CONSIDERATIONS OF ALTERNATIVE METHODS ON TESTING PROPOSALS IN YOUR REGISTRATION

Please complete this form and provide information for each of the points below.

If you have more than one testing proposal, please copy and paste the three bullet points within the same document and complete the details as appropriate for each testing proposal.

This document will be published on ECHA website along with the third party consultation on the testing proposal(s).

Public substance name: 1-(4-(ACETYLOXY)-3-((ACETYLOXY)METHYL)PHENYL)ETHANONE
EC number: 921-042-4
CAS number: NS

Date of considerations: 3 March 2020

- **Hazard endpoint for which vertebrate testing was proposed:**

  Genetic toxicity in vivo with the registered substance;

- **Considerations that the general adaptation possibilities of Annex XI of the REACH Regulation were not adequate to generate the necessary information** (instruction: please address all points below):

  - available GLP studies
    The following GLP-compliant in vitro and in vivo studies have been considered prior to making the test proposal which is being addressed by this document:

    In vitro: Gene mutation (Bacterial reverse mutation assay): S. typhimurium TA100: positive with and without metabolic activation at 50µg/plate (equivalent to OECD 471); In vitro: Chromosome aberration study. Human peripheral blood lymphocytes: positive at 24 hrs without metabolic activation at 40µg/ml (According to OECD 473); In vitro: Gene mutation (Mouse lymphoma assay): positive at 4 hours with and without metabolic activation (80 and 20µg/ml respectively).(According to OECD 476)

  - available non-GLP studies
    Non-GLP-compliant in vivo genetic toxicity studies are not available

  - historical human data
    No historical human data that could address the remaining concern are available

  - (Q)SAR
    (Q)SAR tools sufficiently addressing in vivo genetic testing are not available

  - *in vitro* methods
    The following *in vitro* test methods have been considered prior to making test proposal which is being addressed by this document:

    OECD 471 – positive data available for the registered substance

OECD 473 – positive data available for the registered substance
OECD 476 – Positive data available for the registered substance

- weight of evidence
  Three separate in vitro tests reported mutagenic potential for the substance. When tested in the Ames Assay the substance induced base pair changes in the S. typhimurium TA100 strain at low concentrations (50µg/plate), both with and without S9 metabolic activation; this implies a direct acting mutagen, possibly by an alkylating mechanism. Further testing in human peripheral lymphocytes without metabolic activation found a significant increase in the number of affected cells exposed to 40µg/ml for 4 hours, indicating clastogenic properties of the substance. This was confirmed in the in vitro mouse lymphoma assay where genotoxic effects were observed following 4 hours exposure to the substance, resulting in a high ratio of small colony formation with and without metabolic activation (80 and 20µg/ ml respectively). The decreased sensitivity of the cells which were exposed to the substance in the presence of S9 would indicate that the substance is a direct acting genotoxin. The available in vitro data suggest there is potential for in vivo mutagenicity, however there are no available data to fill this data gap via a weight of evidence approach. Further studies in vivo (OECD 474 Mammalian Erythrocyte Micronucleus Test and OECD 489 In Vivo Mammalian Alkaline Comet Assay) need to be conducted to confirm the potential of the substance as a somatic genotoxin.

- grouping and read-across: Not applicable
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- substance-tailored exposure driven testing: Not applicable

- [approaches in addition to above [if applicable]

- other reasons [if applicable]

- **Considerations that the specific adaptation possibilities of Annexes VI to X (and column 2 thereof) were not applicable**:
  The substance is not classified for carcinogenicity or mutagenicity therefore genetic toxicity testing cannot be waived. Annex VIII Section 8.4 states: 'Appropriate in vivo mutagenicity studies shall be considered in case of a positive result in any of the genotoxicity studies in Annex VII or VIII'. As there is no appropriate in vivo mutagenicity study to follow up the positive in vitro mutagenicity results, further testing is needed.