

## GENETIC TOXICOLOGY

### Bacterial Reverse Mutation Test - Ames Test

The Ames Test is used to detect potential point mutations, which involve substitution, addition or deletion of one or a few DNA base pairs. This test indicates the genotoxic potential of new substances and can be complemented with other *in vitro* or *in vivo* genotoxicity assays. CIEnP offers the Ames Test according to OECD 471, in GLP or non-GLP conditions.

**Species:** *Salmonella typhimurium* Strains TA97a, TA98, TA100, TA102 and TA1535;  
**Concentrations of Teste Substance:** 5 concentrations (depends on solubility and cytotoxicity);  
**Number of replicates/group:** 3 (triplicate);  
**Control groups:** positive and negative controls for each strain;

**Method:** plate incorporation method (with and without metabolizing system, Aroclor-1254 induced rat liver S9);  
**Main read-outs:** signs of cytotoxicity, signs of precipitation, individual plate counts, number of revertant colonies per plate and standard deviation.

### Validation Data

**Tabela:** Number of revertant colonies of different strains of *Salmonella typhimurium*, without (A) and with (B) metabolizing system (Aroclor-1254 induced rat liver S9). 4-nitroquinoline-N-oxide, sodium azide, 2-Aminofluorene and 2-Aminoanthracene were used as reference items (positive control groups).

Without metabolizing system (S9)					
Strain	Treatment	[ ] µg/placa	Mean	SD	MI*
TA 97a	Vehicle	0	96,7	15,8	-
	4-NQO**	0.5	453,3	38,5	4,7
TA 98	Vehicle	0	20,7	3,0	-
	4-NQO**	0.5	371,7	20,2	17,9
TA 100	Vehicle	0	141,0	18,5	-
	AZS***	1.5	702,0	81,0	4,9
TA 102	Vehicle	0	373,0	10,4	-
	4-NQO**	0.5	1203,0	34,1	3,2
TA 1535	Vehicle	0	16,3	3,5	-
	AZS***	1.5	567,0	71,4	34,7

\* MI: Mutagenic Index  
 \*\* 4-NQO: Positive Control 4-nitroquinoline-N-oxide  
 \*\*\* AZS: Positive Control Sodium Azide  
 SD: Standard Deviation

With metabolizing system (S9)					
Strain	Treatment	[ ] µg/placa	Mean	SD	MI*
TA 97a	Vehicle	0	81,3	22,5	-
	2-AF**	50	1069,3	49,6	13,0
TA 98	Vehicle	0	21,7	2,1	-
	2-AF**	50	3042,0	490,0	140,4
TA 100	Vehicle	0	114,3	16,0	-
	2-AF**	50	2106,0	54,0	18,4
TA 102	Vehicle	0	558,7	56,8	-
	2-AA***	5	1135,3	28,8	2,0
TA 1535	Vehicle	0	15,0	4,4	-
	2-AA***	2.5	148,7	12,7	9,9

\* MI: Mutagenic Index  
 \*\* 2-AF: Positive Control 2-Aminofluorene  
 \*\*\* 2-AA: Positive Control 2-Aminoanthracene  
 SD: Standard Deviation

To avoid bias and to allow reproducibility and reliability all *in vitro* experiments were conducted according to the 471 OECD guidance<sup>1</sup>. The project includes study plan and final report. Raw data are inspected by quality assurance unity.

#### References:

<sup>1</sup> OECD Guideline for Testing of Chemicals: Bacterial Reverse Mutation Test. TG 471. Adopted July 1997.