Cytotoxicity assessment of Fast-Act™ by *in vitro* exposure of human immune cells.

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Plain-language Summary:

In this preliminary study, Fast-Act™ was incubated *in vitro* with cultured human immune cells. The viability of these cells were assessed after 24 and 72 hours of exposure, using a standard method employing a compound that is converted to a coloured product by living cells. Fast-Act™ was not directly toxic to the cells under these *in vitro* exposure conditions.

Detailed Description of Methodology:

Particulate preparation: Prior to incubation, Fast-Act™ was pre-washed in water to remove alkaline MgO, i.e. Fast-Act™ dry powder (20 mg) was suspended in 1 mL of MilliQ water, vortexed to dissolve MgO and centrifuged, with the resultant pellet of solid material (predominantly micro-sized TiO_2 particles) resuspended in 1 mL. When aliquots of the pre-washed Fast-Act™ stock solution were added to cell culture media (ATCC-modified RPMI-1640 + 10% serum), phenol-red in the media indicated that there was a concentration-dependent increase in alkalinity (presumably from traces of dissolved MgO). Physiological pH of 7.4 was restored in wells containing cells by 24 & 72 hours, but not in wells without cells.

Cell culture system: The human immune cells used in this study were the THP-1 human monocyte cell line (ATCC No. TIB-202™; derived from an acute monocytic leukemia). Cells ($1.0x10^5$ cells/well) were exposed in quadruplicate to pre-washed Fast-Act™ at final concentrations of 1, 10, 100 and 250 µg/mL, in a 96-well flat-bottom plate and a final volume of 200 µL per well, for 24 and 72 hours (at 37ºC, 5% CO_2 in a humidified incubator). At 4 hours before the end of the exposure period, 40 µL of "CellTiter 96® AQ_{ueous} solution" (Promega, Madison, USA; containing the soluble tetrazolium MTS salt) was added to the wells, incubated for a further 4 hours (at 37ºC, 5% CO_2) and then the light absorption at 490 nm measured using a microtitre-plate reader.

Results and Conclusions:

The results are expressed below as a percentage of the viability of control (unexposed) cells at each time point. The viability of exposed cells did not significantly fall below the 100% control value, indicating that washed Fast-Act™ particles at *in vitro* concentrations up to 250 µg/mL (0.025% w/v; 250 parts per million, ppm) did not cause direct cytotoxicity in this human immune cell line under these conditions.