COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD CONSUMER PRODUCTS AND THE ENVIRONMENT

STATEMENT ON PHOTOGENOTOXICITY TESTING

REFERRAL

1. HSE CRD has asked the Committee on Mutagenicity (COM) for a view on photogenotoxicity testing and in particular, the molar absorption coefficient which should trigger photogenotoxicity assessment and the photogenotoxicity testing strategy that should be adopted in the absence of any OECD/EU photogenotoxicity test. The referral has arisen as a result of proposed EU data requirements for the phototoxicity testing of chemical pesticide active ingredients.

INTRODUCTION

2. Photochemical activation by non-ionising radiation (in the range 290-700 nm) may result in photochemical reactions which can include photogenotoxicity and if exposure is sufficient and prolonged, photocarcinogenicity. A number of *in vitro* photogenotoxicity tests have been developed based mainly on the existing 'dark' versions of these assays. Positive results in *in vitro* tests may trigger *in vivo* testing such as photocomet and photomicronucleus in animal models dermally exposed to non-ionising radiation and dosed with the test chemical (either dermally or orally).[1] The COM considered photogenotoxicity tests in the context of their proposed use to predict photocarcinogenicity.

3. The reactive processes giving rise to photogenotoxicity are the same as those giving rise to cellular damage or phototoxicity.[1] On this basis, there should be a good correlation between the results of *in vitro* tests for phototoxicity and photogenotoxicity. This aspect is considered further under the section on the use of photogenotoxicity tests in safety assessment (paragraphs 11-12).

4. The COM considered a number of recent publications as part of the horizon scanning exercise for 2011[1-4] and the guidance document from European Medicines Agency 'Questions and Answers' on the 'Note for Guidance on Photosafety Testing' at the March 2012 COM meeting. Some additional publications were retrieved for the drafting of this summary statement. The COM agreed that the conclusions reached by the International Workshops on Genotoxicity Testing (IWGT) working group in 2009 and published in Mutation Research in 2011 were particularly relevant to COM discussions.[1]

TRIGGERS FOR PHOTOSAFETY ASSESSMENT

5. The COM agreed that the triggers for photosafety assessment would apply to photogenotoxicity testing.

Molar Extinction Coefficient (MEC)

6. The MEC is a constant for any given molecule under a specific set of conditions (e.g. solvent, pH, buffering additives, temperature, wavelength) and the efficiency with which a molecule can absorb a photon of light of specific energy (defined by wavelength). The COM were aware that in OECD TG 432 (*in vitro* 3T3 NRU phototoxicity test) a value of 10 $Lmol^{-1}$ cm^{-1} is cited as a trigger for photosafety evaluation. MEC values have been published for a wide range of compounds with human photosafety issues. All of these compounds had absorbance intensities significantly above 1000 $Lmol^{-1}$ cm^{-1}.[2] In a separate analysis, the lowest MEC for an *in vitro* phototoxic compound was 4100 $Lmol^{-1}$ cm^{-1} and for an *in vivo* phototoxic compound was 5100 $Lmol^{-1}$ cm^{-1}.[1]

7. Overall, the COM agreed that an MEC value of 1000 $Lmol^{-1}$ cm^{-1} was a conservative limit which could be used to identify chemicals for photosafety assessment.

Exposure in Skin and/or Eyes

8. Photosafety guidance for pharmaceuticals recognises that photosafety liability is the result of a chemical's interaction with UVR and/or visible light and exposure in skin and/or eyes. The minimum exposure level in skin and/or eyes below which regulatory testing for photosafety would not be warranted is unknown. This is the subject of ongoing research.[1,2] The COM agreed that the results of this research using pharmaceuticals with known phototoxic properties would be relevant across all other chemical sectors (e.g. pesticides, industrial chemicals etc).

Chemical Photoreactivity and the Relationship with Photogenotoxicity

9. Phototoxicity has been shown to be associated with photoreactivity (i.e. production of singlet oxygen, superoxide and molecular photostability).[5] In a subsequent publication from the same group of investigators, the use of chemical reactivity measurements on the mechanistic prediction of phototoxic and/or photogenotoxic liability was studied using data from 3T3 NRU phototoxicity and photochromosome aberration (in CHO cells) tests.[4] The results showed a >90% concordance for 3T3 NRU phototoxic positives for chemicals which resulted in one or more of the three photoreactive products following exposure to UVR. The concordance between positive phototoxicity and photogenotoxicity was 57%. Chemicals known to be strong

photogenotoxins were predicted to be positive in photoreactivity assays. A number of chemicals were negative for photogenotoxicity despite being positive for photochemical reactivity and *in vitro* phototoxicity. This apparent lack of concordance between phototoxicity and photogenotoxicity was considered to be likely to be due differences in UVR irradiation modality between the assays.

10. The IWGT working group considered that more independently generated data were needed before a conclusion could be reached on the use of photochemical reactivity indices as potential triggers for photosafety testing. The COM concurred with this view with regard to the use of photochemical reactivity indices to predict potential photogenotoxicity.

USE OF PHOTOGENOTOXICITY TESTS IN SAFETY ASSESSMENT

11. Many of the *in vitro* photogenotoxicity assays in common use are based on the standard 'dark' versions of these assays.[6] Photoclastogenicity tests (tests for chromosomal aberrations or micronuclei formation) have been shown to give rise to misleading positive results. The term pseudophotoclastogenicity has been used to describe chemicals which do not absorb UV radiation or visible light between 290-700 nm but give positive results in photoclastogenicity tests.[1,7,8] The COM concurred with the IWGT working group and agreed photoclastogenicity assays should no longer be recommended for photogenotoxicity testing.

12. There are no *in vivo* photogenotoxicity assays currently recommended by Regulatory Agencies. Some developmental work on *in vivo* skin and or eye photocomet and photomicronucleus assays in both rats and mice has been undertaken.[1,9,10]

COM DISCUSION AND CONCLUSIONS

13. The Committee agreed with the conclusions reached by the IWGT working group regarding the use of photogenotoxicity data in the overall safety assessment of chemicals. Thus, there should be no requirement for photosafety assessment of chemicals with an MEC below 1000 $Lmol^{-1}$ cm^{-1}. A tiered approach to testing should be used and thus if an *in vitro* 3T3 NRU phototoxicity test was negative there would be no need for photogenotoxicity testing. Also, the Committee agreed with the IWGT working group conclusion that photogenotoxicity testing had a negligible impact in the overall assessment for potential of photocarcinogenicity. Follow up *in vivo* testing in the case of chemicals which gave positive results for 3T3 NRU assay would involve *in vivo* phototoxicity testing. It would not be necessary to undertake photocarcinogenicity testing if *in vivo* phototoxicity studies were negative.

14. The COM reached the following conclusions

i) Photogenotoxicity testing need not be undertaken routinely as part of a photosafety assessment and would only be required when the MEC was >1000 $Lmol^{-1}$ cm^{-1}.

ii) The prediction of photocarcinogenic potential should involve *in vitro* and *in vivo* phototoxicity tests where permitted, but this would only apply to chemicals with an MEC >1000 $Lmol^{-1}$ cm^{-1} which absorb light and which are likely to be present in/on skin and/or eye.

iii) We recommend a revision of OECD guideline TG 432 to require phototoxicity assessment if the ultraviolet/visible molar extinction /absorption coefficient of the active substance and its major metabolites is greater than 1000 $Lmol^{-1}$ cm^{-1}.

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