Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Tetramethylene dimethacrylate

EC Number: 218-218-1
CAS Number: 2082-81-7

CLH-O-0000007058-72-01/F

Adopted

26 November 2021

26 November 2021

CLH-O-0000007058-72-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **Tetramethylene dimethacrylate**

EC Number: **218-218-1**

CAS Number: **2082-81-7**

The proposal was submitted by **Finland** and received by RAC on **13 July 2020.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Finland has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **9 November 2020**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **22 January 2021**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Bogusław Barański**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **26 November 2021** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	Tetramethylene dimethacrylate	218-218-1	2082-81-7	Skin Sens. 1B	H317	GHS07 Wng	H317			
RAC opinion	TBD	Tetramethylene dimethacrylate	218-218-1	2082-81-7	Skin Sens. 1B	H317	GHS07 Wng	H317			
Resulting Annex VI entry if agreed by COM	TBD	Tetramethylene dimethacrylate	218-218-1	2082-81-7	Skin Sens. 1B	H317	GHS07 Wng	H317			

GROUNDS FOR ADOPTION OF THE OPINION

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) assessed the skin sensitising property of tetramethylene dimethylacrylate using the available human data and the results of five animal studies: one murine local lymph node assay (LLNA) and four guinea pig studies.

Animal studies

1.The **LLNA** was conducted in accordance with OECD TG 429 (2010) and principles of GLP (Anonymous 2014) and is considered as reliable (Reliability score 1) and as key study by DS. A pre-test was performed in two animals with concentrations of 50 and 100% to determine the highest non-irritant test concentration. The mouse treated with the undiluted test substance showed slightly reduced spontaneous activity, and an erythema of the ear skin was observed in both animals (score 1 in the mouse treated with 50% concentration, score 1-2 in the mouse treated with 100% concentration). Furthermore, scabby ears were observed on day 5 in the animal treated with the undiluted test substance. In the main study, three treated groups of five CBA/CaOlaHsd female mice aged 8-9 weeks and weighing 17.8-22.3 g (mean 20.3 g ± 1.2 g) were used. The animals were treated by topical application to the dorsal surface of left and right ears with test concentrations of 25, 50 and 100% in acetone/olive oil (4+1, v/v).

The control group of five mice received vehicle only. Five days after the topical application, all mice were given 250 µl of 19.5 µCi 3H-methyl thymidine (corresponds to 78 µCi/ml 3H-methyl thymidine) by intravenous injection via the tail vein. The proliferative capacity of the cells was determined by the incorporation of 3H-methyl thymidine measured on a β-scintillation counter. No mortality was observed during the study period. All treated animals showed unspecific clinical signs on day 3, including reduced spontaneous activity, ruffled fur and hunched posture. In this study, Stimulation Indices of 2.74, 3.76, and 5.72 were determined at concentrations of 25, 50 and 100%, respectively and EC3 value was 31.4% (w/v).

2. The first **guinea-pig study** (Anonymous 1984a) was conducted according to OECD TG 406 but GLP conditions were not specified. The DS has assigned to this study reliability score of 3. The female guinea pigs (no. of animals not specified) were induced on day 0 with 1% intradermal injections of tetramethylene dimethylacrylate. Purity of the test substance is not specified in the study report. On day 7, approximately 250 mg of 10% sodium lauryl sulphate in petrolatum was gently massaged into the neck and left uncovered for 24 hours. Epicutaneous application of 5% tetramethylene dimethacrylate followed on day 8, and the dressing containing the test solution was left in place for 48 hours. The vehicle controls received the same treatment, but with an equivalent amount of petrolatum. Challenge exposure was performed on day 21 using an occlusive epicutaneous application with a 25% concentration, and readings were made on days 23 and 24 (after 48 and 72 hours, respectively). The vehicle controls received identical treatment. Positive control not specified. The test substance was not found to be skin sensitising in the study.

3. The second guinea-pig study, a non-guideline Freund´s complete adjuvant test (FCAT), GLP conditions not specified, was conducted on groups of eight albino female guinea pigs (Reliability

score 3) (Anonymous 1983a). The purity of tetramethylene dimethacrylate was 97%. According to the authors, sensitisation to impurities cannot be completely excluded. There were four to six animals in the control group in the FCAT. A pre-test with FCA-treated animals preceded both studies. The animals were induced with intradermal injections of 0.5 M tetramethylene dimethacrylate which, according to the authors, corresponds to a 13% concentration. The 3 M (78%) concentration was used for challenge and rechallenge exposures. Aramek mixture of methyl ethyl ketone:arachis oil 2:1 was used as a vehicle for the closed patch induction and for challenge tests. There is no information on mortality or clinical signs. After challenge 8/8 animals were sensitised on day 21, after rechallenge 5/8 animals were positive on day 35.

4. In the third guinea pig study (Reliability score 2) the sensitisation potential of tetramethylene dimethacrylate was examined in a non-guideline guinea pig maximisation test with no specified GLP (Anonymous 1983b). The study was done with Himalayan white spotted female guinea pigs with 10 animals in the treatment group and 6 animals in the control group. The animals were induced with intradermal injections of 0.5 M tetramethylene dimethacrylate which, according to the authors, corresponds to a 13% concentration. Undiluted substance was for topical induction exposure (day 7). The 1 M (26%) concentration was used for challenge and rechallenge exposures on days 21 and 36. Petrolatum or 80% ethanol was used as a vehicle for the topical induction. For challenge tests Aramek mixture of methyl ethyl ketone:arachis oil 2:1 was used as a vehicle. There is no information on mortality or clinical signs. After challenge on day 21 0/10 animals were sensitised, after rechallenge on day 35 2/10 animals were positive. According to the authors, a third challenge has been performed on day 49 which confirmed the results of the rechallenge, but the data are not shown in the publication.

5. In the fourth guinea pig study (Reliability score 2) 10 female Dunkin-Hartley guinea pigs each received tetramethylene dimethacrylate at concentration of 2% (w/w) in olive oil/acetone for intradermal induction, 50% in petrolatum for topical induction, and 1% (w/w) in petrolatum for challenge and rechallenge exposures (test item amount equivalent of ca. 0.015 g) (Anonymous, 1984b). Ten animals were used in the control group. Before topical induction, a pre-treatment with 10% sodium lauryl sulphate (w/w) in petrolatum was used. Positive control is not specified. A booster dose was applied intradermally on the neck using the same concentration and vehicle 48 hours after the first challenge. The rechallenge occurred one week after the first challenge. None of the animals were sensitised in this test, but it is not documented whether the scores were obtained after the first or the second challenge. No clinical observations or macroscopical findings are described in the study report.

Human data

A total of 26 clinical studies have been identified for tetramethylene dimethacrylate (**Error! Reference source not found.**). The studies comprised a total of 128 patients who tested positive to the substance. In all studies, the diagnostic method was patch testing. Data on level and frequency of skin exposure to tetramethylene dimethacrylate is scarce.
Diagnostic patch testing is conducted in order to diagnose contact allergy to a substance and was performed according to international standards by dermatologists (Johansen et al. 2015). The results of such tests are usually reported as number of patients/subjects with positive reactions in relation to the total number of tested (frequency of positive patch tests). An important factor of assessing prevalence of positive reactions in diagnostic patch test is how the group of patients is defined, i.e., if they are selected in some way or not. Selected patients can be, for instance, patients with dermatitis suspected of having contact with acrylic compounds or special

occupational groups (aimed testing). Consecutive or unselected patients are groups of patients for whom allergic contact dermatitis is generally suspected.
There are no studies on diagnostic patch tests with tetramethylene dimethacrylate in general population or unselected clinical patients.

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
CASE REPORTS ON SINGLE CASES				
Case report	Tetramethylene dimethacrylate (2%, Chemotechnique's test substance i.e. in petrolatum)	A 38-year-old female was sensitised to a glue used in the attachment of car rear-view mirrors to the windscreen (with 6 years of work history). She developed a dry and fissured dermatitis on fingers and palms of both hands. The dermatitis spread within a couple of weeks to lower arms, chest, neck and face, and she developed rhinitis, paresthesia of fingertips and gastrointestinal complaints.	13 acrylic compounds provoked mild to extreme allergic reactions in a patch test. Positive reaction to test substance (++ on day 2, ++ on day 3, ++ on day 4). Tetramethylene dimethacrylate was not mentioned in the safety data sheet of the glue or detected in chemical analysis.	Kanerva *et al*. (1995)
Case report	Tetramethylene dimethacrylate (2%, vehicle not specified)	A 47-year-old atopic female cosmetician developed dermatitis on her thumb within some weeks after starting to work with photobonded nails. The dermatitis spread to both hands, and after stronger exposure to UV-gel 3 months later, she developed a severe hand and face dermatitis.	Allergic reactions to 15 (meth)acrylates, a total of 31 were tested Allergic reactions to the test substance (+ was the strongest reading on days 2, 3 and 4) Tetramethylene dimethacrylate was not detected in chemical analyses of the nail products.	Kanerva *et al*. (1996)
Case report	Tetramethylene dimethacrylate (concentration and vehicle not defined)	47-year-old woman had used acrylic nails for 10 years. She presented with periungual dermatitis of all the fingers. Symptoms had begun 6 months earlier.	She tested positive to 11 acrylic compounds including the test substance. Tetramethylene dimethacrylate reaction was + at 96 hours.	Paley *et al*. (2006)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
PATIENT SERIES				
Patient series	Tetramethylene dimethacrylate (2% in petrolatum)	7 patients occupationally sensitized to methacrylate-based dental composite products	1 patient reacted positively to the test substance out of 5 patients tested (20%). The test substance was not mentioned in safety data sheets of the products.	Kanerva *et al*. (1989)
Patient series	Tetramethylene dimethacrylate (2% in petrolatum), purity 97%	126 dental technicians were tested with (meth)acrylates in 1995-1999 in Department of Dermatology, Städtische Kliniken (Dortmund, DE)	Positive reaction to the test substance in 6 of 126 patients (4.8%), all the reactions were assessed clinically relevant i.e. the sensitised persons had handled tetramethylene dimethacrylate-containing products. Authors considered that the test substance was a weak sensitiser in comparison to methyl methacrylate due to low number of positive reactions despite common exposure.	Peiler *et al*. (2000)
Patient series	Tetramethylene dimethacrylate (2% in petrolatum)	A retrospective study of 13 833 patients tested for contact allergy at the Department of Dermatology, Catholic University (Leuven, BE) in 1978-1999 It is unclear how many patients were tested with (meth)acrylates.	Positive reaction to the test substance in 5 of 72 patients (6.9%) who were positive to some (meth)acrylate.	Geukens & Goossens (2001)
Patient series	Tetramethylene dimethacrylate (2% in petrolatum)	The incidence of allergic contact dermatitis was studied in 79 dentists and 46 dental nurses who were referred to the	In dentists sensitised to acrylic resins, 8 of 20 patients (40%) reacted positively to the test substance. There	Kiec-Swierczynska & Krecisz (2002)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		Institute of Occupational Medicine (Lodz, PL) in 1990-2000. All were tested with the European standard set, dental screening test and additional allergens.	were no positive reactions to the test substance in dental nurses.	
Patient series	Tetramethylene dimethacrylate (2% in petrolatum)	90 patients suspected of having dermatitis caused by (meth)acrylates were patch tested at the Department of Occupational and Environmental Dermatology (Malmö, SE) in 1995-2004	24 patients reacted to some (meth)acrylate. 16 of these patients were tested with the test substance, and 3 of them tested positive (18.8%). It is unclear how many patients in total were tested with tetramethylene dimethacrylate.	Goon *et al*. (2007)
Patient series	Tetramethylene dimethacrylate (2% in petrolatum)	473 patients were tested with a (meth)acrylate series at Finnish Institute of Occupational Health (Helsinki, FI) in 1994-2006. 32 patients with allergic reaction to some (meth) acrylate and working in dental professions (dentist, dental nurse, dental technician) were identified.	Positive reactions to the test substance in 3 cases: 1 dentist (++ reaction), 1 dental nurse (++ reaction) and 1 dental technician (+ reaction). Tetramethylene dimethacrylate was not mentioned in safety data sheets of the products used by these 3 patients.	Aalto-Korte *et al*. (2007)
Patient series	Tetramethylene dimethacrylate (2% in petrolatum)	473 patients were tested with a (meth)acrylate series at Finnish Institute of Occupational Health (Helsinki, FI) in 1994-2006. Among 61 patients with allergic reaction to some	Positive reaction to the test substance in 4 (40%) of 10 patients (++ in three patients, +++ in one patient). All 4 patients had handled methacrylate-	Aalto-Korte *et al*. (2008)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		(meth)acrylate, 10 patients with present occupational exposure to acrylic glues were identified.	based glues but tetramethylene dimethacrylate was not mentioned in the safety data sheets of the glues.	
Patient series	Tetramethylene dimethacrylate (0.1% in petrolatum)	A retrospective study on 43 patients diagnosed with allergic contact dermatitis caused by (meth)acrylates in long-lasting nail polish at dermatology departments of 4 Spanish hospitals in 2013-2016	Positive reaction to the test substance in 1 patient out of 7 (20%) tested with the substance within the group of 43 patients.	Gatica-Ortega (2017)
Patient series	Tetramethylene dimethacrylate (2% in petrolatum)	A retrospective study on 16 nail technicians with methacrylate allergy who had been patch tested at the Department of Dermatology (Gävle and Malmö, SE) in 2007-2016	Positive reaction to the test substance in 2 of 16 patients (12.5%).	Fisch *et al*. (2019)
Patient series	Tetramethylene dimethacrylate (2% in petrolatum)	A retrospective study on patients suspected of nail manicure-related sensitisation to (meth)acrylates at dermatology departments of 3 Spanish hospitals in 2008-2017 A total of 208 patients were tested with (meth)acrylates.	66 patients reacted positively to at least one (meth)acrylate and the sensitisation was due to nail products. In this group, positive reaction to the test substance in 6 of 26 patients (23.1%) tested with the substance.	Marrero-Alemán *et al*. (2019)
CROSS-SECTIONAL STUDIES ON RISK OCCUPATIONS				
Cross-sectional study	Tetramethylene dimethacrylate (2% in petrolatum)	A questionnaire was sent to 1132 dental technicians and 173 answered. 55 cases were patch tested.	Tetramethylene dimethacrylate was positive in 1 (2%) case of those tested (N=55).	Rustemeyer & Frosch (1996)
Cross-sectional study	Tetramethylene dimethacrylate (Chemotechnique's	49 out of 1038 dental technicians voluntarily participated in a	Positive reaction to the test substance in 1 case, 2.1% of those tested.	Lee *et al*. (2001)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
	test substance i.e. 2% in petrolatum)	study on patch testing at the Department of Dermatology in the Catholic University of Korea (Seoul, KR)	7 patients were positive to some acrylic substance. The test substance-positive case constituted 14% of this group.	
CLINICAL PATCH TEST DATA ON SELECTED PATIENTS (AIMED TESTING WITH ACRYLIC COMPOUNDS)				
Patch test data, selected patients	Tetramethylene dimethacrylate (2% in petrolatum)	A retrospective study on 23 patients patch tested with (meth)acrylate series at the Nofer Institute of Occupational Medicine, Lodz (PL) in 1990-1994	Positive reactions to the test substance in 2 (9.5%) dentists out of 21 patients tested with the substance.	Kiec-Swierczynska (1996)
Patch test data, selected patients	Tetramethylene dimethacrylate (2% in petrolatum)	The incidence of allergic reactions to certain methacrylates by the Information Network of Departments of Dermatology (Göttingen, DE) in 1992-1995	Positive reaction to the test substance in 13 of 2971 patients (0.4%).	Schnuch (1996)
Patch test data, selected patients	Tetramethylene dimethacrylate (2%; Chemotechnique's test substance i.e. in petrolatum)	A retrospective study on patients tested with (meth)acrylate patch test series at the Section of Dermatology in the Finnish Institute of Occupational Heath in 1885-1995	Positive reaction to the test substance in 10 of 274 (3.6%) patients tested with the substance. 48 patients reacted positively to some (meth)acrylate. The test substance-positive cases constituted 20.8% of these.	Kanerva *et al*. (1997)
Patch test data, selected patients	Tetramethylene dimethacrylate (2%, Chemotechnique's test substance i.e. in petrolatum)	A retrospective study of patch test records at the Section of Dermatology, University of Manchester (Salford, UK) in 1983-1998 440 patients with a history of exposure to (meth)acrylates were identified and patch tested with (meth)acrylates	Positive reaction to the test substance in 7 of 255 patients (2.7%) tested with the substance.	Tucker & Beck (1999)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Patch test data, selected patients	Tetramethylene dimethacrylate (concentration or vehicle not stated)	A retrospective study on patients patch tested with dental screening series in 7 dermatology clinics in Finland in 1994-1998	There were 13 (0.5%) allergic reactions to the test substance in the 2408 patients tested. The frequency of allergic reactions varied between 0.1% and 2.2% in different clinics.	Kanerva *et al*. (2001)
Patch test data, selected patients	Tetramethylene dimethacrylate (2% in petrolatum)	109 patients (all dental personnel) were tested with a dental screening series at the Department of Occupational and Environmental Dermatology (Stockholm, SE) in 1995-1998	Positive reaction to the test substance in 6 (5.5%) of 109 patients tested with (meth)acrylates. 24 patients had allergic reactions to some (meth)acrylate. The 6 test substance-positive cases constituted 25% of these.	Wrangsjö *et al*. (2001)
Patch test data, selected patients	Tetramethylene dimethacrylate (2% in petrolatum)	A retrospective study of patch test records of 1632 patients tested with dental patient and/or dental personnel series at the Department of Occupational and Environmental Dermatology in Malmö University Central Hospital (SE) in 1995-2004	Positive reaction to the test substance in 9 (0.5%) out of 1642 patients tested. 48 patients reacted positively to at least one (meth)acrylate. The test substance-positive cases constituted 18.8% of these patients.	Goon *et al*. (2006)
Patch test data, selected patients	Tetramethylene dimethacrylate (2% in petrolatum)	A retrospective study on 451 patients suspected of having occupational contact dermatitis and tested with a (meth)acrylate series at Finnish Institute of Occupational Health (Helsinki, FI) in 1994-2009	Positive reaction to the test substance in 9 patients (2.0%) 66 patients reacted positively to at least one (meth)acrylate. The test substance-positive cases constituted 13.6% of this group.	Aalto-Korte *et al*. (2010) Includes the patients in Aalto-Korte *et al*. (2008) and Aalto-Korte *et al*. (2007)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Patch test data, selected patients	Tetramethylene dimethacrylate (2%; Chemotechnique's test substance i.e. in petrolatum)	A retrospective study on patients tested with (meth)acrylate series at the Department of Dermatology, University Medical Centre in Groningen (NL) in 1993-2012	Positive reactions in 6 of 151 (4.0%) patients tested with the substance. 24 patients reacted positively to some (meth)acrylate. The positive reactions to tetramethylene dimethacrylate constituted 25% of these.	Christoffers *et al*. (2013)
Patch test data, selected patients	Tetramethylene dimethacrylate (2% in petrolatum)	122 patients were tested with an extended series of (meth)acrylates at the Department of Dermatology (Coimbra, PT) in 2006-2013	Positive reaction to the test substance in 5 (4.1%) patients. 37 patients reacted positively to (meth)acrylates. The tetramethylene dimethacrylate-positive cases constituted 13.5% of these.	Ramos *et al*. (2014)
Patch test data, selected patients	Tetramethylene dimethacrylate (2% in petrolatum)	475 patients were tested with a (meth)acrylate series at the Cutaneous Allergy Unit (Birmingham, UK) in 2002-2015	Positive reactions to the test substance in 10 (2.1%) patients tested with the substance. 52 patients reacted positively to (meth)acrylates. The positive reactions to tetramethylene dimethacrylate constituted 19% of these.	Spencer *et al*. (2016)

Tetramethylene dimethacrylate has been commonly tested as part of the (meth)acrylate series since the 1980s. Its established test concentration is 2% in petrolatum. A total of 11 diagnostic patch test studies on selected patients could be identified for the substance. The frequency of positive reactions varied between 0.4% and 9.5% (median 2.7%).

No strict workplace studies could be identified for tetramethylene dimethacrylate. However, two cross-sectional studies on dental technicians who are at risk of developing a contact allergy due to exposure to acrylic compounds at work, share a similar design. Only the workers with skin symptoms were patch tested in these studies. Frequency of positive patch test reactions to the

substance was 2% in both studies (1/55 and 1/49 of the tested patients; Rustemeyer & Frosch 1996 and Lee *et al*. 2001, respectively).

The rest of the identified studies were either case reports of single cases (n=4) or reports describing patient series (n=10) without clearly stating the frequency of reaction to tetramethylene dimethacrylate in all patients tested during the same time period.

Specific exposure to the substance was described by Peiler *et al*. (2000) in all six dental technicians who tested positive to it. In the 1990s in Germany, tetramethylene dimethacrylate was commonly found in the products used by dental technicians and virtually all workers were exposed to the substance. The authors considered that tetramethylene dimethacrylate was a weak sensitiser compared to methyl methacrylate because the frequency of contact allergy was low (4.8%), despite common exposure. Dental technicians' skin exposure to tetramethylene dimethacrylate may also vary within countries, as for instance in Finland only two dental technicians out of eight had used products containing the substance (Aalto-Korte *et al*. 2007).

Based on the available data, the DS has proposed classification as **Skin Sens. 1B** with hazard statement **H317: May cause an allergic skin reaction**.

Comments received during consultation

One MSCA supported proposed classification as **Skin Sens. 1B** with hazard statement **H317: May cause an allergic skin reaction** based on results of the key animal study, and human data as supportive evidence.

One Company-Importer agreed with the harmonised classification as **Skin Sens. 1B, H317**, mainly based on animal data, namely LLNA data. This Company noted that human data support the classification and labelling in a weight of evidence approach and do not allow a sub-categorisation due to the absence of exposure information.

One MSCA noted that in the view of the DS the outcome of an LLNA indicates that tetramethylene dimethylacrylate should be classified as skin sensitiser in sub-category 1B and ask the DS to assess in more detail the clinical findings that have been observed during the study, such as:

- "Trying to burrow oneself in the bedding" observed one hour after the third application,
- "Ruffled fur", "Hunched posture" and "Reduced spontaneous activity % observed on 3rd day after application of the substance on surface of ears at concentrations of 25 and 50% and on day 3 and 4 after application of substance at concentration of 100%"
- "Eyelid closure" and "Abnormal walk" observed on 3rd day after application of the substance on surface of ears at concentrations of 50 and 100%

In the opinion of the MSCA, it is crucial to discuss the above-mentioned clinical findings in more detail because they may have an influence on the acceptability of the LLNA to be used as basis for sub-categorisation. Assessment of these findings is advisable because OECD testing guideline 429 specifies with respect to dose selection "that the highest concentration maximises exposure while avoiding systemic toxicity" (see OECD TG 429, par. 18).

In response to this comment the DS noted that the assessment relies on the full study report of the LLNA and that it does not have access to more detailed information. An acute dermal toxicity study conducted with the substance is not available. There is only a supporting study available on a closely related read-across substance 1,3-BDDMA. The study is poorly reported. No clinical signs or other effects were observed. The acute dermal LD50 of 1,3-BDDMA is reported to be >3000 mg/kg bw in rabbit. Acute oral toxicity LD50 of 1,3-BDDMA (rat, combined) is reported to be 10 066 mg/kg bw. The study has been performed according to the OECD TG 401.

As the substance is not acutely toxic by the oral route this supports findings that 1,3-BDDMA is not acutely toxic by the dermal route either.

In the first LLNA study (Anonymous 2014), the unspecific clinical symptoms reduced spontaneous activity, ruffled fur and hunched posture may in general indicate mild systemic toxicity. These effects were observed in all treated animals on day 3 (25%: 1h after the third application; 50% and 100%: 1h before and 1h after the third application). Furthermore, the animals in mid and high dose groups showed eyelid closure and abnormal walk. No marked reduction in body weight nor mortality was observed during the study period. According to the authors, it cannot be confirmed whether these symptoms were signs of systemic toxicity or mere reactions to the irritant nature of the test substance. However, the study was considered valid by the authors. In the registration dossier the study is reliable without restrictions with Klimisch score 1. Skin irritation in test animals was not excessive as the erythema scores varied between 1 and 2 (<3). It cannot be concluded if the effects observed were reactions to the irritant nature of the substance. Without any more detailed information on the clinical signs and, taking into account that there was no relevant body weight loss, it is difficult to conclude on systemic toxicity either. Nevertheless, the DS noted that slight clinical signs were observed in the study and that they might indicate systemic toxicity.

One MSCA noted that based on the weight of evidence, including both human and animal data, it should be concluded that a classification as skin sensitiser is warranted for tetramethylene dimethacrylate. In relation to sub-categorization, MSCA is of the opinion that, when available, adequate human data should always be preferred over animal data to conclude on classification. The MSCA is of the view that sufficient information is available to conclude on exposure of the substance, at least for some categories of workers. MSCA considers that both frequency of occurrence of skin sensitisation and frequency of exposure of worker should be concluded to be high. Similarly, the workers in the field of long-lasting nail polishing might be considered highly exposed to tetramethylene dimethacrylate. Based on human data, MSCA is of the opinion that tetramethylene dimethacrylate should be classified as Skin Sens. 1 without sub-categorization, because in line with the CLP guidelines, relatively high frequency of occurrence of skin sensitisation and relatively high frequency of exposure (score 5-6) support such decision. The MSCA also noted that in the key LLNA the animals showed clinical signs indicating acute systemic toxicity from 50% and 100% concentrations (eyelid closure and abnormal walk on day 3, and ruffled fur on day 4; reduced spontaneous activity on day 4 at the highest dose) while according to the OECD 429 guidance on LLNA, the highest concentration should be selected in order to "maximise exposure while avoiding systemic toxicity and/or excessive local skin irritation". Therefore the dose selection of this LLNA using concentrations of 25%, 50% and 100% is questionable.

In response on evaluation of human data, the DS pointed out that the assessment of human exposure was not included in the CLH report because there are no adequate data available to allow a reliable evaluation of the exposure to the specific substance. There is a lack of data on the products containing the substance. Therefore, it is not possible to know the concentration or dose humans are exposed to. The same applies for information of repeated exposure and the number of exposures. In view of the DS, only assumptions can be made on human exposure as there is no reported information of the exact exposure. Therefore, basing an evaluation on assumptions and to use it to conclude on the classification requires great care.

Regarding the LLNA the DS has agreed that the test concentrations were high. In the pre-test with 2 animals on day 4, the mice treated with the undiluted test substance showed transiently a slightly reduced spontaneous activity. An erythema of the ear skin was observed in both animals (at 50%: score 1 on days 3-6; at 100%: score 1 on days 2, 3 and 6, and score 2

on days 4-5). Furthermore, scabby ears were observed on day 5 in the animal treated with 100% test substance. Increase in ear thickness on day 6 was 6% and 3% in mouse treated with 50 and 100 % test substance, respectively. No relevant change in body weights was observed. According to the study authors "The highest concentration tested was the highest level that could be achieved whilst avoiding systemic toxicity and excessive local skin irritation as confirmed in the pre-test". The concentrations of 25, 50 and 100% were selected for the main test. According to the OECD TG 429: "Excessive local skin irritation is indicated by an erythema score ≥3 and/or an increase in ear thickness of ≥25% on any day of measurement". No excessive local skin irritation was observed in pre-test animals as erythema scores were 1-2 (<3) and increase in ear thickness was not more than 6% (<25%). The DS notes the substance has self-classification as Skin Irrit. 2, however, according to data in the registration dossier the substance is not a skin irritant. OECD TG 429 states also that "The highest dose selected for the main LLNA study will be the next lower dose in the pre-screen concentration series that does not induce systemic toxicity and/or excessive local skin irritation". It is unclear why the concentration of 100% was selected for the main test. In the main test all treated animals showed a slight or moderate erythema of the ear skin (at 25%: score 1 on days 3-4; at 50%: score 2 on days 3-5; at 100%: score 1 on days 2 and 6) but there was no excessive skin irritation.

The unspecific clinical symptoms reduced spontaneous activity, ruffled fur and hunched posture were observed in all treated animals on day 3 (at 25%: 1h after the third application; at 50% and 100%: 1h before and 1h after the third application). Furthermore, the animals in mid and high dose groups showed eyelid closure and abnormal walk. A loss in body weight or mortality was not observed in any of animals treated with test substance during the study period. According to the authors, it cannot be confirmed whether these symptoms were signs of systemic toxicity or mere reactions to the irritant nature of the test substance. The study was considered valid by the authors. In the registration dossier the study is reliable without restrictions with Klimisch score 1. It cannot be concluded if the effects observed in LLNA were reactions to the irritant nature of the substance. Without any more detailed information on the clinical signs, and taking into account that there was no relevant body weight loss, it is difficult to conclude on systemic toxicity either. The DS notes that slight clinical signs were observed in the study and they might indicate systemic toxicity.

Assessment and comparison with the classification criteria

According to Regulation (EC) 1272/2008, point 3.4.2.2.4.2.: "*Evidence from animal studies is usually much more reliable than evidence from human exposure. However, in cases where evidence is available from both sources, and there is conflict between the results, the quality and reliability of the evidence from both sources must be assessed in order to resolve the question of classification on a case-by-case basis. Normally, human data are not generated in controlled experiments with volunteers for the purpose of hazard classification but rather as part of risk assessment to confirm lack of effects seen in animal tests. Consequently, positive human data on skin sensitisation are usually derived from case-control or other, less defined studies. Evaluation of human data must therefore be carried out with caution as the frequency of cases reflect, in addition to the inherent properties of the substances, factors such as the exposure situation, bioavailability, individual predisposition and preventive measures taken.*"

Animal data

In case of tetramethylene dimethacrylate both human data and animal data were provided, but in line with the above statement the animal data are analysed first. Results of five animal studies are available: one LLNA and four guinea pig studies. The LLNA (Anonymous 2014) was assessed with reliability index 1 and used by the DS as a key study.

In the public discussion reliability of this LLNA has been questioned due to high doses or concentrations used in the test. It has been pointed out that in the pre-test and the main study tetramethylene dimethacrylate was inducing toxic symptoms in treated mice (at 25, 50 and 100% reduced spontaneous activity, ruffled fur and hunched posture, and at 50 and 100% additionally eyelid closure and abnormal walk on day 3). It is noted that none of the symptoms indicating narcotic effects of tetramethylene dimethacrylate were reported in treated mice 5 and 6 days after exposure, and that this is indicating the effects were reversible. No effect on survival and body weight gain were observed and therefore the symptoms may be considered as an evidence of slight systemic toxicity. Such a conclusion is supported by OECD TG 429 recommendations on excessive systemic toxicity findings: "*the following clinical observations may indicate systemic toxicity when used as part of an integrated assessment and therefore may indicate the maximum dose level to use in the main LLNA: changes in nervous system function (e.g. pilo-erection, ataxia, tremors, and convulsions); changes in behaviour (e.g. aggressiveness, change in grooming activity, marked change in activity level); changes in respiratory patterns (i.e. changes in frequency and intensity of breathing such as dyspnea, gasping, and rales), and changes in food and water consumption. In addition, signs of lethargy and/or unresponsiveness and any clinical signs of more than slight or momentary pain and distress, or a >5% reduction in body weight from Day 1 to Day 6, and mortality should be considered in the evaluation of systemic toxicity. Moribund animals or animals obviously in pain or showing signs of severe and enduring distress should be humanely killed* ". The authors of the study did not report such symptoms. The symptoms observed in mice in this LLNA are considered as an evidence of slight toxicity which is not expected to affect assessment of skin sensitisation in this test.

On the other hand, the study authors were unable to decide whether these symptoms were signs of systemic toxicity or mere reactions to the irritant nature of the test substance. The DS has provided additional information indicating that intensity of irritation was relatively low (score 1 and 2), and an increase in ear thickness on day 6 was 6% and 3% in animals treated with 50 and 100 % test substance, respectively. According to the OECD TG 429, "Excessive local skin irritation is indicated by an erythema score ≥3 and/or an increase in ear thickness of ≥25% on any day of measurement". No excessive local skin irritation was observed in animals as erythema scores were 1-2 (<3) and increase in ear thickness was not more than 6% (<25%). Taking into account the above analysis RAC considers that the LLNA is valid and its results can be used for evaluation of classification of tetramethylene dimethacrylate.

In the current Guidance on the Application of CLP Criteria (point 3.4.2.2.2) it is noted that classification into sub-categories is only possible if the data are sufficient.Care should be taken when classifying substances into category 1B when category 1A cannot be excluded. In such cases classification into category 1 should be considered.

In order to classify a substance into sub-category 1A based on a Local lymph node assay, a value of EC3 should be ≤ 2 % while that for the subcategory 1B should be > 2 %. In order to classify in sub-category 1B (if the EC3 is > 2 %), there is also a need for data demonstrating that a substance at a concentration of ≤ 2 % will not induce a SI ≥ 3 meeting the CLP criteria for sub-category 1A. The results of LLNA (Anonymous, 2014) indicate that tetramethylene dimethacrylate did not induce a Stimulation Index above 3 at concentration of 25%, and therefore it will not induce such a Stimulation Index a concentration 10 times lower, therefore classification of this substance to category 1A can be excluded and sub-categorization is possible. Tetramethylene dimethacrylate has induced Stimulation Index above 3 at concentration 50% and 100%, with EC3 meeting classification criteria for category 1B (calculated to be 31.4%). Since

classification in subcategory 1A can be excluded, tetramethylene dimethacrylate warrants classification to category 1B based on results of LLNA.

Only one out of four skin sensitisation studies on guinea pigs (Anonymous 1983a) with reliability score 3 was positive. In the study all 8 animals given in intradermal induction tetramethylene dimethacrylate at concentration of 13% had positive response in the challenge test at concentration of 78% providing supportive evidence for skin sensitisation properties of tetramethylene dimethacrylate. Since only one concentration was used, this study does not provide data for sub-categorization.

Three other guinea pig studies (Anonymous 1983b with reliability score 2; Anonymous 1984a with reliability score 3; Anonymous 1984b with reliability score 2) did not disclose skin sensitising potential of tetramethylene dimethacrylate, what might be interpreted that skin sensitising potency of this substance is low.

Human data

According to the classification criteria listed in points 3.4.2.2.2.1 and 3.4.2.2.2.2 of Regulation (EC) 1272/2008, the human evidence for sub-categories 1A and 1B can include the following type of data (ECHA 2017b, Section 3.4.2.2.3.1.), respectively:

	Human data
Sub-category 1A	(a) positive responses at ≤ 500 µg/cm2 (HRIPT, HMT – induction threshold); (b) diagnostic patch test data where there is a relatively high and substantial incidence of reactions in a defined population in relation to relatively low exposure; (c) other epidemiological evidence where there is a relatively high and substantial incidence of allergic contact dermatitis in relation to relatively low exposure.
Sub-category 1B	(a) positive responses at > 500 µg/cm2 (HRIPT, HMT – induction threshold); (b) diagnostic patch test data where there is a relatively low but substantial incidence of reactions in a defined population in relation to relatively high exposure; (c) other epidemiological evidence where there is a relatively low but substantial incidence of allergic contact dermatitis in relation to relatively high exposure.

HRIPT: Human Repeat Insult Patch Test; HMT: Human Maximisation Test

The Guidance on the Application of the CLP Criteria further outlines how high or low frequency of occurrence of skin sensitisation shall be assessed (ECHA 2017b, Section 3.4.2.2.3.1., Table 3.2):

Human diagnostic patch test data	High frequency	Low/moderate frequency	Tetramethylene dimethacrylate
General population studies	≥ 0.2 %	< 0.2 %	No studies
Dermatitis patients (unselected, consecutive)	≥ 1.0 %	< 1.0 %	No studies
Selected dermatitis patients (aimed testing, usually special test series)	≥ 2.0 %	< 2.0 %	11 studies 0.4%-9.5% (median 2.8%)
Workplace studies: 1: all or randomly selected workers 2: selected workers with known exposure or dermatitis	 ≥ 0.4 % ≥ 1.0 %	 < 0.4 % < 1.0 %	 No studies 2 studies: 2%
Number of published cases	≥ 100 cases	< 100 cases	128 patch-test-positive cases

There are no studies on general population or on unselected consecutive dermatitis patients.

Frequencies of positive patch tests in 11 selected dermatitis patient materials (aimed testing) vary between 0.4% and 9.5% (median 2.7%) but are mostly above the limit of high frequency (≥ 2.0 %).

There are no workplace studies on all or randomly selected workers. In two cross-sectional studies on dental technicians, mimicking workplace studies (on selected workers), the frequency of positive patch tests was 2%, i.e., above the cut-off value of 1.0% for high frequency.

The number of published patch-test-positive cases, 128, also exceeds the cut-off value for high frequency (≥ 100).

Positive patch test reactions to tetramethylene dimethacrylate are relatively common in patients sensitised to methacrylates, but specific exposure to the substance in sensitised or tested patients has rarely been described in the literature. Both the exposure and the lack of exposure to tetramethylene dimethacrylate are typically difficult to assess in clinical work due to the unavailability of chemical analyses. Positive test reactions may also arise from cross-reactivity to other methacrylates, yet true exposure to tetramethylene dimethacrylate in clinical patients cannot be excluded. Of the identified literature, only Peiler *et al*. (2000) confirmed exposure to the substance in all six dental technicians who gave a positive reaction to it.

After analysis of human data, RAC concours with the DS that the frequency of positive reactions to tetramethylene dimethacrylate in diagnostic patch tests (median 2.8%) are above 2.0 %, the guidance threshold value for high frequency. However, there is no adequate information enabling the assessment of true exposure of humans to the substance. According to the Guidance on the Application of the CLP Criteria: "*the concept of 'guidance' should be applied generally to all of the numeric criteria – they represent indicators derived from expert opinion and are not to be taken as proven absolute values. Application of this guidance should permit sub-categorisation where the human data on exposure and sensitisation is clear*". In this case data on dermal exposure leading to skin sensitisation do not exist. Therefore, it is not possible to sub-categorise potency based on human data. On the other hand, according to Regulation (EC) 1272/2008, point 3.4.2.2.4.2.: "*Evidence from animal studies is usually much more reliable than evidence*

from human exposure. However, in cases where evidence is available from both sources, and there is conflict between the results, the quality and reliability of the evidence from both sources must be assessed in order to resolve the question of classification on a case-by-case basis." In case of tetramethylene dimethacrylate both animal and human data provide sufficient evidence on skin sensitisation, and there is no conflict between results of animal and human data. However, only animal data provide a clear information on level of exposure needed to induce skin sensitisation while a judgement on the exposure level is not possible based on human data. In the opinion of RAC tetramethylene dimethacrylate warrants a classification as **Skin Sens. 1B; H317** based on results of the key LLNA study. The other positive Guinea pig studies and studies on humans support the classification of tetramethylene dimethacrylate as a skin sensitiser, although they are not conclusive for sub-categorization.

After analysis of human data, RAC concours with the DS that the frequency of positive reactions to tetramethylene dimethacrylate in diagnostic patch tests can be considered high. However, there is no adequate information enabling the assessment of true exposure to the substance. Animal data is sufficient for sub-categorization, and human data supports the classification of tetramethylene dimethacrylate as a skin sensitiser. Based on the key LLNA, sub-category 1A can be excluded and **sub-category 1B is justified**.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).