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How do we derisk NDSRIs - not by seeing new sights, but by looking with new eyes?

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Introduction

On September 2024, the much anticipated update to the FDA's Guidance for Industry, "Control of Nitrosamine Impurities in Human Drugs" was published. This update is being called the Revision 2 by the Agency, the original guidance being published in 2020 and updated in February 2021. In this guidance, the FDA has established the much needed bridge with the FDA guidance for industry, *Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities* (NDSRIs) (August 2023) which has been referred to as **RAIL guidance** and the information in the **FDA webpage** on nitrosamines, **https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cder-nitrosamine-impurity-acceptable-intake-limits#compound.** It is clear from the current version that the FDA would like the sponsors to take a holistic view of controlling nitrosamines in pharmaceuticals based on all the information provided in the guidance documents and website. And that is where this story begins. While Revision 2 of "Control of Nitrosamine Impurities in Human Drugs" was sending waves around the industry, a significant change in the content of the website went unnoticed by many. In the section, "Recommended Safety Testing Methods for Nitrosamine Impurities" of the website, an update that merits looking with new eyes.

Discussion

The section "**Recommended Safety Testing Methods for Nitrosamine Impurities**" in the FDA webpage now states the following:

"*In addition to the enhanced Ames test, the **Agency is currently requesting a second in vitro mammalian cell mutation assay and in vitro metabolism data (including human hepatocyte or microsome) to support an AI limit of 1500 ng/day.** Negative results in the in vitro mutation tests along with metabolism data are considered supportive. …. Specifically, a negative result in an **in vivo mutagenicity study may not be supportive of an AI limit equal to the qualification thresholds stated in ICH guidances** for industry Q3A(R2) Impurities in New Drug Substances (June 2008) and Q3B(R2) Impurities in New Drug Products (August 2006). FDA acknowledges that these recommendations may differ from those of other drug regulatory agencies."*

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This information came as a surprise to many since till recently, the pharmaceutical industry has been under the impression that negative results in an Enhanced Ames Test (EAT) performed per the Agency's directions should be adequate to justify an acceptable intake of 1500 ng/day for an NDSRI which has tighter AI based on CPCA. Additionally, the communication from several agencies till date led the industry to believe that an *in vivo* study in a transgenic rodent gene-mutation model should be adequate to justify the control of a nitrosamines above 1500 ng/day, even it may not be at levels based on ICH Q3A(R2) and ICH Q3B(R2).

Now, the question arises as to what kind of additional *in vitro* assays could be used by a sponsor to justify increasing the AI of an NDSRI at 1500 ng/day. While there is no way to read the minds of the regulators, here are a few possibilities. The *in vitro* mammalian cell gene mutation test (OECD 490) could be used to detect gene mutations induced by an NDSRI. This test can include two distinct *in vitro* mammalian gene mutation assays requiring two specific *tk* heterozygous cells lines, L5178Y *tk*+/-3.7.2C cells for the mouse lymphoma assay (MLA) and TK6 *tk*+/- cells for the TK6 assay. Genetic events detected using the *tk* locus include both gene mutations and chromosomal events. This test is positive for simple alkyl nitrosamines such NDMA and NDEA.

OECD 490 could be used in combination with other mammalian *in vitro* assays that have been positive for NDMA including the *in vitro* chromosomal aberration test (OECD 473). This test is capable of identifying agents that cause structural chromosome aberrations in cultured mammalian somatic cells. This test is positive for simple alkyl nitrosamines such NDMA and NDEA.

In vitro metabolism data mentioned by the FDA would probably be experimental in nature and likely focused on Phase I enzymatic activity and the production and identification of potential active metabolites and/or electrophiles from a NDSRI.

Conclusion

The information provided here are just "food for thought" as we await further clarification on what the agency expects in terms of *in vitro* studies beyond the enhanced Ames to derisk NDSRIs. But our best guess is that OECD 490 would be a reasonable complementary mammalian cell study to the enhanced Ames assay. *In vitro* metabolism data requirement is much less defined and could include a number of different endpoints – there is currently no "off the shelf" OECD test guideline for such *in vitro* studies, which adds to the complexity of this requirement.

Regarding the *in vivo* studies, there may be several possibilities and that's a whole different story for another day.