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Brussels, 4 August 2017

Re: Comments on the Proposal for Harmonised Classification & Labelling (CLH) of Trimethoxyvinylsilane (EC No. 220-449-8; CAS No. 2768-02-7) as Skin Sens. 1B H317)

Substance Name:	Trimethoxyvinylsilane
EC Number:	220-449-7
CAS Number:	2768-02-7

Dear Sir or Madam:

I am writing in response to the public consultation on the proposed hazard classification of Trimethoxyvinylsilane (EC No. 220-449-8).

General comment

In its CLH report 'Proposal for Harmonized Classification and Labelling' (Vers. 2; Date: 170329), the Swedish Chemical Agency proposes to classify trimethoxyvinylsilane (EC no. 220-449-8) as Skin Sens. 1B (H317) under Regulation (EC) No 1272/2008 ('CLP Regulation'). The Swedish Chemical Agency evaluated the quality and reliability of a total of 5 *in vivo* skin sensitisation studies available on the substance in guinea pigs, 2 according to the Buehler test and 3 according to the maximisation test protocol. Only one of the 5 studies, a Buehler test, resulted in a positive outcome indicating a skin sensitisation potential for trimethoxyvinylsilane. Based on a self-developed model, the CLH report submitter considered a single positive Buehler test to be more sensitive than the 3 guinea pig maximisation and 1 Buehler tests on their own right and in combination.

It is my view that the CLH proposal to classify trimethoxyvinylsilane as Skin Sens. 1B is neither based on a thorough evaluation of the science nor on an appropriate application of the CLP criteria for skin sensitisation and should therefore be reconsidered.

My assessment is based on the fact that the consistent absence of a skin sensitisation potential of trimethoxyvinylsilane in four animal studies was not considered in an appropriate weight of evidence manner. Moreover, the model used by the CLH report submitter to argue the higher sensitivity of the single positive Buehler assay versus the 3 guinea pig maximisation and 1 Buehler tests is flawed and the actual calculations to rank order the reliability of the available animal studies are based on a chain of unsupported and partly faulty assumptions. More detail is provided in section 'specific comments/skin sensitisation'.

Specific comments/Skin sensitisation

Three of the four *in vivo* studies which the CLH report submitter did not consider in its final evaluation (i.e., studies II, III, IV) are of good quality and largely in line with OECD TG 406. Where deviations from OECD TG 406 occurred (as per the description of CLH report submitter), these are minor with no expected impact on the study outcome. Since the full study reports are not publicly available for review, I recommend checking the quality assurance procedure of the contract laboratory of study III, before prematurely concluding that the absence of a positive control in the actual study limits its reliability. To reduce the amount of laboratory animals used for toxicity testing, contract laboratories do not always include a positive control into each skin sensitisation study, but regularly conduct positive control testing to assure the sensitivity of the different skin sensitization protocols ran within the contract laboratory. The procedure is typically stated in the study report. I agree with the Swedish Chemical Agency that, due to the observed precipitation and polymerisation of the test substance, the outcome of study V should be regarded with a certain degree of uncertainty. However, it should still be considered in an overall weight of evidence context as the result of study V matches those of studies II, III and IV.

The model which the CLH report submitter developed to estimate 'internal doses' resulting from the *in vivo* studies with trimethoxyvinylsilane presents an interesting approach to take account of potential hydrolysis of the substance under actual testing conditions, but is flawed with regard to estimating exposure and ranking the reliability of the studies for hazard classification purposes. If at all, it establishes a sort of bioavailable concentration based on a chain of unsupported assumptions, but not an internal dose. Thereby it ignores the accepted concept of dose metrics in the acquisition of skin sensitisation which has been established by Kimber *et al*. (2008). Moreover, the model does not take into account the basic principle of the GPMT to maximise exposure by intradermally injecting the test substance, thereby bypassing the skin barrier, and to increase the sensitivity of the animal (compared to the Buehler test) by concurrent injection of Freund's complete adjuvant.

Following a thorough review of the scientific evidence along with the immunologic basis for skin sensitization, Kimber *et al*. (2008) concluded that, for topically applied substances, the dose of chemical per unit area of skin rather than the total amount of the chemical delivered is the key metric in terms of effectiveness with which sensitisation is acquired. Kimber *et al*. (2008) stated further that the only exception to this rule is when very small topical application sites are considered. This is however not the case for the Buehler or maximisations test where topical patches of 4-8 cm^2 are being applied.

The model proposed by the CLH dossier submitter is significantly hampered by not taking into account the total mass of the test substances per unit area of skin applied in the induction phase of the respective studies. This should be straightforward to determine for topical applications in the Buehler test and the topical induction of the GPMT, but it is yet unclear how to consider the first phase of the GPMT, the intradermal injection of the test substance alone and test substance mixed with the Freund's complete adjuvant in this context. When comparing outcomes from a Buehler test with those of the GPMT, it is particularly this intradermal induction step which increases the GPMT's effectiveness in triggering a sensitisation response and makes, along with the longer induction patch application (48 hrs in the GPMT vs 6 hrs in the Buehler assay), the GPMT more sensitive compared to the Buehler test. This is scientifically widely recognised (see for example van Loweren *et al*., 2008) and the reason why the regulatory community prefers the GPMT over the Buehler test for skin sensitisation

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hazard identification purposes. Not considering these differences in dosing with its impact on the sensitivity of the assays is a major flaw in the CLH dossier submitter's model.

Notwithstanding the lack of consideration of the sensitivity differences between the two guinea pig assays, another significant flaw in the CLH dossier submitter's is the assumption that trimethoxyvinylsilane is rapidly and completely taken up by the skin (i.e., 100%). According to Shen J. *et al*. (2014), for chemical substances with a molecular weight of 150 Da and a log Kow of 1.1 (i.e., the same parameters considered by the CLH dossier submitter for Trimethoxyvinylsilane), a maximum flux of 1.0 – 10 $\mu g/cm^2/h$ is considered leading to a proposed default absorption of 40% per 24 hrs of skin exposure. According to OECD TG 406, the induction patches in a Buehler test are applied for 6 hrs, while in a GPMT the induction patches are applied for 48 hrs. If linearity is assumed, one could estimate an absorption of 10% of the topically applied trimethoxyvinylsilane in the Buehler test and 80% of the topical induction dose in the GPMT.

Using these different absorptions in the dossier submitter's algorithm with the same assumptions of hydrolysis (Table 20) would lead to a completely different picture: For study I (positive Buehler test), one would calculate an 'estimated internal induction dose' of 9.3%. In study III (negative GPMT), one would calculate an 'estimated internal induction dose' of 52%. For the 2nd negative GPMT, study IV, an 'estimated internal induction dose' of 45% would be calculated. When looking at these very different 'intake' values, the observed sensitisation rate of 65% for study I at lower intake level is not consistent with the 3 negative GPMT studies and appears a chance finding.

This calculation example is meant for illustrative purposes and should not be considered as support for the dossier submitter's model for the mechanistic reasons discussed before. Also, trimethoxyvinylsilane's hydrolysis rate of 5% or 50% upon contact with skin moisture or via the vehicles is purely speculative and should not be used in support of a skin sensitisation hazard classification proposal as long as not understood.

In conclusion, the CLH proposal to classify trimethoxyvinylsilane as Skin Sens. 1B is neither based on a thorough evaluation of the science nor an appropriate application of the CLP criteria for skin sensitisation. The weight of evidence from five *in vivo* skin sensitisation studies in guinea pigs according to the Buehler and the maximisation test protocol suggests that trimethoxyvinylsilane should not be classified as skin sensitiser. This is due to the consistent absence of a skin sensitisation potential of trimethoxyvinylsilane in four animal studies, 3 GPMT's and 1 Buehler assay versus a positive finding in a single Buehler test. The model used by the CLH report submitter to argue the higher sensitivity of the single positive Buehler assay versus the 3 guinea pig maximisation and 1 Buehler tests is flawed and the actual calculations to rank order the reliability of the available animal studies based on a chain of unsupported and partly faulty assumptions. Finally, I suggest including the review and consideration of available human exposure data in any update of the CLH report on the classification of trimethoxyvinylsilane for skin sensitisation.

Dr. Thomas Petry, DABT, ERT

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