

Dear colleagues!

Our comments for «Draft COM guidelines»:

#### Part I

Based on **own experience** and literature data, we consider it appropriate to include comet assay as core test for *in vitro* genotoxicity testing for the following reasons:

- In comet assay dead or dying cells can be identified on the same slides by their specific morphology. Such cells exhibit a large diffuse tail with invisible or almost invisible head, so-called “ghost” cells, “clouds” or “hedgehogs”, while a bimodal distribution of cells with control level DNA migration and such cells or cells highly damaged DNA indicate only cytotoxic rather than a genotoxic effect. Also, the available data indicate that *in vitro* comet assay is not prone to false positive calls due to cytotoxicity [Hartmann et al, 2001, 2003]. Thus, comet assay in addition to the evaluation of genotoxicity may provide important confirmatory cytotoxicity information.
- Ames test for some substances with antibacterial activity (e.g. human medicines, components of household or cosmetics) may fail to detect mutagenic activity (i.e. misleading negative results). As stated in draft paper, *in vitro* comet assay can also detect substances that induce gene mutations. Considering this, inclusion of comet assay in a core tests may be useful to avoid underestimation of mutagenic hazard in such cases.
- It was shown *in vitro* and *in vivo* high sensitivity for comet assay. The carcinogens that were positive in comet assay formed 88%, the non-carcinogens that were negative in comet test formed 64% [Anderson et al, 1998]. High positive response (94%) for rodent genotoxic carcinogens and a high negative response (80%) for rodent non-carcinogens were shown [Sasaki et al, 2000]. Also, 49 of 54 (91%) rodent carcinogens that *in vivo* do not induce micronuclei were positive in the comet assay. Although few non-carcinogens have been tested using comet assay available data suggest it relative high specificity. Thus, it is obvious that the comet assay in combination with Ames test and MN test will improve the specificity and sensitivity of *in vitro* genotoxicity testing.

#### Part II

- In Russia *in vivo* genotoxicity testing strategy include two tests for assessment tissue specificity: Comet assay and multi-tissue micronucleus test.
- Multi-tissue micronucleus test is well designed in number of tissue: bone marrow, forestomach, stomach, intestine, colon, lung, urine bladder, liver (in partially hepatoectomised rats), spermatides, blood erythrocytes (Sycheva L.P. et al. 2002, 2007).
- Comet assay and multi-tissue micronucleus test may be used together in the same experiment for assessment some end points of genotoxicity of chemicals.
- Comet assay and multi-tissue micronucleus test may be integrating into subacute or subchronic toxicity studies.
- In our studies multi-tissue micronucleus test used as multi-tissue caryologic test with analysis not only micronuclei, but some another cytogenetic (micronuclei, protrusions, nuclear bridges in cells), proliferation (binucleated, polynucleated cells, mitotic index) and apoptosis end points (cells with nucleus destruction). It is important for elucidation of the mode of genotoxicity action.

Literature:

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