Introduction to specific guidelines

125. This introduction applies to all assays covered by this guidance document (GD), although it should be noted that guidance for test guidelines (TGs) that have not received full validation by the OECD, or are in the process of OECD validation, remains provisional until those assays have been fully validated with endocrine active substances (EASs) and the TG published.

126. As indicated earlier, the information given in Section C is intended to provide guidance on the interpretation of data from individual assays, and on a possible next step for obtaining additional data, if required by a given user. It is important to understand that the guidance should be used flexibly in the light of local regulatory circumstances and available data – it is not a rigid prescription, but should be considered as a decision-support tool. The guidance in Section C adopts a form of weight of evidence (WOE) approach that uses all available data and expert judgement, but it should be noted that other WOE methodologies are available (see Section B.5).

127. Discussion of each assay takes the form of textual guidance which describes the basis of the assay and any special considerations or limitations, when and why the assay is likely to be used, and what broad conclusions may be appropriate when one is in possession of positive, negative or equivocal results. This is followed by a table (known as a "building block") that elaborates that guidance for each of a number of data scenarios. Thus, for each type of assay result, the guidance varies depending on the type and amount of pre-existing data (both *in vitro* and *in vivo*). The intention has been to cover all the major possible scenarios, but the document cannot address all eventualities. Furthermore, it is implicit that expert advice will need to be consulted at many points in these building blocks – they are not recipes which can be followed blindly. Note that some scenarios are much less likely to occur than others – for example, it is unlikely (but still possible) that a higher tier procedure such as a fish life cycle test will have been performed in the absence of various screening assays. A large range of possible scenarios has, therefore, been described for the sake of completeness.

128. When considering a possible "next step" in evidence gathering that could follow from a particular result in an *in vitro* assay, guidance is given in the next section about suitable *in vivo* testing with vertebrate species. It is, of course, important to ensure that an *in vitro* assay has been conducted at realistic exposure levels before concluding on the possible need for *in vivo* testing. Some guidance is also given concerning possible mammalian tests that might be conducted following positive non-mammalian tests, and vice versa. Experience using these assays, particularly in the United States Environmental Protection Agency's (US EPA) Endocrine Disruptor Screening Program (EDSP) Tier 1, has demonstrated a high degree of cross-species sensitivity (Ankley and Gray, 2013). A positive result in an endocrine disrupter-responsive mammalian assay could be interpreted as an alert about possible related effects in non-mammalian wildlife, and the reverse also applies (although mammalian assays will often have been performed before any with non-mammals). Positive effects in mammalian assays should generally be regarded as a trigger for

some non-mammalian testing if the hazards experienced by the non-mammalian group are to be taken into account. On the other hand, insufficient data yet exist to be confident that negative mammalian data imply an absence of effects in non-mammalian wildlife, even when assuming the pathway under investigation is present and relatively well conserved.

129. It will be apparent that the underlying approach when implementing this guidance is to consider the weight of available evidence – situations in which a single assay provides conclusive evidence that a chemical is an ED may not be common, although there will be exceptions. For example, feminised anogenital distance in male offspring (observed in OECD TG 443, TG 421/422, TG 414 or TG 416) may be considered as conclusive evidence of an endocrine disrupting effect. OECD GD 43 (Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment; OECD, 2008) states: "A statistically significant change in [anogenital distance] that cannot be explained by the size of the animal indicates effects of the exposure and should be used for setting the [no observed adverse effect level]." It is vital to consider all relevant data on the test chemical, including their quantity, type and quality. For example, without adequate mechanistic data from (quantitative) structure activity relationships (QSARs), *in vitro* and/or other *in vivo* assays, or from the *in vivo* assay under consideration, it will often not be possible to conclude with confidence that any apical effects have been caused by an endocrine mode of action. Indeed, any linkage between mechanistic data and apical responses will probably have to be assessed according to the weight of evidence and is unlikely to be confirmed absolutely. Another example of the use of WOE concerns *in vivo* screening assays which may indicate that a chemical can interfere with the endocrine system in intact animals, but will sometimes not be able to provide data on apical effects, or supply information which could be used on its own in a full hazard identification/characterisation of endocrine disruption. In such situations, more complete apical data may have to be obtained from a higher tier test, which will then be evaluated in conjunction with the screening data. Note, however, that negative data from a higher tier test should generally be given more weight than positive data from a lower tier screen, assuming the same class of vertebrates has been employed at both tiers, the quality of the data is good, the suspected mechanism or mode of action is adequately covered by apical endpoints, and a sensitive life stage has been used in the higher tier negative test.

130. The guidance in this document is considered reliable for estrogen/androgen/thyroid/steroidogenesis (E,A,T,S) modalities, and in certain invertebrates, juvenile hormone, ecdysone or retinoid-related activity. However, it is recognised that other endocrine modes of action exist and that some assays have not yet been fully validated (e.g. see OECD [2012]). The field of endocrine disruption continues to develop, so for that reason, this is still a "living document" which will be subject to amendment as new data are generated, new modalities are described and new assays are published as test guidelines.

131. Users of this GD should be aware that comparisons of no-effect doses or concentrations from different types of test may be very difficult or impossible. This is obvious if one is trying to compare an oral dose in a mammalian or avian test with an ambient concentration in an aquatic test. However, caution should also be used when making comparisons within these two major types of test if different methods have been used to calculate the no-effect dose or concentration (e.g. if test concentrations in one test were nominal and in the other were measured).

References

Ankley, G.T. and L.E. Gray (2013), "Cross-species conservation of endocrine pathways: A critical analysis of tier 1 fish and rat screening assays with 12 model chemicals", *Environmental Toxicology and Chemistry*, Vol. 32/5, pp. 1084-1087, https://doi.org/10.1002/etc.2151.

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