**GSH-depletion by rubber oligomer C13H23Br**

**Introduction:**

According to the information, given in the OECD toolbox, the initial event (adverse outcome pathway, AOP)for skin sensitizing is protein binding Three in-chemico assays are suggested to assess sensitizing potential, Glutathione (GSH) depletion assay, adduct formation with LC-MS and DPRA assay (direct peptide reactivity assay,Cys and Lys reactivity).

Within this project, GSH depletion and GSH adduct formation were investigated.

**Method:**

GSH: 600 ug/ml in PBS pH 7.4

C13H23Br: 500 ug/ml in CH3CN

Mix 500 ul GSH solution with 500 ul C13H23Br solution (final molar ratio 1mM GSH to 1 mM C13H23Br, final solvent composition 50% CH3CN in aqueous PBS pH 7.4)

Reaction up to 20 h at room temperature.

Dilution of samples 1:10 with 50%CH3CN in PBS pH 7.4Analysis of GSH and GSH adduct with LC-MS, ESI positive, ion traces m/z 308 for GSH and m/z 486 for GSH-C13H23Br adduct.

Blank control: Mix 500 ul GSH solution with 500 ul CH3CN.

The reaction was performed in basic conformity with published methods (Natsch 2008, Gerberick 2007), but with some modifications (e.g. 50% organic solvent instead of 25%).

**Results:**

C13H23Br was able to deplete GSH almost complete after reaction at room temperature for 20 h. The reaction product of GSH and C13H23Br was identified by high resolution MS. The amount of adduct raised with reaction time (see diagram below).

In the blank control, after 20 h a GSH depletion of 30% was observed. Obviously, the reaction conditions have to be optimized to avoid GSH autoxidation.

**Conclusion:**

It has to be mentioned, that the method used was not in full compliance with the published methods. However, it could be demonstrated, that C13H23Br is able to deplete GSH and to form GSH adducts. Therefore, it seems plausible to classify C13H23Br as sensitizer.

Further investigations(in-vitro assays like gene expression in keratinocytes (LuSens)or dendritic cell activity assay( h-CLAT and MUSST) or in vivo assays like lymph node assay (LLNA) or Guinea pig maximizing test (GPMT)) should be applied to confirm or reject this classification. In extractable and leachable studies, a SCT of 5ug/d has to be appliedfor sensitizers(PQRI 2013).