



S2(R1)

***GUIDANCE ON
GENOTOXICITY TESTING
AND DATA INTERPRETATION
FOR PHARMACEUTICALS
INTENDED FOR HUMAN USE***

International Conference on Harmonisation of Technical
Requirements for Registration of Pharmaceuticals for Human Use



Background

- *Too many positives in the in vitro mammalian cells assay systems that may not be relevant to human risk*
- *Taking into consideration of 3R ' s for genotoxicity assays whenever possible “without impacting” the scientific value of the tests and the evaluation of the human risk.*

Objective of the Guideline

- This guidance replaces and combines the ICH S2A and S2B guidelines. The purpose of the revision is to optimize the standard genetic toxicology battery for prediction of potential human risks, and to provide guidance on interpretation of results, with the ultimate goal of improving risk characterization for carcinogenic effects that have their basis in changes in the genetic material. The revised guidance describes internationally agreed upon standards for follow-up testing and interpretation of positive results *in vitro* and *in vivo* in the standard genetic toxicology battery, including assessment of non-relevant findings.

Scope/Content of the Guideline

- The primary focus of this guidance is testing of “small molecule” drug substances, and not biologics as defined in the ICH S6 guidance.

Summary of major points of the revisions

- S2A and S2B guidances merged into one
- Options provided for the test battery
 - Battery with in vitro mammalian cell assay
 - Battery without in vitro mammalian cell assay but two in vivo endpoints

Summary of major points of the revisions

- *In vitro* mammalian cell assay
 - Reduction in top concentration from 10 mM to 1 mM
 - Tightened acceptable cytotoxicity limits
 - No longer require testing of precipitating concentrations
 - The *in vitro* MN is accepted as a third alternative in the *in vitro* assays
- *In vivo* cytogenetic assay
 - Rat blood is now acceptable for *in vivo* MN analysis

Cytotoxicity in *in vitro* mammalian assays

- *In vitro* metaphase chromosome aberrations or micronuclei
 - it is not necessary to exceed a reduction of about 50% in cell growth
- Mouse lymphoma *tk* mutation assay
 - reduction of about 80% in RTG (relative total growth)

Summary of major points of the revisions, continued

- *In vitro* bacterial mutation assay no longer requires duplicate assay
- Integration of genotoxicity endpoints into routine toxicology studies
 - Stringent criteria defined for acceptability of top dose

Criteria for acceptable dose/exposure in (sub)chronic study

- Maximum feasible dose (MFD) based on physico-chemical properties of the drug in the vehicle
- Limit dose of 1000 mg/kg for studies of 14 days or longer, if this is tolerated
- Exposure:
 - a. plateau/saturation in exposure
 - b. accumulation

Criteria for acceptable dose/exposure in (sub)chronic study, continued

- Reduced exposure with time would usually disqualify study
- Top dose is $\geq 50\%$ of the top dose that would be used for acute administration if such acute data are available for other reasons

Summary of major points of the revisions, continued

- Advice on choice of second *in vivo* genotoxicity endpoint
 - includes Comet assay, decreases emphasis on UDS assay
- Provided advice on weight of evidence and data evaluation to determine relevance of positive findings

Benefits of revisions: The 3 R's

- No longer require concurrent positive controls in every *in vivo* assay
- Integration of genotoxicity into toxicology assays
- Reduction in “non-relevant” *in vitro* results will reduce number of follow-up *in vivo* assays

Benefits of revisions:

- Incorporates accumulated knowledge specific to testing of pharmaceuticals
- Takes advantage of new technologies
- More options in the test battery

Benefits of revisions:

- Reduction in delays caused by dealing with “non-relevant” *in vitro* positive genotoxicity results
- More efficient use of resources

Conclusion

- S2A and S2B guidances merged into one
- Options provided for the test battery
- Reduction of irrelevant positives in *in vitro* mammalian cell assay
- *In vivo* assays can be incorporated into repeat dose toxicological assays
- No longer require concurrent positive controls in every *in vivo* assay
- *In vitro* bacterial mutation assay no longer requires duplicate assay