

### C.3.3. Repeated Dose 28-Day Oral Toxicity Study in Rodents (OECD TG 407)

Status: Assay validated by the OECD.

695. Modalities detected: (anti)estrogen, (anti)androgen, thyroid, steroidogenesis.
696. Endpoints: Mandatory: Weight of adrenals, testes, epididymides, prostate + seminal vesicles with coagulating glands. Histopathologic changes in testes, epididymides, prostate + seminal vesicles with coagulating glands, ovary, uterus/cervix, vagina, thyroid gland and adrenals.
697. Optional: Weight of uterus, ovaries, thyroid. Estrous cyclicity. Histopathologic changes in mammary glands and pituitary. Circulating levels of T3, T4, TSH.

#### Background to the assay

698. This assay determines the general toxicity of chemicals in rodents after 28 days of oral dosing (e.g. effects on liver, kidneys, heart, lungs); it also provides information on effects on the nervous, immune and reproductive systems. This is the primary purpose of this assay. The preferred species is the rat. Route of administration is oral, by gavage, via the diet or in drinking water. The original OECD TG 407 was adopted in 1981. A revised version was adopted in 1995, to obtain additional information in particular on neurotoxicity and immunotoxicity. It then underwent a validation study in the rat (OECD, 2006) where more parameters suitable for the detection of endocrine disruptors (EDs) were included and the test guideline (TG) was updated in October 2008 (most recent version). Following the validation study, many of the parameters were included in the updated guideline, as either mandatory or optional endpoints. It is important that the collection of endocrine endpoints does not interfere with the primary purpose (e.g. collection of blood for hormones should ideally be carried out at a comparable time of day in case of diurnal variations but blood collection for clinical chemistry should take precedence).

699. Experience with of serum hormone determinations in Levels 4 and 5 rodent assays has revealed that their detection/measurement in rodent studies can be challenging. A recent workshop was “Practicability of Hormonal Measurements” was organised by the BfR (Germany) and the finding from this workshop will be published (Kucheryavenko et al., 2018). The OECD Expert Group on Reproductive and Developmental Toxicity recommends that to demonstrate proficiency for thyroid hormones measurement, a laboratory should be able to show results from a separate study using a positive control substance. Laboratories may also submit their calibration curves, standard curves, as well as data on the levels of quantification and detection. This group is also establishing a historical control database with thyroid toxicant positive controls.

700. OECD TG 407 is considered to be an apical assay (i.e. it contains endpoints that may be changed by a number of different modes of action [MOA] and may not be specific to endocrine active substances [EASs]). The animals are young adults with intact hypothalamus-pituitary-gonadal/thyroid axes and therefore are a relevant model for human

health, although the sensitivity of the assay for EASs is less than that of the UT and H assays. The validation of the assay for endocrine endpoints showed that this assay is relatively insensitive and would only detect chemicals that were moderate and strong EDs for (anti)estrogenicity and (anti)androgenicity (e.g. ethinylestradiol and flutamide). However, it did detect EDs that were weak and strong modulators of thyroid hormone-related effects (e.g. propylthiouracil, T4 and methyl testosterone). It may also detect steroidogenesis inhibition, although only one (potent) chemical was used in the validation study (CGS 18320B) (OECD, 2006). Endocrine modalities other than estrogen/androgen/thyroid/steroidogenesis (E,A,T,S) may also be detected, although these have not been validated.

### When/why the assay may be used

701. This assay is likely to be used as a preliminary study for longer term studies (e.g. 90-day studies or carcinogenicity studies, where the endocrine endpoints give additional information on the potential of the chemical to interact with the endocrine system). This assay is also necessary as a standard information requirement in certain chemical legislations (e.g. REACH for chemicals manufactured or imported in quantities of ten tonnes or more). It may also be used for chemicals where chronic exposure scenarios are not anticipated. Depending on the number of doses used, the assay may be used for hazard assessment (when one or two doses are used) or for hazard characterisation if a more detailed dose response curve is available. It should be noted that as this assay is not primarily designed to detect endocrine disruption, a higher degree of systemic toxicity is typically induced than is the case with the other Level 3 and 4 assays. The possibly confounding effect of systemic toxicity on endocrine endpoints therefore needs to be considered.

702. In order to provide information relevant for assessing whether or not a chemical may fulfil the WHO/IPCS (2002) definition of an ED, the study design has to be sufficiently robust to demonstrate the presence or absence of effects. In the dose selection, the investigator should also consider and ensure that data generated are adequate to fulfil the regulatory requirement across OECD countries as appropriate (e.g. hazard and risk assessment and labelling, ED assessment, etc.). The top dose or concentration should be sufficiently high to give clear systemic (i.e. non endocrine-specific) toxicity in order to ensure that a wide range of exposures (high to low) is tested. However, endocrine effects observed solely in the presence of clear systemic toxicity should be interpreted with caution and may be disregarded when sufficiently justified to be caused by secondary effects which are unlikely to be due to endocrine activity. The reason for this advice is a concern that some endocrine active substance (EAS) sensitive assays are being run at doses/concentrations of EASs that are too low to trigger direct impacts on the endocrine system. This guidance document is not the place to address this issue directly, but it should be considered when EAS-sensitive TGs are revised in the future. In addition, the number and spacing of dose/concentration levels should also be adequate to fulfil the objectives of the study (e.g. to demonstrate dose response relationships if this is required).

### Introduction to the table of scenarios

703. [Table C.3.3](#) gives guidance on a further step to take in the event of a positive (+) or negative (-) result and in the presence of positive (+), negative (-) or equivocal/absent (Eq/0) existing results. “Existing results” are subdivided into “mechanism” and “effects” data (third and fourth columns). The table is divided horizontally into a series of scenarios that represent all the combinations of these events.

704. The results of OECD TG 407 are given in the second column. As OECD TG 407 is not a screening test where a yes/no (qualitative) answer is obtained, criteria for positive results for the endocrine endpoints are not given in the TG. Results for the endpoints would be considered both individually and as a whole. It is not possible to provide guidance on all endpoints individually and therefore the endpoints have been pragmatically divided into “apical” and “indicators of hormonal activity”. Both groups have similar biological importance, although the “indicators of hormonal activity” in the mammalian assays are serum hormones and are generally, but not always, more variable than “apical endpoints”.

705. “Apical” endpoints are weights of testes, epididymides, prostate (+ seminal vesicles with coagulating glands), ovary, uterus, histopathologic changes in testes, epididymides, prostate, seminal vesicles, coagulating glands, ovary, uterus/cervix, vagina, thyroid and estrous cyclicity. “Indicators of hormonal activity” are hormones (T3, T4 and TSH).

706. Three possible outcomes for a positive result are therefore envisaged in [Table C.3.3](#):

1. indicators of hormonal activity and apical endpoints positive
2. indicators of hormonal activity positive and apical endpoints negative
3. indicators of hormonal activity negative and apical endpoints positive.

707. A positive result for apical endpoints could be significant reductions in reproductive organ weights, accompanied by treatment-related histopathologic changes. A positive result for indicators of hormonal activity could be biologically significant changes in thyroid hormone profiles. The indicators of hormonal activity are optional endpoints for this TG and therefore they may not be measured. Alternatively, other endpoints not specified in the guideline (e.g. reproductive hormones), may be measured and if positive would contribute to the overall assessment of a positive result. The apical endpoints for the detection of effects on male and female reproductive organs tended to be less sensitive than the indicators of hormonal activity in the validation of OECD TG 407 and therefore changes are more likely to be indicative of an ED, although the results in entirety should be considered rather than single isolated changes. This was not true for the thyroid though, where changes in thyroid histopathology were always as sensitive, or more sensitive, than changes in thyroid hormone/TSH levels. The guidance on histopathologic changes in endocrine tests (OECD, 2009) may be helpful in interpretation. A positive result for indicators of hormonal activity alone should be considered with caution, although it is possible that these endpoints may have detected weak effects that were not detected by the apical endpoints in this study but may then be detected in longer term studies.

708. A negative result for OECD TG 407 is taken to be the absence of changes in both endocrine-relevant indicators of hormonal activity and apical endpoints. In the absence of other pertinent lines of evidence, negative results in this test alone cannot be taken as evidence that the chemical is not an ED. Further studies will be required as confirmation.

709. Equivocal results for the guideline are not considered in [Table C.3.3](#), partly for brevity but also because equivocal results are by nature uncertain. A decision must eventually be reached about whether the endocrine endpoints tend to be positive or negative or whether the result must be put to one side and the test repeated (using the same or a different test guideline). Factors which may have interfered with the result (e.g. composition of the diet used, environmental influences), should be considered. Apparent equivocal results may arise because of the low sensitivity of the assay for (anti-)estrogens/androgens.

## Existing data to be considered

710. Existing “mechanism” *in vitro* data are assumed to be available from estrogen receptor (ER; ER binding and ER STTA), androgen receptor (AR; AR binding and AR STTA) and steroidogenesis-based assays (Level 2). Assays may also be available for interference with thyroid modalities. In practice, it is possible that data from all of these assays may not be available, so judgement will need to be used to decide which assays to perform. Although the current *in vitro* test guidelines do not incorporate metabolic activation, published information on use of metabolic activation systems is available in Jacobs et al. (2008; 2013) and OECD (2008a). These methods, however, have not yet been validated.

711. Existing “effects” data refer to *in vivo* effects that may come from UT or H assays where a non-physiological animal model is used. In these cases, it should be remembered that these assays are specifically designed to be sensitive to EASs, compared to OECD TG 407. Other data such as repeat dose oral toxicity studies, reproduction/developmental toxicity screen tests may be available, although it is unlikely that OECD TG 407 will be performed if higher tier data are already available as OECD TG 407 offers no advantage over these assays. Data may also be available on effects in mammalian and non-mammalian vertebrate species, although caution should be used when extrapolating between taxa. A chemical causing endocrine effects in non-mammalian environmental species (fish, for example) may also have endocrine effects in mammals, but the physiological consequences of the effects are likely to be different.

712. When considering the results of the OECD TG 407 assay, all available data should be used in order to reach a conclusion and take a weight of evidence approach. This may include high throughput screening data, read-across data from structural analogues and quantitative structure activity relationship (QSAR). Several QSAR models for ER and AR binding/activation are now available (see [Sections B.1.1.1](#) and [B.1.1.2](#)).

## Scenarios: Positive and negative results combined with existing data

713. The scenarios (A to R) presented in [Table C.3.3](#) represent all the possibilities of positive or negative results in combination with the presence or absence of existing data. The action taken will also depend on the regulatory environment, but the considerations given here are generally science based. Although the OECD TG 407 assay uses rodents, the well-conserved nature of the hormonal pathways across taxa indicate that results on endocrine endpoints in this assay may be relevant to other vertebrate species. Effects in laboratory mammal tests are also highly relevant for environmental mammalian species. Wherever possible, the recommended “next step which could be taken” avoids unnecessary animal testing. However, sometimes conducting an animal test will be indicated and then the relevance of species, strain and exposure route should always be considered. The sensitivity and physiological function of the hormone under investigation in the test species should also be considered. In general, lower level tests should be conducted before higher level tests in order to avoid unnecessary animal usage, unless it is apparent that a Level 5 test will be required anyway or will be needed to establish the evidence to conclude on ED properties. Information on some endocrine-related tumours may be detected more comprehensively in carcinogenicity studies (OECD TG 451/453) (Level 4); for example, detection of certain types of thyroid tumors in the absence of reproductive or developmental effects, as well as substances causing tumors in other endocrine-sensitive tissues. At Level 5, the Extended One-Generation Reproduction Toxicity Study (EOGRTS – OECD TG 443)

is the most sensitive reproduction assay for detecting endocrine disruption because it includes evaluation of a number of endocrine endpoints not included in the two-generation study (OECD TG 416) adopted in 2001. It is recognised, however, that some jurisdictions may require a two-generation study. Further considerations, specific to each scenario are given in the table.

714. Scenarios A to C represent positive results in the OECD TG 407 assay in the presence of positive *in vitro* mechanistic data and positive, negative or equivocal *in vivo* effects data. Each positive OECD TG 407 result scenario is divided into the [three possible outcomes](#) given above. A positive result in the *in vitro* assays in combination with a positive OECD TG 407 assay is moderate or strong evidence for E,A,T,S-mediated activity that may or may not be supported by the *in vivo* effects data. In the absence of robust upper-level data, the next step may be to conduct an upper-level test. In the presence of robust *in vivo* data, there may be sufficient evidence to conclude concern for endocrine disruption and therefore no need for further testing. Positive results in the OECD TG 407 assay may also indicate the potential for endocrine mediated effects in lower vertebrates. These could be followed up with partial life cycle tests such as the Fish Sexual Development Test (FSDT), the Larval Amphibian Growth and Development Assay (LAGDA) or the Medaka Extended One-Generation Reproduction Test (MEOGRT) if the evidence is strong enough. *In vivo* assays/tests with negative results should be interpreted with caution as they may either indicate that the tests used do not have sufficient power to detect weak effects or, alternatively, that the effects do not present a concern for endocrine disruption. The possibility of other (non-E,A,T,S) mechanisms should also not be overlooked (e.g. involving other receptors or endocrine axes).

715. Scenarios D to F represent positive results in the OECD TG 407 assay in the presence of negative *in vitro* mechanistic data and positive, negative or equivocal *in vivo* effects data. Each positive OECD TG 407 result scenario is divided into the [three possible outcomes](#) given above. Negative results in the *in vitro* assays should be viewed with caution in case a metabolite is responsible for the positive OECD TG 407 assay. Unless the metabolic profile of the test substance is known, one option may be to conduct these assays with an added metabolising system. If the metabolic profile is known, then a higher level *in vivo* test may be advisable. The choice of tests will depend on the available *in vivo* effects data. Positive results in the OECD TG 407 assay may also indicate the potential for endocrine mediated effects in lower vertebrates. As in scenarios A to C, *in vivo* assays/tests with negative results should be interpreted with caution as they may either indicate that the tests used do not have sufficient power to detect weak effects or, alternatively, that the effects do not present a concern for endocrine disruption.

716. Scenarios G to I represent positive results in the OECD TG 407 assay in the presence of various combinations of missing or equivocal data. Positive results in the OECD TG 407 assay may also indicate the potential for endocrine mediated effects in lower vertebrates. Each positive OECD TG 407 result scenario is divided into the [three possible outcomes](#) given above. The next step to take in these eventualities will depend on the nature of the other available data and the jurisdiction in which it is being used. In some cases, equivocal data may be viewed as positive whilst in others it may or may not contribute to the weight of evidence. The interpretation may also depend on the MOA in question and why the data are considered equivocal, e.g. a study that is equivocal for thyroid effects may still be of value in evaluating (anti)androgenic effects. In all three scenarios, the recommended first step is to obtain reliable mechanistic (*in vitro*) data rather than proceed further with *in vivo* testing. Equivocal and missing data are alternative scenarios and two possibilities for the next step are given in most cases, but the nature of equivocal data means

that decisions need to be taken on a case-by-case basis. In all cases, the role of metabolism, route of exposure and data from structural analogues should be considered before deciding on the next step.

717. Scenarios J to L represent negative results in the OECD TG 407 assay in the presence of positive *in vitro* mechanistic data and positive, negative or equivocal *in vivo* effects data. As a negative result for OECD TG 407 is taken to be negative findings for both indicators of hormonal activity and apical endpoints then (unlike the situation with positive outcomes), there is only one possible negative outcome. Negative outcomes in OECD TG 407 should be viewed with caution because of the power of the assay to detect (anti)estrogens and androgens. All three scenarios could also arise from a chemical that is positive in *in vitro* assays, but is metabolised to a non-active metabolite leading to negative results in the OECD TG 407 assay. This should be considered first when investigating the next step. Endocrine active potency may also explain differences between *in vitro* and *in vivo* results (e.g. a chemical with weak endocrine activity may give a positive result *in vitro* but may be negative *in vivo*). Positive *in vivo* effects data may involve other E,A,T,S, non-E,A,T,S mechanisms (e.g. involving other receptors or endocrine axes), more sensitive endpoints, greater statistical power or life stages that are more sensitive to the substance than the young adult animals in OECD TG 407.

718. Scenarios M to O represent negative results in the OECD TG 407 assay in the presence of negative *in vitro* mechanistic data and positive, negative or equivocal *in vivo* effects data. Negative results for all tests (Scenario N) may be sufficient to enable a conclusion of no concern for endocrine disruption. This will depend on the weight of evidence and may not be possible. Where there are positive *in vivo* effects data, there could still be an E,A,T,S-related mechanism, the effects may be related to length of exposure, route of exposure or exposure at different life stages. Other E,A,T,S or non-E,A,T,S mechanisms may also be involved.

719. Scenarios P to R represent negative results in the OECD TG 407 assay in the presence of various combinations of missing or equivocal data. As with the positive result scenarios above (see [Paragraph 712](#)) the next step to take in these eventualities will have to be decided on a case-by-case basis. However, the recommended first step is generally to obtain reliable mechanistic (*in vitro*) data rather than proceed further with *in vivo* testing. In all cases, the role of metabolism, route of exposure and data from structural analogues should be considered before deciding on the next step.

720. In all scenarios (A to R), the next step to take to strengthen weight of evidence will depend on the existing information. [Table C.3.3](#) is meant to provide a succinct guide and may not cover all circumstances or possibilities. The scenarios may also suggest that chemicals have simple or single MOA, when in practice they may have multiple endocrine and non-endocrine MOA. In some cases, for example, two opposite modes of simultaneous action (e.g. estrogenic and anti-estrogenic) could, depending on dose, lead to a minimisation or abolition of effects, while in others two different MOA (e.g. estrogenic and anti-androgenic) could potentially reinforce effects. Endocrine pathways interact, mixed effects are common and there are many pathways that cannot be distinguished with currently available TGs. If multiple MOA are suspected, either from the existing results or based on QSAR/read-across/integrated approaches, this should be investigated further if needed for regulatory decision making.

## References

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Table C.3.3. **Repeated Dose 28-Day Oral Toxicity Study in Rodents (OECD TG 407):  
Guidance for scenarios of combinations of results with existing data**

This table represents possible conclusions to be drawn from assay data, and a next step which could be taken if further evidence is required about possible endocrine disrupting properties and/or effects. The guidance offered is not meant to be prescriptive, but provides science-based considerations. It encourages the use of all available data and expert judgement in a weight of evidence approach. Regional and national interpretation of results and “next steps” may vary.

The conclusions are grouped into a series of scenarios (A-R), each scenario representing a different combination of assay results, existing *in vitro* data and existing *in vivo* data. The symbol “+” indicates that the data in question represent a positive result, “-” indicates a negative result, and “Eq/0” indicates that the data are either equivocal or are not available.

Existing results \* “Mechanism (*in vitro* mechanistic data)” assumes that mechanistic data are available from estrogen receptor (ER-), androgen receptor (AR-) and steroidogenesis-based assays (Level 2). Thyroid hormone receptor (TR) and other assays concerning mechanisms of thyroid disruption may be available, but they are not in common use. In practice, data from all assays may not be available and therefore this must be taken into account when deciding on the “next step”. Quantitative structure activity relationship (QSAR) predictions of estrogen and androgen binding/activation may be made for some substances.

Existing results \*\* “Effects (*in vivo* effects of concern)” assumes effects have been observed in other *in vivo* screens/tests which give rise to concern that the test chemical may be an endocrine disrupter. These may be other repeated dose toxicity tests, Uterotrophic Bioassays and Hershberger Bioassays or read-across from chemical analogues.

\*\*\* *Note*: three possible outcomes for a positive result are given:

1. indicators of hormonal activity and apical endpoints positive
2. indicators of hormonal activity positive and apical endpoints negative
3. indicators of hormonal activity negative and apical endpoints positive.

“Apical endpoints” are weights of testes, epididymides, prostate (+ seminal vesicles with coagulating glands), ovary, uterus, histopathologic changes in testes, epididymides, prostate, seminal vesicles, coagulating glands, ovary, uterus/cervix, vagina, thyroid and estrous cyclicity.

“Indicators of hormonal activity” are hormones (T3, T4 and TSH).



Scenarios	Result of OECD TG 407 (rodent 28-day assay)	Existing results		Possible conclusions: 1) Indicators of hormonal activity and apical endpoints positive 2) Indicators of hormonal activity positive and apical endpoints negative 3) Indicators of hormonal activity negative and apical endpoints positive	Next step which could be taken to strengthen weight of evidence if necessary	Other considerations
		Mechanism ( <i>in vitro</i> mechanistic data)*	Effects ( <i>in vivo</i> effects of concern)**			
A	+ ***	+	+	1) Moderate or strong (anti)-E,A,T,S activity. Increased evidence of (anti)-E,A,T,S activity. 2) Possible evidence of (anti)-E,A,T,S activity, apical endpoints may be less sensitive or unaffected. Increased evidence of (anti)-E,A,T,S activity. 3) Moderate or strong (anti)-E,A,T,S activity, indicators of hormonal activity may be less sensitive or unaffected. Increased evidence of (anti)-E,A,T,S activity.	Perform assay from Level 5 (e.g. Extended One-Generation Reproduction Toxicity Study [EOGRTS,] or two-generation) assay.	If existing data are from a Level 5 assay, there may be sufficient information to conclude evidence of concern for endocrine disruption (the EOGRTS provides the most information; however, for endocrine disrupting chemicals [EDCs] with a carcinogenic potential, OECD TG 451-3 may be more sensitive). Effects on indicators of hormonal activity alone may be indicative of changes not detected by apical endpoints. Effects on apical endpoints alone may indicate E,A,T,S modalities or other mechanisms. Consider route of exposures for effects data and possible implications of absorption, distribution, metabolism and excretion (ADME) characteristics of the chemical. Endocrine activity possible in lower vertebrates. Consider performing a Fish Sexual Development Test (FSDT), a Larval Amphibian Growth and Development Assay (LAGDA) or a Medaka Extended One-Generation Reproduction Test (MEOGRT).
B	+	+	-	1) Moderate or strong (anti)-E,A,T,S activity. Increased evidence of (anti)-E,A,T,S activity. 2) Possible evidence of (anti)-E,A,T,S activity, apical endpoints may be less sensitive or unaffected. Increased evidence of (anti)-E,A,T,S activity. 3) Moderate or strong (anti)-E,A,T,S activity, indicators of hormonal activity may be less sensitive or unaffected. Increased evidence of (anti)-E,A,T,S activity.	Perform assay from Level 5 (e.g. EOGRTS or two-generation) assay.	If existing data are from an adequate Level 5 assay, question why there are differences. Consider route of exposures and possible implications of ADME characteristics of the chemical. If existing data are from a less sensitive assay, a higher level test may be required. Effects on indicators of hormonal activity alone may be indicative of changes not detected by apical endpoints. Effects on apical endpoints alone may indicate E,A,T,S modalities or other mechanisms. Endocrine activity possible in lower vertebrates. Consider performing an FSDT, LAGDA or MEOGRT.
C	+	+	Eq/0	1) Moderate or strong (anti)-E,A,T,S activity. Increased evidence of (anti)-E,A,T,S activity. 2) Possible evidence of (anti)-E,A,T,S activity, apical endpoints may be less sensitive or unaffected. Increased evidence of (anti)-E,A,T,S activity. 3) Moderate or strong (anti)-E,A,T,S activity, indicators of hormonal activity may be less sensitive or unaffected. Increased evidence of (anti)-E,A,T,S activity.	Perform assay from Level 5 (e.g. EOGRTS or two-generation) assay.	Check data on chemical analogues. Effects on indicators of hormonal activity alone may be indicative of changes not detected by apical endpoints. Effects on apical endpoints alone may indicate E,A,T,S modalities or other mechanisms. Consider route of exposure for OECD TG 407 and follow-up assay. Possible implications of ADME characteristics of the chemical. Endocrine activity possible in lower vertebrates. Consider performing an FSDT, LAGDA or MEOGRT. Equivocal results may indicate chemical has multiple modes of action (MOA).

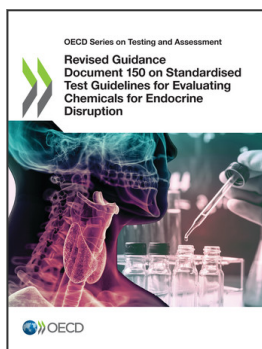
Scenarios	Result of OECD TG 407 (rodent 28-day) assay	Existing results		Possible conclusions: 1) Indicators of hormonal activity and apical endpoints positive 2) Indicators of hormonal activity positive and apical endpoints negative 3) Indicators of hormonal activity negative and apical endpoints positive	Next step which could be taken to strengthen weight of evidence if necessary	Other considerations
		Mechanism (in vitro mechanistic data)*	Effects (in vivo effects of concern)**			
D	+	-	+	<p>1) Moderate or strong (anti)-E,A,T,S activity. Acts via non-ER,AR,TR, S mechanism or requires metabolic activation for activity.</p> <p>2) Possible evidence of (anti)-E,A,T,S activity, apical endpoints may be less sensitive or unaffected. Acts via non-ER, AR, TR, S mechanism or requires metabolic activation for activity.</p> <p>3) Moderate or strong (anti)-E,A,T,S activity, indicators of hormonal activity may be less sensitive or unaffected. Acts via non-ER, AR, TR, S mechanism or requires metabolic activation for activity.</p>	Perform <i>in vitro</i> ER, AR, TR, S assays with added metabolising system.	<p>If existing data are from an adequate Level 5 assay, there is sufficient information to conclude evidence of concern for endocrine disruption (the EOGRTS provides the most information; however, for EDCs with a carcinogenic potential, OECD TG 451-3 may be more sensitive). Effects on indicators of hormonal activity alone may be indicative of changes not detected by apical endpoints.</p> <p>Effects on apical endpoints alone may indicate E,A,T,S modalities or other mechanisms.</p> <p>Consider route of exposures for effects data and possible implications of ADME characteristics of the chemical.</p> <p>Further mechanistic studies may help determine MOA.</p> <p>Endocrine activity possible in lower vertebrates. Consider performing an FSDT, LAGDA or MEOGRT.</p>
E	+	-	-	<p>1) Moderate (anti)-E,A,T,S activity. Acts via non-ER, AR, TR, S mechanism or requires metabolic activation for activity. Route of exposure may account for the differences between OECD TG 407 and existing data.</p> <p>2) Possible evidence of (anti)-E,A,T,S activity, apical endpoints may be less sensitive or unaffected. Acts via non-ER, AR, TR, S mechanism or requires metabolic activation for activity. Weak activity does not result in adverse effects.</p> <p>3) Moderate (anti)-E,A,T,S activity, indicators of hormonal activity may be less sensitive or unaffected. Acts via non-ER, AR, TR, S mechanism or requires metabolic activation for activity. Weak activity does not result in adverse effects.</p>	<p>Perform <i>in vitro</i> ER, AR, TR, S assays with added metabolising system.</p> <p>OR</p> <p>Perform assay from Level 5 (e.g. EOGRTS or two-generation assay).</p>	<p>If existing data are from an adequate Level 5 assay, question why there are differences.</p> <p>Consider route of exposures and possible implications of ADME characteristics of the chemical.</p> <p>If existing data are from a less sensitive assay, a higher level test may be required.</p> <p>Effects on indicators of hormonal activity alone may be indicative of changes not detected by apical endpoints.</p> <p>Effects on apical endpoints alone may indicate other mechanisms.</p> <p>Endocrine activity possible in lower vertebrates. Consider performing an FSDT, LAGDA or MEOGRT.</p>

Scenarios	Result of OECD TG 407 (rodent 28-day) assay	Existing results		Possible conclusions: 1) Indicators of hormonal activity and apical endpoints positive 2) Indicators of hormonal activity positive and apical endpoints negative 3) Indicators of hormonal activity negative and apical endpoints positive	Next step which could be taken to strengthen weight of evidence if necessary	Other considerations
		Mechanism ( <i>in vitro</i> mechanistic data)*	Effects ( <i>in vivo</i> effects of concern)**			
F	+	–	Eq/0	<p>1) Moderate (anti)-E,A,T,S activity. Acts via non-ER, AR, TR, S mechanism or requires metabolic activation for activity.</p> <p>2) Possible evidence of (anti)-E,A,T,S activity, apical endpoints may be less sensitive or unaffected. Acts via non-ER,AR,TR, S mechanism or requires metabolic activation for activity.</p> <p>3) Moderate (anti)-E,A,T,S activity, indicators of hormonal activity may be less sensitive or unaffected. Acts via non-ER, AR, TR, S mechanism or requires metabolic activation for activity.</p>	<p>Perform <i>in vitro</i> ER, AR, TR, S assays with added metabolising system</p> <p>OR</p> <p>Perform assay from Level 5 (e.g. EOGRTS or two-generation assay).</p>	<p>Effects on indicators of hormonal activity alone may be indicative of changes not detected by apical endpoints.</p> <p>Effects on apical endpoints alone may indicate other mechanisms. Check data on chemical analogues.</p> <p>Further mechanistic studies may help determine MOA.</p> <p>Level 5 studies will provide hazard data.</p> <p>Endocrine activity possible in lower vertebrates. Consider performing an FSDT, LAGDA or MEOGRT.</p> <p>Equivocal results may indicate chemical has multiple MOA.</p>
G	+	Eq/0	+	<p>1) Moderate or strong (anti)-E,A,T,S activity. Increased evidence of (anti)-E,A,T,S activity. May act via ER, AR, TR, S mechanism (metabolic activation needed).</p> <p>2) Possible evidence of (anti)-E,A,T,S activity, apical endpoints may be less sensitive or unaffected. Increased evidence of (anti)-E,A,T,S activity. May act via ER, AR, TR, S mechanism (metabolic activation needed).</p> <p>3) Moderate or strong (anti)-E,A,T,S activity, indicators of hormonal activity may be less sensitive or unaffected. Increased evidence of (anti)-E,A,T,S activity. May act via ER, AR, TR, S mechanism (metabolic activation needed).</p>	<p>Perform <i>in vitro</i> ER, AR, TR, S assays (for the “0” scenario), otherwise Eq result available)</p> <p>OR</p> <p>Perform <i>in vitro</i> ER, AR, TR, S assays with added metabolising system.</p>	<p>If existing data are from a Level 5 assay, there may be sufficient information to conclude evidence of concern for endocrine disruption (the EOGRTS provides the most information; however, for EDCs with a carcinogenic potential, OECD TG 451-3 may be more sensitive).</p> <p>Effects on indicators of hormonal activity alone may be indicative of changes not detected by apical endpoints.</p> <p>Effects on apical endpoints alone may indicate E,A,T,S modalities or other mechanisms.</p> <p>Check data on chemical analogues.</p> <p>Further mechanistic studies may help determine MOA.</p> <p>Consider route of exposures for effects data and possible implications of ADME characteristics of the chemical.</p> <p>Endocrine activity possible in lower vertebrates. Consider performing an FSDT, LAGDA or MEOGRT.</p> <p>Equivocal results may indicate chemical has multiple MOA.</p>

Scenarios	Result of OECD TG 407 (rodent 28-day assay)	Existing results		Possible conclusions: 1) Indicators of hormonal activity and apical endpoints positive 2) Indicators of hormonal activity positive and apical endpoints negative 3) Indicators of hormonal activity negative and apical endpoints positive	Next step which could be taken to strengthen weight of evidence if necessary	Other considerations
		Mechanism ( <i>in vitro</i> mechanistic data)*	Effects ( <i>in vivo</i> effects of concern)**			
H	+	Eq/0	–	<p>1) Moderate (anti)-E,A,T,S activity. Acts via unknown mechanism or may require metabolic activation for activity. Route of exposure may account for the differences between OECD TG 407 and existing data.</p> <p>2) Possible evidence of (anti)-E,A,T,S activity, apical endpoints may be less sensitive or unaffected. Acts via unknown mechanism. Weak activity does not result in adverse effects.</p> <p>3) Moderate (anti)-E,A,T,S activity, indicators of hormonal activity may be less sensitive or unaffected. Acts via unknown mechanism. Weak activity does not result in adverse effects.</p>	For the "0" scenario, perform <i>in vitro</i> ER, AR, TR, S assays, maybe with added metabolising system (otherwise Eq result available).	<p>If existing data are from an adequate Level 5 assay, question why there are differences.</p> <p>Consider route of exposures and possible implications of ADME characteristics of the chemical.</p> <p>If existing data are from a less sensitive assay, a higher level test may be required.</p> <p>Effects on indicators of hormonal activity alone may be indicative of subtle changes not detected by apical endpoints.</p> <p>Effects on apical endpoints alone may indicate E,A,T,S modalities or other mechanisms.</p> <p>Check data on chemical analogues.</p> <p>Further mechanistic studies may help determine MOA.</p> <p>Endocrine activity possible in lower vertebrates. Consider performing an FSDT, LAGDA or MEOGRT.</p> <p>Equivocal results may indicate chemical has multiple MOA.</p>
I	+	Eq/0	Eq/0	<p>1) Moderate or strong (anti)-E,A,T,S activity. Acts via unknown mechanism. Unknown potential for adverse effects.</p> <p>2) Possible evidence of (anti)-E,A,T,S activity, apical endpoints may be less sensitive or unaffected. Acts via unknown mechanism. Unknown potential for adverse effects.</p> <p>3) Moderate or strong (anti)-E,A,T,S activity, indicators of hormonal activity may be less sensitive or unaffected. Acts via unknown mechanism. Unknown potential for adverse effects.</p> <p>There may be a need for metabolic activation.</p>	<p>Perform <i>in vitro</i> ER, AR, TR, S assays with added metabolising system</p> <p>OR</p> <p>Perform assay from Level 5 (e.g. EOGRTS or two-generation assay).</p>	<p>Effects on indicators of hormonal activity alone may be indicative of changes not detected by apical endpoints.</p> <p>Effects on apical endpoints alone may indicate E,A,T,S modalities or other mechanisms.</p> <p>Check data on chemical analogues.</p> <p>Further mechanistic studies may help determine MOA.</p> <p>Endocrine activity possible in lower vertebrates. Consider performing an FSDT, LAGDA or MEOGRT.</p> <p>Equivocal results may indicate chemical has multiple MOA.</p>
J	–	+	+	<p>No evidence for moderate or strong (anti)-E,A,T,S activity in OECD TG 407. Weak (anti)-E,A,S activity not detected by this assay.</p> <p>Metabolism or potency explains the difference from existing <i>in vitro</i> and <i>in vivo</i> data.</p>	<p>Perform <i>in vitro</i> ER, AR, TR, S assays with added metabolising system</p> <p>OR</p> <p>Perform assay from Level 5 (e.g. EOGRTS or two-generation assay).</p>	<p>If existing data are from an adequate Level 5 assay, question why there are differences.</p> <p>Effects seen in existing studies may be in a more sensitive life stage.</p> <p>Consider route of exposures and possible implications of ADME characteristics of the chemical.</p> <p>Further mechanistic studies may help determine MOA.</p>

Scenarios	Result of OECD TG 407 (rodent 28-day) assay	Existing results		Possible conclusions: 1) Indicators of hormonal activity and apical endpoints positive 2) Indicators of hormonal activity positive and apical endpoints negative 3) Indicators of hormonal activity negative and apical endpoints positive	Next step which could be taken to strengthen weight of evidence if necessary	Other considerations
		Mechanism ( <i>in vitro</i> mechanistic data)*	Effects ( <i>in vivo</i> effects of concern)**			
K	–	+	–	No evidence for moderate or strong (anti)-E,A,T,S activity in OECD TG 407. Weak (anti)-E,A,S activity not detected by this assay. Metabolism or potency explains <i>in vitro/in vivo</i> differences.	Perform <i>in vitro</i> ER, AR, TR, S assays with added metabolising system OR Perform assay from Level 5 (e.g. EOGRTS or two-generation assay).	If existing data are from an adequate Level 5 assay, there may be sufficient information to conclude absence of concern for endocrine disruption (the EOGRTS provides the most information; however, for EDCs with a carcinogenic potential, OECD TG 451-3 may be more sensitive). If existing data are from a less sensitive assay, a higher level test may be required. Further mechanistic studies with metabolism may help determine MOA.
L	–	+	Eq/0	No evidence for moderate or strong (anti)-E,A,T,S activity in OECD TG 407. Weak (anti)-E,A,S activity not detected by this assay. Metabolism or potency explains <i>in vitro/in vivo</i> differences. Unknown potential for adverse effects.	Perform <i>in vitro</i> ER, AR, TR, S assays with added metabolising system OR Perform assay from Level 5 (e.g. EOGRTS or two-generation assay).	Metabolic deactivation of chemical may occur <i>in vivo</i> so that possible <i>in vitro</i> activity is not realised. Consider possible routes of exposure implications of metabolism. Equivocal results may indicate chemical has multiple MOA.
M	–	–	+	No evidence for moderate or strong (anti)-E,A,T,S activity in OECD TG 407. Weak (anti)-E,A,S activity not detected by this assay.	Perform assay from Level 5 (e.g. EOGRTS or two-generation assay).	If existing data are from an adequate Level 5 assay, question why there are differences. Effects seen in existing studies may be in a more sensitive life stage. Consider route of exposures and possible implications of ADME characteristics of the chemical.
N	–	–	–	No evidence for moderate or strong (anti)-E,A,T,S activity in OECD TG 407. Weak (anti)-E,A,S activity not detected by this assay. No evidence for (anti)-E,A,T,S activity <i>in vitro</i> . No evidence of adverse effects.	Perform assay from Level 5 (e.g. EOGRTS or two-generation assay).	If existing data are from an adequate Level 5 assay, there may be sufficient information to conclude absence of concern for endocrine disruption (the EOGRTS provides the most information; however, for EDCs with a carcinogenic potential, OECD TG 451-3 may be more sensitive).
O	–	–	Eq/0	No evidence for moderate or strong (anti)-E,A,T,S activity in OECD TG 407. Weak (anti)-E,A,S activity not detected by this assay. No evidence for (anti)-E,A,T,S activity <i>in vitro</i> . Unknown potential for adverse effects.	Perform assay from Level 5 (e.g. EOGRTS or two-generation assay).	Consider route of exposures and possible implications for ADME characteristics of the chemical in follow-up assay.
P	–	Eq/0	+	No evidence for moderate or strong (anti)-E,A,T,S activity in OECD TG 407. Weak (anti)-E,A,S activity not detected by this assay. Potential for adverse effects via unknown mechanism.	Perform <i>in vitro</i> ER, AR, TR, S assays with added metabolising system.	Consider route of exposure for OECD TG 407 assay and possible implications for differences from existing assay. Effects seen in existing studies may be in a more sensitive life stage. Equivocal results may indicate chemical has multiple MOA.

Scenarios	Result of OECD TG 407 (rodent 28-day assay)	Existing results		Possible conclusions: 1) Indicators of hormonal activity and apical endpoints positive 2) Indicators of hormonal activity positive and apical endpoints negative 3) Indicators of hormonal activity negative and apical endpoints positive	Next step which could be taken to strengthen weight of evidence if necessary	Other considerations
		Mechanism (in vitro mechanistic data)*	Effects (in vivo effects of concern)**			
Q	–	Eq/0	–	No evidence for moderate or strong (anti)-E,A,T,S activity in OECD TG 407. Weak (anti)-E,A,S activity not detected by this assay. No evidence of adverse effects.	Perform <i>in vitro</i> ER, AR, TR, S assays with added metabolising system	If existing data are from an adequate Level 5 assay, there may be sufficient information to conclude absence of concern for endocrine disruption (the EOGRTS provides the most information; however, for EDCs with a carcinogenic potential, OECD TG 451-3 may be more sensitive). Further mechanistic studies may strengthen weight of evidence.
R	–	Eq/0	Eq/0	No evidence for moderate or strong (anti)-E,A,T,S activity in OECD TG 407. Weak (anti)-E,A,S activity not detected by this assay.	Perform <i>in vitro</i> ER, AR, TR, S assays, with added metabolising system, otherwise Eq result available.	Further mechanistic studies may strengthen weight of evidence. Check data on chemical analogues. Equivocal results may indicate chemical has multiple MOA.



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