AMES^{plus1} - OECD 471 Bacterial Reverse Mutation Test + Confirmation Test

Test definition

Description	Assessment of the mutagenic or promutagenic potential (genotoxicity) of a test item. Guideline OECD 471 requires the verification with a confirmatory test for negative and equivocal results, which occurs in the > 99% of the cases. In the AMES^{plus1} the confirmatory test is included and performed in parallel, reducing time to delivery of the final results, increasing data robustness, while allowing a competitive price.
GLP compliance	Yes
Experimental system	Bacteria: *Salmonella typhimurium, Escherichia coli* – to be selected by Sponsor.

Test item / administration

Test item (TI)	An amount of 1g is deemed necessary to complete the 1st Ames test and the 2nd confirmation Ames test.
Dose	Five different doses are analysed: an initial dose of 5 mg/plate or 5 μL/plate and four additional doses at 1:3 serial dilutions. Exact doses will be established in base of the solubility and cytotoxicity data/information available.
Formulation analysis	Not included. Upon request, at the start of the experimental part, a total of five samples accounting for all concentrations/mixes to be used may be shipped to the Sponsor for formulation analysis.

Experimental design / scope

Experimental approach	Study is divided in three different stages: 1. Sterility, (solubility) and cytotoxicity assays of the TI 2. 1st AMES test 3. 2nd AMES test – confirmation test

Preliminary tests

Sterility test	The Ames test has to be performed with a sterile test item. Otherwise false positive results may occur. TI will be added to agar plates and bacterial growth will be observed. Non-sterile TI will lead to the cancellation of the assay.
Solubility test	Optional – upon request, a standard solvent panel (water, ethanol, DMSO and corn oil) will be used to evaluate maximum solubility of TI at ≤ 50mg/mL.
Cytotoxicity test	The potential cytotoxicity of the TI will be analyzed on one strain (normally *S. Typhimurium* TA 100) and using five concentrations up to and including 5 mg/plate or below if limited by solubility.

1st AMES test

General design

	Bacteria		Positive control	
	Species	**Strain**	**Without S9**	**With S9**
	S. typhimurium	TA 98	2-nitrofluorene	2-aminoanthracene
	S. typhimurium	TA 100	Sodium azide	2-aminoanthracene
	S. typhimurium	TA 1535	Sodium azide	2-aminoanthracene
	S. typhimurium	TA 1537	9-aminoacridine	2-aminoanthracene
	(*) *S. typhimurium*	TA 102	Mitomycin C	2-aminoanthracene
	(*) *E. coli*	WP2 uvrA (pKM101)	4-nitroquinoline	2-aminoanthracene

(*) The fifth bacterial strain is to be selected by the Sponsor – please tick the box with your option in the previous page.

Number of replicas	Three
S9 activation	Included – to assess the pro-mutagenic potential of TI (i.e. mutagenic potential of TI-metabolites resulting from the action of the S9 liver metabolic activation system).

2nd AMES - confirmation test

General design	To confirm results from the 1st AMES test, a confirmatory test (2nd AMES - confirmation test) is performed in the same bacterial strains and conditions but following the preincubation method.

Data report

Results	Classification of TI in view of the number of revertant colonies per plate. Historical data from the vehicle and positive control will be included in the report.
Duration of the study	6 weeks from start of experimental procedure until final report.
Final report	One hard copy and pdf format. Full QA audited report. Archive, only documents, two years included.