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EUROPEAN CHEMICALS AGENCY

Technical Discussion Session on the Scientific Adequacy of in vivo Mutagenicity Assays, the Transgenic Rodent Gene Mutation Assay and the Unscheduled DNA Synthesis Assay

Report
Helsinki, 4 October 2012

LEGAL NOTICE

This report summarises the scientific views that were expressed at the Technical Discussion Session. The conclusions of this report are without prejudice to any on-going regulatory work by ECHA. The opinions expressed in this report may not reflect an official position of the European Chemicals Agency or the organisations that participated in the session.

Further information on the Technical Discussion Session is available in the internet at http://echa.europa.eu/en/view-article/-/journal_content/afca5628-6ab2-4fa2-b80e-c4a4e0e61923

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1. Introduction

As part of its activities under REACH, ECHA has to evaluate information on mutagenicity in registration dossiers both in compliance checks and testing proposal examinations. In particular, a higher-tier mutagenicity study may be necessary according to Annex IX and/or Annex X to examine the potential for mutagenicity in somatic cells *in vivo*. Such a study could be proposed by the registrant in a testing proposal or be required by ECHA as an outcome of a compliance check.

There have been discussions on which test to use, partly triggered by the adoption in July 2011 of a new OECD test guideline, the Transgenic Rodent Gene Mutation Assay (TGR, OECD 488), which is an alternative to the longer-established Unscheduled DNA Synthesis Assay (UDS, OECD 486, B.39 Test Methods Regulation). The Agency's Member State Committee identified some scientific uncertainties when considering these two test methods. In order to clarify the scientific considerations in selecting an appropriate *in vivo* somatic cell mutagenicity test, ECHA held a Technical Discussion Session on 4 October 2012.

Remit of the technical discussion session: detection of systemically-available substances which induce gene mutations in somatic cells *in vivo*

This meeting was to discuss the TGR and UDS, the only two in vivo assays that currently (1) have adopted OECD guidelines, and (2) are considered suitable to cover the gene mutation endpoint in vivo under REACH according the ECHA Guidance on information requirements (Volume 4, chapter R7a, May 2008). The discussion was restricted to focus on the TGR and UDS in order to have a comprehensive and structured discussion of relevant scientific issues.

Mutagenicity is the induction of mutations in DNA, and gene mutations may be a consequence of effects on single DNA bases (point mutations) or of larger changes, including deletions and rearrangements of DNA. There are different methods for detecting larger changes (rearrangements) and smaller changes (e.g. point mutations), and the technical discussion session was restricted to considering the detection of the latter type of gene mutation. There are different considerations and consequences for mutations in somatic cells and in germ cells, and the technical discussion session was also restricted to looking at somatic cell gene mutation. It should also be emphasised that some substances are highly reactive, and can cause gene mutations at the site of contact to the body. This topic of identifying chemicals that induce mutations at the site of contact was not addressed in the meeting. The discussions were regarding mutagenicity where it can be assumed, or where we have no data to exclude the assumption, that the substance is systemically available within the body after administration of the substance.

2. The technical discussion session

Forty-four experts from sixteen Member States or Associated Member State Competent Authorities, the European Commission, the European Medicines Agency, the European Food Safety Authority, industry, consultants, contract research organisations and non-governmental organisations visited ECHA for this one-day session on the fourth of October, 2012.

Experts provided detailed accounts on the science, limitations and practicalities of the two assays. Scientific comparisons and an in-depth analysis of concordance with carcinogenicity of the two assays were presented. The industry experience and perspective on the two tests was presented by one of ECHA's accredited stakeholder organisations, ECETOC. ECHA's sister agencies, the European Medicines Agency and the European Food Safety Authority presented their views on the two tests as well as case studies demonstrating their scientific applicability.

The presentations were followed by a discussion which focused on the question of whether each assay is adequate to detect substances that induce gene mutations in vivo and if there were specific conditions which modified the applicability of the assays. Furthermore, it was

discussed whether certain conditions could be defined under which either one of the two tests, the TGR or the UDS, should preferably be performed.

During the discussion, **the remit of the meeting** was agreed:

> Which of the two assays can address most suitably the gene mutation endpoint in vivo for detecting systemically-available chemicals that induce gene mutations in somatic cells in vivo.

2.1 Difference between 'adequate' and 'preferable'

In evaluating the science underpinning the use of the UDS and TGR, it is helpful to distinguish between two different questions. These are:

- Is an assay adequate (or inadequate)?
- Is an assay to be preferred over another assay (e.g. under specific circumstances)?

During the discussion session it was concluded that the term "adequacy" should be understood as "fit for purpose", and the purpose is to detect systemically-available chemicals that induce gene mutations in somatic cells in vivo.

2.2 Report on the conclusions from the technical discussion session

ECHA reports the following conclusions about what scientific views were expressed by the experts attending the workshop. The opinions expressed in this report may not reflect an official position of the European Chemicals Agency or the organisations that participated in the session.

(a) Adequacy of TGR and UDS to detect systemically-available chemicals that induce gene mutations in somatic cells *in vivo*

After hearing the experts, it was concluded that the TGR is adequate to detect chemicals that induce gene mutations and that it is theoretically applicable to all tissues; however, practical limitations were mentioned.

It was concluded that it is a majority view that the UDS is adequate to detect some substances that induce gene mutations in the liver and that substance specific reasons can justify the use of the UDS. ECHA came to the conclusion that there was a majority view that the UDS is not adequate for other tissues than the liver. Furthermore, consequences for historically available data were discussed, however, no clear conclusions could be drawn.

(b) Is one assay preferred over the other for detecting systemically-available chemicals that induce gene mutations in somatic cells *in vivo*?

In order to detect systemically-available chemicals that induce gene mutations in somatic cells in vivo, the TGR is usually preferred over the UDS.

However, the UDS might be equally adequate in some cases and substance-specific considerations should be taken into account.

The TGR is a new OECD Testing Guideline, and data gathered by using the OECD 488 guideline is limited compared to other testing guidelines (limited negative control data).