

COMMENTS FROM ANSES
ON THE GUIDANCE FOR GENOTOXICITY TESTING AND MUTAGENIC HAZARD
ASSESSMENT OF CHEMICAL SUBSTANCES
11th February 2011

Page	Chapter (Line)	Sentence	Comment
7	§14 (L 27-28)	For most chemicals negative results from the two Stage 1 core tests should be sufficient to reach conclusion on the presence or absence of mutagenic potential.	<p>- If this sentence is focused on negative results, it should be reworded as follows: « For most chemicals negative results from the two Stage 1 core tests should be sufficient to reach conclusion on the presence or absence of mutagenic potential ».</p> <p>-There is some discrepancy between this statement and the one made at §17, L 19-22 p8, indicating that “Few chemicals are active only <i>in vivo</i>...”)”because of metabolic activation, influence of the gut flora etc., implying that negative results obtained <i>in vitro</i> are not sufficient to conclude for an absence of mutagenic potential.</p> <p>- The <i>in vitro</i> chromosomal aberration test in mammalian cells is a major endpoint for detecting genotoxic potential and it is not part of the core battery test. It is not fully agreeable to us that the core battery test is limited to the 2 <i>in vitro</i> tests. We are in favor to include as well the <i>in vitro</i> chromosomal aberration test performed with mammalian cells.</p>
9	§19 (L 21-22)	Such SAR data may also be helpful in identifying misleading genotoxicity test results.	<p>Is the correct meaning of this sentence L 21-22 as “SAR data are more informative or trustable than those obtained in <i>in vitro</i> tests”.</p> <p>If not, could it be clarified?</p>
10	§20 (L 4-9)	If (from steps 1-3) there are no genotoxicity data available , then publicly available (Q)SAR databases can provide a preliminary assessment of genotoxic potential (see guidance given below in paragraphs 23-27) and helpful information to aid in deciding priorities for genotoxicity testing. In addition the Threshold of Toxicological	<p>This principle should be extended to the case where no adequate data are available.</p> <p>Therefore, we suggest to slightly modify the beginning of the sentence as follows: « If (from steps 1-3) there are no adequate genotoxicity data available,... »</p>

		Concern (TTC) concept can also provide helpful information to aid in considering testing priorities.	
10	§20 (L 12)	For genotoxic chemicals the TTC is set at the low exposure level of 1.5 µg/kg bw/day (Kroes et al., 2004).	The TTC is not expressed as <i>per kg of body weight</i> but <i>per person</i> . Moreover, the TTC is set at 0.15 µg/person/day (corresponding to a 10 ⁻⁶ lifetime risk of cancer) for chemicals instead of 1.5 µg/kg bw/day for food.
11	§23	Physico-chemical and Toxicological Properties	Regarding the purity of the test chemical, would it be acceptable to add some precision in this chapter 23? For instance, indicate that the purity of a test chemical has to be taken in account since it vary like being : -as pure as possible (the impurities being tested separately) - different to another source of the same test chemical (consider the technical equivalence of different sources)
11	§23 (L 15)	The physico-chemical properties of the test substance (for example pKa, partition coefficient, solubility, and stability in solvents/vehicles) ..	The physico-chemical properties of the test substance (for example pKa, partition coefficient, solubility, volatility and stability in solvents/vehicles) .. Please include volatility in the text.
12	§24	Structure Activity Relationships	Would it be possible to include a table compiling the respective sensitivity and specificity of the various models presented in this chapter? A column listing the number of test chemicals leading to the corresponding sensitivity and specificity would be of value.
15	§29 (L 1-5)	With regard to chemicals for which there are limited, possibly inadequate, or no genotoxicity test data available, a (Q)SAR prediction of mutagenicity (within the domain of applicability of the system(s) used) should be taken as preliminary evidence for potential or lack of potential mutagenicity .	In connexion with a previous sentence of this paragraph (lines 29-32, page 14) which states that « (Q)SAR [...] cannot replace the need to undertake the <i>in vitro</i> and <i>in vivo</i> genotoxicity tests currently required to derive conclusions on mutagenic hazard », we suggest to add the following words (in bold) : « [...] should be taken as preliminary evidence for potential or lack of potential mutagenicity, and be confirmed by adequate <i>in vitro</i> and/or <i>in vivo</i> assays ».
19	§40 (L 25-26)	“There is no convincing evidence that any rodent carcinogen or <i>in vivo</i> genotoxin would be “missed” by using an <i>in vitro</i> genotoxicity	The proposed approach to improve the <i>in vitro</i> detection of positive mutagen, namely by using structure based metabolism predictions, use of genetically

	§41 (L 30-32)	test battery). “It is most likely that the few occasions where <i>in vitro</i> test strategies fail to detect mutagenic activity will be due to the absence of appropriate metabolic activity <i>in vitro</i> ”	modified target organisms and use of exogenous metabolic activation or P450 recombinant systems, should systematically be considered when running the tests in order to avoid possible false negative results.
21	§43 (L 9-11)	Thus the available evidence supports an upper concentration limit of 1mM for mammalian cells but there is a need to reach international consensus on this proposal before making a recommendation on its application to genotoxicity tests using mammalian cells.	We agree with the need of an international consensus before proposing any modification of test guidelines. This consensus should be based on the analysis of the outcomes due to the reduction of the top concentration when evaluating the well-known genotoxic chemicals (i.e. reviewing a large data base of chemicals for which the <i>in vitro/in vivo</i> correlation is well characterized).
21-22	§44 (L 32 p21 L 10 p22)	The COM agrees that it is not necessary to undertake independent confirmatory <i>in vitro</i> tests when clear negative or positive results have been obtained provided the following criteria are satisfied: <ul style="list-style-type: none"> • [...] • the result is neither clearly negative nor clearly positive (i.e. is considered to be equivocal) by appropriate statistical and biological criteria. 	There is an obvious discrepancy that needs to be clarified here.
25	§53 (L 15)	...than cell lines proficient for p53 activity such as TK6, HepG2 and human lymphocytes.	It appears that there is a limited number of data obtained in the HepG2 cell line for genotoxic endpoint, and we consider it important to gain more knowledge of the sensitivity of this cell line reg. genotoxic testing.
27	§57 (L 25-31)	An IWGT (Galloway et al. 2010) has agreed that the preferred measure of cytotoxicity....rather than simple cell counts.	It would be worth to give reference to the use of mitotic index for assessing cytotoxic effects in the <i>in vitro</i> micronucleus test.
30	§62 (L 15-19)	The recommended two core genotoxicity tests in Stage 1 are the <i>in vitro</i> bacterial gene mutation test and <i>in vitro</i> micronucleus test (MNvit). These recommended assays provide sufficient information for the genotoxicity assessment of most chemicals.	It would be worth reminding the sensitivity and specificity of the combination of these two tests. We propose therefore to complete the second sentence as follows: « These recommended assays, when combined , provide sufficient information for the genotoxicity assessment of most chemicals and allow a better sensitivity

			(94%) and specificity (12%), in comparison with other combinations (see annex 1) ».
33	§67 (L 13-17)	“... evidence to suggest that <i>in vivo</i> comet assay and rodent transgenic mutation assays have better sensitivity and specificity for the identification of rodent carcinogens compared with the rat liver USD test..”	From our point of view, it is not unanimously accepted nor demonstrated in the document that <i>in vivo</i> comet and rodent transgenic mutation assays have better sensitivity and specificity for the identification of rodent carcinogens compared with the rat liver UDS test. Evidence cited in the document is mainly based on a review from Kirkland and Speit (2008).
34	§70 (L 15-16)	Examples of irrelevant modes of action in micronucleus tests, for instance, include compound induced hypothermia in rodents and compound induced increases in cell division of bone marrow erythroblasts (Tweats et al. 2007a)	We consider that <u>hyperthermia</u> would also be considered, since it has been shown that increasing the atmosphere temperature leads to increase the micronucleus frequency (Shuey <i>et al.</i> 2007)
39	§80 (L 25-26)	There is now consensus agreement on a protocol for most tissues which would be consistent with an OECD guideline (Burlinson et al., 2007).	To the best of our knowledge, some recommendations are given in three publications (Tice et al., 2000; Hartmann et al., 2004 and Burlinson et al., 2007). Therefore, we don't see it correct to conclude that there is a consensus agreement.
42	Table 1	Investigation of DNA adducts	The ELISA method could also be mentioned (Vidyasagar T. <i>et al.</i> 1997, van Schooten FJ. <i>et al.</i> 1990)
45	Annex 1	Structure Activity Assessments	Please, add a definition of what “concordance” means when mentioned in the case of DEREK and TOPKAT.
48	Annex 1	Untitled table	Adding <i>in vitro</i> prior to MN would make the understanding straightforward.
49	Annex 2	Chromosomal aberrations	In addition to the typological error, it is not clear why the <i>in vivo</i> chromosomal aberration assay in rodents is not cited, unlike the micronucleus assay.
	Figure 2	Screening (stage 0) and <i>in vitro</i> tests	In the lower and left hand clear box, it is written “If high, moderate <u>or sustainable</u> exposure, consider...” To keep consistency with previous wording, we suggest to modify it by “ If high, moderate and prolonged exposure, consider ...”