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The Xenometrix Ames MPF™ assay:
Compliance with OECD Guideline 471 for Testing of Chemicals

The Xenometrix Ames MPF™ assay is a liquid microplate modification of the Ames test based on the fluctuation and preincubation method.

The first parts of the OECD Guideline, i.e. introduction (**points 1–3**) and initial considerations (**points 4–7**) apply entirely to the Xenometrix Ames MPF™ assay.

Principle of the Test Method

Point 8 describes the procedures of the plate incorporation and preincubation methods. The procedure of the Ames MPF™ is the same except for the use of liquid media and multiwell plates. Colony counting is replaced by a colorimetric read-out.

Point 9 specifies the commonly used procedures, among which the fluctuation and preincubation methods are mentioned.

Point 10: "The procedures… pertain primarily to the standard plate incorporation and preincubation methods". It is also recognized that alternative procedures are acceptable but they need to be scientifically justified.

The justification for the use of the Ames MPF™ assay is four-fold:

1. In early development many compounds are available only in small quantities. The Ames MPF™ assay consumes three times less test compound than the standard plate incorporation test.
2. Several publications and posters have shown excellent correlation between the Ames liquid microplate method and the standard plate incorporation test.
3. The Ames MPF™ assay needs considerably less hands-on-time due to the use of multichannel pipettes and ready-to-use media.
4. The 90-minute exposure procedure in the Ames MPF™ assay using a medium with limited histidine and biotin (or tryptophan) corresponds to the preincubation method.

Description of the method

Point 11, (cells of the late exponential phase) and **point 12** (incubation temperature 37°C) are identical to those of the Ames MPF™ assay procedure.

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Point 13: "At least 5 strains of bacteria should be used". Xenometrix offers strains of the recommended combination: TA98, TA100, TA1535, TA1537, *E. coli* WP2 *uvrA* combined with *E. coli* WP2 (pkM101) and the strain *E. coli* WP2 *uvrA*[pKM101].

Point 14 describes the use of established stock culture preparations and the quality control procedures of the strains. The Xenometrix strains are cultured and quality controlled in-house according to the guideline. A certificate of analysis is available for each individual lot. In addition, Xenometrix has sequenced the *his* locus (*S. typhimurium*) or *trp* locus (*E. coli*) of its frozen stocks, and found them consistent with the published mutations.

Point 15 describes the agar preparation for the standard procedure. The Ames MPF™ assay uses a liquid medium and there is no need for minimal and overlay agars. The composition of the MPF™ exposure medium corresponds to that suggested under point 15.

Point 16 explains preparation and use of the metabolic activation system. Preparation and use of S9 in the Ames MPF™ assay are identical to those of the guideline.

Point 17 describes the test substance preparation which is identical.

Test conditions (**points 18–26**): all points are all in line with the Ames MPF™ procedure, except for the exposure concentrations which are expressed as mg/mL rather than mg/plate (points 19 and 21).

Procedure

Point 27 describes the treatment with test substance in the plate incorporation procedure which is not applicable for the Ames MPF™ assay.

Point 28 refers to the treatment with test substance in the preincubation method which corresponds to the Ames MPF™ exposure procedure.

Points 29 (triplicates), **30** (volatile compounds), and **31** (incubation temperature, incubation time) are in line or can be performed with the Ames MPF™ procedure.

The treatment of results

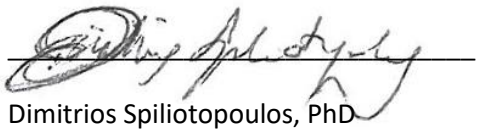
Points 32-34: Data of the liquid Ames MPF™ assay are expressed as revertant wells out of 48 per compound concentration (mg/mL), and not as revertant colonies per plate. All other conditions described under points 32-34 all are fulfilled by the Ames MPF™ assay.

Ad **point 34**, Modification of study parameters for confirmation of equivocal and negative results: The Ames MPF™ assay might be repeated using a different concentration spacing or different metabolic

activation conditions, or the Ames MPF™ procedure might be applied as a modified procedure to the Ames plate incorporation test.

Evaluation and interpretation of the results and test report

The Ames MPF™ data reporting applies to all points described in this section except for the results being expressed as mg (or µg) per mL.



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