

Conclusions

In conclusion it can be stated that during the mutagenicity test described and under the experimental conditions reported, a substantial but not reproducible dose dependent increase of the mutation frequency was observed in the main experiments with and without metabolic activation.

However, the applicant indicated that interpretation of the study followed the methodology laid out by the IWGT (Moore et al 2003) by use of the global evaluation factor (GEF) to assess increases in mutant frequency for significance. In this interpretation a test substance is considered positive if the GEF is exceeded and a positive trend test is also observed. However, an important caveat to this as described by the IWGT where effects are seen only at higher cytotoxicity levels, i.e. lower RTG: "It is generally advisable to conduct one or more additional experiments to better define the assay response (particularly in the (30 – 10%) RTG cytotoxicity range".

In the absence of metabolic activation.

Experiment 1. The mutant frequency was raised above the GEF value threshold only at a concentration approaching the cytotoxicity limit (20 µg/mL) with RTG values of 16.7 and 16.1% in cultures I and II. Concentrations above this were too cytotoxic (RTG less than 10%) and so should not be considered as part of the mutagenicity assessment. In line with the IWGT recommendations these conditions were further investigated in experiments 2 and 3. In experiment 2 concentrations of 20 µg/mL and above were too cytotoxic and in experiment 3 concentrations of 15 µg/mL and above were too cytotoxic. In experiments 2 and 3 in the absence of metabolic activation no relevant increases in GEF above the threshold were observed. Hence, the relevance of the response in experiment 1 must be considered questionable and overall the lack of reproducibility and cytotoxic mediated response would indicate these effects not be of biological significance.

In the presence of metabolic activation, no increase in mutant frequency above the GEF threshold was seen under any condition and in any experiment that was reproducible in both cultures and with an acceptable RTG (>10%). Here again increases in mutant frequency was associated with principally cytotoxic (RTG <10%) concentrations. Hence on this basis, the lack of reproducibility and influence of cytotoxicity, the sporadic increases in mutant frequency are considered to be not biologically relevant.

The RMS agrees that the nonreproducible increase in mutation frequency mainly at (near) toxic doses, are not biologically relevant. Therefore, R613636 is considered non-mutagenic in this mouse lymphoma assay.

Moore, M.M., Honma, M., Clements, J., Bolcsfoldi, G., Burlinson, B., Cifone, M., Clarke, J., Delongchamp, R., Durward, R., Fellows, M., Gollapudi, B., Hou, S., Jenkinson, P., Lloyd, M., Majeska, J., Myhr, B., O'Donovan, M., Omori, T., Riach, C., San, R., Stankowski Jr., L.F., Thakur, A.K., Van Goethem, F., Wakuri, S., Yoshimura, I., Assay: Follow-up Meeting of the International Workshop on Genotoxicity Testing Aberdeen, Scotland, 2003 Assay Acceptance Criteria, Positive Controls, and Data Evaluation. *Environmental and Molecular Mutagenesis* 47: 1-5 (2006) Mouse Lymphoma Thymidine Kinase Gene Mutation