Minority opinion regarding the classification of silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica; pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide (SAS-HDMS) as STOT RE 2; H373 (lung, inhalation)

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The minority opinion regarding the classification of this substance is the following:

In the two rat inhalation studies originally present in the CLH dossier (a pilot 14-day study and the main 90-day study), effects on the lungs were seen. In the 90-day key study (Reuzel et al., 1991), with 35 mg/m^3 as only dose tested, some of the effects on lungs showed only a slow or even no recovery, e.g. granuloma-like lesions and increased septal cellularity were still observed 39 and 52 weeks after treatment, respectively. Focal interstitial fibrosis was first observed 13 weeks after exposure in males, but was no longer seen after 39 weeks recovery.

During the public consultation a re-analysis of the lung tissue slides of the Reuzel et al. (1991) study was provided, concluding that focal interstitial fibrosis, an irreversible disease, was not present in the lungs of the treated rats at any time point. Fibrogenesis, a reversible process, was considered the main finding, along with extensive local inflammation in the lung (Weber *et al*., 2018). But, as noted in the RAC opinion, some critical comments can be made on this re-analysis and the length of the recovery period:

- the re-evaluation did not concern all animals, and only one lung section per animal;
- for re-evaluation, the almost 30-year old slides were de-coverslipped, restained (with standard hematoxylin and eosin (H&E) staining) and then coverslipped again, whereby the de-coverslipping may potentially have damaged the original tissue samples;
- the standard H&E staining is considered of lower sensitivity than e.g. Van Gieson staining in showing an increase in fibre tissue;
- the claimed recovery pertains to unusually long recovery periods for a 13-week rat study (13-52 weeks, as compared to 4 weeks as recommended in the OECD test guideline).

In deciding on the classification, RAC took also note of the results of some additional repeated dose inhalation studies reviewed in Becker *et al.* (2013) and ECETOC (2006), but were not part of the CLH dossier. Notably, interstitial fibrosis was also seen in an 1-yr inhalation study with monkeys and in an 8-12 month inhalation study with female rats, and in both studies the fibrosis did not resolve during recovery. Despite ECETOC rating these two studies a low reliability score (corresponding to Klimisch 4), these studies support that fibrosis is not an uncommon finding for this substance. Although strictly the effective dose in the Reuzel et al. (1991) study fits STOT RE 2, this dose is very close to the guidance value for STOT RE 1 (20 mg/m^3). Unfortunately, Reuzel et al. (1991) did not test below 35 mg/m^3, and also most other studies did not test below the (extrapolated) guidance value for STOT RE 1. There is in fact only one study that has studied the effects of SAS-HMDS at low dose levels. This is a 90-day rat inhalation study by Wacker (1998), as reviewed in ECETOC (2006) and given a reliability score corresponding to Klimisch 1. In this study, no fibrosis was seen at doses of 0.51, 2.05 or 10.01 mg/m^3, but rats did show several lung effects at 10 mg/m^3, including histiocytosis in lung draining mediastinal lymph nodes. This (severe) finding was reported to be reversible, but again only after a longer than usual recovery period. It is noted that in the Reuzel et al. (1991) study the lungs and

associated mediastinal lymph nodes contained relatively high levels of silicon, the amount of which decreased only slowly during the recovery period.

Given that a study with a reliability rating corresponding to Klimisch 1 must be seen as a good quality study, that this study showed severe lung effects at a dose below the guidance value for STOT RE 1, and that the key study by Reuzel et al. (1991) does not contradict this, overall classification as STOT RE 1; H372 (lung, inhalation) is considered more appropriate than STOT RE 2; H373 (lung, inhalation).