C.2.13. Medaka Extended One-Generation Reproduction Test (MEOGRT) (OECD TG 240)

Status: Assay validated by the OECD.

468. Modality detected/endpoints: This fish life cycle test was specifically designed to investigate the apical effects of endocrine disrupters, and has several endpoints which can be considered diagnostic of some types of estrogen/androgen/thyroid/steroidogenesis (E,A,T,S) activity. This gives it an advantage over other currently standardised life cycle tests, and its use for evaluating endocrine disruptors (EDs) is to be preferred to the Fish Life Cycle Toxicity Test (see Section C.2.21) which, although sensitive to the apical effects of some EDs, contains no endocrine-sensitive endpoints. In view of the inclusion of certain ED-specific endpoints, the MEOGRT can contribute useful evidence about the probable causality of apical effects, which is a key issue in the definition of EDs.

Background to the assay

469. This assay is a comprehensive test using medaka (*Oryzias latipes*) exposed continuously from the adult stage of the first generation (F0) to the newly hatched stage of the third generation (F2). In other words, it includes two phases of reproductive activity, and two phases of embryonic development and hatching, separated by a full phase of growth and sexual development. It begins with pairs of sexually mature F0 fish (at least 12 weeks post-fertilisation, or wpf) reproducing for 3 weeks, brings their F1 offspring to sexual maturity (15 weeks), then allows the F1 adults to breed, and finally follows their offspring (F2) to hatching (up to 18 days post-fertilisation, or dpf). The main emphasis of the assay concerns population-relevant apical endpoints (e.g. survival, development, growth and reproduction). However, in order to obtain mechanistic information, additional endpoints include measurements of vitellogenin (either as protein – VTG, or as mRNA coding for vitellogenin – *vtg*), secondary sex characteristics, phenotypic sex compared with genetic sex, and gonadal histopathology. Histopathology of liver and kidney may also be measured in order to distinguish between endocrine effects and possible systemic or other toxicity. While the assay is able to distinguish large deviations from the expected 50:50 sex ratio of F1 offspring, it has less power than the Fish Sexual Development Test (FSDT) to distinguish small deviations due to the relatively small number of fish per replicate (12).

470. It should be noted that the MEOGRT is a relatively new test (adopted by the OECD in 2015) which has not yet been widely used (Watanabe et al., 2017). Furthermore, due to the test's cost and complexity, the validation process involved fewer laboratories than for many simpler assays. A recent publication (Flynn et al., 2017) which evaluated nine validation studies of the MEOGRT found that only one complied with all the biological validity criteria, so caution should be used when assessing MEOGRT data. There is a significant risk of test failure because of its length and difficulty. Nevertheless, development of the assay has built on experience with shorter assays involving medaka (e.g. the Fish Short-Term Reproduction Assay [FSTRA] and the FSDT), and earlier versions of it have been used for research purposes. It is possible that for some applications (e.g. when testing

highly bioaccumulative chemicals for trans-generational effects or if epigenetic effects are suspected) it might be feasible to extend the MEOGRT to the reproduction phase of the F2 generation, but at present there is insufficient information to warrant this. Currently, however, few testing laboratories have experience with the MEOGRT, and an extended version has not been standardised or validated.

471. Only medaka is recommended for use in this test design. A related assay using zebrafish (*Danio rerio*), the Zebrafish Extended One-Generation Test (ZEOGRT), is currently being validated by the OECD (see Section C.2.22), but is not expected to be adopted for several years.

When/why the assay may be used

472. Although the MEOGRT could, in principle, be used at any stage in the hazard assessment process, the most likely use scenario will be when there are already some data available to suggest possible endocrine disruption properties. In other words, the MEOGRT will generally be used to investigate whether such potential properties result in adverse apical effects on development, growth or reproduction over an entire life cycle. It is unlikely (and undesirable) that the MEOGRT will be the first ED-responsive test procedure to be applied to a chemical. Furthermore, the conduct of a ZEOGRT in addition to a MEOGRT is not likely to be necessary (for example, to address perceived sensitivity differences). Before either assay is initiated, careful thought should be given to which is more appropriate in the circumstances. For example, if previous data are available with zebrafish and the ZEOGRT is sufficiently powerful for the expected endpoint of concern, then conducting a ZEOGRT may be the correct choice. However, if a genetic sex marker or secondary sexual characters are desired, it may be more beneficial to consider a MEOGRT.

473. This is a comprehensive test which examines a range of potentially adverse apical effects, but also considers several ED-specific endpoints. It is therefore suitable for helping to define whether a test chemical is an ED, and the results could be used in an environmental hazard identification/characterisation for fish. Given the high degree of endocrine system conservation across the vertebrates, adverse endocrine-linked effects in the MEOGRT may also indicate the possibility of related activity in other organisms such as amphibians, reptiles, birds or mammals.

474. 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 test guidelines (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).

Existing data to be considered

475. Existing data available before deployment of the MEOGRT for endocrine disruption hazard assessment are likely to include information on possible modes of action (MOA) from quantitative structure activity relationships (QSARs), adverse outcome pathways (AOP) and/or *in vitro* screens. These may be accompanied by *in vivo* fish assay data from EASZY, the Juvenile Medaka Anti-Androgen Screening Assay, OECD TG 229 and/or OECD TG 230, and may also include data from TG 234 (FSDT). In addition, existing information on endocrine-related effects from other vertebrates (up to and including mammals, e.g. positive findings for endocrine endpoints in mammalian repeat dose toxicity or reproductive studies) should also be considered, given the commonality of endocrine mechanisms in these taxa. It would not be advisable or ethically desirable to conduct a MEOGRT without mechanistic or *in vivo* screening data because it would then be less straightforward to link any apical effects with endocrine disruption. Furthermore, data from OECD TG 229 and/or TG 234 (FSDT), especially if obtained with medaka, could be of use in focusing attention in the MEOGRT on particularly vulnerable parts of the life cycle. Given the high ethical and financial cost of the MEOGRT, it is important to make full use of existing endocrine-related data, both before the test is begun and during data evaluation.

Scenarios: Positive and negative results combined with existing data

476. The scenarios (A to R) presented in Table C.2.13 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. 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. Further considerations specific to each scenario are given in the table.

477. Positive results obtained with one of the MEOGRT apical endpoints result in the conclusion that the test chemical is able to cause adverse effects *in vivo* (Table C.2.13, Scenarios A-I), but not necessarily that it is an ED. Note that if doubt exists about the test performance (e.g. highly unusual results in controls), a comparison with historical control data with respect to overall test performance might be helpful. However, the nature of these effects and any existing data will require careful consideration. If *in vitro* and/or *in vivo* data already exist which reveal possible endocrine disrupting properties (Scenarios A, B and D), a positive apical endpoint in the MEOGRT could lead to a conclusion that the test chemical is an actual ED if adverse population effects are expected as a consequence. This conclusion will, of course, be reinforced if mechanistic endpoints in the MEOGRT itself also respond. The probability that the test chemical is an ED will also be strengthened considerably if the endocrine modality identified in the present or earlier tests is plausibly linked to the responding endpoint. For example, if the chemical has estrogenic properties (such as the induction of vitellogenin in males) and observations indicate reduced fecundity of the F0 or F1 adults in the MEOGRT, this gives added confidence in this conclusion. On the other hand, it may be harder to argue a plausible link between estrogenic properties on the one hand, and an endpoint such as growth or survival on the other, although it is known that some estrogens are able to cause changes in growth rates (Knacker et al., 2010). In this

example, an effect solely on growth or survival, while potentially of concern from the viewpoint of environmental hazard identification/characterisation, would not on its own lead to a conclusion that the chemical is an ED in fish.

478. If a plausible link of a responding MEOGRT apical endpoint with identified endocrine activity can be made, regulatory authorities may conclude that sufficient evidence is available to categorise the chemical as an ED (i.e. interference with the endocrine system has caused adverse effects *in vivo*), and no further information might then be required. It may also be necessary to consider whether or not effects observed are relevant at the population level (e.g. reproduction, growth, development). On the other hand, if data from prior endocrine screens and tests are negative, including negative mechanistic data from the MEOGRT itself (Scenario E), a positive apical response in the MEOGRT would not in general support the hypothesis that the chemical is an ED in fish (although a change in sex ratio may have been caused by an ED). The chemical could, of course, still be subjected to an environmental hazard identification/characterisation.

479. The scenarios in which the MEOGRT gives a negative apical result (Table C.2.13, Scenarios J-R) lead to a tentative conclusion that the test chemical is not an ED in fish, and this conclusion is strengthened considerably if prior screens, or the MEOGRT itself, have failed to reveal endocrine activity (Scenario N). In the latter circumstances, regulatory authorities would be justified in concluding that no further action is needed. On the other hand, if one or more of those screens was positive (Scenarios J-M and P), the bioconcentration factor (BCF) of the chemical should be checked. If the BCF indicates that the chemical is strongly bioaccumulative and reaches equilibrium slowly, it would be worth considering the conduct of an extended MEOGRT (but no TG is available for this), although as indicated above, there is little evidence at present that EDs with a high BCF would be consistently more potent in such a test. If a chemical which screened positive is not bioaccumulative, the probable reasons for lack of effects in the MEOGRT might be metabolism to an inactive chemical, or failure to reach the active site, and no further action would be indicated.

480. In each of the above scenarios, it is possible that existing data will be equivocal (Table C.2.13, Scenarios C, F-I, L and O-R), or there may be no existing data. This will weaken the conclusions which can be drawn about a positive apical endpoint in the MEOGRT, and this is reflected in Table C.2.13. However, as indicated above, it would be undesirable to proceed with a MEOGRT if prior data on endocrine activity are equivocal or absent, and if there are no other effect- or exposure-related reasons for considering such a comprehensive test. On the other hand, if the MEOGRT shows a positive apical endpoint, it would be essential to obtain some reliable mechanistic data before reaching a conclusion about whether or not the chemical is an ED in fish. There is also the possibility that equivocal mechanistic data may be the result of multiple modes of endocrine action. Under some circumstances, two opposite modes of simultaneous action (e.g. estrogenic and anti-estrogenic) could, depending on dose, lead to a minimisation or abolition of adverse effects, while in others two different MOA (e.g. estrogenic and anti-androgenic) could potentially reinforce effects on certain apical endpoints. If multiple MOA are suspected, either from the existing results or based on QSAR/read-across/integrated approaches, this situation should be investigated further if needed for regulatory decision making.

481. The scenario in which the results of the MEOGRT are themselves equivocal has not been dealt with in Table C.2.13, for reasons of brevity. In this context, an equivocal result might be an inconsistent concentration-response (e.g. no effect at a high concentration but effects at a lower concentration), or a result which borders on statistical significance.

Without knowing the exact circumstances, reliable advice cannot be given, but the opinions of an experienced ecotoxicologist should be sought. However, if a comprehensive set of prior screens are all negative, it is doubtful whether further action is needed, because the chemical is unlikely to be an ED. If an endocrine screen is positive, some types of equivocal MEOGRT apical results would have to be taken more seriously. For example, a non-monotonic concentration-response would not necessarily rule out the test chemical as an ED in fish. An example of this would be a chemical like ethinylestradiol, which causes adverse effects (increased fecundity) on fish reproduction at low doses, but reduced reproductive success at very high doses, thus potentially giving a U-shaped response curve (e.g. Jobling et al., 2004). Ideally, concentrations causing systemic toxicity of this type should not be tested in MEOGRT, but such toxicity may have been missed in earlier screens.

482. In summary, positive apical results in the MEOGRT indicate that a chemical is a probable ED if they can be plausibly linked to an endocrine MOA established on the basis of prior mechanistic screening or concurrent observation of mechanistic effects or their biochemical/physiological manifestations. If such screening data are unavailable or negative, it should not be concluded that a positive MEOGRT is the result of endocrine disruption (although it is likely that biased sex ratio will be the result of ED). On the other hand, a negative MEOGRT combined with a sufficiently comprehensive set of negative screening data could lead to a firm conclusion that a chemical is not an ED in fish. A negative MEOGRT set against a background of a positive screen might, however, raise concerns (e.g. if the chemical is strongly bioaccumulative or known to be involved in epigenesist). In this case an extended MEOGRT could be considered, although this is not covered by OECD TG 240, and its effectiveness in this regard is unproven.

References

Flynn, K. et al. (2017), "Summary of the development the US Environmental Protection Agency's Medaka Extended One-Generation Reproduction Test (MEOGRT) using data from 9 multigenerational medaka tests", *Environmental Toxicology and Chemistry*, Vol. 36/12, pp. 3387-3403, https://doi.org/10.1002/etc.3923.

Jobling, S. et al. (2004), "Comparative responses of molluscs and fish to environmental estrogens and an estrogenic effluent", *Aquatic Toxicology*, Vol. 66/2, pp. 207-222, https://doi.org/10.1016/j.aquatox.2004.01.002.

Knacker, T. et al. (2010), "Environmental effect assessment for sexual endocrine-disrupting chemicals: Fish testing strategy", *Integrated Environmental Assessment and Management*, Vol. 6/4, pp. 653-662, https://doi.org/10.1002/ieam.92.

Watanabe, H. et al. (2017), "Medaka Extended One-Generation Reproduction Test (MEOGRT) evaluating 4-nonylphenol", *Environmental Toxicology and Chemistry*, Vol. 36/12, pp. 3254-3266, https://doi.org/10.1002/etc.3895.

WHO/IPCS (2002), "Global assessment of the state-of-the-science of endocrine disrupters", Damstra, T. et al. (eds.) WHO/PCS/EDC/02.2, World Health Organization, Geneva, www.who.int/ipcs/publications/new_issues/endocrine_disruptors/en.

Table C.2.13. **Medaka Extended One-Generation Reproduction Test (MEOGRT) (OECD TG 240): 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 mechanistic data and existing *in vivo* effects 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.

Results of the MEOGRT: * Apical results of the MEOGRT include effects on survival, growth, development, sex ratio and reproduction. The other MEOGRT endpoints, including vitellogenin, secondary sex characteristics, sex ratio (again) and gonadal histopathology, can be indicative of endocrine mechanisms which may have caused the apical effect.

Existing results: ** "Mechanism (*in vitro* and/or *in vivo* 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 also be available. 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. There is no evidence at present that equivalent *in vitro* assays with systems derived from fish offer advantages over their mammalian counterparts.

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.

Scenario	Apical result of MEOGRT*	Existing results		Possible conclusions: 1. Indicators of endocrine activity and apical endpoints positive 2. Indicators of endocrine activity positive and apical endpoints negative 3. Indicators of endocrine activity negative and apical endpoint positive	Next step which could be taken to strengthen weight of evidence if necessary	Other considerations
		Mechanism (*in vitro* and/or *in vivo* mechanistic data)**	Effects (*in vivo* effects of concern)***			
A	+	+	+	1) Strong evidence for adverse effects in fish and other organisms by an endocrine mechanism. 2) Strong evidence for endocrine effects, but they do not appear adverse in fish. 3) Strong evidence for adverse effects in fish and other organisms. There is a possibility that the apical endpoint sex ratio is more sensitive to the test chemical than the mechanistic endpoint vitellogenin (VTG), or mechanism may hypothetically not be via direct interaction with estrogen receptor (ER), androgen receptor (AR) or by aromatase inhibition, even though it is noted that currently there is no evidence for sex ratio change in fish caused by other mechanisms than those mentioned here at otherwise non-toxic concentrations of chemicals.	Further evidence is probably not required.	If the affected apical endpoint in the MEOGRT cannot be plausibly linked to the known modality, the test chemical is unlikely to be an endocrine disruptor (ED). The MEOGRT is unlikely to detect epigenetic effects. If these are suspected, extending the test beyond F2 hatching could be considered, although this would depart from OECD TG 240.
B	+	+	–	1) Strong evidence for adverse effects in fish by an endocrine mechanism. 2) Strong evidence for endocrine effects in fish, but they do not appear adverse. 3) Strong evidence for adverse effects in fish and other organisms. There is a possibility that the apical endpoint sex ratio is more sensitive to the test chemical than the mechanistic endpoint VTG, or mechanism may hypothetically not be via direct interaction with ER, AR or by aromatase inhibition, even though it is noted that currently there is no evidence for sex ratio change in fish caused by other mechanisms than those mentioned here at otherwise non-toxic concentrations of chemicals.	Further evidence is probably not required.	If the affected apical endpoint in the MEOGRT cannot be plausibly linked to the known modality, the test chemical is unlikely to be an ED. The MEOGRT is unlikely to detect epigenetic effects. If these are suspected, extending the test beyond F2 hatching could be considered, although this would depart from TG 240.
C	+	+	Eq/0**	1) Strong evidence for adverse effects in fish by an endocrine mechanism. 2) Strong evidence for endocrine effects in fish, but they do not appear adverse. 3) Strong evidence for adverse effects in fish and other organisms. There is a possibility that the apical endpoint sex ratio is more sensitive to the test chemical than the mechanistic endpoint VTG, or mechanism may hypothetically not be via direct interaction with ER, AR or by aromatase inhibition, even though it is noted that currently there is no evidence for sex ratio change in fish caused by other mechanisms than those mentioned here at otherwise non-toxic concentrations of chemicals.	Further evidence is probably not required.	If the affected apical endpoint in the MEOGRT cannot be plausibly linked to the known modality, the test chemical is unlikely to be an ED. The MEOGRT is unlikely to detect epigenetic effects. If these are suspected, extending the test beyond F2 hatching could be considered, although this would depart from OECD TG 240. It should be borne in mind that equivocal data may be due to a variety of causes, including experimental error, very weak endocrine activity or multiple modes of action (MOA). If the latter case is suspected, it may be necessary to investigate the matter further and/or increase the weight given to the mechanistic information.

Scenario	Apical result of MEOGRT*	Existing results		Possible conclusions: 1. Indicators of endocrine activity and apical endpoints positive 2. Indicators of endocrine activity positive and apical endpoints negative 3. Indicators of endocrine activity negative and apical endpoint positive	Next step which could be taken to strengthen weight of evidence if necessary	Other considerations
		Mechanism (*in vitro* and/or *in vivo* mechanistic data)**	Effects (*in vivo* effects of concern)***			
D	+	–	+	1) Strong evidence for adverse effects in fish and other organisms, possibly by an unknown endocrine mechanism. 2) Medium-strong evidence for endocrine effects in fish, but they do not appear to be adverse. 3) Strong evidence for adverse effects in more than one organism, but mechanism may not be by endocrine disruption.	Further evidence is probably not required.	If the affected apical endpoint in the MEOGRT cannot be plausibly linked to the known modality, the test chemical is unlikely to be an ED. The MEOGRT is unlikely to detect epigenetic effects. If these are suspected, extending the test beyond F2 hatching could be considered, although this would depart from OECD TG 240.
E	+	–	–	1) Strong evidence for adverse effects in fish, possibly by an unknown endocrine mechanism. 2) Medium-strong evidence for endocrine effects in fish, but they do not appear to be adverse. 3) Strong evidence for adverse effects in fish, but mechanism may not be by endocrine disruption.[1]	Further evidence is probably not required.	It is possible that the effects observed in the MEOGRT have been caused by an unknown endocrine mechanism. This would not, however, prevent the chemical being subjected to hazard identification/characterisation. The MEOGRT is unlikely to detect epigenetic effects. If these are suspected, extending the test beyond F2 hatching could be considered, although this would depart from OECD TG 240.
F	+	–	Eq/0	1) Strong evidence for adverse effects in fish, possibly by an unknown endocrine mechanism. 2) Medium-strong evidence for endocrine effects in fish, but they do not appear to be adverse. 3) Strong evidence for adverse effects in fish, but mechanism may not be by endocrine disruption.	Further evidence is probably not required.	It is possible that the effects observed in the MEOGRT have been caused by an unknown endocrine mechanism – equivocal existing *in vivo* data may throw some light on this. The absence of data on a possible endocrine mechanism would, however, not prevent the chemical being subjected to hazard identification/characterisation. The MEOGRT is unlikely to detect epigenetic effects. If these are suspected, extending the test beyond F2 hatching could be considered, although this would depart from OECD TG 240. It should be borne in mind that equivocal data may be due to a variety of causes, including experimental error, very weak endocrine activity or multiple MOA. If the latter case is suspected, it may be necessary to investigate the matter further and/or increase the weight given to the mechanistic information.

Note: 1. However, note that if biased sex ratio is observed, it is likely to have been caused by an endocrine disrupting chemical.

Scenario	Apical result of MEOGRT*	Existing results		Possible conclusions: 1. Indicators of endocrine activity and apical endpoints positive 2. Indicators of endocrine activity positive and apical endpoints negative 3. Indicators of endocrine activity negative and apical endpoint positive	Next step which could be taken to strengthen weight of evidence if necessary	Other considerations
		Mechanism (*in vitro* and/or *in vivo* mechanistic data)**	Effects (*in vivo* effects of concern)***			
G	+	Eq/0	+	1) Strong evidence for adverse effects in more than one organism, possibly by an unknown endocrine mechanism. 2) Medium-strong evidence for endocrine effects, but they do not appear to be adverse in fish. 3) Strong evidence for adverse effects in more than one organism, but mechanism may not be by endocrine disruption.	If reliable mechanistic data are not available, it would be desirable to obtain some.	The test chemical is probably an ED if a modality identified in the newly commissioned mechanistic screens, or in the existing *in vivo* data, can be plausibly linked to the affected endpoint. The MEOGRT is unlikely to detect epigenetic effects. If these are suspected, extending the test beyond F2 hatching could be considered, although this would depart from OECD TG 240. It should be borne in mind that equivocal data may be due to a variety of causes, including experimental error, very weak endocrine activity or multiple MOA. If the latter case is suspected, it may be necessary to investigate the matter further and/or increase the weight given to the mechanistic information.
H	+	Eq/0	–	1) Strong evidence for adverse effects in fish, possibly by an unknown endocrine mechanism. 2) Medium-strong evidence for endocrine effects in fish, but they do not appear to be adverse. 3) Strong evidence for adverse effects in fish, but mechanism may not be by endocrine disruption.	If reliable mechanistic data are not available, it would be desirable to obtain some.	The test chemical is probably an ED if a modality identified in the newly commissioned mechanistic screens can be plausibly linked to the affected endpoint. The MEOGRT is unlikely to detect epigenetic effects. If these are suspected, extending the test beyond F2 hatching could be considered, although this would depart from OECD TG 240. It should be borne in mind that equivocal data may be due to a variety of causes, including experimental error, very weak endocrine activity or multiple MOA. If the latter case is suspected, it may be necessary to investigate the matter further and/or increase the weight given to the mechanistic information.
I	+	Eq/0	Eq/0	1) Strong evidence for adverse effects in fish, possibly by an unknown endocrine mechanism. 2) Moderate-strong evidence for endocrine effects in fish, but they do not appear to be adverse. 3) Strong evidence for adverse effects in fish, but mechanism may not be by endocrine disruption.	If reliable mechanistic data are not available, it would be desirable to obtain some.	The test chemical is probably an ED if a modality identified in the newly commissioned mechanistic screens can be plausibly linked to the affected endpoint. The MEOGRT is unlikely to detect epigenetic effects. If these are suspected, extending the test beyond F2 hatching could be considered, although this would depart from OECD TG 240. It should be borne in mind that equivocal data may be due to a variety of causes, including experimental error, very weak endocrine activity or multiple MOA. If the latter case is suspected, it may be necessary to investigate the matter further and/or increase the weight given to the mechanistic information.

Scenario	Apical result of MEOGRT*	Existing results		Possible conclusions: 1. Indicators of endocrine activity and apical endpoints positive 2. Indicators of endocrine activity positive and apical endpoints negative 3. Indicators of endocrine activity negative and apical endpoint positive	Next step which could be taken to strengthen weight of evidence if necessary	Other considerations
		Mechanism (*in vitro* and/or *in vivo* mechanistic data)**	Effects (*in vivo* effects of concern)***			
J	–	+	+	The chemical is probably not an ED in fish, unless this conclusion is contradicted by existing *in vivo* data.	If the chemical is strongly bioaccumulative, or if epigenetic effects are suspected, an extended version of the MEOGRT could be considered, although this would depart from OECD TG 240.	If any effects in an extended MEOGRT can be plausibly linked with mechanistic data, the test chemical is probably an ED.
K	–	+	–	The chemical is probably not an ED in fish.	If the chemical is strongly bioaccumulative, or if epigenetic effects are suspected, an extended version of the MEOGRT could be considered, although this would depart from OECD TG 240.	If any effects in an extended MEOGRT can be plausibly linked with mechanistic data, the test chemical is probably an ED.
L	–	+	Eq/0	The chemical is probably not an ED in fish.	If the chemical is strongly bioaccumulative, or if epigenetic effects are suspected, an extended version of the MEOGRT could be considered, although this would depart from OECD TG 240.	If any effects in an extended MEOGRT can be plausibly linked with mechanistic data, the test chemical is probably an ED. It should be borne in mind that equivocal data may be due to a variety of causes, including experimental error, very weak endocrine activity or multiple MOA. If the latter case is suspected, it may be necessary to investigate the matter further and/or increase the weight given to the mechanistic information.
M	–	–	+	The chemical is probably not an ED in fish.	If the chemical is strongly bioaccumulative, or if epigenetic effects are suspected, an extended version of the MEOGRT could be considered, although this would depart from OECD TG 240.	If any effects in an extended MEOGRT can be plausibly linked with *in vivo* data which provide information on ED properties, the test chemical is probably an ED, but likely not by a mechanism covered by the existing *in vitro* screens.
N	–	–	–	The chemical is probably not an ED.	Further evidence is probably not required.	–
O	–	–	Eq/0	The chemical is probably not an ED in fish.	Further evidence is probably not required.	It should be borne in mind that equivocal data may be due to a variety of causes, including experimental error, very weak endocrine activity or multiple MOA. If the latter case is suspected, it may be necessary to investigate the matter further and/or increase the weight given to any mechanistic information.

Scenario	Apical result of MEOGRT*	Existing results		Possible conclusions: 1. Indicators of endocrine activity and apical endpoints positive 2. Indicators of endocrine activity positive and apical endpoints negative 3. Indicators of endocrine activity negative and apical endpoint positive	Next step which could be taken to strengthen weight of evidence if necessary	Other considerations
		Mechanism (*in vitro* and/or *in vivo* mechanistic data)**	Effects (*in vivo* effects of concern)***			
P	–	Eq/0	+	The chemical is probably not an ED in fish.	If reliable mechanistic data are not available, it would be desirable to obtain some.	If the newly commissioned mechanistic data are positive and the chemical is strongly bioaccumulative, or if epigenetic effects are suspected, consider conducting an extended MEOGRT, although this would depart from OECD TG 240. It should be borne in mind that equivocal data may be due to a variety of causes, including experimental error, very weak endocrine activity or multiple MOA. If the latter case is suspected, it may be necessary to investigate the matter further and/or increase the weight given to the mechanistic information.
Q	–	Eq/0	–	The chemical is probably not an ED, but confidence in this conclusion is reduced by the lack of clear mechanistic data.	Further evidence is probably not required, but confidence in the conclusion would be increased by the provision of reliable negative mechanistic data.	If the newly commissioned mechanistic data are positive and the chemical is strongly bioaccumulative, or if epigenetic effects are suspected, consider conducting an extended MEOGRT, although this would depart from OECD TG 240. It should be borne in mind that equivocal data may be due to a variety of causes, including experimental error, very weak endocrine activity or multiple MOA. If the latter case is suspected, it may be necessary to investigate the matter further and/or increase the weight given to the mechanistic information.
R	–	Eq/0	Eq/0	The chemical may not be an ED, but confidence in this conclusion is reduced by the lack of clear mechanistic and existing *in vivo* data.	Further evidence is probably not required, but confidence in the conclusion would be increased by the provision of reliable negative mechanistic data.	If the newly commissioned mechanistic data are positive and the chemical is strongly bioaccumulative, or if epigenetic effects are suspected, consider conducting an extended MEOGRT, although this would depart from OECD TG 240. It should be borne in mind that equivocal data may be due to a variety of causes, including experimental error, very weak endocrine activity or multiple MOA. If the latter case is suspected, it may be necessary to investigate the matter further and/or increase the weight given to the mechanistic information.