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*Paragraph 50, page 23:*

*“There have been developments to automate and minimise the amount of test substance required for the Ames test (e.g. Spiral Salmonella mutagenicity assay (Claxton et al., 2001) and Ames II<sup>TM</sup> test (Fluckigettr-Isler et al., 2004)). These methods have not been developed to a point where they can be routinely be used for regulatory submissions”.*

**Comment 1 [Para. 50, p. 23].**

We do not agree with the designation of the Ames II test as “not been developed to a point where it can be routinely used for regulatory submissions”. There is extensive experience with the liquid microplate format employed in the Ames II test in laboratories in Europe and elsewhere [4, 6, 7, 8, 9, 10], and a number of publications demonstrating its excellent agreement with results from the standard Ames plate and preincubation tests [1, 3, 5, 10]. The concordances between the Ames II and standard plate or preincubation assay formats correspond to concordances found when comparing inter-laboratory results in the plate incorporation assay. These results demonstrate that the change to a liquid microplate format does not negatively affect the sensitivity and specificity of the Ames tester strains

The Ames II assay produced by Xenometrix AG, using TA98 and TAMix is a fluctuation method of the type listed in the OECD guideline 471. This method is also available for the standard strains TA100, TA1535, TA1537, *E.coli* wp2 [pKM101], *E.coli* wp2 *uvrA*, (Ames MPF), and data presented in several posters demonstrate an excellent concordance with published results for these strains [11, 12, 13]. The assay (Ames II and Ames MPF) is manufactured and performed in compliance with the criteria of the guideline OECD 471 (i.e., the compound is tested in triplicates for each dose level, in presence and absence of metabolic activation S9, in 6 different concentrations starting with top dose of 5mg/ml, with positive and negative controls using the regulatory required *S. typhimurium* strains (Ames MPF) listed in the OECD 471 guideline). Rather than the standard *Salmonella* and *E. coli* strains, the Ames II version uses the single-base-change sensitive *Salmonella* culture TA7001-TA7006 which includes strains with G:C and A:T base-pairs at the site of mutation.

The Ames II assay has been subject to state-of-the art validation studies with industry laboratories and the results were subsequently published. [3, 5]

Based on the available published data a more positive phrasing is warranted than to say that the Ames II assay "...has not been developed to a point where it can be routinely used for regulatory submissions". Ames II data are being routinely used for screening purposes by pharmaceutical and chemical companies in Europe. Available data suggest that miniaturized formats such as the Ames II and the Ames MPF format have identical sensitivities and specificities as the conventional plate incorporation or preincubation assay.

It should also be noted that Sanofi Aventis has registered two compounds with the US FDA and one compound with the Agence Française de Sécurité Sanitaire des Produits de Santé (AFFSAPS) in France based on "Ames data" obtained performing the Xenometrix Ames II assay. The registration of the compounds by Sanofi Aventis was communicated officially by Knut Braun, Sanofi Aventis, at the 40th Annual Meeting EMS (Environmental Mutagen Society), St. Louis, USA, October 24th, 2009, "The use of Ames II Screening Assay for Drug Development".

### **Comment 2 [p 46 – 47]**

The inclusion of a short list of "screening tests" in a separate Table, pg. 46) is highly selective and does not list all the tests addressed in paras. 30 - 32 (p. 15-16). This selectivity in the Table, although not stated, implies that these tests have an advantage over the others not mentioned, despite the caveats expressed in the text. Either all the tests addressed in the text should be listed, or the table is not needed.

### **Comment 3.**

Flückiger's name is misspelled in the references "Flückiger-Isler et al. 2005" throughout the document.

### Publications Ames II:

1. P. Gee, et al. Comparison of responses of base-specific Salmonella tester strains with the traditional strains for identifying mutagens: the results of a validation study. *Mutation Res* (1998) 412, 115-130.
2. W. Piegorsch, et al. (2000) Statistical modeling and analyses of a base-specific Salmonella mutagenicity assay. *Mutation Res* (2000) 467, 11-19.
3. S. Flückiger-Isler, et al. Assessment of the performance of the Ames II assay: a collaborative study with 19 coded compounds. *Mutation Res* (2004) 558, 181-197.
4. E. Lorge et al. Genetic toxicity assessment: employing the best science for human safety evaluation part IV: a strategy in genotoxicity testing in drug development: some examples. *Toxicological Sciences* (2007) 98 (1), 39-42.
5. M. Kamber, et al. Comparison of the Ames II and traditional Ames test responses with respect to mutagenicity, strain specificities, need for metabolism and correlation with rodent carcinogenicity. *Mutagenesis* (2009) 24, 359-366.
6. C.V. Chandrasekaran et al. Evaluation of the genotoxic and acute oral toxicity of standardized extract of *Andrographis paniculata* (KalmCold™). *Food and Chemical Toxicology* (2009) 47, 1892-1902.
7. C.V. Chandrasekaran et al. *In vitro* efficacy and safety of poly-herbal formulations. *Toxicology in Vitro* (2010) 24, 885-897

8. G. de Aragão Umbuzeira et al. Comparison of the Salmonella/Microsome Microsuspension assay with the new microplate fluctuation protocol for testing the mutagenicity of environmental samples. *Environmental and Molecular Mutagenesis* (2010) 51, 31-38

Poster abstracts Ames II/Ames MPF:

9. K. Braun. Automation of the Ames II assay: high through-put screening of mutagenic substances. MipTec ICAR Basel, Switzerland (2001).
10. V. Gervais et al. Assessment of a screening experience with the Ames II test and future prospects. EEMS Aberdeen, Scotland (2003)
11. S. Flückiger-Isler, M. Kamber. The Ames MPF™ assay: novel mutagenicity testing in liquid microplate format using *S. typhimurium* TA98 and TA100. EEMS Prague, Czech Republic (2006)
12. S. Flückiger-Isler, M. Kamber. The Ames MPF™ assays: Novel mutagenicity testing in liquid microplate format using *S. typhimurium* TA98, TA100, TA1535 and TA1537. SOT Charlotte, USA (2007)
13. S. Flückiger-Isler, M. Kamber. The Ames MPF™ PENTA I assay: Mutagenicity testing in liquid format using OECD guideline 471 compliant strains *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2 uvrA plus *E. coli* WP2 [pKM101]. ICEM Florence, Italy (2009).