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ENVIRONMENT DIRECTORATE
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THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

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Test Guidelines Programme

Draft Summary Record: 28th Meeting of the Working Group of the National Coordinators of the Test Guidelines Programme

19-22 April 2016
OECD Conference Centre, Room CC15, 2 rue André-Pascal, 75775 Paris cédex 16, France

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GENERAL AND PROGRAMME-RELATED ISSUES

1. Opening of the meeting, adoption of the draft agenda

1. The WNT meeting was open by the Chair, Tim Singer (Canada), and was attended by forty six participants from member countries, the European commission (EC), People's Republic of China, BIAC and ICAPO. The draft agenda, dated 7 March 2016, was adopted without changes.

2. Room document 1 [ENV/JM/TG/RD(2016)1] containing all late comments received until 8 April 2016 was brought to the attention of the WNT.

2. Approval of the draft summary record of WNT-27

3. The WNT approved the draft Summary Record of their previous meeting ENV/JM/TG/M(2015)1.

3. Follow-up from the last WNT meeting publications, useful resources and material for the WNT and expert groups

4. The Secretariat reported on information and documents published since the last WNT (public page on avian toxicity testing work at OECD: http://www.oecd.org/chemicalsafety/testing/avian-toxicity-testing.htm), and work on-going to link Test Guidelines with documents in the Series on Testing and Assessment (e.g., validation reports and guidance documents). Also, the Secretariat noted that the public page providing software and Excel sheets for recording test data in support of Test Guidelines will be updated to include a disclaimer about the validation and quality assurance status of these tools for those using them in a GLP environment.

4. Follow-up on issues related to intellectual property in Test Guidelines

5. The Secretariat presented the agenda document ENV/JM/TG(2016)26 including a text proposal to clarify to the public the OECD expectations and best practice as regards licensing and standards containing elements of intellectual property. The text was approved with two minor modifications and made available on-line soon after the meeting on the OECD public site (http://www.oecd.org/env/ehs/testing/oecdguidelinesforthetestingofchemicals.htm).

5. Preparation of Joint Meeting discussions in November 2016 on governance of IATA activities at OECD

6. The Secretariat presented the agenda document ENV/JM/TG(2016)2 outlining a proposal for clearer governance of the OECD activities on integrated approaches to testing and assessment (IATA), as requested by the Joint Meeting in February 2016. A few text clarifications were made to the types of IATA activities, diagram 1, paragraph 19 on the intended goal of testing strategies in relation to the 3Rs, and a new paragraph 20 was drafted and agreed during the meeting.

7. On 25 April a revised version of the document was submitted on OLIS [see ENV/JM/TG(2016)2/REV1]. The revised document will be discussed by the Task Force on Hazard Assessment as well, and a consolidated proposal made to the Joint Meeting in November 2016.

6. Issue of re-testing and redundant testing discussed at the Joint Meeting in February 2016

8. The Secretariat presented document ENV/JM/TG(2016)33 describing issues related to the use of OECD Test Guidelines that potentially lead to duplicative and unnecessary testing, and elaborating proposals to improve clarity in the sound use of Test Guidelines. Especially, it should be made clear that the regulatory requirements determine which test method to use. An earlier version of the document was the basis of discussion at the Joint Meeting in February 2016.

9. The WNT asked for several modifications to the table to avoid misguiding users on Test Guidelines and alternatives, and to give more visibility to background documents providing overviews in specific areas, and which should be read first. All Test Guidelines will be presented in one column only and a second column should provide succinct notes and considerations. ICAPO proposed an example of what the table could look like for the case of skin irritation and skin corrosion. This example was well received by the WNT. ICAPO and BIAC agreed to continue revising the table for other toxicity testing areas for review by the WNT following the meeting. Eventually the text and examples, once agreed by the WNT will be made available on the public site for reference.

APPROVAL OF NEW AND UPDATED TEST GUIDELINES AND GUIDANCE DOCUMENTS

7. Draft new Test Guidelines on molluscs partial life-cycle toxicity tests

ENV/JM/TG(2016)3, ENV/JM/TG(2016)4, ENV/JM/TG(2016)5, ENV/JM/TG(2016)6, ENV/JM/TG/RD(2016)2, ENV/JM/TG/RD(2016)3

10. The Secretariat briefly introduced the two draft new Test Guidelines for Mollusc reproductive toxicity tests, i.e. using *Lymnea stagnalis* [ENV /JM /TG(2016)3] and using *Potamopyrgus antipodarum* [ENV /JM /TG(2016)5] respectively. No late comments were received for these draft TGs or the validation reports.

11. The WNT approved the two Test Guidelines, and their validation reports, with the following modifications.

- Lymnaea, Paragraph 10: the validity criteria for dissolved oxygen content and for water mean temperature apply to both the control and the exposure groups.
- Lymnaea, Annex 1: the definition for reproductive output has been aligned with the wording in paragraph 49 and becomes: **Production of clutches per surviving parent organism over the test duration (28 days)**~~Offspring production by parental animals within the test period~~
- Potamopyrgus, Paragraph 9: the validity criteria for dissolved oxygen content and for water mean temperature apply to both the control and the exposure groups.
- Potamopyrgus, paragraph 11: reference to identification methods is included, i.e.: **Städler et al. (6) and Warwick (7) describe distinct genetic and anatomical markers of haplotype t / morphotype "Warwick A".**
- Potamopyrgus, paragraph 19: a sentence to avoid possible bias due to differences in reproduction at the start of the test has been included, i.e.: **Additionally, the coefficient of variation for the mean embryo number should not exceed 40%.**
- Potamopyrgus, Annex 1, Temperature and light regime: The light intensity should be in the range ~~250~~-500+/- **100** lx.

8. Draft updated Test Guidelines 220-222-226-232 on Soil Toxicity Testing

12. The Secretariat briefly introduced the proposed updated Test Guidelines on soil toxicity testing, modified for the criteria determining their applicability for volatile test chemicals [ENV /JM /TG(2016)7, ENV /JM /TG(2016)8, ENV/JM/TG(2016)9, ENV/JM/TG(2016)10].

13. A request to update the reference to ISO standards was accepted. Another request was made to extend the changes proposed in the above mentioned Test Guidelines to the Test Guideline 228 on dipteran dung flies development test, although not strictly a 'soil' toxicity test, room document ENV/JM/TG/RD(2016)17 was produced showing similar changes in the dung flies test as in the other four soil toxicity Test Guidelines. All five test Guidelines were agreed without further modifications during the meeting, except the updated ISO references.

9. Draft updated Test Guideline 223 on Avian Acute Oral Toxicity

14. The Secretariat briefly introduced changes proposed to TG 223 on avian acute toxicity [ENV/JM/TG(2016)11]. A number of late comments and requests for modifications were available in ENV/JM/TG/RD(2016)1. The main changes concerned the mortality in the control group to consider the study valid.

15. The WNT agreed to the following changes to the draft updated TG 223 to reconcile the various views and bring more clarity to the text :

- Paragraph 12: "**If there is any more than 10% mortality in the control group, the study is considered invalid.** ~~If there are any control mortalities, when five control birds are used, the study is considered invalid.~~ No additional control birds should be added during the course of the study, except as otherwise noted in paragraph **25 or** 45. "
- Paragraph 17 (end): "**Further details on the care of bird species can be found in (Felasa 2007)**."
- Standard language for testing mixtures for regulatory purpose and for reporting the identity of the test chemical was also added.

10. Draft new Guidance Document on Honeybee Larval Toxicity following Repeated Dose Exposure

16. The lead country and Secretariat presented document ENV/JM/TG(2016)12 providing guidance on toxicity to honeybees following repeated exposure. Following a number of late comments submitted and visible in ENV/JM/TG/RD(2016)1, the Secretariat and lead country proposed the following modifications, which were all agreed by the WNT:

- Paragraph 4: "(…) Mortality and other observations/abnormal effects are recorded daily from ~~D3~~**D4** to D8 and on D15 of the test, and emergence rate should be recorded on D22. (…)";
- Paragraph 7: the validity criteria apply: "**across all replicates**" was added for each of the three validity criterion;
- Paragraph 10: "(…) The pieces of dental roll ~~(not mandatory) may be~~ **are** removed from the wells. The container is then placed into an incubator at 34-35 ºC."
- Paragraph 11: "(…) The boxes are transferred into an incubator at 34-35°C with a relative humidity of ~~50% +/- 10%~~ **within the range 50 - 80%.**
- Figure 5: Figure 5 will be updated to reflect the amendment mentioned for paragraph 11, i.e. to reflect the humidity range of 50-80% during the emergence stage.
- Paragraph 12: "(…) It is recommended checking bee colonies for signs of variability and test performance before starting the season by conducting a pre-test with a representative number of larvae per potential test colony."
- Paragraph 20: "(…) **Nevertheless, one should be aware that the analytical measurement of the test chemical concentration in the diet is more difficult because of the presence of royal jelly.** ~~Note: the analytical measurement of the test chemical concentration in the diet is difficult because of the presence of royal jelly).~~ **Any difficulties with analytics and their justification should be reported in the test report.**
- Paragraph 29: "(…) The presence of uneaten food on D8 should be qualitatively recorded~~, i.e. range of % of unconsumed diet~~. "
- Paragraph 31: "On D22, the number of emerged adults and non-emerged bees are counted (see paragraph 33) and the test is terminated by freezing the plates at ≤ -10C**, or preferably ≤-80ºC or using other humane methods**."

- Paragraph 32: "Following the first chemical exposure on D3, mortalities are checked and recorded at the time of feeding from D4 to D8 and on D15. An immobile larva or a larva which does not react to the contact of the grafting tool or paintbrush, **or which does not show signs of respiration under a stereomicroscope** is noted as dead. (…)"
- Paragraph 34: "Other observations, e.g. larval appearance and size, behaviour, morphological differences and any other adverse effects after emergence (in comparison with controls) should be recorded qualitatively. **Bees showing severely impaired behaviour or other severe effect after emergence that suggest bees are in pain should be euthanised immediately using the most humane method.** The presence of uneaten food on D8 should be qualitatively recorded."
- Paragraph 35: "Data are summarised (e.g. in a tabular form), showing for each treatment group, as well as control and reference chemical groups, the number of larvae used, mortalities and adverse effects: larval mortalities from D3 to D8, pupal mortalities from D8 to D15 and emergence rate on D22 ~~(i.e. bees that did not leave their cell)~~. (…)"
- Paragraph 36: "The NOEC/NOED is determined on D22 for adult emergence (see Annex 2 for definitions). In case no effects are detected at all test concentrations/doses the NOEC/NOED will be considered to be **higher or equal to** the highest concentration tested. If, in a limit test, the effect at the tested concentration/dose is not statistically significantly different from the control, ~~the NOEC/NOED is considered to be the tested concentration/dose. In that case~~, it should be indicated that the NOEC/NOED is **higher or equal to** the concentration/dose tested.
- Results section: "The mortality from D3 to D8, on D15 and emergence rate on D22, NOEC/NOED and/or EC/ED, and/or any EC/ED for adult emergence on D22, and a graph of the fitted model, the slope of the concentration-response curve **and its corresponding 95% confidence limits and the criteria for goodness of fit** ~~and its standard error~~; statistical/mathematical procedures used for the determination of the NOEC/NOED and EC/EC if appropriate;"
- Results section: addition of "**Any difficulties with analytics and their justifications.**"
- Annex 3: pictures of pupae at D8 and D15 under control conditions will be provided by the lead country and added to Annex 3 in the TG to facilitate comparison with the existing pictures of pupae at D8 and D15 under treated conditions in Annex 3.

17. BIAC expressed concern having a 70% emergence rate as a validity criterion; however Secretariat and lead country France reminded that emergence rates routinely higher than 90% were reported by Canada [ENV/JM/RD(2016)6] and rates higher than 70% have been obtained in the ring trial.

11. Draft updated Test Guidelines 412 and 413 on 28-d and 90-d Repeated Inhalation Toxicity Studies

18. The Secretariat presented the outcome of discussions and the current status of revisions of TG 412 and TG 413 to adapt them to the safety testing of manufactured nanoparticles [see ENV /JM /TG(2016)13 /REV and ENV /JM /TG(2016)14 /REV]. At the time of the meeting, there were still a number of issues that remained unresolved. The WNT managed to clearly identify these issues: upper limit concentration, range-finding study, testing in one sex in the main study, conducting histopathology, bronchoalveolar lavage and lung burden in the same animal, measuring lung burden, and the feasibility study. The WNT provided a common position on each issue and a recommendation for further work by the expert group (see Annex 1).

19. It was agreed at the WNT that an expert meeting should be convened in September or October to work on the finalisation of TG 412 and TG 413, as well as other companion documents if possible (Guidance documents 39 and 125, TG 403 and TG 436). The dates and venue (United States, Netherlands or OECD) will be confirmed soon after the WNT via an invitation letter. Expectations are that the draft Test Guidelines will be submitted for approval at the next meeting of the WNT in 2017.

20. ECHA asked whether the Joint Expert Group could consider indicating what is/are the most important parameter(s) that should be measured in the Repeated Inhalation Toxicity Studies (TG412 and TG413). In addition, ECHA also invited the Joint Expert Group to consider whether the appropriateness of developing parallel TGs that only address nanomaterials.

12. Draft new Test Guideline 433 on Acute Inhalation Toxicity- Fixed Concentration Procedure

21. The draft TG 433 presented in ENV /JM /TG(2016)15 received many late comments, gathered in ENV/JM/TG/RD(2016)1. The main issue still unresolved concerns signs of evident toxicity that are not sufficiently defined regarding their predictivity. Also, the selection of chemicals used in the desk study was criticised as being over-representing gases and vapours compared to particles, while those elicit specific effects that are not necessarily well captured under the current signs of toxicity selected. Finally the number of animals was also part of the issues raised in the discussion.

22. The WNT agreed that there is still support for pursuing efforts to find a solution and recommended that a teleconference be organised between the United Kingdom, the United States and the Netherlands to address those remaining issues. The United Kingdom was hoping this could be done in a short timeframe, with the view to get the draft TG 433 approved via written procedure, otherwise at WNT in 2017.

13. Draft Guidance Document on Bridging or Waiving Acute Mammalian Toxicity Studies

23. The Secretariat introduced agenda document ENV/JM/TG(2016)16 providing guidance for bridging or waiving acute mammalian toxicity studies. The following changes to the document were made and agreed based on late comments received and available in ENV/JM/TG/RD(2016)1 or requests made at the meeting itself:

- Paragraph 1: "(…) Another approach to reducing or eliminating animal testing is to use existing hazard information that is informative for the acute toxicity endpoint for the test chemical; this would include the use of hazard information for one or multiple similar test chemicals to characterize the hazard for another (often referred to as ~~bridging or~~ read-across) **or for mixtures, the use of recognized calculation approaches and bridging concepts**. (…)"
- Paragraph 6: "(…) The added value of the toxicological information for risk management can be a further consideration in some cases. **For example, ICH guidance has removed the requirement for traditional acute toxicity studies due to their limited value for predicting consequences of overdose in humans; ICH guidance points to dose-escalation studies or short duration dose-ranging studies as alternate sources for acute toxicity information (ICH reference here). However, this does not directly apply in cases where repeated dose toxicity studies are not available, and when information on acute toxicity may be relevant.** (…)"
- Paragraph 13: "(…) A waiver will be considered if the oral LD_{50} of the test chemical is predicted to be greater than 2000 mg/kg bw based on the results of a validated and/or accepted alternative test or test battery provided the test system was shown to have high sensitivity and the applicability domain is inclusive of the chemistry under investigation. ~~Current in vitro cytotoxicity tests are generally insufficient as stand-alone methods due to their limited predictive ability for test chemicals that require metabolic activation or for test chemicals that affect specific modes of action~~. (…)"
- Acute dermal toxicity, paragraph 16: a reference to the EPA and REACH recently released policies will be inserted at the end of the paragraph.
- Paragraph 26: "The OECD inhalation test guidelines and Guidance Document 39 recommend that **corrosive test chemicals should be assessed and tested following expert judgement on a case-by-case basis and where** testing corrosive chemicals is required, it should be carried out at targeted concentrations that are low enough to not cause marked pain and distress, yet sufficient to extend the concentration-response curve to levels that reach the regulatory and scientific objectives of the test. (…) ~~In addition to the appropriate acute inhalation classification and labelling indicated for a diluted preparation of a corrosive test chemical, consideration should be given to retaining a corrosion hazard statement such as "corrosive" or "corrosive to the respiratory tract" for the undiluted test chemical~~."
- Paragraph 28: "The determination of corrosion is based on validated and/or accepted in vivo, in vitro or other data, or in the absence of any other information, when a test chemical has a pH less than or equal to 2 or greater than or equal to 11.5 together with high buffering capacity **when relevant** (OECD, 2014b). Such test chemicals will be considered as Category 1 dermal corrosives under the GHS for labelling purposes. It cannot be ruled out that some test chemicals may be over-predicted based solely on pH considerations. Accordingly, using the acid/alkali reserve method, **especially for classification of mixtures containing acidic or alkaline substances** (Young at el, 1988), or testing with in vitro methods can be performed as an alternate approach for test chemicals with strong acidity or alkalinity.

- Paragraph 34: "(…) The determination of corrosion is based on validated and/or accepted in vivo, in vitro or other data, or in the absence of any other information, when a test chemical has a pH less than 2 or greater than 11.5 together with high buffering capacity **when relevant** (OECD, **2014b**). In this case, the test chemical should be considered in GHS Category 1 for serious eye damage. **In fact, the potential for eye damage is reflected in the GHS hazard statement for a test chemical that is corrosive to skin which states "Causes severe skin burns and eye damage**".
- Paragraph 41: "The determination of corrosion is based on validated and/or accepted in vivo, in vitro or other data, or in the absence of any other information, when a test chemical has a pH less than 2 or greater than 11.5 together with high buffering capacity **when relevant (OECD, 2014b)**.

14. Draft new Test Guideline on in vitro skin sensitisation: human Cell Line Activation Test (hCLAT)

24. The Secretariat presented document [ENV /JM /TG(2016)17] on a new Test Guideline on in vitro skin sensitisation: human Cell Line Activation Test (hCLAT). The draft Test Guideline was approved with the following changes (shaded text was added, text stroked through was removed):

- Paragraph 27: […] Then, 1.2-fold serial dilutions are made using the corresponding solvent/vehicle to obtain the stock solutions (eight concentrations ranging from ~~0.335 × CV75 to 1.2 × CV75~~**100×1.2 × CV75 to 100×0.335 × CV75 (for saline or medium) or from 500×1.2 × CV75 to 500×0.335 × CV75 (for DMSO)**) to be tested in the h-CLAT method […]
- Paragraph 36: Negative results are acceptable only for test chemicals exhibiting a cell viability of less than 90% at **the highest concentration tested (i.e.** 1.2 × CV75 **according to the serial dilution scheme described in paragraph 27)**~~(or highest concentration)~~. If the cell viability at 1.2 × CV75 is equal or above 90% the negative result should be discarded. […]

15. Draft Guidance Document on OECD Genetic Toxicity Test Guidelines

25. The Secretariat presented document [ENV /JM /TG(2016)18] on an overview of the set of OECD Genetic Toxicology Test Guidelines and updates performed in 2014-2015. The WNT supported changes that had been proposed by the Secretariat in advance of the meeting, including a change in the title to be more accurate and better conform with the scope of the document; actually the document describes the consensus based changes to TGs and is not a Guidance Document per say. It was recognised that it is difficult to go beyond what is now available. The WNT recommended however to consider two axes for guidance development in the future:

- Initiate discussion on future work priority setting on improvement of OECD TGs on genetic toxicology, including describing what the current set of OECD TGs does, and does not cover, identification of areas where further testing development would be needed, identification of gaps, identifications of areas where science is mature enough to start TG development.
- Consider developing guidance on TG related issues such as what might be indicative of systemic exposure, the influence of fasting vs non fasting animals, and other topics included in document [ENV/JM/TG/RD(2016)12].

26. Interested countries are encouraged to develop a SPSF based on these recommendations.

27. The draft document was approved with the following changes (shaded text was added, text stroked through was removed):

- Title: ~~Guidance Document on Revisions to OECD Genetic Toxicology Test Guideline~~**Overview of the set of OECD Genetic Toxicology Test Guidelines and updates performed in 2014-2015**
- […] Regulatory authorities have established various ways to do this, including a prohibition of *in vivo* tests in the European Union (EU) Regulation for cosmetic ingredients (EC, 2009), and the need to submit a testing proposal prior to **some**~~any~~ vertebrate test~~ing~~ **specified** under REACH **(EC, 2006)**. […]
- Paragraph 6: […] During the 18 months, under the Mutual Acceptance of Data (MAD), assays **that were planned before may**~~ean~~ continue to be conducted and the results should be accepted by regulatory agencies. After the 18 months, results that were previously generated, prior to the **effective** deletion date

shall continue to be accepted, but ~~OECD recommends that~~ no new test should be initiated using the deleted test guideline.

- Table 1: column added with the reference to each TG.
- Paragraph 11: […] **For the purpose of this Document, the use of working definitions for mutagenicity and genotoxicity has been proposed. These definitions are presented below.** ~~Various iterations of the definitions were extensively discussed by the Lead Country Experts responsible for this Guidance Document who agreed on the following general definitions, which can be used in the context of the OECD TGs.*~~
- Paragraph 12: […] Mutagenicity results in events that alter the DNA and/or chromosomal number or structure that are irreversible and, therefore, capable of being passed to subsequent cell generations if they are not lethal to the cell in which they occur, **or, if they occur in germ cells, to the offspring.** […]
- Paragraph 28: […] Normal growing colonies are considered indicative ~~(but not exclusively predictive)~~ of chemicals inducing point and other small-scale mutations; whereas, slow growing colonies are considered indicative of chemicals that induce chromosomal damage […]
- Paragraph 46: Micronuclei can be measured in other tissues, provided that the cells have proliferated before tissue collection and can be properly sampled (**Hayashi et al., 2007**; Uno 2015a and b). However, this TG is restricted to measurement of effects in the bone marrow **that are subsequently detected in the bone marrow per se** or **in** the peripheral blood because of the lack of validation of tests applied to other tissues. […]
- Paragraph 58: […] Currently, **testing of** fresh tissue samples **is recommended** ~~must be analysed because~~ freezing/thawing of sampled tissues and subsequent performance of the comet assay is not regarded as fully validated **(see also paragraph 91)**.
- Paragraph 71: […] The reason for the difference in sampling times for chromosomal aberration and micronucleus analysis is that more time is needed for the cells to divide in order to see micronuclei in the daughter cells. **In addition the *in vitro* micronucleus TG (487) permits the application of extended sampling times if it is known, or suspected, that the test chemical affects the cell cycling time (e.g. when testing nucleoside analogues). Sampling times may be extended by up to a total 3.0 to 4.0 cell cycle lengths after the beginning of treatment, but care should be taken to ensure that the cells are still actively dividing during the extended sampling time.**
- Paragraph 73: […] It is now recommended that, while the use of duplicate cultures is advisable, either replicate or single treated cultures may be used at each concentration tested **provided the same total number of cells are scored in the cytogenetic assays for either single or duplicate cultures**. […]
- Paragraph 74: […] Human liver S9 preparations are sometimes used **especially in the case of follow-up studies on chemicals with species-specific metabolic differences, such as aromatic amines** (Cox et al., 2016). […]
- Paragraph 83: […] Examples are absorption, distribution, metabolism, and excretion (ADME or toxicokinetic) data **obtained from previous or subsequent studies** ~~requested by other guidelines/ guidances~~. […]
- Paragraph 91: […] **It is thus described** ~~Descriptions of current considerations for conducting this test are~~ in Annex 3 **of the TG which presents the current limitations of the assay. This annex indicates that if used, the laboratory should demonstrate competency in freezing methodologies and confirm acceptable low ranges of % tail DNA in target tissues of vehicle treated animals, and that positive responses can still be detected. In the literature, the freezing of tissues has been described using different methods. However, currently there is no agreement on how to best freeze and thaw tissues, and how to assess whether a potentially altered response may affect the sensitivity of the test**. ~~of the Test Guideline and should be consulted. It should also be noted that there is currently limited experience with the regulatory use of this test.~~
- Paragraph 116: For *in vitro* tests and most *in vivo* somatic cell assays, a selection of positive (at least two *in vivo)*and negative control substances should be investigated under all experimental conditions of the specific test (e.g. short- and long-term treatments for *in vitro* assays, as applicable) and give responses consistent with the published literature. ~~It should be based on at least 10, but preferably 20 experiments, that demonstrate that the assay conforms to published positive and negative control norms.~~ **The literature suggests that a minimum of 10 experiments may be necessary but would preferably consist of at least 20 experiments conducted under comparable experimental conditions** (Hayashi *et*

al., 2011). **It is noted that this recommendation appears in most of the test guidelines but is absent from two in vivo test guidelines (488 and 489).**

- Paragraph 129: […] All control data of each individual genetic toxicology test, strain *etc*. during a certain time period (*e.g.* 5 years), or from the last tests performed (*e.g*. the last 10 or 20 tests) should ~~initially~~ be accumulated to create the historical control data set. […]
- Appendix D:
  - **Cytogenetic assay: a test that detects damage to chromosomes (i.e. cytogenetic damage) either as chromosome anomalies *per se* that can be visualized microscopically at metaphase (i.e. TGs 473, 475, 483), or as micronuclei that can be detected microscopically, or with flow cytometry, (i.e. TGs 474, 487). Chromosomal aberrations include breaks in chromosomes that result in deletion, duplication or rearrangement of chromosome segments, or a change (gain or loss) in chromosome number (i.e. aneuploidy).**
  - **Erythroblast:** an early stage of erythrocyte development, ~~immediately~~ preceding the immature erythrocyte, where the cell still contains a nucleus.
  - **Frameshift mutation**: a gene mutation characterized by the addition or deletion of single or multiple **(different from three or multiples of three)** base pairs in the DNA molecule.
  - **Gene mutation assay: a test that detects heritable (to daughter cells or organisms) alterations to the gene specific to the particular assay. These alterations can either activate or inactivate the specific gene. Gene mutation include changes in a single or multiple nucleotide base pairs which can be substitution of one base for another or addition or deletion of one or more bases in the base pair sequence and in some assays, the deletion of the entire gene.**

16. Draft new Test Guideline on a Stably Transfected Human Androgen Receptor Transcriptional Activation Assay for Detection of Androgenic Agonist and Antagonist Activity

28. The Secretariat presented document [ENV/JM/TG(2016)19] on a new Test Guideline on an androgen receptor transactivation assay (ARTA) and the related validation report [ENV/JM/TG(2016)32].

29. Comments were made by the WNT in relation to the solubility issues (e.g. how to test solubility of a mixture). It was reported that the VMG NA has also identified this general issue as requiring further work and guidance. This is expected to be addressed in a chapter of the GIVIMP (see paragraph 41, project 4.104) in preparation. BIAC noted that the draft TG requires test chemicals to be tested up to potentially 1mM, depending on their solubility, while only few chemicals were tested above 10 µM in the validation study (i.e. only one chemical in the second validation phase of the assay).

30. The draft Test Guideline was approved with the following changes (shaded text was added, text stroked through was removed):

- Paragraph 21: The acceptability criteria of three concurrent reference standards can ensure the accuracy of quantitative sensitivity of the assay, but for the purposes of qualitative assessment, ~~small~~ deviations **from acceptable ranges of the reference standards (as specified in tables 1-2 and 1-4)** ~~quality criteria (see tables 1-1 or 1-3) are met,~~ could be allowed if the **quality criteria (see tables 1-1 or 1-3) are met, however the reference standards should be included with each experiment and the results should be judged according to the parameters indicated in tables 1-2 and 1-4 and the** concentration-response curve of the positive reference standards **should be** ~~are~~ sigmoidal.
- Tables 1-2 and 1-4: an additional column was added indicating how the reference standards should be judged
- Legend of figure 2: **YES: No precipitation, NO: Precipitation**
- Paragraph 40: Repetition of definitive tests for the same chemical should be conducted on different days using freshly prepared assay reagents and dilutions of the test chemicals, to ensure independence. **In cases where multiple chemicals are concurrently tested within a single run, maintaining the same plate design, while changing the order in which chemicals are added to the test wells would be preferable**.

- Paragraph 51: […] Be reproducible **in triplicate wells (CV<20%)**
- Annex 2:
  - Paragraph 2: To ensure validity of this approach, **the agonistic activity of the** following needs to be tested in the same plate:
    - Agonistic activity of the unknown chemical with /without1µM of HF **(in triplicate)**
    - VC (in triplicate)
    - ~~Unknown chemical (in triplicate)~~
    - 1 µM HF(in triplicate)
    - 500 pM of DHT (in triplicate) as ~~agonist~~ PC_{AGO}
    - ~~Unknown chemical + 1 µM HF (in triplicate)~~
  - Paragraph 3:
    - If the agonistic activity at any concentrations tested is inhibited by the treatment **with 1µM of HF (**~~of~~ AR antagonist), the difference in the responses between the **wells** non-treated **with the AR antagonist** and **wells** treated ~~wells~~ with the AR antagonist is calculated. This difference should be considered as the true response and should be used for the calculation of the appropriate parameters to enable a classification decision to be made.
    - **True response = (Response without HF) - (Response with HF)**

31. The draft validation report [ENV/JM/TG(2016)32] was endorsed with only editorial modifications.

17. Draft updated Performance-Based Test Guideline 455 for Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists and Antagonists

32. The Secretariat presented document [ENV /JM /TG(2016)20] on the draft revised TG 455, updated with the inclusion of a new test method based on the ERα CALUX cell line (in Annex 4), and document [ENV/JM/TG(2016)31], the validation report of the ERα CALUX based test method. The draft Test Guideline was approved and the validation report endorsed without modification.

33. It was noted that more guidance may be useful on how to validate me too tests. Aware of potential issues in that matter, a short document has recently been developed by one co-chair of the VMG NA. It is directed to developers of new test methods and recommends in particular engaging discussion with the VMG NA from the stage of the validation plan development.

19. Corrections and deletion of OECD Test Guidelines

34. The WNT approved the deletion of TG 457 (BG1Luc Estrogen Receptor Transactivation Test Method for Identifying Estrogen Receptor Agonists and Antagonists) since it is now merged with TG 455 (PBTG for Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists and Antagonists).

35. The WNT approved the correction to TG 490, presented in document [ENV/JM/TG(2016)28].

36. The Secretariat presented document [ENV/JM/TG(2016)27] introducing changes and correction to TGs 421 and 422. The draft revised Test Guidelines were approved with the following changes compared to those presented in document [ENV/JM/TG(2016)27] (shaded text was added, text stroked through was removed):

- Paragraphs 34 / 41: If litter size is not adjusted, two pups per litter are sacrificed on day 4 after birth and blood samples are taken for measurement of serum thyroid hormone concentrations. **If possible the two pups per litter should be female pups to reserve male pups for nipple retention evaluations except in the event that removing these pups leaves no remaining females for assessment at termination.** […]
- Paragraphs 42 / 56: - from at least two pups per litter on day 4 after birth, **if the number of pups allows (see paragraphs *33-34 / 40-41*)**
- Change specific to para 46 in TG 421: The testes and epididymides as well as **prostate and seminal vesicles with coagulating glands as a whole, of all male adult animals should be trimmed of any**

adherent tissue, as appropriate, and their wet weight taken as soon as possible after dissection to avoid drying. In addition, optional organ weights could include levator ani plus bulbocavernosus muscle complex, Cowper's glands and glans penis **in males and paired ovaries (wet weight) and uterus (including cervix) in females; if included, these weights should be collected as soon as possible after dissection.**~~of all male adult animals should be weighed.~~

- Change specific to para 63 in TG 422: The testes and epididymides as well as ~~levator ani plus bulbocavernosus muscle complex, Cowper's glands~~ prostate **and** seminal vesicles with coagulating glands as a whole ~~and glans penis~~ of all **male** adult ~~males~~ **animals** should be trimmed of any adherent tissue, as appropriate, and their wet weight taken as soon as possible after dissection to avoid drying ~~and the~~. **In addition, optional organ weights could include levator ani plus bulbocavernosus muscle complex, Cowper's glands and glans penis in males and paired ovaries (wet weight) and uterus (including cervix) in females; if included, these weights should be collected as soon as possible after dissection. The** ovaries, testes, epididymides, accessory sex organs, and all organs showing macroscopic lesions of all adult animals, should be preserved.
- Paragraphs 48 / 65: […] The tunica albuginea ~~should~~ **may** be gently and shallowly punctured at the both poles of the organ with a needle to permit rapid penetration of the fixative. […]

WORKPLAN: CURRENT PROJECTS AND PROPOSALS FOR FUTURE PROJECTS

20. Action plan towards the possible deletion of TG 415 and TG 416

37. The Secretariat introduced agenda document ENV/JM/TG(2016)30 which outlined an action plan towards the deletion of TG 415 (One-generation reproductive toxicity test) and TG 416 (Two-generation reproductive toxicity test). The topic was already addressed under "Any other business" at previous meetings with the view to gauge the readiness to start such discussions. Denmark, who had been active on the preparation of the discussion at previous meetings, noted they had contacts with the European Medicines Agency (EMA) prior to the WNT So that Denmark could know whether there is alignment in Europe across chemicals legislations. It was the understanding of Denmark that EMA may have a different appreciation of TG 443 potential to replace TG 416 compared to other chemicals legislations, which may be the result of a lack of communication between experts in relevant field.

38. In the ensuing discussion at the WNT, some members reaffirmed that there was still scepticism in their countries with the replacement of TG 416 by TG 443 and that a comparative analysis was necessary to increase certainty. There were questions about the exact protocol of the extended one-generation that will be used in the comparative analysis planned in the United States; the US confirmed that a modified version of TG 443 will be used, but no timelines are yet communicated on the analysis. The WNT discussed the possibility of deleting TG 415 separately from TG 416 given the difficulties foreseen with the deletion of TG 416; but currently there appears to be preference to keep the deletion of both TGs grouped. The WNT supported the action plan in the agenda document; Denmark, the Netherlands, Japan, the United States and ICAPO will work together on the finalisation of the survey questions and the preparation of an SPSF.

21. New Standard Project Submission Forms (SPSFs)

39. The WNT approved most project proposals submitted in November 2015 (see Annex 2); some of them had been revised in February 2016 following WNT written comments [see ENV/JM/TG(2016)1/REV1]. Some proposals were discussed at the meeting and the following outcome was agreed:

- SPSF on juvenile medaka anti-androgen screen: the WNT recommended and agreed to re-open the guidance document No. 148 (Guidance Document on the Androgenised Female Stickleback Screen) and adapt it to include the two species and protocol variations, as the aim of the assays are quite similar, i.e. detection of anti-androegenic chemical. Japan submitted a revised SPSF.
- SPSF on GARDSkin for sensitisation: the WNT approved the proposal and noted that the highest priority is for test methods that allow potency evaluation.
- SPSF on update of TG 442B (LLNA-Brdu): the WNT confirmed that comments had been adequately addressed by Korea and agreed to include the project on the work plan.

- SPSF on a miniaturised Ames test (TG 471): proposing countries informed the meeting of the change of scope of the project proposal to start with a Detailed Review Paper of all miniaturised systems. Another SPSF might be developed as a next step to integrate some of these miniaturised systems in TG 471. Also, the Netherlands and the United States will co-lead the project together with Belgium. The WNT agreed to include the revised project proposal on the work plan.
- SPSF on the deletion of TG 478: several countries were opposed to the deletion of TG 478, which measures effects such as heritable genetic damage that cannot be detected in other assays. The European Commission noted that they will move one with removing reference to TG 478 in the European legislation.
- SPSF juvenile rodent assay: the WNT did not agree to include this project proposal to develop a new Test Guideline of juvenile rodent assay on the work plan. Italy, supported by the United Kingdom and Denmark, expressed interest in having a general discussion on tools and methodologies available to regulators to address protect children health. Countries recommended to Italy to explore specificities of childhood that are not well covered and evaluated in current OECD Test Guidelines. This could then feed discussion on concrete action(s) for the Test Guidelines Programme.
- SPSF on critical deviations from ecotoxicity Test Guidelines. The WNT did not agree to include this project on the work plan. Several comments were made in the review period and still need to be clarified.

40. One SPSF was a proposal to update TG 431 for which a dedicated agenda document ENV/JM/TG(2016)21 was prepared, showing the proposed updates, and received approval at the WNT.

REPORT ON PROGRESS WITH RELVANT ACTIVITIES AND RECENT MEETINGS

22. Review and updates to the work plan

41. The WNT briefly reviewed projects currently on the work plan [ENV /JM /TG(2016)22], and lead countries provided updates as necessary. The following points were made:

- project 2.55 led by the European Commission on the revision of Guidance Document 23 on difficult to test substances (part 1 of the project) will now be co-led with the United States, given the extensive commenting that has been done on several parts of the document, not originally targeted by the project.
- Germany noted a correction to make to project 3.7 (last bullet to be removed).
- The United States provided the Secretariat with written text to update project 3.11, mentioned the termination and removal of project 4.52 on the cytosensor microphysiometer test method due to the unclear regulatory need, and finally announced some delays with project 5.6 on the development of a guidance document on bed bugs treatment due to a public commenting period in the United States.
- Sweden mentioned financial support received from the European Commission on project 4.97 to develop a Detailed Review Paper on the retinoid pathway.
- Austria provided some details on on-going work with project 2.45 on the IATA for fish acute toxicity, integrating TG 236 (embryo test), and mentioning the collaboration with ECHA, Germany and BIAC, and delays with the tasks.
- For project 4.104, the EC indicated that a first draft guidance document should be available mid-2016, and a meeting of the expert group might be convened later in 2016.
- The United Kingdom informed the WNT that projects on the work plan related to environmental fate and ecotoxicity testing led by the United Kingdom are now on hold and invited other countries to take the lead if they were in a capacity to do so.
- For project 4.49 (update of TG 402), the United Kingdom expects that the project can be finalised in written procedure before the next WNT. The draft updated TG 402 and responses to the first commenting round in 2015 will be provided to the WNT and expert group on acute mammalian toxicity for another review round. A teleconference will be held as necessary to finalise the project, and hopefully get approval of the WNT on an updated TG 402.

42. All updates will be made shortly after the meeting and presented to the WNT, with a request for approval of the revised work plan.

23. Outcome of the EAGMST meeting and status of the AOPs for endorsement by WNT and TFHA

43. The Secretariat presented document [ENV/JM/TG(2016)23] which compiles the 5 AOPs that were submitted to the WNT and Task Force on Hazard Assessment (TFHA) for endorsement in February 2016, as well as document [ENV/JM/TG(2016)24], presenting the comments received on these AOPs and the responses from the Secretariat and AOP authors.

44. The WNT agreed on the disclaimer below to clarify what endorsement by the WNT means. This text will be displayed on the OECD public webpage where AOPs will be published following Joint Meeting endorsement

- Disclaimer:
  "This AOP has been developed under the auspices of the OECD AOP Development Programme, overseen by the EAGMST, which is an advisory group under the WNT. The AOP has been reviewed internally by the EAGMST, externally by experts nominated by the WNT, and has been endorsed by the WNT.
  Through endorsement of this AOP, the WNT expresses confidence in the scientific review process that the AOP has undergone and accepts the recommendation of the EAGMST that the AOP be disseminated publicly. Endorsement does not necessarily indicate that the AOP is now considered a tool for direct regulatory application."

45. The 5 AOPs were endorsed by the WNT. It was clarified that they were also endorsed by the TFHA since no comment was received from the TFHA after the request for endorsement by written procedure.

24. Focus session on AOPs, with participation from the training team

Kristie Sullivan from ICAPO made a presentation of training activities on-going to extend knowledge and understanding of the AOP Development Programme, as outlined in document ENV /JM /TG(2016)25. She highlighted that training activities have been organised in 2015-2016 for regulatory agencies and more training can be done if requested. In relation to a discussion started on the regulatory application of AOPs, it was noted by the training team that while EAGMST tends to separate the development from the application of AOPs and focus on the former, the suggestions and ideas of the WNT on how AOPs can be used for regulatory purposes are welcome. Some members of the WNT emphasised the need to establish quality criteria for one to envisage the regulatory application of AOPs; others noted that criteria should be informed based on experience. Importantly, it was reflected in the discussion that the long term challenge is to understand how to integrate mechanistic information into chemicals assessments; it is also important to determine when there is enough information without further testing to be able to conclude on a hazard or exclude with limited uncertainty any potential hazards that have not been measured. It was indicated by the Secretariat that the case-study group is already applying the AOP concept for read-across chemicals assessments.

26. Outcome of the EDTA Advisory Group meeting

46. The Secretariat made a short presentation of the outcome of the last EDTA Advisory Group meeting held in October 2015 [see ENV/JM/TG/EDTA/M(2015)2], and raised the question on the need and appropriate timing to organise a meeting of the EDTA AG, and informed the WNT about chemical case studies on endocrine disrupters being discussed under the Task Force on Hazard Assessment (in the scope of IATA-related activities).

47. Various members of the WNT reported several on-going activities and events related to endocrine disrupters testing and assessment in Europe and the US that will need to be shared and discussed at the OECD in 2016. There was support for having a meeting in 2016, with a focus on science and information exchange. Concern was expressed in relation to resources in countries to participate in case-study discussions in other fora that the EDTA AG.

48. It was agreed that the meeting of the EDTA Advisory Group will be maintained in October 2016, with a focus on science for regulatory application. Progress with case studies under the Task Force on Hazard Assessment should also be communicated to the EDTA AG at the meeting.

27. Renewal of the Mandate of the WNT

49. The proposed mandate of the WNT for 2017-2020, presented in ENV/JM/TG(2016)29 was approved without changes.

28. Any other business

50. Germany asked that more time be dedicated at future WNT meetings to discuss project proposals (SPSF), and Denmark suggested that the discussion on project proposals be advanced on meeting agenda; the Secretariat took note of these requests.

51. The EC asked about the possibility to re-open discussion on the deletion of TG 212 (fish sac-fry test), mentioning that the test was no longer scientifically valid and ethically defensible for regulatory use, especially in Europe; Japan is not in favour of the deletion of TG 212 as it is still in use; the Chair concluded that further national consultation would be needed to have more clarity on the case of TG 212.

52. The Secretariat noted that the standard text agreed by the WNT on the testing of mixtures for regulatory purposes is often subject of comments, or misunderstood, when draft Test Guidelines are circulated for comments. Denmark agreed to assist the Secretariat to consider this further and if needed to attempt developing a proposal for improving clarity.

53. The Secretariat also draw attention of the WNT on the need to have a national position in compilations of comments, in particular when several experts are commenting and various views are expressed. The Secretariat also asked the WNT to think of a rule for the availability of presentation made at expert meetings.

29. Renewal of the bureau

54. The bureau (Tim Singer as Chair, Martin Paparella and Hajime Kojima as co-Chairs) was renewed for another year.

30. Conclusion of the meeting; dates of the next meeting

55. The next meeting of the WNT will take place on 25-28 April 2017 at OECD.

Annex 1: List of Participants

The list of participants is available as a separate document coded ENV/JM/TG/PL(2016)1.