

**COMMENTS ON**  
**GUIDANCE ON A STRATEGY FOR GENOTOXICITY TESTING AND**  
**MUTAGENIC HAZARD ASSESSMENT OF CHEMICAL SUBSTANCES**  
**COM DRAFT PAPER**

**COMMENTS FROM:**

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**GUM - Gesellschaft für Umwelt- Mutationsforschung**  
**Task force Strategies of Mutagenicity Testing**

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## 1. GENERAL COMMENTS

The GUM task force “Strategies of Mutagenicity Testing” appreciates the opportunity to submit comments on the COM Guidance draft paper. Overall, we consider the Guidance paper an important and valuable contribution to the regulatory genotoxicity testing area. Also, we fully endorse the main elements of the proposed strategy, consisting of a two-staged approach supported by appropriate screening and in silico data (stage 0).

Our main criticism relates to the rationale of selecting non-core tests for stage 1 tests. Specifically, we question the relevance of including the in vitro Comet Assay as a follow-up test to clarify positive or equivocal Stage 1 core test results. Moreover, we propose to include the HPRT gene mutation test in the list of acceptable non-core Stage 1 tests. The rationale for our criticism is as follows:

- The strategic use of the in vitro Comet Assay as “adjunct” to recommended core-tests is not plausible. In case of negative Ames and MNvit data, neither a negative nor a positive in vitro comet assay would add any value. In case the Ames test and/or MNvit test is(are) positive a follow-up with the in vitro Comet Assay as an indicator assay that detects primary DNA alterations appears inappropriate even in instances where in vivo testing is not permitted. Therefore, we recommend to eliminate the in vitro Comet Assay from the list of appropriate Stage 1 non-core tests.
- The reason for not including the HPRT-Test as non-core test is not clear. The HPRT-test is being used routinely in the cosmetic, chemical, biocide and agrochemical sectors as part of the basic testing strategy, where three genotoxicity tests have to be carried out. The HPRT test is a robust test system in which numbers of compounds were investigated for genotoxicity. In particular for following-up equivocal or positive Ames Test results, the HPRT test as specific gene mutation test is at least equally suited as the Mouse Lymphoma Assay. We therefore recommend to include the HPRT test in the list of appropriate Stage 1 non-core tests.

## 2. SPECIFIC COMMENTS

Page, line	Comment	Proposed change (if applicable)
Throughout the document	<p>The term “mutation” is exclusively used for “gene mutations”</p> <p>“numerical chromosome damage” is inappropriate</p> <p>Three levels of genetic damage is inappropriate</p>	<p>Nomenclature should be used consistently:</p> <p>When the text refers to gene mutations, this term should be used instead of “mutation” throughout the text.</p> <p>Use “numerical chromosome mutation” instead of damage</p> <p>Use “three classes of mutation”</p>
p.8, line1-5	<p>TTC may give information on whether genotoxicity testing is needed at all rather than whether testing beyond a (negative) Stage 1 is needed. Only the latter is dealt with in chapter 14; reference to TTC therefore seems to be inadequate.</p>	<p>Either delete reference to TTC or take as a separate point, i.e., even Stage 1 tests might not be needed below TTC.</p>
9, line 17	<p>After adequacy add: “and quality”. This is key for evaluating existing data and should be included in the assessment</p>	
9, line 26	<p>MoGA should be explained as it is firstly mentioned here</p>	
10, line 12	<p>The statement that “for genotoxic chemicals the TTC is set at the low exposure level of 1.5 ug/kg bw/day” in conjunction with the citation “Kroes et al., 2004” is not correct.</p> <p>For one, the value is based on daily intake and not on body weight, i.e. it should read 1.5 µg/day.</p> <p>Kroes et al. (2004) showed that estimated lifetime cancer risks for most of the compounds with different structural alerts exceeded 10<sup>-6</sup> at an intake of 1.5 µg/day. In contrast at an intake of 0.15 µg/day this level of risk was exceeded by only about 5% of several other chemical classes, including aromatic amines, aromatic nitrates etc. Hence, for genotoxic chemicals the low TTC level of 0.15 µg/day is applicable. Only for the application of the TTC value for potential genotoxicity/carcinogenicity to genotoxic impurities in medicinal products a higher value of 1.5 µg/day was suggested</p>	<p>Specify the TTC value to 0.15 µg/day for genotoxic chemicals (at the basis of a 10<sup>-6</sup> lifetime risk).</p>

Page, line	Comment	Proposed change (if applicable)
	using a higher cancer risk estimate (10–5 lifetime risk) to allow for the fact that exposure is of direct benefit to the exposed individual.	
11, Chapter 23.	References should be given for the specific recommendations (e.g. auto-oxidation in culture medium). Moreover, we propose to add a sentence about reactions with the solvent. For example, cis-platin does react with DMSO but this is not common knowledge and several studies have been published without taking into account this fact (Fischer et al., 2008).	
17, Chapter 36	<i>Two-test battery: In general, if clear negative results are obtained in all in vitro tests undertaken, it can be concluded that the chemical has no mutagenic activity.</i> We appreciate the strict statement, but it might be open to criticism in light of a few examples like ethylcarbamate and benzene.	Include reference Tweats et al., 2007b (ref. page 8, chapter 17) to acknowledge the existence of “in vivo only” mutagens
p.18, line 3/4	(Negative) in vitro genotox tests usually overrule SAR	...for classes/structures when knowledge suggests that standard test(s) (protocols) would be inappropriate to detect relevant genotoxicity
18, line 31-32	Six in vitro genotoxicity tests had been proposed by SCCNFP for hair dyes and had been heavily criticized (not proposed) by Kirkland et al (2005b)	... or a complex approach involving up to six in vitro genotoxicity tests (SCCNFP/0720/03) which has been critically evaluated by Kirkland et al. (2005b)
20, line 30	Evidenced should be provided that the two test battery is detecting carcinogens with the probability than the three test strategy if the concentrations are reduced to 1mM or 500 ug/ml. This could consist of a Re-analysis of the data presented in Parry et al., 2010 by limiting the evaluation on the ability of a combination of the Ames test and the micronucleus test in vitro to detect rodent carcinogens.	
22, line 27	HPRT test should be included as adequate assays to follow-up equivocal Ames test results.	Add after mouse lymphoma mutation assay: or HPRT test satisfied:
p.27, line 29-31	The statement that in vitro metaphase analysis and in vitro MN assay have similar overall performance as part of a strategy is contradictory to the conclusion that MN is more appropriate for aneugenicity testing.	

Page, line	Comment	Proposed change (if applicable)
29, line 13	<p>The determination of cross-linking agents with the help of the comet assay is not that new. Initial work was already published in 1992 by Olive et al. Fundamental results were then published by Pfuhrer &amp; Wolf, 1996 and Merk &amp; Speit (1999).</p> <p>The same is true for the comet-FISH. First technique was firstly described in 1997 by Santos et al.. The term “comet-FISH” was introduced in 2000 by Rapp et al. by studying the stability of human chromosomes following UVA irradiation.</p>	adapt References
31, line 10	First-site of contact tissue and such organs which are relevant based on toxicokinetic point of view (e.g., accumulation) should be added.	<p>Specify point 4:</p> <p>Investigation of in vivo mutagenicity where there is high or moderate and prolonged exposure (e.g., first-site of contact tissues, evidence for tissue accumulation from toxicokinetic studies)</p>
34, line 26	<p><i>Mode of action / thresholds (e.g. repair of DNA adducts formed from small molecular weight alkylating agents</i></p> <p>Small molecular weight alkyl-DNA adducts are not necessarily well repaired (i.e. adducts resulting from exposure to ENU or MNU) and large adducts are not necessarily poorly repaired.</p>	... formed from <u>many</u> small molecular weight..
35, line 23	<i>typo mouse bone marrow micronucleus</i>	
p.36, line 13/14	“German Speaking section of the European Environmental Mutagen Society”	Should read: GUM (German speaking section of the European Environmental Mutagen Society)
p.38, line 9	Heading “Rodent Bone Marrow MN and CA Assays...”; restriction to Bone Marrow only is not justified, should include “Blood” in heading	
p.38, line 23-27	Given the increasing use of the peripheral blood MN assay in regulatory testing this assay needs more attention in this paragraph and updated references	Include references from previous IWGT discussions on this topic and/or cited references therein.
39, line 27	<p><i>The comet assay can be used in a wide range of species with <b>any</b> tissue including germ cells and can be applied to site-of-contact tests</i></p> <p>Some tissues are really difficult to proceed, such as lung and skin. Thus, it might</p>	... with <b>many</b> tissues

Page, line	Comment	Proposed change (if applicable)
	be better to change the wording e.g. “multiple tissues”.	
41; 11	Wrong name	Eichenlaub-Ritter (also in reference list)
p.49, Table	Row “Rodent Bone Marrow...” in 2. column “aneuploidy” is missing; Rodent Bone Marrow Chromosomal aberration assay is not included in table	Add “aneuploidy” and include Rodent Bone Marrow Chromosomal aberration
p.59, line 9	Wrong name	Rothfuss

### References:

Santos SJ, Singh NP, Natarajan AT. Fluorescence in situ hybridization with comets. *Exp Cell Res.* 1997 May 1;232(2):407-11

Rapp A, Bock C, Dittmar H, Greulich KO. UV-A breakage sensitivity of human chromosomes as measured by COMET-FISH depends on gene density and not on the chromosome size. *J Photochem Photobiol B.* 2000 Jul;56(2-3):109-17.

Stephanie J. Fischer, Linda M. Benson, Abdul Fauq, Stephen Naylor, Anthony J. Windebank, Cisplatin and dimethyl sulfoxide react to form an adducted compound with reduced cytotoxicity and neurotoxicity, *NeuroToxicology*, Volume 29, Issue 3, May 2008, Pages 444-452, ISSN 0161-813X, DOI: 10.1016/j.neuro.2008.02.010.