C.2.7. *Daphnia magna* Reproduction Test (OECD TG 211)

Status: Assay validated by the OECD.

374. Modality detected/endpoints: This *in vivo* assay with *Daphnia magna* was not primarily designed to detect endocrine-active substances, but can be responsive to juvenile hormone (JH) agonists which lead to the production of male offspring (an optional endpoint described in Annex 7 of OECD TG 211). However, it is important to note that *D. magna* can also produce male offspring in response to such natural factors as short photoperiod, temperature fluctuations, decreased food density and increased F0 population density. The lack of internationally validated mechanistic assays for endocrine activity in crustaceans may prevent firm conclusions about whether test chemicals are endocrine disrupters (EDs) in this taxon, although *in vitro* assays for JH and ecdysteroid (Ec) activity are available in the literature. However, the data from the test may nevertheless be of value for classification and hazard identification/characterisation.

Background to the assay

375. This *in vivo* assay with parthenogenetic female *Daphnia magna* is widely used to evaluate the chronic effects of non-endocrine active chemicals, but if it is used to test a JH agonist, it can lead to the production of male neonates. An adverse outcome pathway for this process is under development[1] – significant male production in a population could potentially lead to its decline. However, due to the relatively short-term nature of OECD TG 211, the endpoint of male production should not be considered as an adverse apical endpoint without further investigation in longer term tests.

376. Used in this mode, OECD TG 211 should only be considered as a non-specific screen for *in vivo* JH activity, and a positive result should ideally be followed up with longer term tests such as the *Daphnia* Multi Generation Test (DMGT). However, OECD TG 211 is resource-intensive, and a cheaper option for screening *in vivo* JH activity would be to use the Short-Term Juvenile Hormone Activity Screening Assay (SJHASA). At present, however, OECD TG 211 is the only validated *in vivo* assay which is able to identify potential JH activity.

When/why the assay may be used

377. Although OECD TG 211 could, in principle, be used at any stage in the hazard assessment process, the most likely use scenario will be when there are relatively few data available about the possible JH-disrupting properties of a chemical. The results from this assay are most likely to be available after deployment of a battery of *in vitro* screens, or as a supplement to existing data which suggest possible JH-related activity. Given the significant degree of endocrine system conservation across the arthropods, endocrine-linked effects in OECD TG 211 may also indicate the possibility of related activity in other arthropods such as copepods, decapods and insects.

378. It is possible that no endocrine-relevant data are available before OECD TG 211 is deployed (e.g. if OECD TG 211 has been used as a primary screen, even though the SJHASA may be more appropriate), but in that case a positive result in the screen should probably be followed up with relevant *in vitro* screening, if available, to investigate the suspected mode of action (MOA) in more detail. However, it should be noted that there are no standardised *in vitro* screens for JH agonists, although some are described in the scientific literature (e.g. Cherbas, Koehler and Cherbas [1989]).

Existing data to be considered

379. Existing information on endocrine-related effects from other arthropods should also be considered before deployment of OECD TG 211, given the commonality of endocrine mechanisms in these taxa. Existing data available might also include one or more of a range of *in silico* or *in vitro* results which suggest that JH disruption may occur *in vivo* (but note the limitations of this approach, as indicated above). Such indicators of possible JH activity might include quantitative structure activity relationship (QSAR) predictions of JH activity, "read-across" from *in vivo* results obtained with structurally related chemicals or positive results from an *in vitro* screen for JH agonist activity.

380. 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).

Scenarios: Positive and negative results combined with existing data

381. In the context of this section, the terms positive and negative refer solely to the production or otherwise of male neonates. The scenarios (A to R) presented in Table C.2.7 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.

382. Positive results obtained with the OECD TG 211 (Table C.2.7, Scenarios A-I) result in the conclusion that the test chemical is a possible JH disrupter *in vivo*, at least in crustaceans. However, as indicated above, although a positive response of OECD TG 211 indicates that the chemical is a possible JH agonist, a result of this type would generally need to be followed up with a more comprehensive screen. The most appropriate choice for this is the DMGT (a draft OECD TG). However, if countries need further evidence concerning growth and sexual development etc., a Harpacticoid Copepod Development and Reproduction Test (OECD GD 201) and/or the Sediment-Water Chironomid Life Cycle Toxicity Test (OECD TG 233) would be able to provide a precise no-observed-effect-concentration/x% effect concentration (NOEC/ECx) for adverse effects. This may be particularly important because *Daphnia* are parthenogenic, while *Amphiascus* and *Chironomus* reproduce sexually. In other words, in order to strengthen weight of evidence, a positive result in OECD TG 211 could be followed by a DMGT at Level 5. Existing data suggesting endocrine-specific activity (e.g. positive *in vitro* data, or positive *in vivo* data from other species) will strengthen the case for additional testing still further.

383. The situation in which OECD TG 211 gives a negative result (Table C.2.7, Scenarios J-R) needs careful consideration of any existing data. If these data suggest that the chemical is endocrine active both *in vitro* and *in vivo* (Scenario J), then it is possible that OECD TG 211 is simply insufficiently sensitive.

384. If OECD TG 211 and existing *in vivo* data are all negative, but *in vitro* data reveal some JH activity (Scenario K), the probability is that the test chemical is not sufficiently potent to produce JH agonism *in vivo* in arthropods, or it may be rapidly metabolised. In such a situation, further testing is probably not necessary. However, if the chemical is known to bioaccumulate slowly, it may be that exposures in the *in vivo* tests have been insufficiently prolonged, in which case longer term testing with OECD TG 233 might be justified.

385. On the other hand, if OECD TG 211 and the *in vitro* tests are negative (Scenario M), but there are positive existing *in vivo* data, the nature of those existing data should be considered. Unless the existing data are from another crustacean, the chemical is possibly not a JH agonist acting in crustaceans, but it may be more potent in species (e.g. insects) or life stages that have not been tested. In this situation, the existing *in vivo* data should be used to guide decisions about whether to conduct any further testing.

386. Finally, a negative OECD TG 211, set against a background of negative *in vitro* and *in vivo* data (Scenario N), suggests that the test chemical is probably not a JH agonist *in vitro* or *in vivo*, and further action is unnecessary.

387. In each of the above scenarios, it is possible that existing data will be equivocal, or there may be no existing data. This will weaken the conclusions which can be drawn about a negative OECD TG 211, and this is reflected in Table C.2.7. However, a lack of mechanistic data on JH activity should ideally be rectified before any further *in vivo* testing is finally conducted, although as indicated above, *in vitro* JH screens have not yet been internationally standardised. On the other hand, if OECD TG 211 is positive, further *in vivo* testing would generally be needed to quantify any adverse effects and/or to establish a NOEC or ECx for such effects, even if all existing data are equivocal, or if there are no existing data. Again, however, it may be useful to obtain some mechanistic information before conducting further *in vivo* testing. There is also the possibility that equivocal mechanistic data may be the result of multiple modes of endocrine action. Under rare circumstances, two opposite modes of simultaneous action (e.g. JH agonistic and antagonistic) could, depending on dose, lead to a minimisation or abolition of adverse

effects, while in others two different MOA could potentially reinforce effects on the OECD TG 211 endpoint. 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.

388. The scenario in which the results of OECD TG 211 are themselves equivocal has not been dealt with in Table C.2.7, 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. Clearly, however, such equivocal results do not necessarily rule out the existence of *in vivo* endocrine activity. If possible reasons for false negatives are suspected, OECD TG 211 could be repeated (e.g. conduct it at lower concentrations which avoid systemic toxicity). However, note that a repeat screen in the event of systemic toxicity would not be needed providing at least one tested concentration was not subject to such effects. It should also be borne in mind that changing environmental conditions such as shortening photoperiod, temperature and food shortages can also cause the production of male neonates in *D. magna*, so if these have accidentally occurred during the test, the results may constitute false positives and should be treated as suspect.

389. In summary, positive results in OECD TG 211 may indicate that a chemical is endocrine active *in vivo* via JH agonism. This suggests that more comprehensive *in vivo* testing would be needed if the intention is to derive a long-term NOEC/ECx and/or to confirm whether or not the chemical is an actual endocrine disrupter in arthropods due to the occurrence of adverse effects. Negative results in OECD TG 211 do not necessarily mean that the chemical is not a potential ED – a judgement about the endocrine disruption potential in other arthropods (especially sexually reproducing species) and the possible need for additional testing will have to be made based on a weight of evidence evaluation of existing *in vitro* and *in vivo* data.

Note

1. See: https://aopwiki.org/wiki/index.php/Aop:201.

References

Cherbas, L., M.M.D. Koehler and P. Cherbas (1989), "Effects of juvenile hormone on the ecdysone response of *Drosophila* Kc cells", *Developmental Genetics*, Vol. 10/3, pp. 177-188, https://doi.org/10.1002/dvg.1020100307.

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.2.7. ***Daphnia magna*** **Reproduction Test (OECD TG 211): 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. Note that the terms positive and negative refer solely to the optional male-production endpoint of TG 211.

Existing results: * "Mechanism (*in vitro* mechanistic data)" assumes that mechanistic data are available from available from juvenile hormone (JH-) based assays. JH assays concerning mechanisms of JH disruption may be available, but have not yet been internationally standardised. In practice, data from all assays may not be available and therefore this must be taken into account when deciding on the "next step".

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 a JH disrupter.

Scenarios	Result of OECD TG 211 (male endpoint only)	Existing results		Possible conclusions	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)**			
A	+	+	+	Strong evidence for *in vivo* juvenile hormone (JH) activity in crustaceans, plus possible JH effects in other arthropods.	Consider performing a *Daphnia* Multigeneration Test (DMGT – draft OECD TG).	Based on the limited scope of current *in vitro* screens, the positive *in vitro* data suggest that the test chemical is a JH agonist. The DMGT will show if sex ratio bias towards males carries over into the F2 generation and some regulatory authorities may consider that this provides sufficient information on adverse apical effects in crustaceans. However, as *Daphnia* are parthenogenetic, it would be desirable to perform an additional apical test with sexually reproducing crustaceans and/or insects (e.g. the Harpacticoid Copepod Development and Reproduction Test – OECD GD 201; and/or the Sediment-Water Chironomid Life Cycle Toxicity Test – OECD TG 233).
B	+	+	–	Strong evidence for *in vivo* JH activity in crustaceans.	Consider performing a DMGT (draft OECD TG).	Based on the limited scope of current *in vitro* screens, the positive *in vitro* data suggest that the test chemical is a JH agonist. The DMGT will show if sex ratio bias towards males carries over into the F2 generation and some regulatory authorities may consider that this provides sufficient information on adverse apical effects in crustaceans. However, as *Daphnia* are parthenogenetic, it would be desirable to perform an additional apical test with sexually reproducing crustaceans and/or insects (e.g. the Harpacticoid Copepod Development and Reproduction Test – OECD GD 201; and/or the Sediment-Water Chironomid Life Cycle Toxicity Test – OECD TG 233).
C	+	+	Eq/0	Strong evidence for *in vivo* JH activity in crustaceans.	Consider performing a DMGT (draft OECD TG).	Based on the limited scope of current *in vitro* screens, the positive *in vitro* data suggest that the test chemical is a JH agonist. The DMGT will show if sex ratio bias towards males carries over into the F2 generation and some regulatory authorities may consider that this provides sufficient information on adverse apical effects in crustaceans. However, as *Daphnia* are parthenogenetic, it would be desirable to perform an additional apical test with sexually reproducing crustaceans and/or insects (e.g. the Harpacticoid Copepod Development and Reproduction Test – OECD GD 201; and/or the Sediment-Water Chironomid Life Cycle Toxicity Test – OECD TG 233). 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.

Scenarios	Result of OECD TG 211 (male endpoint only)	Existing results		Possible conclusions	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	+	–	+	Moderate evidence for *in vivo* JH activity in crustaceans, plus possible JH effects in other arthropods.	Consider performing a DMGT (draft OECD TG).	The lack of *in vitro* JH activity is not evidence against any JH activity, due to the limited nature of current *in vitro* JH screens. The DMGT will show if sex ratio bias towards males carries over into the F2 generation and some regulatory authorities may consider that this provides sufficient information on adverse apical effects in crustaceans. However, as *Daphnia* are parthenogenetic, it would be desirable to perform an additional apical test with sexually reproducing crustaceans and/or insects (e.g. the Harpacticoid Copepod Development and Reproduction Test – OECD GD 201; and/or the Sediment-Water Chironomid Life Cycle Toxicity Test – OECD TG 233).
E	+	–	–	Some evidence for *in vivo* JH activity in crustaceans.	Consider performing a DMGT (draft OECD TG).	The lack of *in vitro* JH activity is not evidence against any JH activity, due to the limited nature of current *in vitro* JH screens. The DMGT will show if sex ratio bias towards males carries over into the F2 generation and some regulatory authorities may consider that this provides sufficient information on adverse apical effects in crustaceans. However, as *Daphnia* are parthenogenetic, it would be desirable to perform an additional apical test with sexually reproducing crustaceans and/or insects (e.g. the Harpacticoid Copepod Development and Reproduction Test – OECD GD 201; and/or the Sediment-Water Chironomid Life Cycle Toxicity Test – OECD TG 233).
F	+	–	Eq/0	Some evidence for *in vivo* JH activity in crustaceans.	Consider performing a DMGT (draft OECD TG). Given the absence or equivocal nature of existing *in vivo* data, it might also be sensible to conduct a JH-responsive insect assay (e.g. the Sediment-Water Chironomid Life Cycle Toxicity Test – OECD TG 233).	The lack of *in vitro* JH activity is not evidence against any JH activity, due to the limited nature of current *in vitro* JH screens. The DMGT will show if sex ratio bias towards males carries over into the F2 generation and some regulatory authorities may consider that this provides sufficient information on adverse apical effects in crustaceans. However, as *Daphnia* are parthenogenetic, it would be desirable to perform an additional apical test with sexually reproducing crustaceans (e.g. the Harpacticoid Copepod Development and Reproduction Test – OECD GD 201) 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.

Scenarios	Result of OECD TG 211 (male endpoint only)	Existing results		Possible conclusions	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)**			
G	+	Eq/0	+	Moderate evidence for *in vivo* JH activity in crustaceans, plus possible JH effects in other arthropods.	Consider performing a DMGT (draft OECD TG). Given the absence or equivocal nature of the *in vitro* mechanistic data, it might also be helpful to conduct an *in vitro* screen for JH activity.	If a new *in vitro* mechanistic assay is conducted, note that a negative result does not mean that the test material has no JH activity. The DMGT will show if sex ratio bias towards males carries over into the F2 generation and some regulatory authorities may consider that this provides sufficient information on adverse apical effects in crustaceans. However, as *Daphnia* are parthenogenetic, it would be desirable to perform an additional apical test with sexually reproducing crustaceans (e.g. the Harpacticoid Copepod Development and Reproduction Test – OECD GD 201 and/or the Sediment-Water Chironomid Life Cycle Toxicity Test – OECD TG 233). 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	–	Some evidence for *in vivo* JH activity in crustaceans.	Consider performing a DMGT (draft OECD TG). Given the absence or equivocal nature of the *in vitro* mechanistic data, it might also be helpful to conduct an *in vitro* screen for JH activity.	The DMGT will show if sex ratio bias towards males carries over into the F2 generation and some regulatory authorities may consider that this provides sufficient information on adverse apical effects in crustaceans. However, as *Daphnia* are parthenogenetic, it would be desirable to perform an additional apical test with sexually reproducing crustaceans (e.g. the Harpacticoid Copepod Development and Reproduction Test – OECD GD 201 and/or the Sediment-Water Chironomid Life Cycle Toxicity Test – OECD TG 233). 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	Some evidence for *in vivo* JH activity in crustaceans.	Consider performing a DMGT (draft OECD TG). Given the absence or equivocal nature of the *in vitro* mechanistic data, it might also be helpful to conduct an *in vitro* screen for JH activity.	If a new *in vitro* mechanistic assay is conducted, note that a negative result does not mean that the test material has no JH activity. The DMGT will show if sex ratio bias towards males carries over into the F2 generation and some regulatory authorities may consider that this provides sufficient information on adverse apical effects in crustaceans. However, as *Daphnia* are parthenogenetic, it would be desirable to perform an additional apical test with sexually reproducing crustaceans (e.g. the Harpacticoid Copepod Development and Reproduction Test – OECD GD 201 and/or the Sediment-Water Chironomid Life Cycle Toxicity Test – OECD TG 233). 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.

Scenarios	Result of OECD TG 211 (male endpoint only)	Existing results		Possible conclusions	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)**			
J	–	+	+	The test chemical is probably a JH agonist without activity in crustaceans, although it is possible that *Daphnia magna* responds atypically in this case.	Some regulatory authorities may conclude that no further evidence is required. However, it might be desirable to obtain data from insects (e.g. the Sediment-Water Chironomid Life Cycle Toxicity Test – OECD TG 233) if these are not already available.	Based on the limited scope of current *in vitro* screens, the positive *in vitro* data suggest that the test chemical is a JH agonist.
K	–	+	–	The test chemical is likely to have JH activity; however, without demonstrating sufficient activity to disrupt physiological processes *in vivo*.	If there is no activity in crustaceans or insects, further evidence is probably not needed.	Based on the limited scope of current *in vitro* screens, the positive *in vitro* data suggest that the test chemical is a JH agonist.
L	–	+	Eq/0	The test chemical is likely to have JH activity; however, without demonstrating sufficient activity to disrupt physiological processes *in vivo*.	Some regulatory authorities may conclude that no further evidence is required, but if insect data are absent, it might be desirable to conduct a Sediment-Water Chironomid Life Cycle Toxicity Test – OECD TG 233.	Based on the limited scope of current *in vitro* screens, the positive *in vitro* data suggest that the test chemical is a JH agonist. 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 test chemical is probably without JH activity in crustaceans, although it is possible that *Daphnia magna* responds atypically in this case.	Some regulatory authorities may conclude that no further evidence is required. However, it might be desirable to obtain data from insects (e.g. the Sediment-Water Chironomid Life Cycle Toxicity Test – OECD TG 233) if these are not already available.	The lack of *in vitro* JH activity is not evidence against any JH activity, due to the limited nature of current *in vitro* JH screens. However, it is possible that the existing effects may not be due to JH activity.
N	–	–	–	The test chemical is probably without JH activity in arthropods.	No further action is necessary.	–
O	–	–	Eq/0	The test chemical is probably without JH activity in arthropods.	Some regulatory authorities may conclude that no further evidence is required. However, it might be desirable to obtain data from insects (e.g. the Sediment-Water Chironomid Life Cycle Toxicity Test – OECD TG 233) if these are not already available.	The lack of *in vitro* JH activity is not evidence against any JH activity, due to the limited nature of current *in vitro* JH screens. 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.

Scenarios	Result of OECD TG 211 (male endpoint only)	Existing results		Possible conclusions	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)**			
P	–	Eq/0	+	The test chemical is probably without JH activity in crustaceans, although it is possible that *Daphnia magna* responds atypically in this case.	Some regulatory authorities may conclude that no further evidence is required. Also, if clear *in vitro* mechanistic data are missing, it might be desirable to obtain some.	If a new *in vitro* mechanistic assay is conducted, note that a negative result does not mean that the test material has no JH activity. However, it is possible that the existing effects may not be due to JH activity. 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 test chemical is probably without JH activity in arthropods.	No further action is necessary.	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 test chemical is probably without JH activity in crustaceans and possibly insects.	Some regulatory authorities may conclude that no further evidence is required. However, it might be desirable to obtain data from insects (e.g. the Sediment-Water Chironomid Life Cycle Toxicity Test – OECD TG 233) if these are not already available.	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.