COM Ongoing Work 2021

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COM Guidance Series Update

2.1 The updating of the overarching COM Guidance document on a strategy for the genotoxicity testing of chemicals was finalised in 2021. Amendments to the overarching COM Guidance document had previously been considered at Committee meetings in July 2018 (paper MUT/2018/09), October 2018 (paper MUT/2018/13), February 2019 (MUT/2019/01), October 2019 (MUT/2019/12), February 2020 (MUT/2020/03), June 2020 (MUT/2020/09) and November 2020 (MUT/2020/16). An additional sub-group meeting was held in January 2021 to complete review of comments left outstanding following the November 2020 meeting.

2.2 Following consideration of paper MUT/2021/01 the update of the overarching COM Guidance document on a strategy for the genotoxicity testing of chemicals was agreed by members, signed off by Chair action and published on the COM website. It was intended that this would be updated in the future as part of a rolling revision.

Guidance Statement – Germ Cell Mutagens

2.3 Drafts of a stand-alone guidance statement on genotoxicity testing strategies for germ cell mutagens were considered at the Committee meeting in February 2019 (MUT/2019/05), in October 2019 (MUT/2019/12), in June 2020 (MUT/2020/11) and November (MUT/2020/17). In 2021, members considered paper MUT/2021/02, which presented changes suggested following the November 2020 meeting. Following agreed amendments, the finalised document was signed off by Chair’s action and published on the COM website.

Guidance Statement – 3D Models

2.4 Drafts of a stand-alone guidance statement on the use of 3D models for genotoxicity testing were considered at the Committee meetings in February 2019 (MUT/2019/04), October 2019 (MUT/2019/12), June 2020 (MUT/2020/11) and

November (MUT/2020/18). In 2021, members considered paper MUT/2021/03, which included suggested changes following the meeting in November 2020. Following agreed amendments, the finalised document was signed off by Chair's action and published on the COM website.

Guidance on Genotoxicity Testing Strategies for Nanomaterials

2.5 Genotoxicity testing of nanomaterials (NMs) was recognised by the Committee as a rapidly developing area. Paper MUT/2021/09 presented a draft COM Guidance on the genotoxicity testing strategy for NMs. This was prepared to a format previously agreed by COM at the meeting in November 2020 (MUT/2020/19). Members considered that it was important to add a note to clarify that 'Stage 0' of the COM recommended approach for genotoxicity testing would not apply to NMs. A question was raised regarding whether COM should recommend a positive control for NM testing. This was not considered feasible at present as this would probably need to be both assay and cell line specific, due to differing sensitivities. Members requested that this information be added to the document. It was also agreed that a note should be added to consider the most appropriate dispersion technique for a specific NM. Following these amendments, members agreed that a final version of the document could be signed off by Chair's Action and published on the COM website. It is recognised by the Committee that this is a rapidly developing area and updates will be carried out as new information becomes available.

Guidance Statement on Testing for Impurities – Update

2.6 The COM published a guidance statement in 2012 on a strategy for genotoxicity testing and mutagenic hazard assessment of impurities in chemical substances. Since 2012, there have been a number of initiatives in this area and as part of the ongoing update of the COM Guidance Statement series, members agreed that the Guidance document should be updated. A draft revised document was presented at the Committee meeting in November 2020 (MUT/2020/21) and following comments and suggestions from members a revised draft statement was produced (MUT/2021/04) and presented at the February 2021 meeting. During review it was suggested that the impurities guidance statement and QSAR guidance statement could be merged as there was overlap between the two

areas.

COM Guidance Statement on the Use of QSAR Models

2.7 A range of Quantitative Structure-Activity Relationship (QSAR) models have been developed to predict genotoxicity. The COM has previously agreed that where no genotoxicity data are available, the intrinsic chemical and toxicological properties of a chemical must be considered prior to developing a genotoxicity testing programme, as reported in “Guidance On A Strategy For Genotoxicity Testing Of Chemical Substances” (COM, 2011) and as updated in 2021. This guidance describes a staged approach to testing consisting of stages 0 (preliminary considerations including physico-chemical properties), 1 (in vitro genotoxicity tests) and 2 (in vivo genotoxicity tests). QSARs are incorporated into Stage 0 of the COM guidance.

Alternatives to animal testing and the usefulness of computational methods in the prediction of genotoxicity are areas of increasing research. QSAR models and their predictions currently cannot replace the need to undertake the in vitro and in vivo genotoxicity tests required to derive conclusions on mutagenic hazard except in specific regulatory settings. As the development and use of QSAR is a rapidly developing field, it was agreed that the current text in the COM overarching guidance document should be reduced and a larger ‘stand-alone’ guidance statement be prepared which could be updated as needed.

2.8 A draft document - ‘Guidance Statement on the use of QSAR models to predict genotoxicity’ was prepared and discussed by COM in February 2019 (MUT/2019/03). Following amendments, a revised paper was discussed in February 2020 (MUT/2020/02) and November 2020 (MUT/2020/20). No agreement was reached as to whether the draft guidance statement was ‘fit-for-purpose’, and it was also suggested that QSARs could be incorporated into the COM guidance on impurities, as this is where it is likely to be used.

2.9 Following a further draft COM Guidance on QSARs (MUT/2021/05) considered at the February 2021 meeting, a sub-group discussion with some COM members was held in September 2021 to plan a way forward. It was suggested that, based on current acceptance and use of QSARs, incorporation of examples of use and reporting of data should be included in the updated impurities guidance document, with a link to the OECD portal provided to give the most current perspective/tools etc. A more general description (taken from the current

draft document) would then be re-introduced into the COM overarching guidance document to support the Stage 0 testing text.

2.10 Members agreed that it was important for any COM guidance to highlight applications of QSAR, rather than providing a list of QSAR models and approaches.

Toxicogenomics and Risk Assessment: Application of Transcriptomics and Next Generation Sequencing to Genotoxicity and Carcinogenicity Assessment

2.11 At the COM meeting in February 2021, during discussions of some preliminary literature on ‘toxicogenomics and risk assessment’ (MUT/2021/06), members noted that this field could at present be considered to comprise two different major elements; the more highly established field of transcriptomics, and the newer area of next-generation sequencing technologies. It was felt that it would be useful for a document to be prepared providing a preliminary overview of these two areas and their potential applications to risk assessment in the fields of mutagenicity and carcinogenicity. Discussion paper MUT/2021/08 provided an overview of these two areas, summarising narrative from three recently published review articles.

2.12 Members noted that overall, this was a fast-developing area. For this reason, it may be difficult for the COM to establish a specific guidance document, as this would rapidly become out of date. However, members also considered that this is a very important area in the development in genotoxicity assessment and should be kept under evaluation by the Committee.

2.13 Some major areas of work in this field were highlighted. These included: Current efforts to obtain mutational signatures and match these to environmental exposures, which was noted as an area that the COM would probably wish to focus on further; Progression of work on TGx-DDI (a transcriptomic biomarker for genotoxicity), noting that data is being passed to regulators with the aim to be able to provide guidance; Development of duplex sequencing at Health Canada, which is starting to be useful for investigations of germ-cell mutagenesis and for dose-response analysis; Use of cancer-driver mutations via the ‘CarcSeq’ method at FDA.

2.14 In terms of document progression, a more detailed paper could be envisaged, noting techniques and methodologies that are becoming available, and describing some examples of how these techniques may be becoming applicable to investigation of genotoxicity. It was agreed that further development of any paper from COM concerning the use of toxicogenomics for risk assessment purposes would be discussed by a small sub-group of interested members.

Presentation by Professor Michael K Skinner – Washington State University, USA – Environmental Toxicant Induced Epigenetic Transgenerational Inheritance of Disease. Generational Toxicology – Open to COC and COT Members

2.15 At the February 2021 meeting, Professor Skinner from Washington State University (Washington, USA) presented a talk entitled 'Environmental Toxicant Induced Epigenetic Transgenerational Inheritance of Disease: Generational Toxicology'. This was also open to COC and COT members.

2.16 As an introduction, Professor Mike Skinner highlighted that it is difficult to explain all disease based solely on the genome and that that environmental factors also play a role on the occurrence of disease. What is observed is not completely explained by the paradigm of the genome affecting gene expression, which in turn affects physiology and the development of disease. For example, the development of disease in identical twins is reported to vary when identical twins live in different regions. This indicates that other factors are involved in addition to individual DNA sequence.

2.17 Professor Mike Skinner summarised animal studies that showed adverse effects in future generations (i.e., F2 and later generations, where the germline was not directly exposed to the initial test chemical) arising from an initial chemical exposure in pregnant females. The observed adverse effects arose from epigenetic changes. Epigenetic effects could arise from chemical induced changes in DNA methylation, histone modifications and effects on RNA (i.e., not involving a change in the DNA sequence). Such chemical induced epigenetic changes can result in modification of gene expression.

2.17 Professor Skinner noted that if a gestating F0 female animal is exposed to a particular chemical, then the F3 generation would be first generation that did not receive a direct test chemical germline exposure. Chemical induced effects seen in the F3 generation and subsequent generation could be due to epigenetic effects or inherited changes in gene expression arising from the initial gestating exposure of the F0 female. This would be an example of transgenerational inheritance. If a non-pregnant female or a male animal was exposed to the test chemical, then the F2 generation would be the first generation that did not receive direct germline chemical exposure. Chemical induced effects in this generation could arise from inherited epigenetic changes (this would be an example of transgenerational inheritance).

2.18 A number of examples of results of chemical exposure in animals were reported where 90% of treated animals showed adverse effects in the F3 generation resulting from an initial F0 gestating female exposure. For example, vinclozolin (agricultural fungicide), TCDD/Dioxin, DDT, bisphenol A and diethyl hexyl phthalate produced adverse effects in the F1 generation and in the F3 generation. Flutamide (anti-androgenic pharmaceutical) produced adverse effects in F1, but not in F3 generation. However, atrazine (agricultural herbicide) and glyphosate (herbicide) did not induce adverse effects in F1 but did in F3 (transgenerational effect). Examples of chemically induced transgenerational disease effects included spermatogenic defects, male infertility, prostate disease, premature ovarian failure, ovarian polycystic ovarian disease, birth defects, kidney disease, obesity, behavioural effects and immune effects.

2.19 Other types of exposures can also induce epigenetic and transgenerational effects, such as extreme temperature, drought, high fat diet or caloric restriction, smoking and alcohol. Studies were described where various transgenerational epimutations and clusters were detected in the sperm genome in the F3 generation following initial chemical exposure, such as with vinclozolin and DDT.

2.20 One of the most sensitive periods of exposure is during fetal gonadal sex determination when the germ line is undergoing epigenetic programming and DNA re-methylation occurs. The suggestion that environmental toxicants can re-programme the germ line to induce epigenetic transgenerational inheritance of disease, is a new paradigm in disease aetiology, and indicates the need to assess generational toxicology in the future.

2.21 Key take home messages from the presentation included: the germline (eggs and sperm) are where epigenetic changes are critical because they get passed on in a transgenerational manner; this epigenetic transgenerational

inheritance does not involve an inherited change in the DNA sequence; and a recommendation that adverse transgenerational effects need to be investigated in chemical health risk assessment. It was suggested that animal studies would be required to do this because current *in vitro* studies would not be suitable.

2.22 In discussions following the presentation, clarification was sought by members around how assessment of intragenerational effects may be included in current testing regimes. At the present time this can only be achieved through laboratory animal studies where the third generation needs to be evaluated, with minimum study length of between 1 and 1.5 years. It is not feasible to assess the germ cells of affected individuals because the shifts in developmental programming need to be established before the effects of the exposure are seen. A large proportion of the changes seen in earlier generations are due to direct exposure.

2.23 At present, transgenerational effects have been shown for many toxic compounds and so such testing is likely to be needed on a routine basis. There are no *in vitro* approaches that are effective to replace *in vivo* assays. It was considered possible that thresholds existed for the level of DNA methylation sites, below which long-term disease was avoided.

2.24 Diet was discussed as a major factor that had previously been linked with epigenetic changes. For a generational impact to occur the dietary influences have to be quite severe (for example, calorific restriction or high fat diets), with small shifts in diet not having an impact. Timing of exposure was also found to be key, with exposure during the early fetal life period being critical. Environmental toxicants were considered to have an effect at similar levels to calorific restriction. The importance of epidemiology studies in supporting animal data and showing causality was also discussed. Epigenetic biomarkers are needed for use in epidemiological studies, and these have not been developed.

2.25 The Chair thanked the speaker on behalf of the Committee for an interesting and informative presentation. In conclusion, it was agreed that the COM would keep an active watching brief on developments in the area, particularly in relation to inclusion in toxicity testing regimes.

Presentation on Toxicogenomics in Toxicology Testing by Dr Scott Auerbach, Division of the National Toxicology Program, National Institute

of Environmental Health Sciences, USA

2.26 At the June 2021 COM meeting, Dr Scott Auerbach provided a presentation on toxicogenomics in toxicology testing. Dr Auerbach noted that functional omics technologies are a powerful tool for the characterisation of chemical effects in biological systems. Historically the primary use of omics technologies, transcriptomics in particular, has been to characterise chemical mode of action to understand toxicological mechanisms and human relevance. More recently effort has been put into use of transcriptomics as a means to identify a biological effect point of departure that roughly approximates a point of departure derived from much more resource intensive studies such as the two-year cancer bioassay.

2.27 The presentation discussed how transcriptomics has been used for qualitative characterisation of chemical effects and how it is being modelled to derive a genomic-based point of departure. In addition, some of the current scientific challenges that need to be addressed to facilitate more widespread use of genomic point of departure values for health-based guidance value determination were also discussed.

2.28 Following the presentation, the sensitivity of the methodology was queried as some genotoxic compounds may not have a strong genotoxicity signal over the shorter exposure time. This is addressed by the inclusion of doses of test substance up to the maximum tolerated dose during screening which should produce a signal if it is genotoxic. The limitation of precision of toxicogenomics in its ability to determine what proportion of cells are affected to produce the measured ‘fold’ change was highlighted. This was anticipated to be a chemical specific issue as those only affecting a small number of focal points (e.g., nitrosamines) would take longer to produce a signal than chemicals affecting multiple sites (e.g., 3,3',4,4'-Tetrachloroazobenzene) and should be taken into account to avoid inaccuracies. The use of gene-set dose response data (as a point of departure) with benchmark dose modelling was also discussed. There is no standard model to use with such data as the adverse effect size (BMR) for a particular gene is not known for many chemicals. It is also not possible at this time to take into account the effect of co-variables, which is an important consideration for human data, however this is being actively addressed by a number of groups.

Presentation on OECD development of the Mini-Ames

Dr Robert Smith, Covance

2.29 Dr Robert Smith, the UK representative on the OECD expert group developing the mini-Ames test, gave a presentation and summary of the activities of the OECD expert group on the miniaturised bacterial mutation assay.

2.30 New approaches to the or Ames test (OECD TG 471) are being explored, such as miniaturised assays, as they offer higher throughput with a significant reduction in the amount of test material required, resources and cost.

2.31 Several miniaturised versions have been developed and are already extensively used for screening purposes during product development/candidate selection or for impurity assessment/qualification. These have some differences when compared to the standard Ames assay and are not described in any existing OECD Test Guideline. Differences include the use of multi-well plates, use of liquid media rather than agar plates, the number of bacterial strains used, and the use of reduced numbers of bacterial cells (and volumes, etc.).

2.32 Following the presentation, members considered the possibility that data obtained from Ames II^{TM} assays run by inexperienced laboratories may have influenced the findings of the Detailed Review Paper (DRP). However, there had been a requirement for laboratories to show proficiency prior to submitting data for inclusion. Although there was good concordance between the 4 assays evaluated (6 and 24-well agar plates, micro-fluctuation and Ames II^{TM} assays) there was some remaining discussion around comparison of top doses, as the microfluctuation assay expressed doses as µg/ml and the Ames assay as µg/plate. It was also considered that exposure might be enhanced for the fluctuation assay, as fewer cells are present. The effect of pre-incubation in the fluctuation assay was queried and had been associated with a small increase in sensitivity and specificity. The maximum limit on concentration per well/plate was considered by members to be a critical factor for take-up of the assays once finalised. The OECD had produced a DRP on the evaluation of various mini-Ames assays cited in the literature compared with the standard Ames test. The OECD DRP was circulated to COM members for comment.