# JWH-018 Genotoxicity

## GreenScreen HC Summary:

| Test Compound | Cytotoxicity | Genotoxicity | Comment |
|---|---|---|---|
| Methyl-methanesulfonate | NO | YES | Positive control |
| JWH-018 | NO | NO | |

## GreenScreen HC Genotoxicity Individual Data:

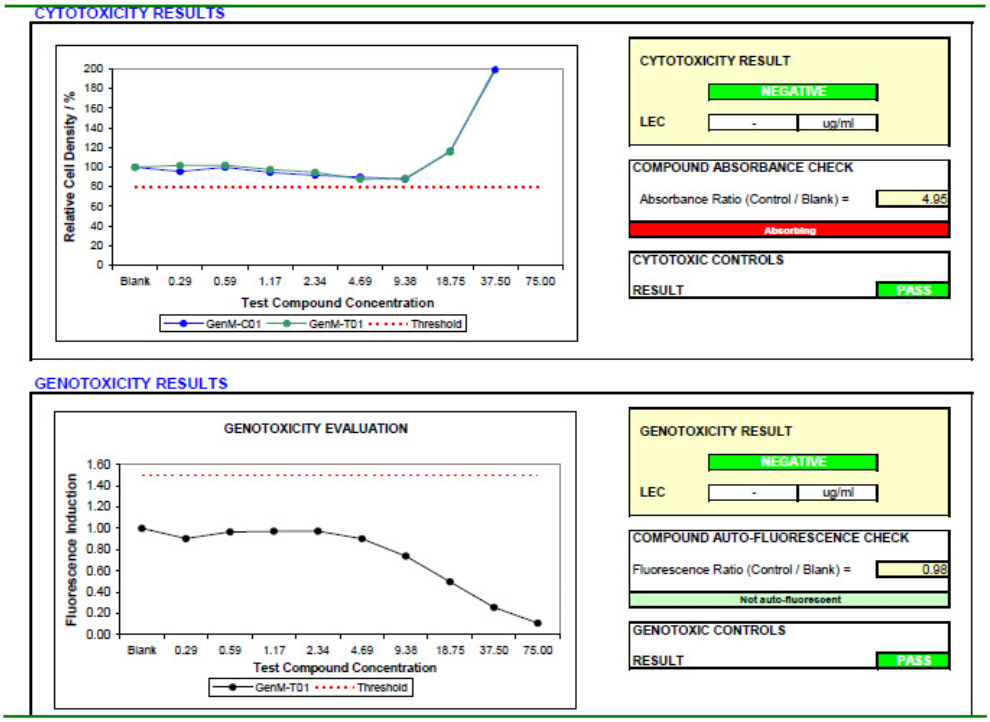


***Figure 1.*** *GreenScreen HC negative genotoxicity results with associated cytotoxicity data for compound JWH-018*

## GreenScreen HC Mammalian Genotox with S9 Experimental Conditions:

| Compound ID | Incubation | Reference compound | Analytical method |
|---|---|---|---|
| JWH-018 | 3 hr with S9 with 45 hr recovery | MMS | FACS |

## GreenScreen HC with S9 Genotoxicity Summary:

| Test Compound | High Concentration Tested (9g/mL) | Cytotoxicity | Genotoxicity | LEC[a] (9g/mL) | Comment |
|---|---|---|---|---|---|
| Cyclophosphamide | 25 | NO | YES | 5 | Positive control |
| JWH-018 | 75 | YES | NO | -- | |

[a]LEC is lowest effective concentration

## GreenScreen HC with S9 Genotoxicity Individual Data:

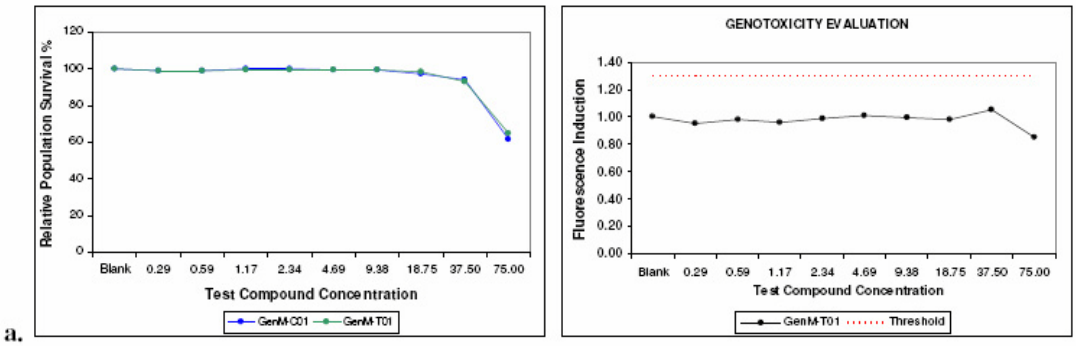


***Figure 2.*** *GreenScreen HC S9 negative genotoxicity results with associated cytotoxicity data for compound JWH-018*

## Standard Methods:

GreenScreen HC, with S9 Genotoxicity Assay: A dilution series of each test compound is generated in a 96-well microplate. Two strains of cultured human lymphoblastoid TK6 cells are used: the test strain (GenM-T01) and the non-fluorescent control strain (GenM-C01). The latter is used to allow correction for any autofluorescence from the test compounds. Incorporated in the test strain is a patented green fluorescent protein (GFP) reporter system, that exploits the proper regulation of the GADD45a gene, which mediates the adaptive response to genotoxic stress.

Exposure to a genotoxic compound increases expression of GFP and hence the induction of cellular fluorescence in the test strain. Compounds are incubated with both cell strains in the presence of 1% v/v S9 fraction mix in Exposure Medium at 37°C (5% CO2, 95% humidity), for 3 hours. After this treatment time, cells are washed in phosphate buffered saline solution, harvested by centrifugation and allowed to recover in Recovery Medium for 45 hours at 37°C, with 5% CO2 and 95% humidity.

Cyclophosphamide, a commonly used control in genotoxicity studies utilizing metabolic activation, is used as the positive control in this assay.

After the recovery period, the GFP fluorescence signal and cell viability (assessed by propidium iodide uptake) are measured, using a flow cytometer (BD FACSCalibur system). The data is analysed to produce a results summary with data presented, both in tabulated and graphical formats, giving a semi-quantitative assessment of cytotoxicity and genotoxicity for the test compound, in combination with S9 fraction.