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ecNGS analysis of induced mutagenesis; case studies with noteworthy nitrosamines

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The N-nitrosamines, NDMA and NDEA, are environmental mutagens that have been identified as contamination impurities in some commonly used drugs, resulting in several product recalls. These nitrosamines were evaluated in transgenic gene mutation assays (Muta™mouse or Big Blue® rats, respectively). NDMA mutation was determined at the transgenic lacZ-locus in liver and the endogenous Pig-a gene in peripheral blood, following 28-day dosing. Acute treatments were included to investigate the accumulation and/or additivity of individual dose effects on NDMA mutation induction. NDEA mutation was determined at the transgenic cII-locus. Liver was selected because it is the most sensitive organ for tumour and mutation induction in rodents. There were dose-dependent increases in liver lacZ (NDMA) and cII (NDEA) mean mutation frequencies following 28-day repeat dosing, or after a single dose of NDMA (10 mg/kg). The No Observed Genotoxic Effect Levels (NOGEL) were determined for both nitrosamines. NDEA liver tissue samples were subsequently analysed for DNA mutations using DS (Duplex Sequencing, Twinstrand-Bio) and genome-representative panels of loci to determine mutation frequency directly. The results showed broad agreement with the cII mutation frequency data, with DS revealing slightly higher sensitivity. NDMA samples are currently being analysed. NDMA and NDEA mutagenesis will be discussed in terms of historically defined mutation signatures for both compounds obtained in traditional mutation assays and tri-nucleotide signatures that have been identified for alkylating agents in the catalogue of somatic mutations in cancer (COSMIC). As DS does not rely on phenotypic selection, it will be interesting to compare results and ascertain whether any selection bias exists in the historical data.

Keywords: NDMA, NDEA, Mutation, Duplex-Sequencing, Transgenic gene mutation assays.