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: [3-(2,3-epoxypropoxy)propyl]diethoxymethylsilane
EC Number (omit if confidential): 220-780-8
CAS Number (omit if confidential): 2897-60-1

Date of considerations: 19 September 2016

- **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:

Gene mutation (Bacterial reverse mutation assay / Ames test): positive with and without activation in *Salmonella typhimurium* strains TA 100, TA 1535 and *E. coli* WP2 *uvrA* (OECD TG 471) (BioReliance, 2000).

Cytogenicity in mammalian cells: positive with and without metabolic activation in human blood lymphocytes (OECD TG 473) (SafePharm, 2004cb).

Two micronucleus assays in mouse (oral gavage): Negative (OECD TG 474) (BioReliance, 2001 and Shin-Etsu, 2009).

    - available non-GLP studies:

No non-GLP studies were available.

    - historical human data:

No historical human data were available.

    - (Q)SAR:

QSAR is not considered to be appropriate because there is no existing QSAR method which can discriminate between *in vivo* cytogenicity and mutagenicity, which is the remaining uncertainty of the data set.

- *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 or 490 - consideration has been given to conducting *in vitro* mammalian cell mutagenicity testing of the registered substance. Firstly, this endpoint is not required following a positive *in vitro* cytogenicity result according to Annex VIII of the REACH Regulation. Secondly, it is considered likely that the mechanism of mutagenicity to bacterial cells also applies to mammalian cells *in vitro*. Although an *in vivo* Comet assay is expected to be carried out on the surrogate substance 3-(2,3-epoxypropoxy)propyltrimethoxysilane, CAS 2530-83-8, EC 219-784-2 (awaiting a decision from ECHA), read-across of this study is not considered appropriate in view of the differences between the substances (discussed further below), therefore a new *in vivo* study with [3-(2,3-epoxypropoxy)propyl]diethoxymethylsilane (EC Number: 220-780-8, CAS Number: 2897-60-1) is considered the most appropriate follow-up to the positive result in bacteria.

The available *in vitro* methods have therefore been considered as a part of the tiered approach.

- weight of evidence:

The available *in vitro* data suggest there is potential that the substance is mutagenic and clastogenic. The positive result for clastogenicity was not confirmed in two *in vivo* micronucleus assays with the registered substance, however there is data gap for *in vivo* mutagenicity. Therefore, further information is required on its potential for mutagenicity and an *in vivo* Comet assay is proposed.

- grouping and read-across:

The *in vivo* mutagenicity data available for analogue substances has been considered. The substances considered are:
[3-(2,3-epoxypropoxy)propyl]triethoxysilane, CAS 2602-34-8: no *in vivo* data
[3-(2,3-epoxypropoxy)propyl]trimethoxysilane, CAS 2530-83-8: three *in vivo* micronucleus studies (conflicting results); *in vivo* Comet assay proposed.

The results from micronucleus assays for the potential analogue substance 3-(2,3-epoxypropoxy)propyltrimethoxysilane, CAS 2530-83-8, EC 219-784-2 are conflicting. A Comet assay has been proposed to follow up positive *in vitro* mutagenicity results for 3-(2,3-epoxypropoxy)propyltrimethoxysilane, CAS 2530-83-8, EC 219-784-2. Both the registered substance and the potential analogue substance 3-(2,3-epoxypropoxy)propyltrimethoxysilane, CAS 2530-83-8, EC 219-784-2 are subject to hydrolysis, but the rates of hydrolysis and the final hydrolysis products differ. Read-across of the proposed study on 3-(2,3-epoxypropoxy)propyltrimethoxysilane, CAS 2530-83-8, EC 219-784-2 has been rejected because of the conflicting *in vivo* micronucleus results and differences in hydrolysis rates and final hydrolysis products, therefore it is considered that testing is more appropriate than read-across.

The only data gap for genetic toxicity is that for an *in vivo* study to follow up the positive *in vitro* mutagenicity results, therefore no read-across for genetic toxicity is included in the dossier.

Data have been read-across from [3-(2,3-epoxypropoxy)propyl]trimethoxysilane, CAS 2530-83-8 to fill data gaps for toxicity to reproduction and developmental toxicity, supported by repeated dose data. No adverse effects were observed in 28-day oral repeated dose toxicity studies with the registered substance [3-(2,3-epoxypropoxy)propyl]diethoxymethylsilane or the analogue substance [3-(2,3-epoxypropoxy)propyl]trimethoxysilane.

- substance-tailored exposure driven testing: not 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.