxicity but includes also DNT and DIT. NOAEL for developmental toxicity is also to be indicated separately to increase clarity.

Summarise the NOAEL values, also showing based on which effects, for:

- **P0 male reproduction (sexual function and fertility):**

Click or tap here to enter text.

- **F1/P1 male reproduction (sexual function and fertility):**

Click or tap here to enter text.

- **P0 female reproduction (sexual function and fertility):**

Click or tap here to enter text.

- **F1/P1 female reproduction (sexual function and fertility):**

Click or tap here to enter text.

- **F1 offspring (pup) toxicity:**

Click or tap here to enter text.

- **F2 offspring (pup) toxicity:**

Click or tap here to enter text.

- **Developmental neurotoxicity:**

Click or tap here to enter text.

- **Developmental immunotoxicity:**

Click or tap here to enter text.

- **F1 developmental toxicity:**

Click or tap here to enter text.

- **F2 developmental toxicity:**

Click or tap here to enter text.

- **P0 male general/systemic toxicity:**

Click or tap here to enter text.

- **F1/P1 (adult) male general/systemic toxicity:**

Click or tap here to enter text.

- **P0 female general/systemic toxicity:**

Click or tap here to enter text.

- **F1/P1 (adult) female general/systemic toxicity:**

Click or tap here to enter text.

Are the NOAEL values in the full study report and IUCLID dossier in line with your assessment?
☐Yes
☐No

If NO, summarise the registrant's NOAELs with his terminology and justifications as reported in the full study report and IUCLID dossier.
Click or tap here to enter text.

Based on your assessment, indicate the lowest NOAEL value related to reproduction (sexual function and fertility, or developmental toxicity) and the lowest NOAEL value for general/systemic toxicity
Click or tap here to enter text.

Based on your assessment, indicate the lowest no-effect value (NOEL or NOAEL) related to endocrine activity and the lowest NOEL/NOAEL value for general/systemic toxicity
Click or tap here to enter text.

Identify the most sensitive parameter (as NOEL and NOAEL) of this study design for this substance.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

2.5. Classification and labelling

Please note that this section is related only to the EOGRTS analysis and does not provide information on overall classification for e.g. reproductive toxicity or specific target organ toxicity of this substance. Classification is always a weight of evidence exercise where all available relevant information shall be considered.

In CLP there is no specific limit dose stated for reproductive toxicity above which the production of an adverse effect is considered to be outside the criteria which lead to classification.

The registrant self-classified the substance for sexual function and fertility and or development and/or effects on or via lactation as
Leave empty if the registrant has not classified.
☐Repr. 1B H360
☐Repr. 1B H360F
☐Repr. 1B H360D
☐Repr. 1B H360FD
☐Repr. 1B H360Fd
☐Repr. 1B H360Df
☐Repr. 2 H361
☐Repr. 2 H361f
☐Repr. 2 H361d
☐Repr. 2 H361fd
☐Lact

You propose a need for classification for the following hazard classes:
Leave empty if there is no need.
☐Repr. 1B H360
☐Repr. 1B H360F
☐Repr. 1B H360D
☐Repr. 1B H360FD
☐Repr. 1B H360Fd
☐Repr. 1B H360Df
☐Repr. 2 H361
☐Repr. 2 H361f
☐Repr. 2 H361d
☐Repr. 2 H361fd
☐Lact

2.5.1. Sexual function and fertility

Please note that effects on sexual maturation (preputial separation and vaginal opening) are effects on sexual function and fertility rather than on development according to CLP although reported under effects on the offspring.

CONDUCTED STUDY

Have the results of the EOGRTS influenced the self-classification for sexual function and fertility?
☐Yes, the results alone justify the classification
☐Yes, the classification proposed by the registrant became more stringent
☐Yes, the classification proposed by the registrant became less stringent
☐No, the results did not have an impact on initial classification

Briefly summarise the registrant's justification for classifying / not classifying for sexual function and fertility.
This justification is usually provided in the study summary in IUCLID.
Click or tap here to enter text.

EVALUATION

Is the EOGRTS showing clear evidence, some evidence or no evidence of adverse effects on sexual function and fertility?
☐Clear evidence
☐Some evidence
☐No evidence

If there is clear or some evidence of adverse effects on sexual function and fertility and these effects co-occur with other toxic effects, are the effects on sexual function and fertility considered to be solely secondary-non-specific consequences of other toxicity?
Adverse effects on sexual function and fertility seen only at dose levels causing marked systemic toxicity (e.g. lethality, dramatic reduction in absolute bw, coma) are not relevant for classification. Parental toxicity that is less than marked should not influence the classification for reproductive toxicity.
☐Yes
☐No

If the effects do not warrant Repr. 1B classification for sexual function and fertility, was the top dose level sufficiently high allowing a conclusion that the substance does not possess a hazard for sexual function and fertility (on those parameters that have been tested)?
The data may be considered inconclusive for assessing sexual function and fertility in accordance with CLP when the top dose is significantly below 1000 mg/kg bw/day, and more severe parental toxicity is not expected to interfere with the interpretation of the effects on sexual function and fertility. The top dose should not induce severe suffering such as prostration, severe inappetence or excessive mortality (>10%) in parental animals.
☐Yes
☐No

Please add a short justification for why you think that the EOGRTS contributes or does not contribute to classification for sexual function and fertility.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

2.5.2. Developmental toxicity

Please note that developmental neurotoxicity and developmental immunotoxicity are part of developmental toxicity. Developmental effects can be manifested at any time point in the life span of the organism that has been exposed to the substance during prenatal development and/or postnatally to the time of sexual maturation. Therefore, effects in the F1 offspring observed at any time point may be developmental effects, although reported under general/organ toxicity (other toxicity).

CONDUCTED STUDY

Have the results of the EOGRTS influenced this self-classification for developmental toxicity?
☐Yes, the results alone justify the classification
☐Yes, the classification proposed by the registrant became more stringent
☐Yes, the classification proposed by the registrant became less stringent
☐No, the results did not have an impact on initial classification

Briefly summarise the registrant's justification for classifying / not classifying for developmental toxicity.
This justification is usually provided in the study summary in IUCLID.
Click or tap here to enter text.

EVALUATION

Is the EOGRTS showing clear evidence, some evidence or no evidence of adverse effects on development?
☐Clear evidence
☐Some evidence
☐No evidence

If there is clear or some evidence of adverse effects on development and these effects co-occur with other toxic effects, are the effects on development considered to be solely secondary non-specific consequences of other toxicity?
Developmental effects which occur even in the presence of other toxicity (e.g. maternal toxicity) are considered to be evidence of developmental toxicity unless it can be unequivocally demonstrated on a case-by case-basis that the developmental effects are solely secondary to maternal toxicity. When the substance is so toxic that maternal death of severe inanition results, or the dams are prostrate and incapable of nursing the pups, it is reasonable to assume that developmental toxicity is produced solely as a secondary non-specific consequence of maternal toxicity and discount the developmental effects. Maternal mortality greater than 10 % is considered excessive and the data for that dose level shall not normally be considered for further evaluation.
☐Yes
☐No

If the effects do not warrant Repr. 1B classification for development, was the top dose level sufficiently high allowing a conclusion that the substance does not possess a hazard for development (on those parameters that have been tested)?
The data may be considered inconclusive for assessing development in accordance with CLP when the top dose is significantly below 1000 mg/kg bw/day, and more severe parental toxicity is not expected to interfere with the interpretation of the effects on development. The top dose should not induce severe suffering such as prostration, severe inappetence or excessive mortality (>10%) in parental animals.
☐Yes
☐No

Please add a short justification for why you think that the EOGRTS contributes or does not contribute to classification for development.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

2.5.3. Lactation

Do the results in EOGRTS provide clear evidence of adverse effects in the offspring due to transfer of the test item in milk or adverse effect on the quality of the milk?
☐Yes
☐No

Do the results in EOGRTS provide information on absorption, metabolism, distribution and excretion that indicate the likelihood that the substance is present in potentially toxic levels in breast milk?
☐Yes
☐No

2.5.4. Specific target organ toxicity

CONDUCTED STUDY

Has the registrant self-classified / or is there a harmonised classification for the substance as
☐STOT RE 1
☐STOT RE 2
☐STOT SE 1
☐STOT SE 2
☐STOT SE 3 H335
☐STOT SE 3 H336

Briefly summarise the registrant's justification for classifying / not classifying including the specific target organs included in his classification.
This justification is usually provided in the study summary in IUCLID.
Click or tap here to enter text.

Have the results of the EOGRTS influenced this self-classification?
☐Yes, the results alone justify the self-classification
☐Yes, the initial self-classification by the registrant became more stringent
☐Yes, the classification proposed by the registrant became less stringent
☐No, the results did not have an impact on initial classification

EVALUATION

Are there significant and / or severe toxic effects in the EOGRTS that are not specifically addressed by reproductive toxicity (i.e. sexual function and fertility and / or development) and that indicate specific target organ toxicity?
Such effects could be seen in parental P0 animals and include morbidity; death (after repeated dose; death after single exposure relevant for the assessment of acute toxicity); significant functional changes in the central or peripheral nervous systems or other organ systems, any consistent and significant adverse change in clinical biochemistry, haematology, or urinalysis parameters; significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination; multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g., severe fatty change in the liver); evidence of appreciable cell death (including cell degeneration and reduced cell number) in vital organs incapable of regeneration. These effects can be seen after a single or repeated doses and they can be reversible or irreversible, immediate and/or delayed. Also transient narcotic effects and transient respiratory tract irritation after a single exposure

are relevant.
☐Yes
☐No

If yes, are these effects occurring within the guidance value range for classification for STOT SE 1 or 2 (if occurring after a single dose) or STOT RE 1 or 2 (if occurring only after repeated dose)?
☐Yes
☐No

You propose a need for classification for the following hazard classes:
☐STOT RE 1
☐STOT RE 2
☐STOT SE 1
☐STOT SE 2
☐STOT SE 3 H335
☐STOT SE 3 H336
☐other classification

Specific target organs to be included in the hazard statement for STOT SE/RE classification:
Click or tap here to enter text.

Briefly summarise why you think that the data from this study support that the substance should be classified for these hazard classes or any other hazard classes excluding Repr. classification.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

2.6. Identification of additional concern(s) resulting from reported findings above

Considering the reported findings above, do you think that these identify additional concern(s)?
For example, for specific target organ toxicity, reproductive toxicity, endocrine activity, SVHC identification.
☐Yes
☐No

If YES, briefly explain the additional concern(s).
Click or tap here to enter text.

Do you think that dedicated follow-up study(ies) might be needed to clarify this/these concern(s)?
☐Yes
☐No

If YES, what kind of follow-up study(ies) would you consider as appropriate?
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

2.7. Alignment of your conclusions and conclusions in the full study report and IUCLID dossier

Are the conclusions on the observed effect in the full study report and the IUCLID dossier in line with your conclusion on adversity and regulatory NOAEL setting?
For example, the study report may consider an effect for NOEL only but you consider that it sets the NOAEL.
☐Yes
☐No

If NO, briefly explain the difference in interpretation.
Click or tap here to enter text.

Is the reporting in IUCLID representative of the reporting in the full study report?
☐ Yes
☐ No

If NO, briefly explain the deviations.
Click or tap here to enter text.

Is the reporting in IUCLID alone sufficient to draw conclusions on the adversity and regulatory relevance of this observed effect?
☐ Yes
☐ No

If NO, briefly explain why.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

3. Specific methodologies

In this project, specific methodologies of interest are investigated. These have been selected based on the evaluation of test cases. These methodologies are thyroid hormone measurements, follicular/ corpora lutea count, auditory startle, T-cell dependent antibody response (TDAR), anogenital distance and nipple retention.

The applied methodology should always be reported in IUCLID and the full study report. If you see that it is not, please report.

3.1. Thyroid hormone measurements (TSH & T4)

3.1.1. TSH

CONDUCTED STUDY

What methodology was applied?
Consider applied test, sampling times, pooling of blood in pups, positive control included (note: this is not a requirement of the OECD TG 443), detection limits, etc. If references to published protocols are given, please state.
Click or tap here to enter text.

Briefly summarise the results of these measurements.
Click or tap here to enter text.

EVALUATION

Summarise your evaluation of the results in particular with respect to variation in control and dose groups, biological relevance (correlation to organ weights and histopathology), comparison to historical control data (realistic values?), and consistency of the results (e.g. similar results in OECD TG 422 for example).
Click or tap here to enter text.

In your opinion, does the methodology used provide a proper base for toxicological assessment.
☐Yes
☐No

If NO, briefly explain the issues with the applied methodology.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

3.1.2. Thyroxine (T4)

CONDUCTED STUDY

What methodology was applied?
Consider applied test, sampling times, pooling of blood in pups, positive control included (note: this is not a requirement of the OECD TG 443), detection limits, etc. If references to published protocols are given, please state.
Click or tap here to enter text.

Briefly summarise the results of these measurements.

Click or tap here to enter text.

EVALUATION

Summarise your evaluation of the results in particular with respect to variation in control and dose groups, biological relevance (correlation to organ weights and histopathology), comparison to historical control data (realistic values?), and consistency of the results (e.g. similar results in OECD TG 422 for example).
Click or tap here to enter text.

In your opinion, does the methodology used provide a proper base for toxicological assessment.
☐Yes
☐No

If NO, briefly explain the issues with the applied methodology.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

3.2. Follicular / corpora lutea count

CONDUCTED STUDY

What methodology was applied?
Consider applied procedure, comparison to historical control data, etc. If references to published protocols are given, please state.
Click or tap here to enter text.

Briefly summarise the results of these measurements.
Click or tap here to enter text.

EVALUATION

Summarise your evaluation of the results in particular with respect to variation in control and dose groups, biological relevance (correlation to other reproductive toxicity findings), and consistency of the results.
Click or tap here to enter text.

In your opinion, does the methodology used provide a proper base for toxicological assessment.
☐Yes
☐No

If NO, briefly explain the issues with the applied methodology.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

3.3. Auditory startle

CONDUCTED STUDY

What methodology was applied?
Consider applied procedure (e.g. pre-pulse inhibition), comparison to historical control data (and positive control), etc. If references to published protocols are given, please state.
Click or tap here to enter text.

Briefly summarise the results of these measurements.
Click or tap here to enter text.

EVALUATION

Summarise your evaluation of the results in particular with respect to variation in control and dose groups, biological relevance (correlation and consistency to other behavioural findings), habituation, change of amplitude or timing of response with respect to controls and other dose groups.
Click or tap here to enter text.

In your opinion, does the methodology used provide a proper base for toxicological assessment.
☐Yes
☐No

If NO, briefly explain the issues with the applied methodology.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

3.4. TDAR

CONDUCTED STUDY

What methodology was applied?
Consider applied test, sampling times, positive control included (note: there is not a requirement for concurrent positive controls in OECD TG 443), comparison to historical control data, etc. If references to published protocols are given, please state.
Click or tap here to enter text.

Briefly summarise the results of these measurements.
Click or tap here to enter text.

EVALUATION

Summarise your evaluation of the results in particular with respect to variation in control and dose groups (number of responders and non-responders in dose groups and (positive) controls), calculation of mean values and variations for responders and non-responders, biological relevance (correlation to organ weights and histopathology).
Click or tap here to enter text.

In your opinion, does the methodology used provide a proper base for toxicological assessment.
☐Yes
☐No

If NO, briefly explain the issues with the applied methodology.

Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

3.5. Nipple retention

CONDUCTED STUDY

What methodology was applied?
Consider applied test, sampling times, comparison to historical control data, etc. If references to published protocols are given, please state. Also note that retained nipples are usually observed in male rats as biological background. Please note that according to OECD TG 443, no quantitative measure is required ("The presence of nipples/areolae in male pups should be checked on PND 12 or 13."). However, OECD GD 151 clarifies that "A quantitative count in male pups is also recommended as a qualitative assessment only (presence/absence) of nipples/areolae may be rather insensitive particularly when control incidence is high."
Click or tap here to enter text.

Briefly summarise the results of these measurements.
Click or tap here to enter text.

EVALUATION

Summarise your evaluation of the results in particular with respect to variation in control and dose groups, calculation of mean values and variations, biological background.
Click or tap here to enter text.

In your opinion, does the methodology used provide a proper base for toxicological assessment.
☐Yes
☐No

If NO, briefly explain the issues with the applied methodology.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

3.6. Anogenital distance

CONDUCTED STUDY

What methodology was applied?
Consider applied test, sampling times, comparison to historical control data, etc. If references to published protocols are given, please state.
Click or tap here to enter text.

Summarise the method of standardisations.
According to OECD TG 443, the AGD should be normalized to a measure of pup size, preferably the cube root of body weight. According to OECD GDs 151 and 43, a standardized approach for weight versus AGD should be considered when the AGD is used as a covariate in the statistical analysis.
Click or tap here to enter text.

Briefly summarise the results of these measurements.
Click or tap here to enter text.

EVALUATION

Summarise your evaluation of the results in particular with respect to variation in control and dose groups, calculation of mean values and variations.
Click or tap here to enter text.

In your opinion, does the methodology used provide a proper base for toxicological assessment.
☐Yes
☐No

If NO, briefly explain the issues with the applied methodology.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

3.7. [Other methodology]

If you want to discuss an additional methodology, please specify in the header and report here below.

CONDUCTED STUDY

What methodology was applied?
Click or tap here to enter text.

Briefly summarise the results of these measurements.
Click or tap here to enter text.

EVALUATION

Summarise your evaluation of the results.
Click or tap here to enter text.

In your opinion, does the methodology used provide a proper base for toxicological assessment.
☐Yes
☐No

If NO, briefly explain the issues with the applied methodology.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

4. Usefulness of triggered expansions

4.1. Extension of Cohort 1B to produce the F2 generation

Are there any effects observed in P1/F2, which are not observed in P0/F1?
Can be genuine different effects, e.g. reduced fertility in P1 but not in P0.
Can be effects of different nature but on the same parameter, e.g. reduced litter size due to less pups born or due to cannibalism.
☐Yes
☐No

If YES, briefly summarise these new effect(s).
Click or tap here to enter text.

Are there any effects observed in P1/F2 at lower dose levels compared to the identical effects in P0/F1?
☐Yes
☐No

If YES, briefly summarise the more sensitive effect(s).
Discuss also potential differences in actual dose levels.
Click or tap here to enter text.

Are there any effects observed in P1/F2 which are more severe (higher magnitude, incidence, severity or different type) compared to the identical effects at identical effect levels in P0/F1?
☐Yes
☐No

If YES, briefly summarise the more severe effect(s).
Click or tap here to enter text.

Are any of effects discussed above to be considered as adverse?
☐Yes
☐No

If YES, which one, and is it reflected in the NOAEL setting or otherwise relevant in supporting regulatory decision making?
☐Yes
☐No

Do you think that the triggering for the extension of Cohort 1B was useful?
☐Yes
☐No

Briefly explain why you think that the triggering was useful or not useful.
Usefulness may include strengthening interpretation or provide aspects critical for interpretation and/or leading/supporting regulatory decision making.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

4.2. Developmental neurotoxicity Cohorts 2A and 2B

Did the results show effects related to brain development?
☐Yes
☐No

If YES, briefly summarise these new effect(s).
Click or tap here to enter text.

If YES, where the effects observed only in DNT cohorts or also in other animals?
Click or tap here to enter text.

Are any of the effects discussed above to be considered as adverse?
☐Yes
☐No

If YES, which one, and is it reflected in the NOAEL setting or otherwise relevant in supporting regulatory decision making (contributing/warranting classification fro developmental toxicity or identifying as a SVHC)?
Click or tap here to enter text.

Considering your responses above, do you think that the triggering of DNT cohorts was useful?
☐Yes
☐No

Briefly explain why you think that the triggering was useful or not useful.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

4.3. Developmental immunotoxicity Cohort 3

Did the TDAR results show effects related to development of the immune system?
☐Yes
☐No

If YES, briefly summarise these new effect(s).
Click or tap here to enter text.

If YES, where the effects observed only in DIT cohort or also in other animals?
Click or tap here to enter text.

Are any of effects discussed above to be considered as adverse?
☐Yes
☐No

If YES, which one, and is it reflected in the NOAEL setting, or otherwise relevant in supporting regulatory decision making (contributing/warranting classification fro developmental toxicity or identifying as a SVHC)?
Click or tap here to enter text.

Considering your responses above, do you think that the triggering of DIT cohorts was useful?
☐Yes
☐No

Briefly explain why you think that the triggering was useful or not useful.
Click or tap here to enter text.

Any additional comments:
Click or tap here to enter text.

Annex I: Tables of required investigations & reproductive indices

The following tables summarise required investigations according to OECD TG 443 and OECD GD 151. This summary is to help the evaluator during the assessment as reference because it summarises which investigations are conducted at which timepoint in which animals. This table applies in principal to all routes of exposure. However, sometimes specifications for gavage-dosing are given in footnotes.

- **Table I.1: Male reproductive toxicity (sexual function and fertility)**
- **Table I.2: Female reproductive toxicity (sexual function and fertility)**
- **Table I.3: Litter observations**
- **Table I.4: (Developmental) Neurotoxicity**
- **Table I.5: (Developmental) Immunotoxicity**
- **Table I.6: General/ organ toxicity (other toxicity)**
- **Table I.7: Adrenals, Pituitary and Thyroid**
- **Table I.8: Indices relating to male reproductive toxicity**
- **Table I.9: Indices relating to female reproductive toxicity**

Table I.1: Male reproductive toxicity (sexual function and fertility)

Investigation	P0 males	F1 males up to weaning	Male surplus pups after standardisation on PND 4	Male surplus pups not allocated to Cohorts (at weaning)	Cohort 1A (M) 20M/dose Terminated at ca 13 weeks of age	Cohort 1B (M) **without extension** 20M/dose Terminated at ca 14 weeks of age if not mated, 20-25 weeks if mated	Cohort 2A (M) 10M/dose Terminated at ca 11-12 weeks of age	Cohort 2B (M) 10M/dose Terminated at ca 3 weeks of age (at weaning)	Cohort 3 (M) 10M/dose Terminated at ca 8 weeks of age	P1 males (identical to Cohort 1B males)	F2 males up to weaning (identical to F1 males up to weaning)
Clinical observations of theabnormalities of genital organs e.g. hypospadias or cleft penis		As often as is applicable and when weighed.			When animals are weighed	When animals are weighed	When animals are weighed	When animals are weighed	When animals are weighed	When animals are weighed	As often as is applicable and when weighed.
Organ weight: Testes	At termination				At termination	At termination				At termination	
Histopathology of fixed organs: Testes (detailed histopathological examination of one testis cohort 1A)	HD and control; lower doses if treatment related findings. Repro organs of all animals with reduced fertility.				HD and control; lower doses if treatment related findings	If suspected repro or ED and/or if cohort 1A results equivocal				If suspected repro or ED and/or if cohort 1A results equivocal	
Organ weight: Epididymides (total and cauda for the samples used for sperm counts)	At termination				At termination	At termination				At termination	
Histopathology of fixed organs: Epididymides (detailed histopathological examination of one epididymis cohort 1A)	HD and control; lower doses if treatment related findings. Repro organs of all animals with reduced fertility.				HD and control; lower doses if treatment related findings	If suspected repro or ED and/or if cohort 1A results equivocal				If suspected repro or ED and/or if cohort 1A results equivocal	
Organ weight: Prostate (dorsolateral and ventral part combined)	At termination				At termination	At termination				At termination	

Investigation	P0 males	F1 males up to weaning	Male surplus pups after standardisation on PND 4	Male surplus pups not allocated to Cohorts (at weaning)	Cohort 1A (M) 20M/dose Terminated at ca 13 weeks of age	Cohort 1B (M) without extension 20M/dose Terminated at ca 14 weeks of age if not mated, 20-25 weeks if mated	Cohort 2A (M) 10M/dose Terminated at ca 11-12 weeks of age	Cohort 2B (M) 10M/dose Terminated at ca 3 weeks of age (at weaning)	Cohort 3 (M) 10M/dose Terminated at ca 8 weeks of age	P1 males (identical to Cohort 1B males)	F2 males up to weaning (identical to F1 males up to weaning)
Histopathology of fixed organs: Prostate (dorsolateral and ventral)	HD and control; lower doses if treatment related findings. Repro organs of all animals with reduced fertility.				HD and control; lower doses if treatment related findings	If suspected repro or ED and/or if cohort 1A results equivocal				If suspected repro or ED and/or if cohort 1A results equivocal	
Organ weight: Seminal vesicles with coagulating glands and their fluids (as one unit).	At termination				At termination	At termination				At termination	
Histopathology of fixed organs: Seminal vesicles (and coagulating glands)	HD and control; lower doses if treatment related findings. Repro organs of all animals with reduced fertility.				HD and control; lower doses if treatment related findings	If suspected repro or ED and/or if cohort 1A results equivocal				If suspected repro or ED and/or if cohort 1A results equivocal	
Collection of mammary tissues In addition, mammary tissues for these male and female pups may be preserved for further microscopic analysis (see GD 151 (40)). Gross abnormalities and target tissues should be saved for possible histological examination.				10 pups/sex/group at termination		At termination if an identified target organ				At termination if an identified target organ	10 pups/sex/group at termination

Investigation	P0 males	F1 males up to weaning	Male surplus pups after standardisation on PND 4	Male surplus pups not allocated to Cohorts (at weaning)	Cohort 1A (M) 20M/dose Terminated at ca 13 weeks of age	Cohort 1B (M) without extension 20M/dose Terminated at ca 14 weeks of age if not mated, 20-25 weeks if mated	Cohort 2A (M) 10M/dose Terminated at ca 11-12 weeks of age	Cohort 2B (M) 10M/dose Terminated at ca 3 weeks of age (at weaning)	Cohort 3 (M) 10M/dose Terminated at ca 8 weeks of age	P1 males (identical to Cohort 1B males)	F2 males up to weaning (identical to F1 males up to weaning)
Histopathology of fixed organs: Mammary gland (males and females)	HD and control; lower doses if treatment related findings. Repro organs of all animals with reduced fertility.				HD and control; lower doses if treatment related findings	Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal				Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal	
Histopathology of fixed organs: Vas deferens (males)	HD and control; lower doses if treatment related findings. Repro organs of all animals with reduced fertility.				HD and control; lower doses if treatment related findings	Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal				Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal	
Sperm parameters: - Enumeration of cauda epididymis sperm reserves.	At or post termination.				Cohort 1A: At or post termination.						
Sperm parameters: - Evaluation of sperm motility and morphology.	At or post termination.				Cohort 1A: At or post termination.						

Table I.2: Female reproductive toxicity (sexual function and fertility)

Investigation	P0 females	F1 females up to weaning	Female surplus pups after standardisation on PND 4	Female surplus pups not allocated to Cohorts (at weaning)	Cohort 1A (F) 20F/dose Terminated at ca 13 weeks of age	Cohort 1B (F) without extension 20F/dose Terminated at ca 14 weeks of age if not mated, 20-25 weeks if mated	Cohort 2A (F) 10F/dose Terminated at ca 11-12 weeks of age	Cohort 2B (F) 10F/dose Terminated at ca 3 weeks of age (at weaning)	Cohort 3 (F) 10F/dose Terminated at ca 8 weeks of age	P1 females (identical to Cohort 1B females)	F2 females up to weaning (Identical to F1 females up to weaning)
Clinical observations of the abnormalities of genital organs		As often as is applicable and when weighed.			yes	yes	yes	yes	yes	yes	As often as is applicable and when weighed
Oestrous cyclicity (by vaginal cytology) Oestrous cycle stage (by vaginal cytology)	In-Life: Starting at least 2 weeks before mating period until confirmation of mating or end of mating period. At termination.				In-Life: Daily from onset of vaginal patency until 1st oestrus. Daily, for 2 weeks from around PND 75. At termination.		At termination.	At termination.	At termination.	In-Life: If mated: From pairing until confirmation of mating. At termination.	
Mating and pregnancy parameters including: - Precoital interval and duration of pregnancy. - Signs of dystocia, abnormal nesting behaviour, nursing performance.	As often as is applicable.									If mated: As often as is applicable	
Organ weight: Uterus (with oviducts and cervix)	At termination				At termination	At termination				At termination	
Histopathology of fixed organs: Uterus (with oviducts and cervix)	HD and control; lower doses if treatment related findings. Repro organs of all animals with reduced fertility.				HD and control; lower doses if treatment related findings	If suspected repro or ED and/or if cohort 1A results equivocal				If suspected repro or ED and/or if cohort 1A results equivocal	

Investigation	P0 females	F1 females up to weaning	Female surplus pups after standardisation on PND 4	Female surplus pups not allocated to Cohorts (at weaning)	Cohort 1A (F) 20F/dose Terminated at ca 13 weeks of age	Cohort 1B (F) without extension 20F/dose Terminated at ca 14 weeks of age if not mated, 20-25 weeks if mated	Cohort 2A (F) 10F/dose Terminated at ca 11-12 weeks of age	Cohort 2B (F) 10F/dose Terminated at ca 3 weeks of age (at weaning)	Cohort 3 (F) 10F/dose Terminated at ca 8 weeks of age	P1 females (identical to Cohort 1B females)	F2 females up to weaning (Identical to F1 females up to weaning)
Examination of the uteri for presence and number of implantation sites.	At termination									If mated. At termination.	
Organ weight: Ovaries	At termination				At termination	At termination				At termination	
Histopathology of fixed organs: Ovaries	HD and control; lower doses if treatment related findings. Repro organs of all animals with reduced fertility.				HD and control; lower doses if treatment related findings	If suspected repro or ED and/or if cohort 1A results equivocal				If suspected repro or ED and/or if cohort 1A results equivocal	
Quantitative evaluation of primordial and small growing follicles, and corpora lutea in the ovaries of the F1 females					HD and control; lower doses if treatment related findings	If cohort 1A results equivocal				If suspected repro or ED and/or if cohort 1A results equivocal	
Collection of Mammary tissues				10 pups/sex/group at termination		At termination if an identified target organ				At termination if an identified target organ	10 pups/sex/group at termination
Histopathology of fixed organs: Mammary gland (males and females)	HD and control; lower doses if treatment related findings. Repro organs of all animals with reduced fertility.				HD and control; lower doses if treatment related findings	Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal				Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal	

Investigation	P0 females	F1 females up to weaning	Female surplus pups after standardisation on PND 4	Female surplus pups not allocated to Cohorts (at weaning)	Cohort 1A (F) 20F/dose Terminated at ca 13 weeks of age	Cohort 1B (F) without extension 20F/dose Terminated at ca 14 weeks of age if not mated, 20-25 weeks if mated	Cohort 2A (F) 10F/dose Terminated at ca 11-12 weeks of age	Cohort 2B (F) 10F/dose Terminated at ca 3 weeks of age (at weaning)	Cohort 3 (F) 10F/dose Terminated at ca 8 weeks of age	P1 females (identical to Cohort 1B females)	F2 females up to weaning (Identical to F1 females up to weaning)
Histopathology of fixed organs: Vagina	HD and control; lower doses if treatment related findings. Repro organs of all animals with reduced fertility.				HD and control; lower doses if treatment related findings	If suspected repro or ED and/or if cohort 1A results equivocal				If suspected repro or ED and/or if cohort 1A results equivocal	

Table I.3: Litter observations

Investigation	P0	F1 up to weaning	Surplus pups after standardisation on PND 4	Surplus pups not allocated to Cohorts (at weaning)	Cohort 1A 20M+20F/dose Terminated at ca 13 weeks of age	Cohort 1B without extension 20M+20F/dose Terminated at ca 14 weeks of age if not mated, 20-25 weeks if mated	Cohort 2A 10M+10F/dose Terminated at ca 11-12 weeks of age	Cohort 2B 10M+10F/dose Terminated at ca 3 weeks of age (at weaning)	Cohort 3 10M+10F/dose Terminated at ca 8 weeks of age	P1 (identical to Cohort 1B)	F2 up to weaning (identical to F1 up to weaning)
Body weight (see also section "General toxicity above)		In-Life: On PND 0 or PND 1 and regularly thereafter (at least on PND 4, 7, 14 & 21)	In-Life: On PND 0 or PND 1 and regularly thereafter (at least on PND 4, 7, 14 & 21) At termination	In-Life: On PND 0 or PND 1 and regularly thereafter (at least on PND 4, 7, 14 & 21) At termination	In-Life: At least weekly and on day of attainment of vaginal patency or balano-preputial separation. All cohorts at termination.	In-Life: At least weekly and on day of attainment of vaginal patency or balano-preputial separation. All cohorts at termination.	In-Life: At least weekly and on day of attainment of vaginal patency or balano-preputial separation. All cohorts at termination.	All cohorts at termination.	In-Life: At least weekly and on day of attainment of vaginal patency or balano-preputial separation. All cohorts at termination.		In-Life: On PND 0 or PND 1 and regularly thereafter (at least on PND 4, 7, 14 & 21)
Clinical examination of the neonates, e.g. - Qualitative assessment of body temperature, state of activity and reaction to handling.		As often as is applicable and when weighed.									As often as is applicable and when weighed.
Litter examination/parameters including: Number and sex of pups, stillbirths and live births.		As soon as possible after birth. Live pups are to be counted on PND 4, 7, 14 and 21									As soon as possible after birth. Live pups are to be counted on PND 4, 7, 14 and 21

Investigation	P0	F1 up to weaning	Surplus pups after standardisation on PND 4	Surplus pups not allocated to Cohorts (at weaning)	Cohort 1A 20M+20F/dose Terminated at ca 13 weeks of age	Cohort 1B without extension 20M+20F/dose Terminated at ca 14 weeks of age if not mated, 20-25 weeks if mated	Cohort 2A 10M+10F/dose Terminated at ca 11-12 weeks of age	Cohort 2B 10M+10F/dose Terminated at ca 3 weeks of age (at weaning)	Cohort 3 10M+10F/dose Terminated at ca 8 weeks of age	P1 (identical to Cohort 1B)	F2 up to weaning (identical to F1 up to weaning)
Litter examination/parameters including: Presence of gross anomalies (externally visible abnormalities, including cleft palate; subcutaneous haemorrhages; abnormal skin colour or texture; presence of umbilical cord; lack of milk in stomach; presence of dried secretions).		As soon as possible after birth. Live pups are to be counted on PND 4, 7, 14 and 21									As soon as possible after birth. Live pups are to be counted on PND 4, 7, 14 and 21
Anogenital distance in pups (preferred: relative to square root of body weigh)		Between PND 0 and 4 (all pups to be measured on the same PND day).									Between PND 0 and 4 (all pups to be measured on the same PND day).
Presence and number of nipples/areolae in male pups (see GD 151, Section 3).		On PND 12 or 13 (all male pups to be examined on the same PND day); this timing may vary depending on strain									On PND 12 or 13 (all male pups to be examined on the same PND day); this timing may vary depending on strain
Sexual maturity: vaginal patency (females)					All cohorts, except 2B. Daily examination until achieved.	All cohorts, except 2B. Daily examination until achieved.	All cohorts, except 2B. Daily examination until achieved.		All cohorts, except 2B. Daily examination until achieved.		

Investigation	P0	F1 up to weaning	Surplus pups after standardisation on PND 4	Surplus pups not allocated to Cohorts (at weaning)	Cohort 1A 20M+20F/dose Terminated at ca 13 weeks of age	Cohort 1B without extension 20M+20F/dose Terminated at ca 14 weeks of age if not mated, 20-25 weeks if mated	Cohort 2A 10M+10F/dose Terminated at ca 11-12 weeks of age	Cohort 2B 10M+10F/dose Terminated at ca 3 weeks of age (at weaning)	Cohort 3 10M+10F/dose Terminated at ca 8 weeks of age	P1 (identical to Cohort 1B)	F2 up to weaning (identical to F1 up to weaning)
Sexual maturity:[5] balano-preputial separation (males): Day achieved and/or body weight when achieved Note: Male and female sexual maturity should be determined for additional animals in case not all cohorts are included in the study design (3/sex/litter/dose)					All cohorts, except 2B. Daily examination until achieved.	All cohorts, except 2B. Daily examination until achieved.	All cohorts, except 2B. Daily examination until achieved. If not triggered, all animals, including those in cohorts 2 and 3 should be maintained until sexual maturation to ensure that sufficient animals (3/sex/litter/dose) are available for evaluation of critical endpoints	All cohorts, except 2B. Daily examination until achieved. If not triggered, all animals, including those in cohorts 2 and 3 should be maintained until sexual maturation to ensure that sufficient animals (3/sex/litter/dose) are available for evaluation of critical endpoints	All cohorts, except 2B. Daily examination until achieved. If not triggered, all animals, including those in cohorts 2 and 3 should be maintained until sexual maturation to ensure that sufficient animals (3/sex/litter/dose) are available for evaluation of critical endpoints		
Examination of external organs (especially sex organs) for structural abnormalities					All cohorts. At termination.	All cohorts. At termination.	All cohorts. At termination.	All cohorts. At termination.	All cohorts. At termination.	All cohorts. At termination.	

[55] For hazard assessment, sexual maturation is addressed under sexual function and fertility.

Table I.4: (Developmental) Neurotoxicity

Investigation	P0	F1 up to weaning	Surplus pups after standardisation on PND 4	Surplus pups not allocated to Cohorts (at weaning)	Cohort 1A 20M+20F/dose Terminated at ca 13 weeks of age	Cohort 1B without extension 20M+20F/dose Terminated at ca 14 weeks of age if not mated, 20-25 weeks if mated	Cohort 2A 10M+10Fdose Terminated at ca 11-12 weeks of age	Cohort 2B 10M+10Fdose Terminated at ca 3 weeks of age (at weaning)	Cohort 3 10M+10F/dose Terminated at ca 8 weeks of age	P1 (identical to Cohort 1B)	F2 up to weaning (identical to F1 up to weaning)
General observations: - Behavioural changes	Once a day	Once a day			Once a day	Once a day	Once a day	Once a day	Once a day	Once a day	Once a day
Clinical observations including: - Autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). - Changes in gait, posture, response to handling, presence of clonic or tonic movements, stereotypy (e.g. excessive grooming, repetitive circling) or bizarre behaviour (e.g. self-mutilation, walking backwards).	Once a week (e.g. when animals are weighed).	As often as is applicable and when weighed.			When animals are weighed	When animals are weighed	When animals are weighed	When animals are weighed	When animals are weighed	When animals are weighed	As often as is applicable and when weighed.
Clinical examination of the neonates, e.g. - Qualitative assessment of body temperature, state of activity and reaction to handling.		As often as is applicable and when weighed.									As often as is applicable and when weighed.

Investigation	P0	F1 up to weaning	Surplus pups after standardisation on PND 4	Surplus pups not allocated to Cohorts (at weaning)	Cohort 1A 20M+20F/dose Terminated at ca 13 weeks of age	Cohort 1B without extension 20M+20F/dose Terminated at ca 14 weeks of age if not mated, 20-25 weeks if mated	Cohort 2A 10M+10Fdose Terminated at ca 11-12 weeks of age	Cohort 2B 10M+10Fdose Terminated at ca 3 weeks of age (at weaning)	Cohort 3 10M+10F/dose Terminated at ca 8 weeks of age	P1 (identical to Cohort 1B)	F2 up to weaning (identical to F1 up to weaning)
Organ weight: Brain	At termination			10 pups/sex/group at termination	At termination	At termination if an identified target organ	At termination	At termination		At termination if an identified target organ	10 pups/sex/group at termination
Histopathology of fixed organs: Brain	HD and control; lower doses if treatment related findings				HD and control; lower doses if treatment related findings	Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal				Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal	
Brain morphometry							Between PND 75 and 90. HD and control; lower doses if treatment related findings	On PND21 or 22. HD and control; lower doses if treatment related findings			
Assessment of neurohistopathology : (Using qualitative and quantitative methods) - Olfactory bulbs - Cerebral cortex - Hippocampus - Basal ganglia - Thalamus - Hypothalamus - Mid-brain (thecum, tegmentum, cerebral peduncles) - Brain-stem - Cerebellum							Between PND 75 and 90. HD and control; lower doses if treatment related findings	On PND21 or 22. Eyes, peripheral nerve, muscle and spinal cord not required for cohort 2B. HD and control; lower doses if treatment related findings			

Investigation	P0	F1 up to weaning	Surplus pups after standardisation on PND 4	Surplus pups not allocated to Cohorts (at weaning)	Cohort 1A 20M+20F/dose Terminated at ca 13 weeks of age	Cohort 1B without extension 20M+20F/dose Terminated at ca 14 weeks of age if not mated, 20-25 weeks if mated	Cohort 2A 10M+10Fdose Terminated at ca 11-12 weeks of age	Cohort 2B 10M+10Fdose Terminated at ca 3 weeks of age (at weaning)	Cohort 3 10M+10F/dose Terminated at ca 8 weeks of age	P1 (identical to Cohort 1B)	F2 up to weaning (identical to F1 up to weaning)
Histopathology of fixed organs: Periferal nerve	HD and control; lower doses if treatment related findings				HD and control; lower doses if treatment related findings	Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal				Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal	
Assessment of neurohistopathology : (Using qualitative and quantitative methods) - Peripheral nerve							Between PND 75 and 90. HD and control; lower doses if treatment related findings				
Histopathology of fixed organs: Spinal cord	HD and control; lower doses if treatment related findings				HD and control; lower doses if treatment related findings	Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal				Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal	
Assessment of neurohistopathology : (Using qualitative and quantitative methods) - Spinal cord							Between PND 75 and 90. HD and control; lower doses if treatment related findings				
Histopathology of fixed organs: Eye (and optic nerve)	HD and control; lower doses if treatment related findings				HD and control; lower doses if treatment related findings	Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal				Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal	

Investigation	P0	F1 up to weaning	Surplus pups after standardisation on PND 4	Surplus pups not allocated to Cohorts (at weaning)	Cohort 1A 20M+20F/dose Terminated at ca 13 weeks of age	Cohort 1B without extension 20M+20F/dose Terminated at ca 14 weeks of age if not mated, 20-25 weeks if mated	Cohort 2A 10M+10Fdose Terminated at ca 11-12 weeks of age	Cohort 2B 10M+10Fdose Terminated at ca 3 weeks of age (at weaning)	Cohort 3 10M+10F/dose Terminated at ca 8 weeks of age	P1 (identical to Cohort 1B)	F2 up to weaning (identical to F1 up to weaning)
Assessment of neurohistopathology : (Using qualitative and quantitative methods) - Eyes (retina and optic nerve)							Between PND 75 and 90. HD and control; lower doses if treatment related findings				
Assessment of neurotoxicity: Auditory startle test							PND 24±1				
Assessment of neurotoxicity: Functional observation battery							Between PND 63 and 75.				
Assessment of neurotoxicity: Motor activity (determined at least once)							Between PND 63 and 75.				
Assessment of neurohistopathology : (Using qualitative and quantitative methods) - Muscle							Between PND 75 and 90. HD and control; lower doses if treatment related findings				

Table I.5: (Developmental) Immunotoxicity

Investigation	P0	F1 up to weaning	Surplus pups after standardisation on PND 4	Surplus pups not allocated to Cohorts (at weaning)	Cohort 1A 20M+20F/dose Terminated at ca 13 weeks of age	Cohort 1B without extension 20M+20F/dose Terminated at ca 14 weeks of age if not mated, 20-25 weeks if mated	Cohort 2A 10M+10F/dose Terminated at ca 11-12 weeks of age	Cohort 2B 10M+10F/dose Terminated at ca 3 weeks of age (at weaning)	Cohort 3 10M+10F/dose Terminated at ca 8 weeks of age	P1 (identical to Cohort 1B)	F2 up to weaning (identical to F1 up to weaning)
Organ weight: Spleen	At termination			10 pups/sex/group at termination	At termination	At termination if an identified target organ				At termination if an identified target organ	10 pups/sex/group at termination
Histopathology of fixed organs: Spleen	HD and control; lower doses if treatment related findings				HD and control; lower doses if treatment related findings	Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal				Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal	
Organ weight: Thymus	At termination			10 pups/sex/group at termination	At termination	At termination if an identified target organ				At termination if an identified target organ	10 pups/sex/group at termination
Histopathology of fixed organs: Thymus	HD and control; lower doses if treatment related findings				HD and control; lower doses if treatment related findings	Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal				Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal	
Histopathology of fixed organs: Bone marrow	HD and control; lower doses if treatment related findings				HD and control; lower doses if treatment related findings	Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal				Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal	

Investigation	P0	F1 up to weaning	Surplus pups after standardisation on PND 4	Surplus pups not allocated to Cohorts (at weaning)	Cohort 1A 20M+20F/dose Terminated at ca 13 weeks of age	Cohort 1B without extension 20M+20F/dose Terminated at ca 14 weeks of age if not mated, 20-25 weeks if mated	Cohort 2A 10M+10F/dose Terminated at ca 11-12 weeks of age	Cohort 2B 10M+10F/dose Terminated at ca 3 weeks of age (at weaning)	Cohort 3 10M+10F/dose Terminated at ca 8 weeks of age	P1 (identical to Cohort 1B)	F2 up to weaning (identical to F1 up to weaning)
Haematology: Total and differential leukocyte count	10 animals/sex/group at termination (post-fasting).				10 animals/sex/group at termination.						
Assessment of immunotoxicity: - primary IgM antibody response to a T cell dependant antigen (immunization with antigen is part of the test)									On PND 56±3, T-cell dependant antibody response assay on 10 animals/sex/ group.		
Assessment of immunotoxicity: - Splenic lymphocyte subpopulation analysis (CD4+ and CD8+ T lymphocytes, B lymphocytes and NK cells) using one half of the spleen.					10 animals/sex/ group at termination.						
Assessment of immunotoxicity: - Weight of lymph nodes associated with and distant from the route of exposure.					10 animals/sex/ group at termination.						
Assessment of immunotoxicity: - Histopathology on the collected lymph nodes and bone marrow.					10 animals/sex/ group at termination.						

Table I.6: General / organ toxicity (other toxicity)

Investigations on bone marrow, spleen and thymus are listed in table I.5 above ((D)IT) and are not repeated in this table. Investigations on brain, peripheral nerve, spinal cord and eye are listed in Table I.4 above ((D)NT) and are not repeated in this table. Investigations on adrenals, pituitary and thyroid are listed in Table I.7 below and are not repeated in this table.

Please note that this table is not intended to guide the interpretation of the results but to inform the evaluator which investigations are performed with respect to clinical observations, body weight, clinical chemistry, haematology and tissue/organs. Observed effects can inform on specific target organ toxicity and/or reproductive toxicity depending on in which animals and generations these are observed.

Investigation	P0	F1 up to weaning	Surplus pups after standardisation on PND 4	Surplus pups not allocated to Cohorts (at weaning)	Cohort 1A 20M+20F/dose Terminated at ca 13 weeks of age	Cohort 1B without extension 20M+20F/dose Terminated at ca 14 weeks of age if not mated, 20-25 weeks if mated	Cohort 2A 10M+10F/dose Terminated at ca 11-12 weeks of age	Cohort 2B 10M+10F/dose Terminated at ca 3 weeks of age (at weaning)	Cohort 3 10M+10F/dose Terminated at ca 8 weeks of age	P1 (identical to Cohort 1B)	F2 up to weaning (identical to F1 up to weaning)
General observations: - All signs of toxicity - Morbidity - Mortality	Once a day	Once a day			Once a day	Once a day	Once a day	Once a day	Once a day	Once a day	Once a day
Body weight[6]	In-Life: On 1st day of dosing and at least weekly thereafter. Females: During lactation, on the same days as the pups. Females: more regularly post coitum. At termination	In-Life: On PND 0 or PND 1 and regularly thereafter (at least on PND 4, 7, 14 & 21). Also on day when anogenital distance is measured.	At termination (Usually this is not done extra at termination as it is already performed on PND 4 in the animal room)	At termination	In-Life: At least weekly and on day of attainment of vaginal patency or balano-preputial separation. All cohorts at termination.	In-Life: At least weekly and on day of attainment of vaginal patency or balano-preputial separation. All cohorts at termination.	In-Life: At least weekly and on day of attainment of vaginal patency or balano-preputial separation. All cohorts at termination.	All cohorts at termination.	In-Life: At least weekly and on day of attainment of vaginal patency or balano-preputial separation. All cohorts at termination.	In-Life: At least weekly and on day of attainment of vaginal patency or balano-preputial separation. All cohorts at termination.	In-Life: On PND 0 or PND 1 and regularly thereafter (at least on PND 4, 7, 14 & 21) At termination

[6] The dose to each animal should normally be based on the most recent individual body weight determination and adjusted at least

Investigation	P0	F1 up to weaning	Surplus pups after standardisation on PND 4	Surplus pups not allocated to Cohorts (at weaning)	Cohort 1A 20M+20F/dose Terminated at ca 13 weeks of age	Cohort 1B without extension 20M+20F/dose Terminated at ca 14 weeks of age if not mated, 20-25 weeks if mated	Cohort 2A 10M+10F/dose Terminated at ca 11-12 weeks of age	Cohort 2B 10M+10F/dose Terminated at ca 3 weeks of age (at weaning)	Cohort 3 10M+10F/dose Terminated at ca 8 weeks of age	P1 (identical to Cohort 1B)	F2 up to weaning (identical to F1 up to weaning)
Body weight gain based on body weight measurements as outlined above (Not explicitly mentioned in TG/GD)											
Clinical observations including: - Changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions [7]	Once a week (e.g. when animals are weighed).	As often as is applicable and when weighed.			When animals are weighed	When animals are weighed	When animals are weighed	When animals are weighed	When animals are weighed	When animals are weighed	As often as is applicable and when weighed.
Food consumption (or water consumption, if substance administered in the drinking water).	At least weekly (same day as weighing)				At least weekly.	At least weekly.	At least weekly.	At least weekly.	At least weekly.	At least weekly.	
Macroscopic examination of all organs for abnormalities	At termination		Culled pups on PND 4	At termination	At termination	At termination	At termination	At termination	At termination	At termination	At termination

weekly in adult males and adult non-pregnant females, and every two days in pregnant females and F1 animals when administered prior to weaning and during the 2 weeks following weaning.

[7] For the P and the selected F1 animals, a general clinical observation is made once a day. In the case of gavage dosing, the timing of clinical observations should be prior to and post dosing (for possible signs of toxicity associated with peak plasma concentration).

Investigation	P0	F1 up to weaning	Surplus pups after standardisation on PND 4	Surplus pups not allocated to Cohorts (at weaning)	Cohort 1A 20M+20F/dose Terminated at ca 13 weeks of age	Cohort 1B without extension 20M+20F/dose Terminated at ca 14 weeks of age if not mated, 20-25 weeks if mated	Cohort 2A 10M+10F/dose Terminated at ca 11-12 weeks of age	Cohort 2B 10M+10F/dose Terminated at ca 3 weeks of age (at weaning)	Cohort 3 10M+10F/dose Terminated at ca 8 weeks of age	P1 (identical to Cohort 1B)	F2 up to weaning (identical to F1 up to weaning)
Organ weight: Liver	At termination				At termination	At termination if an identified target organ				At termination if an identified target organ	
Histopathology of fixed organs: Liver	HD and control; lower doses if treatment related findings				HD and control; lower doses if treatment related findings	Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal				Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal	
Organ weight: Kidneys	At termination				At termination	At termination if an identified target organ				At termination if an identified target organ	
Histopathology of fixed organs: Kidneys	HD and control; lower doses if treatment related findings				HD and control; lower doses if treatment related findings	Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal				Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal	
Organ weight: Heart	At termination				At termination	At termination if an identified target organ				At termination if an identified target organ	
Histopathology of fixed organs: Heart	HD and control; lower doses if treatment related findings				HD and control; lower doses if treatment related findings	Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal				Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal	
Organ weight: Lung (not mentioned in TG 443 nort GD 151)	At termination if an identified target organ				At termination if an identified target organ	At termination if an identified target organ				At termination if an identified target organ	

Investigation	P0	F1 up to weaning	Surplus pups after standardisation on PND 4	Surplus pups not allocated to Cohorts (at weaning)	Cohort 1A 20M+20F/dose Terminated at ca 13 weeks of age	Cohort 1B without extension 20M+20F/dose Terminated at ca 14 weeks of age if not mated, 20-25 weeks if mated	Cohort 2A 10M+10F/dose Terminated at ca 11-12 weeks of age	Cohort 2B 10M+10F/dose Terminated at ca 3 weeks of age (at weaning)	Cohort 3 10M+10F/dose Terminated at ca 8 weeks of age	P1 (identical to Cohort 1B)	F2 up to weaning (identical to F1 up to weaning)
Histopathology of fixed organs: Lung	HD and control; lower doses if treatment related findings				HD and control; lower doses if treatment related findings	Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal				Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal	
Organ weight: Other known target organs	At termination				At termination	At termination				At termination	
Histopathology of fixed organs: Known target organs	HD and control; lower doses if treatment related findings. Repro organs of all animals with reduced fertility.				HD and control; lower doses if treatment related findings	If suspected repro or ED and/or if cohort 1A results equivocal				If suspected repro or ED and/or if cohort 1A results equivocal	
Organ weight: Other organs as appropriate				10 pups/sex/group at termination							10 pups/sex/group at termination
Histopathology of fixed organs: Muscle	HD and control; lower doses if treatment related findings				HD and control; lower doses if treatment related findings	Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal				Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal	
Histopathology of fixed organs: Gastrointestinal tract	HD and control; lower doses if treatment related findings				HD and control; lower doses if treatment related findings	Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal				Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal	

Investigation	P0	F1 up to weaning	Surplus pups after standardisation on PND 4	Surplus pups not allocated to Cohorts (at weaning)	Cohort 1A 20M+20F/dose Terminated at ca 13 weeks of age	Cohort 1B without extension 20M+20F/dose Terminated at ca 14 weeks of age if not mated, 20-25 weeks if mated	Cohort 2A 10M+10F/dose Terminated at ca 11-12 weeks of age	Cohort 2B 10M+10F/dose Terminated at ca 3 weeks of age (at weaning)	Cohort 3 10M+10F/dose Terminated at ca 8 weeks of age	P1 (identical to Cohort 1B)	F2 up to weaning (identical to F1 up to weaning)
Histopathology of fixed organs: Urinary bladder	HD and control; lower doses if treatment related findings				HD and control; lower doses if treatment related findings	Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal				Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal	
Histopathology of fixed organs: Trachea	HD and control; lower doses if treatment related findings				HD and control; lower doses if treatment related findings	Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal				Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal	
Clinical biochemistry (including): - Glucose	10 animals/sex/group at termination (post-fasting).				10 animals/sex/group at termination.						
Clinical biochemistry (including): - Total cholesterol	10 animals/sex/group at termination (post-fasting).				10 animals/sex/group at termination.						
Clinical biochemistry (including): - Urea	10 animals/sex/group at termination (post-fasting).				10 animals/sex/group at termination.						
Clinical biochemistry (including): - Creatinine	10 animals/sex/group at termination (post-fasting).				10 animals/sex/group at termination.						

Investigation	P0	F1 up to weaning	Surplus pups after standardisation on PND 4	Surplus pups not allocated to Cohorts (at weaning)	Cohort 1A 20M+20F/dose Terminated at ca 13 weeks of age	Cohort 1B without extension 20M+20F/dose Terminated at ca 14 weeks of age if not mated, 20-25 weeks if mated	Cohort 2A 10M+10F/dose Terminated at ca 11-12 weeks of age	Cohort 2B 10M+10F/dose Terminated at ca 3 weeks of age (at weaning)	Cohort 3 10M+10F/dose Terminated at ca 8 weeks of age	P1 (identical to Cohort 1B)	F2 up to weaning (identical to F1 up to weaning)
Clinical biochemistry (including): - Total protein	10 animals/sex/group at termination (post-fasting).				10 animals/sex/group at termination.						
Clinical biochemistry (including): - Albumin	10 animals/sex/group at termination (post-fasting).				10 animals/sex/group at termination.						
Clinical biochemistry (including): - Two enzymes indicative of hepatocellular effects	10 animals/sex/group at termination (post-fasting).				10 animals/sex/group at termination.						
Haematology: Haematocrit	10 animals/sex/group at termination (post-fasting).				10 animals/sex/group at termination.						
Haematology: Haemoglobin concentration	10 animals/sex/group at termination (post-fasting).				10 animals/sex/group at termination.						
Haematology: Erythrocyte count	10 animals/sex/group at termination (post-fasting).				10 animals/sex/group at termination.						

Investigation	P0	F1 up to weaning	Surplus pups after standardisation on PND 4	Surplus pups not allocated to Cohorts (at weaning)	Cohort 1A 20M+20F/dose Terminated at ca 13 weeks of age	Cohort 1B without extension 20M+20F/dose Terminated at ca 14 weeks of age if not mated, 20-25 weeks if mated	Cohort 2A 10M+10F/dose Terminated at ca 11-12 weeks of age	Cohort 2B 10M+10F/dose Terminated at ca 3 weeks of age (at weaning)	Cohort 3 10M+10F/dose Terminated at ca 8 weeks of age	P1 (identical to Cohort 1B)	F2 up to weaning (identical to F1 up to weaning)
Haematology: Blood clotting time/potential	10 animals/sex/group at termination (post-fasting).										
Gross necropsy			At termination	At termination							At termination
Urinalysis (Unless existing data from repeated-dose studies indicate that the parameter is not affected by the test chemical)	Prior to termination				Prior to termination						

Table I.7: Adrenals, Pituitary and Thyroid

Adrenals, pituitary and thyroid are listed separately as being important endocrine organs in addition to specific reproductive organs.

Investigation	P0	F1 up to weaning	Surplus pups after standardisation on PND 4	Surplus pups not allocated to Cohorts (at weaning)	Cohort 1A 20M+20F/dose Terminated at ca 13 weeks of age	Cohort 1B without extension 20M+20F/dose Terminated at ca 14 weeks of age if not mated, 20-25 weeks if mated	Cohort 2A 10M/+10Fdose Terminated at ca 11-12 weeks of age	Cohort 2B 10M/+10Fdose Terminated at ca 3 weeks of age (at weaning)	Cohort 3 10M/+10Fdose Terminated at ca 8 weeks of age	P1 (identical to Cohort 1B)	F2 up to weaning (identical to F1 up to weaning)
Organ weight: Adrenal glands	At termination				At termination	At termination if an identified target organ				At termination if an identified target organ	
Histopathology of fixed organs: Adrenal glands	HD and control; lower doses if treatment related findings				HD and control; lower doses if treatment related findings	Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal				Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal	
Organ weight: Pituitary	At termination				At termination	At termination				At termination	
Histopathology of fixed organs: Pituitary	HD and control; lower doses if treatment related findings				HD and control; lower doses if treatment related findings	If suspected repro or ED and/or if cohort 1A results equivocal				If suspected repro or ED and/or if cohort 1A results equivocal	
Organ weight: Thyroid	Post-fixation				At termination	At termination if an identified target organ				At termination if an identified target organ	
Histopathology of fixed organs: Thyroid (and parathyroid)	HD and control; lower doses if treatment related findings				HD and control; lower doses if treatment related findings	Collected if an identified target organ and investigated if suspected repro or ED and/or if cohort 1A results equivocal				Collected if an identified target organ and investigated If suspected repro or ED and/or if cohort 1A results equivocal	

Investigation	P0	F1 up to weaning	Surplus pups after standardisation on PND 4	Surplus pups not allocated to Cohorts (at weaning)	Cohort 1A 20M+20F/dose Terminated at ca 13 weeks of age	Cohort 1B without extension 20M+20F/dose Terminated at ca 14 weeks of age if not mated, 20-25 weeks if mated	Cohort 2A 10M/+10Fdose Terminated at ca 11-12 weeks of age	Cohort 2B 10M/+10Fdose Terminated at ca 3 weeks of age (at weaning)	Cohort 3 10M/+10Fdose Terminated at ca 8 weeks of age	P1 (identical to Cohort 1B)	F2 up to weaning (identical to F1 up to weaning)
Thyroid hormones (T4 and TSH)	10 animals/sex/group at termination (post fasting) or at a pre-termination bleed.		Optional: Measuring T4 at termination to be considered	10 animals/sex/group at termination	10 animals/sex/group at termination or at a pre-termination bleed.						10 animals/sex/group at termination

Table I.8: Indices relating to male reproduction

OECD GD 151 refers to the following indices in paragraph 89: "Reproductive performance is the ability of male … animals to mate successfully and produce viable offspring. The major indices usually determined are: male … mating indices, male … fertility indices, …. These should be reported in TG 443. Calculation of these indices and discussion on interpretation of reproductive performance can be found in GD 43 (OECD, 2008, paragraph 180)."

OECD GD 43 includes this table:

Index	Calculation	Definition
Male Mating Index	No. of males with confirmed mating X 100 Total No. of males cohabited	Measure of male's ability to mate
Male Fertility Index	No. of males impregnating (siring) a female X 100 Total of No. males cohabited	Measure of male's ability to produce sperm that can fertilise eggs

Table I.9: Indices relating to female reproduction

OECD GD 151 refers to the following indices in para 89: "Reproductive performance is the ability of … female animals to mate successfully and produce viable offspring. The major indices usually determined are: … female mating indices, … female fertility indices, gestation length, gestation index …. These should be reported in TG 443. Calculation of these indices and discussion on interpretation of reproductive performance can be found in GD 43 (OECD, 2008, paragraph 180)."

OECD GD 43 includes this table:

Index	Calculation	Definition
Female Mating Index	No. of sperm positive females X 100 Total No. of females cohabited	Measure of female's ability to mate
Female Fertility Index	No. of pregnant females X 100 No. sperm-positive females	Measure of female's ability to become pregnant
Gestation Index	No. of females with live born pups X 100 No. of pregnant females	Measure of pregnancy that provides at least one live pup