TESTING PROPOSAL ON VERTEBRATE ANIMALS – genetic toxicity in vivo

CONSIDERATIONS THAT THE GENERAL ADAPTATION POSSIBILITIES OF ANNEX XI OF THE REACH REGULATION ARE NOT ADEQUATE TO GENERATE THE NECESSARY INFORMATION [please address all points below]:
- List of all substance-specific available GLP and non-GLP studies considered for this test proposal: Thompson (1999). Reverse mutation assay; According to OECD guideline 471; GLP compliant; positive with and without a mammalian metabolic activation system (S9). Nolan (2000). Mammalian cell gene mutation assay; According to OECD guideline 476; GLP compliant; positive for mutagenicity (with evidence of clastogenicity) with and without S9 in two independent experiments. No substance-specific in vivo genotoxicity studies were identified.
- Historical human data: No substance-specific data identified. - (Q)SAR: It is acknowledged that the results of QSAR modelling are of very limited applicability for inorganic substances (e.g. metals) and organometallics.
- In vitro methods: The available in vitro methods have been considered as part of the tiered approach to testing (see "List of substance-specific available GLP and non-GLP studies considered for this test proposal" above for details). Further in vitro testing, for mammalian cell cytogenicity, is considered unnecessary given the available in vitro positive results.
- Grouping and read-across: No critical supplementary read-across data were identified.
- Weight of evidence (summary of key data): Platinum dinitrate was assessed in a bacterial reverse mutation assay using four Salmonella typhimurium strains (TA98, TA100, TA1535 and TA1537) and Escherichia coli strain WP2 uvrA, in both the presence and absence of S9. Mutagenic activity was observed. The compound was also mutagenic at the TK locus of mouse lymphoma (L5178Y) cells when tested in the absence and presence of S9. Albeit limited in its assessment of chromosome effects, this study also indicated a clastogenic potential for the substance. No in vivo genotoxicity studies were identified for platinum dinitrate.

CONSIDERATIONS THAT THE SPECIFIC ADAPTATION POSSIBILITIES OF ANNEXES VI TO X (AND COLUMN 2 THEREOF) OF THE REACH REGULATION ARE NOT ADEQUATE TO GENERATE THE NECESSARY INFORMATION:
- The substance is not classified for carcinogenicity or mutagenicity therefore genetic toxicity testing cannot be waived. As outlined in the Integrated Testing Strategy (ITS) for mutagenicity (ECHA, 2017b), "if there is a positive result in any of the in vitro studies from Annex VII or VIII and there are no appropriate results available from an in vivo study already, an appropriate in vivo somatic cell genotoxicity study should be proposed." No in vivo genotoxicity data were identified for this substance. Moreover, there are no adaptations for in vivo somatic cell genotoxicity testing according to Column 2 of the REACH Annexes on information requirements (EC, 2014). Hence, the observation of mutagenic activity in both bacteria and mammalian cells necessitates the consideration of further in vivo testing "as a last resort" (ECHA, 2016).

FURTHER INFORMATION ON TESTING PROPOSAL IN ADDITION TO INFORMATION PROVIDED IN THE MATERIALS AND METHODS SECTION:
- Details on study design / methodology proposed: In order to assess the potential to induce genotoxicity in vivo, an alkaline comet assay (OECD Test Guideline 489), with a concomitant micronucleus assay and combined toxicokinetic assessment is proposed. The purpose of the comet assay is to identify substances that cause DNA damage, by detecting single and double stranded breaks. "These strand breaks may be repaired, resulting in no

persistent effect, may be lethal to the cell, or may be fixed into a mutation resulting in a permanent viable change. They may also lead to chromosomal damage which is also associated with many human diseases including cancer" (OECD, 2016). In the Comet assay, it is proposed that somatic cells are sampled from three tissues: the liver (systemically exposed tissue) and the glandular stomach and duodenum (site-of contact tissues). The duodenum tissue will be stored/frozen, and only analysed (Comet measurements taken) if both the liver and glandular stomach provide a negative response. Germ cells will also be collected at the same time, stored/frozen, and Comet measurements taken if either the liver or glandular stomach provide a positive response. It is proposed to conduct this study with platinum dinitrate in rats following oral gavage dosing. Due to the corrosivity of the test material, careful consideration of the top dose is required to ensure significant local effects are not produced in the gastrointestinal tract. Also, careful interpretation into the relevance of the effects seen in the 'site-of contact tissues' will be essential. Bone marrow is selected as the target tissue for micronuclei assessment. Inclusion of a parallel toxicokinetic study is proposed for the purpose of demonstrating that adequate target tissue exposure to the test substance has been achieved.