CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Chemical name:

Tetrahydrofurfuryl methacrylate

EC Number: 219-529-5

CAS Number: 2455-24-5

Index Number: -

Contact details for dossier submitter:

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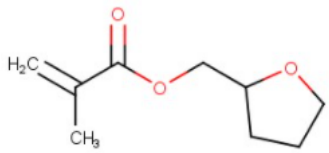
ABBREVIATIONS

AOP	Adverse Outcome Pathway
ATE	Acute Toxicity Estimate
ARE	Antioxidant Response Element
AUC	Area Under the Curve
bw	body weight
CAS	Chemical Abstract Service
CIR	Cosmetic Ingredient Review
CV75	Concentration at which a chemical reaches the cytotoxicity threshold of 75%
d	day
DA	Defined Approaches
DMSO	Dimethylsulfoxid
DPRA	Direct Peptide Reactivity Assay
Drg	Danger
FCA	Freund's Complete Adjuvant
FIOH	Finnish Institute of Occupational Health
GCL	Generic Concentration Limit
GLP	Good Laboratory Practice
HMT	Human Maximization Test
HRIPT	Human repeated insult patch test
IATA	Integrated Approach to Testing and Assessment
IVDK	Informationsverbund dermatologischer Kliniken
KE	Key Event
Kp	Permeability Coefficient
Kow	Partition coefficient octanol/water
LD50	Lethal Dose, 50%
LC50	Lethal Concentration, 50%
m/f	male/female
OECD	Organisation for Economic Co-operation and Development
PI	Propidium Iodide
THFMA	Tetrahydrofurfuryl Methacrylate

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	tetrahydrofuran-2-ylmethyl methacrylate
Other names (usual name, trade name, abbreviation)	2-Propenoic acid, 2-methyl-, (tetrahydro-2-furanyl)methyl ester 2-methyl-2-propenoic_acid, (tetrahydro-2-furanyl)methyl ester tetrahydrofuran-2-ylmethyl methacrylate THFMA
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	219-529-5
EC name (if available and appropriate)	-
CAS number (if available)	2455-24-5
Other identity code (if available)	-
Molecular formula	C9H14O3
Structural formula	 (**source:** European Chemicals Agency, http://echa.europa.eu/)
SMILES notation (if available)	CC(=C)C(=O)OCC1CCCO1
Molecular weight or molecular weight range	170.206
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	-
Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 80 - ≤ 100 % (w/w)

1.2 Composition of the substance

Tetrahydrofurfuryl methacrylate (THFMA) is a mono-constituent substance.

For the substance a boundary composition and several legal entity compositions are registered[1]. Non-confidential information is presented in the tables below. Confidential information is part of Annex I.

Based on registration information two substances contribute to the classification.

Table 2: Constituents (boundary composition)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)
Tetrahydrofurfuryl methacrylate EC 219-529-5	≥ 80 - ≤ 100 % (w/w)	-	Skin Sens. 1, H317 Repr. 1B, H360D Aquatic Chronic 3, H412

Table 3: Impurities (boundary composition), relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Tetrahydrofurfuryl alcohol EC 202-625-6	conf	Eye Irrit. 2, H319 Repr. 1B, H360Df	Eye Irrit. 2, H319 Repr. 1B, H360Df	yes
Methyl methacrylate EC 201-297-1	conf	Flam Liq. 2, H225 Skin Irrit. 2, H315 Skin Sens. 1, H317 STOT SE3, H335 ([2])	Flam Liq. 2, H225 Skin Irrit. 2, H315 Skin Sens. 1, H317 STOT SE3, H335	yes

Table 4: Additives (non-confidential information; legal entity composition)

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
Mequinol EC 205-769-8	-	conf	Acute Tox 4*, H302 Eye Irrit.2, H319 Skin Sens 1, H317	Acute Tox 4, H302 Eye Irrit.2, H319 Skin Sens 1, H317	no

Information on the test substances (if available) are given in the study descriptions.

[1] REACH registration data, accessed 12/2021

[2] A harmonized classification and labelling opinion for Resp. Sens. 1, H334 has been adopted. See Registry of CLH intentions until outcome - ECHA (europa.eu)

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6: For substance with no current entry in Annex VI of CLP

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	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 submitter's proposal	TBD	tetrahydrofurfuryl methacrylate	219-529-5	2455-24-5	Repr. 1B Skin Sens. 1A	H360FD H317	GHS08 GHS07 Dgr	H360FD H317			

Table 5: Reason for not proposing harmonised classification and status under consultation

Hazard class	Reason for no classification	Within the scope of consultation
Explosives	*hazard class not assessed in this dossier*	No
Flammable gases (including chemically unstable gases)	*hazard class not assessed in this dossier*	No
Oxidising gases	*hazard class not assessed in this dossier*	No
Gases under pressure	*hazard class not assessed in this dossier*	No
Flammable liquids	*hazard class not assessed in this dossier*	No
Flammable solids	*hazard class not assessed in this dossier*	No
Self-reactive substances	*hazard class not assessed in this dossier*	No
Pyrophoric liquids	*hazard class not assessed in this dossier*	No
Pyrophoric solids	*hazard class not assessed in this dossier*	No
Self-heating substances	*hazard class not assessed in this dossier*	No
Substances which in contact with water emit flammable gases	*hazard class not assessed in this dossier*	No
Oxidising liquids	*hazard class not assessed in this dossier*	No
Oxidising solids	*hazard class not assessed in this dossier*	No
Organic peroxides	*hazard class not assessed in this dossier*	No
Corrosive to metals	*hazard class not assessed in this dossier*	No
Acute toxicity via oral route	*hazard class not assessed in this dossier*	No
Acute toxicity via dermal route	*hazard class not assessed in this dossier*	No
Acute toxicity via inhalation route	*hazard class not assessed in this dossier*	No
Skin corrosion/irritation	*hazard class not assessed in this dossier*	No
Serious eye damage/eye irritation	*hazard class not assessed in this dossier*	No
Respiratory sensitisation	*hazard class not assessed in this dossier*	No
Skin sensitisation	Skin Sens. 1A, H317	Yes
Germ cell mutagenicity	*hazard class not assessed in this dossier*	No
Carcinogenicity	*hazard class not assessed in this dossier*	No
Reproductive toxicity	Repr. 1B, H360FD	Yes
Specific target organ toxicity-single exposure	*hazard class not assessed in this dossier*	No
Specific target organ toxicity-repeated exposure	*data conclusive but not sufficient for classification*	Yes
Aspiration hazard	*hazard class not assessed in this dossier*	No
Hazardous to the aquatic environment	*hazard class not assessed in this dossier*	No
Hazardous to the ozone layer	*hazard class not assessed in this dossier*	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The substance has no harmonized classification so far.

The substance has 205 C&L notifications with self-classifications (summary) as Skin Sens. 1, H317; Repr. 1B, H360; Aquatic chronic 3, H412 as well as Skin Irrit. 2, H315; Eye Irrit. 2, H319; STOT SE 3, H335 [ECHA dissemination site, accessed 11/2021].

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

[A.] There is no requirement for justification that action is needed at Community level

Harmonized classification for Reproductive Toxicity is needed.

[B.] Justification that action is needed at Community level is required.

Reason for a need for action at Community level: differences in self-classification for the sensitizing property of THFMA.

5 IDENTIFIED USES

Table 6: The following uses are indicated at ECHA dissemination site [accessed 12/2021]:

Categories	Use(s)	Technical function
Manufacture	Manufacture of the substance	-
Formulation	Formulation into mixtures, repacking (into coatings and inks)	-
Uses at industrial sites	Monomer in polymerisation (wet process, dry process) End use in formulations Application of coatings/adhesives formulation Use in adhesives/sealants/coatings	Monomer
Uses by professional workers	End use in formulations End use in adhesives/sealants Application of coatings, adhesives, formulations	Monomer
Consumer Uses	-	-
Article service life	-	-

6 DATA SOURCES

ECHA dissemination site: Tetrahydrofurfuryl methacrylate - Registration Dossier - ECHA (europa.eu)

Also original study reports provided by registrants and scientific literature served as information sources. Please see section 14. References for details.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Colourless to slightly yellowish liquid, ester-like odour	ECHA dissemination site [May, 2021]	-
Melting/freezing point	-	ECHA dissemination site [May, 2021]	OECD 102 No melting point detected. THFMA is reported to undergo glass transition (amorphous solidification) at -113°C (1020 hPa).
Boiling point	222 °C (1020 hPa)	ECHA dissemination site [May, 2021]	OECD 103
Relative density	1.042 (20°C)	ECHA dissemination site [May, 2021]	OECD 109
Vapour pressure	27 Pa (20°C)	ECHA dissemination site [May, 2021]	OECD 104
Surface tension	-	-	-
Water solubility	18 990 mg/L (20°C)	ECHA dissemination site [May, 2021]	OECD 105
Partition coefficient n-octanol/water	1.76 ± 0.08 (22.6°C)	ECHA dissemination site [May, 2021]	OECD 117
Flash point	99 °C (1 013.25 hPa)	ECHA dissemination site [May, 2021]	EU Method A.9, closed cup
Flammability	-	-	-
Explosive properties	Non explosive	ECHA dissemination site [May, 2021]	-
Self-ignition temperature	240 °C (999.8 - 1 007.3 hPa)	ECHA dissemination site [May, 2021]	EU Method A.15
Oxidising properties	Not oxidising	ECHA dissemination site [May, 2021]	-
Granulometry	Not applicable	-	-
Stability in organic solvents and identity of relevant degradation products	-	-	-
Dissociation constant	-	-	-
Viscosity	Kinematic viscosity at 20°C: 2.74 mm²/s at 40°C: 1.84 mm²/s	ECHA dissemination site [May, 2021]	OECD 114

8 EVALUATION OF PHYSICAL HAZARDS

Not addressed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

No toxicokinetic studies for THFMA are available. After oral exposure systemic effects are documented, therefore good absorption can be assumed. For the dermal and the inhalation route of exposure no animal data with THFMA are available. However, in general methacrylate esters are expected to be rapidly absorbed via all routes and distributed (ECHA dissemination site, accessed August 2021).

At ECHA dissemination site a quantitative structure permeability relationships (QSPeRs) prediction for dermal absorption, based on a screening model according to Potts (1992), is documented (Anonymous, 2012). A large number of methacrylate esters, including THFMA, was investigated. With a molecular weight of 170.21 g/mol and a log Kow of 1.35, the predicted flux of THFMA was 28.461 µg/cm²/h. The relative dermal absorption was interpreted to be moderate[3]. No further information is given. According to OECD (2019) the product of the permeability coefficient Kp and solubility in the same vehicle (usually water) provides an estimate of the maximum flux through the skin. Therefore a Kp 0.0015 can be estimated based on a solubility of THFMA of 18 990 mg/L in water and the predicted flux.

However, in a recent OECD 117 study (Anonymous, 2014) the logKow of THFMA was measured to be 1.76. Based on this value the permeability coefficient Kp can be calculated using the formula derived by Potts (1992): Log Kp ($cm.h^{-1}$) = -2.72 + 0.71 Log P – 0.0061 MW. This resulted in a logKp [log(cm/h)] of -2.51 and a Kp of 0.003. Nevertheless, no final conclusion on the skin permeability of THFMA can be drawn because according to OECD (2019) values for dermal absorption estimated via QSAR models have to be taken with care as a number of principal technical problems associated with modelling dermal absorption in silico have so far limited the applicability. One of the biggest challenges is that penetration is influenced not only by molecular and physicochemical properties of the chemical itself but also by the properties of the vehicle and the structure and properties of skin, along with their interactions.

In the registration data the following information based on analogy to alkyl-methacrylate esters is given: Toxicokinetics seem to be similar in man and experimental animals. Methacrylic acid and other short chain alkyl-methacrylate esters are initially hydrolyzed by non-specific carboxylesterases to methacrylic acid and the structurally corresponding alcohol in several tissues, including but not limited to liver, olfactory epithelium, stratum corneum and blood. This has been shown for linear alkyl esters, several ether methacrylates, diesters as well as cycloalkyl and –aryl esters. The carboxylesterases are a group of non-specific enzymes that are widely distributed throughout the body and are known to show high activity within many tissues and organs including the liver, blood, GI tract, nasal epithelium and skin. Recent investigations with related substances (see e.g. registrantion information for 2-ethoxyethyl methacrylate[4]) show a short half-life within the body and effective removal (first pass through liver) of systemically absorbed parent ester. Because of the structural similarity of THFMA to the mentioned esters rapid hydrolysis to Tetrahydrofurfuryl alcohol is expected in the order of minutes (ECHA dissemination site, accessed 08/2021).

Enzymatic studies show that tetrahydrofurfuryl alcohol degradation is initiated by an oxidation of the alcohol via the aldehyde to the corresponding carboxylic acid (Zarnt, 2001). The resulting tetrahydrofuroic acid is either excreted directly via the kidneys, or – by analogy to the structurally similar furfuryl alcohol – in the form of glycine and lysine conjugates (Nomeir, 1992).

An alternative pathway may be GSH conjugation, however, methacrylate esters in general show a low reactivity.

[3] This interpretation is based on the dermal absorption database (several hundred chemicals) developed at the test facility between 1992 and 2012. Ranking: Dermal absorption rate [µg/cm²/h] - predicted absortion from normal exposure: >500 – very high; 100-500 – high; 10-100 – moderate; 0.1-10 - low; 0.001-0.1 – minimal; <0.001 – negligible.

[4] Registration Dossier - ECHA (europa.eu)

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Not addressed in this dossier.

10.2 Acute toxicity - dermal route

Not addressed in this dossier.

10.3 Acute toxicity - inhalation route

Not addressed in this dossier.

10.4 Skin corrosion/irritation

Not addressed in this dossier.

10.5 Serious eye damage/eye irritation

Not addressed in this dossier.

10.6 Respiratory sensitisation

Not addressed in this dossier.

10.7 Skin sensitisation

Table 8: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substances	Dose levels duration of exposure	Results	Reference
Non guideline study 3 (not reliable)	Guinea pigs, Hartley, f	Several substances tested: THFMA Butyl Methacrylate Ethylene Glycol Dimethacrylate HEMA Triethylene Glycol Dimethacrylate Trimethylolpropane Trimethacrylate Vehicle: ethanol:saline (1:4) + FCA	Subcutaneous injection (4x footpads and 1x neck): 0.2% solution in ethanol:saline (1:4) + FCA Epicutan (shaved flank, weekly): 2% in acetone:olive oil (4:1), 20 µl	Negative (all compounds tested)	Parker et al., 1983 [as cited in CIR, 2005]

In a review of the Cosmetic Ingredient Review Expert Panel (CIR, 2005) a study by Parker and Turk (1983) is described. They injected the footpads of female Hartley guinea pigs four times with an emulsion of 2 mg/ml of butyl methacrylate, ethylene glycol dimethacrylate, HEMA, THFMA, triethylene glycol dimethacrylate, or trimethylolpropane trimethacrylate in ethanol:saline (1:4) in Freund′s complete adjuvant (FCA). An additional 0.1 ml of the emulsion was injected into the nape of the neck. The animals received a total of 1mg of the test substance. Seven days later, and weekly thereafter for up to 12 weeks, 0.02 ml of a 2% solution in acetone:olive oil (4:1) (non-irritating) was applied to the shaved flank of the animals, using a different site for each application. Non of the tested substances induced contact sensitization using this protocol (Parker and Turk 1983, cited in CIR, 2005). The study was rated as not reliable as all compounds tested, even clearly sensitizing compounds like e.g. butyl methacrylate, were negative.

In literature several studies describing positive reactions to THFMA in humans are documented. The following table gives an overview. Cross reactions to THFMA after sensitization with other metacrylate compounds can not be excluded in most of the presented studies due to the exposure pattern. In general no information on exposure concentrations to THFMA or frequencies of exposure are available. Exposure is assumed based on the possible contact to THFMA-containing material/mixtures. In addition no information on possible release (migration) is available.

Table 9: Summary table of human data on skin sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Retrospective analysis of patch test records (1983-1998)	THFMA	Patch test concentration 2% (w/w) Finn Chambers® and Scanpor® tape Occlusion for 2 d Reading on day 2 and 4 n=147	Positive 5/147 (3.4%)	Tucker et al., 1999
Retrospective analysis of patch tests to acrylic monomers; dentist personell (1994 – 2006)	THFMA (part of the “methacrylate series”)	Patch test concentration 2% (w/w) in petrolatum Finn Chambers® Reading on day 2, (3), 4/5/6 – depending on day of application n=258	Positive 7/258 (2.7%) Cross reactions to other metacrylates Hand/fingertip dermatitis	Aalto-Korte et al., 2007
Retrospective analysis of patch tests to acrylic monomers; occupational exposure to glues (1994 – 2006)	THFMA (part of the “methacrylate series”)	Patch test concentration 2% (w/w) in petrolatum Finn Chambers® Reading on day 2, (3), 4/5/6 – depending on day of application n=10	Positive 7/10 (70%) Cross reactions to other metacrylates Contact dermatitis	Aalto-Korte et al., 2008
Patch tests; students of dentistry, dental professionals and dental	Several substances tested: THFMA methyl methacrylate	Patch test concentration 0.2% in pet. Formaldehyde 0.1% in aq. IQ Chambers®	THFMA positive in 14/29 (48.3%) unexposed dental patients 13/44 (29.6%) students (3rd and 4th year of dental	Lyapina et al., 2014

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
patients	triethyleneglycol dimethacrylate ethyleneglycol dimethacrylate 2,2-bis[4-(2-hydroxy-3-meth-acryloxypropoxy)phenyl]-propane 2-hydroxyethyl methacrylate formaldehyde	Application for ~2 days Reading on day 2 and 3 Interviews and questionnaire-based survey n= 137	medicine) 9/28 (32.1%) students (6th year of dental medicine) 5/36 (13.9%) dental professionals	
Patch tests; students of dentistry, dental professionals and dental patients	THFMA methyl methacrylate triethyleneglycol dimethacrylate ethyleneglycol dimethacrylate 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl]-propane 2-hydroxyethyl methacrylate glutaraldehyde	Patch test concentration 0.2% in pet. Glutaraldehyde 0.2% in pet. IQ Chambers® Application for ~2 days Reading on day 2 and 3 Interviews and questionnaire-based survey n= 262	THFMA positive in 13/49 (26.5%) dental patients 30/110 (27.3%) students of dental medicine 2/38 (5.3%) students from dental technician school 9/65 (13.8%) dental professionals	Lyapina et al., 2016
Retrospective study, patch tests beauticians	THFMA and other (meth)acrylates	Patch test concentration 2% in pet. Curatest® chambers Exposure time: 2d Readings on day 2 and 4	Positive 31/39 (79.5%) Symptoms: eczema on hands (100%), face dermatitis (37.5%), paraesthesia (23.3%), transient oedema (9.3%); upper respiratory tract symptoms (14.0%) Mean latency: 10.55 months after exposure to long-lasting nail polish and 9.5 years (after exposure to (meth)acrylates of any kind (acrylic or gel nails)	Gatica-Ortega et al., 2017
Case report Female, 38 years old	THFMA ethylene glycol dimethacrylate 2-hydroxyethyl methacrylate hydroxypropyl methacrylate triethylene glycol	Patch test, 2% of test substance in vaseline	positive reactions (+2 and +3) for: THFMA ethylene glycol dimethacrylate 2-hydroxyethyl methacrylate	Kanerva et al., 1995 [cited in CIR, 2005]

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
	dimethacrylate Di-HEMA trimethylhexyl dicarbamate isopropylidenediphenyl bisglycidyl methacrylate		hydroxypropyl methacrylate triethylene glycol dimethacrylate Symptoms: dry and fissured dermatitis on both hands; spread to arms, chest, neck, and face; rhinitis, tenderness of the mucous membranes of the nose; paresthesia of fingertips; reversible while away from work.	
Patch test Dental technicians 1995-1999	Several (meth)acrylates including THFMA	Patch test, 5% of test substance in vaseline n=126 (tested with acrylate series)	THFMA positive 3/126 (2.4%)	Peiler et al., 2000 [cited in DFG, 2001]
Patch test Dental patients (with suspicion of intolerance to dental material)	THFMA	2% of test substance in vaseline	THFMA positive 3/520 (0.6%)	Vilaplana et al., 2000 [cited in DFG, 2001]
Patch test 298 patients (1992 – 2000)	THFMA	2% of test substance in Vaseline Results of reading after 72h	THFMA positive 5/298 (1.7%) (including 2 with occupational exposure to THFMA) 5 equivocal results not included	IVDK, 2001 [cited in DFG, 2001]

In a retrospective study by Tucker (1999), 440 patients with a history of exposure to (meth)acrylates (between January 1983 and March 1998) were identified. Of those, 147 were patch-tested with THFMA (2% (w/w); 2d occlusion time) on the back using Finn Chambers® and Scanpor® tape. 5 out of 147 patients (3.4%) showed positive reactions.

Aalto-Korte (2007) analysed filed patch test series of acrylic monomers (so called “methacrylate series”) at the Finnish Institute of Occupational Health (FIOH) from September 1994 to August 2006. A total of 473 patients were patch tested with the methacrylate series, including 258 working in dentistry (55 dentists, 192 dental nurses and 11 dental technicians). 32 from the total of 473 had at least one allergic reaction and worked in dentistry. THFMA was positive in 6 cases and equivocal in one case. THFMA in general was not found in dental products or mentioned in SDSs provided. The reactions to THFMA were usually connected with multiple reactions to a large number of other methacrylates and are explained by cross-allergy to other methacrylates. The clinical records of one dental nurse were not found; all other 31 patients had hand dermatitis, and 25 of them had fingertip dermatitis.

In a second paper, Aalto-Korte (2008) evaluated the filed methacrylate series patch tests of the FIOH for data on occupational exposure to acrylic glues. 10 patients were identified and all had occupational allergic contact dermatitis from methacrylates in glues. 7/10 (70%) showed positive reactions to 2% THFMA (w/w). These patients always showed wide methacrylate allergy (reactions to 7-10 different methacrylates) while the

three THFMA negative patients reacted only to 3 or 4 methacrylates each. This was explained by cross-allergy to other methacrylates by the study authors. Reactions to methyl methacrylate, ethyl methacrylate, and N-butyl methacrylate were seen only in THFMA-positive patients. THFMA may be a main component of some bi-component acrylic adhesives, however, the substance has not been detected in the analysis of anaerobic sealants at FIOH prior to this study.

Lyapina (2014) evaluated the incidence and risk of cross-sensitization to some methacrylic monomers, including THFMA, and formaldehyde in students of dentistry, dental professionals (occupationally exposed) and dental patients (occupationally unexposed). THFMA is commonly used in crowns and bridges and in the formulation of uv-light-curable adhesives, coatings, paints. 139 participants were patch-tested with methacrylic monomers (0.2% in pet.) (see summary in Table 9) and formaldehyde (0.1% in aq.). Patches (IQ Chambers®) were applied on the back of the tested individuals. No further details are documented in the publication but according to general product information[5] the used IQ Chambers® have an inside area of 64 mm^2 and a recommended loading volume of 25µl. Reading of the tests were performed on day 2, several hours after removing of the patches, with control revision on day 3. Based on the area and volume of the chamber as well as the concentration of the test substance a dose of 70 $\mu g/cm^2$ can be calculated (see also Table 12) for elicitation. Data concerning the prevalence of cross-sensitization to tetrahydrofurfuryl methacrylate and formaldehyde are presented in Table 10. During the interviews numerous female students self-related the positive skin patch test results with THFMA with the use of nail products. This underlines the role of consumer exposure in the onset of contact sensitisation to THFMA (and other methacrylic monomers). The authors concluded that due to the ubiquitous occurrence of formaldehyde and the wide use of composite resins and bonding agents (like THFMA) dental patients are at risk of cross-sensitization to formaldehyde and tested methacrylic monomers.

In a further study by Lyapina (2016) the frequency and the risk of concomitant sensitization to some methacrylic monomers and to glutaraldehyde among students of dental medicine and those from the dental technician school, and dental professionals was investigated. A total of 262 participants was included in the study and tested for six different methacrylic monomers (0.2% in pet.) and glutaraldehyde, a broad-spectrum antimicrobial agent (0.2% in pet.). Patches ((IQ Chambers®) were applied on the back of the tested individuals; no further details are documented in the publication but according to general product information[5] the used IQ Chambers® have an inside area of 64 mm^2 and a recommended loading volume of 25µl. Based on the area and volume of the chamber as well as the concentration of the test substance a dose of 70 $\mu g/cm^2$ can be calculated (see also Table 12). Reading was performed several hours after removing the patches, concretely 48h and 72h after application. 14.6% of students of dental medicin and 12.2% of dental patients showed positive patch tests to THFMA. The highest risk for concomitant sensitization to THFMA and glutaraldehyde was documented for students of dental medicine (12.7%). For further details see Table 11.

Table 10: Results of skin patch test reactions to THFMA and formaldehyde among different groups (Lyapina, 2014).

Group	Neg. reactions to THFMA vs. neg. reaction to formaldehyde	Neg. reactions to THFMA vs. pos. reaction to formaldehyde	Pos. reactions to THFMA vs. neg. reaction to formaldehyde	Pos. reactions to THFMA vs. pos. reaction to formaldehyde	Total
Occupationally unexposed dental patients	11 (38.0%)	4 (13.8%)	7 (24.1%)	7 (24.1%)	29 (100%)
Students (3rd and 4th year of dental medicine)	26 (59.1%)	5 (11.4%)	6 (13.6%)	7 (15.9%)	44 (100%)

[5] Information taken from general product information IQ chamber® Catalogue | Chemotechnique Diagnostics

Students (6th year of dental medicine)	11 (39.3%)	8 (28.5%)	5 (17.9%)	4 (14.3%)	28 (100%)
Dental professionals	27 (70.0%)	4 (11.1%)	3 (8.3%)	2 (5.6%)	36 (100%)
Total	**75 (54.8%)**	**21 (15.3%)**	**21 (15.3%)**	**20 (14.6%)**	**137 (100%)**

Table 11: Results of skin patch test reactions to THFMA and glutaraldehyde among different groups (Lyapina, 2016).

Group	Neg. reactions to THFMA vs. neg. reaction to glutaraldehyde	Neg. reactions to THFMA vs. pos. reaction to glutaraldehyde	Pos. reactions to THFMA vs. neg. reaction to glutaraldehyde	Pos. reactions to THFMA vs. pos. reaction to glutaraldehyde	Total
dental patients	29 (59.2%)	7 (14.3%)	6 (12.2%)	7 (14.3%)	49 (100%)
Students of dental medicine	58 (52.7%)	22 (20.0%)	16 (14.6%)	14 (12.7%)	110 (100%)
Students from dental technician school	30 (78.9%)	6 (15.8%)	1 (2.6%)	1 (2.6%)	38 (100%)
Dental professionals	52 (80.0%)	4 (6.2%)	5 (7.6%)	4 (6.2%)	65 (100%)
Total	**169 (64.4%)**	**39 (14.9%)**	**28 (10.7%)**	**26 (9.9%)**	**262 (100%)**

Table 12: Dose-calculation based on Lyapina, 2014 and 2016.

Chamber Area (according to general product information)	Volume applied (according to general product information)	Test substance THFMA	Density of petrolatum	Calculated dose/cm²
64 mm²	25µl	0.2% in petrolatum	Density is depeding on the fraction; due to missing detailed information an approximated value of 0.9 has been used	70 µg/cm²

In a retrospective study the files of patients (between January 2013 and June 2016) with allergic contact dermatitis caused by (meth)acrylates in long-lasting nail polish who were patch tested in cutaneous allergy units within the dermatology departments of four hospitals in Spain were reviewed (Gatica-Ortega, 2017). During the study period in total 2353 patients were patch tested and a diagnosis of allergic contact dermatitis caused by (meth)acrylates in long-lasting nail polish was made in 43 females (1.82% of all patients; 2.84% of 1514 females tested) with a mean age of 35 years. Not all 43 females were tested with the same acrylate series; THFMA was tested in 39 of them. The allergens that were most frequently positive in the tests were 2-hydroxypropyl methacrylate (41/43), 2-hydroxyethyl methacrylate (39/43) and THFMA (31/39). These three compounds were most frequently identified on the labels of the patients' products. The mean time before the development of allergic contact dermatitis symptoms was 10.55 months (2 weeks to 72 months) from the first exposure to long-lasting nail polish in those beauticians only exposed to this technique, and 9.5 years (range: 2–30 years) from the first exposure to (meth)acrylates of any kind (acrylic or gel nails) in the

remaining patients. Allergic contact dermatitis mostly was seen on both hands (fingers), but usually more severe on the dominant hand. Two clinical stages were observed: an acute phase with itchy vesicular dermatitis, and a more chronic phase with fissured fingertip dermatitis associated with pain. Anatomical sites other than the fingers were found in 18 of 40 (45%) patients: face dermatitis (eyelids and cheeks) in 15 of 40 (37.5%) patients; parts of the forearms that came into contact with contaminated surfaces at work in 5 of 40 (12.5%) patients; the dorsal aspects of the hands in 3 of 40 (7.5%) patients; the thighs in 3 of 40 (7.5%) patients; and the abdomen in 1 of 40 (2.5%) patients. Other symptoms included: paraesthesia in 10 patients (23.3%); transient oedema of the face, eyelids and/or lips in 4 patients (9.3%); and upper respiratory tract symptoms such as throat discomfort, hoarseness or congestion in 6 patients (14.0%). One patient (2.3%) developed generalized acute urticarial lesions. Most patients had some degree of onycholysis, but severe nail dystrophies were not observed.

In a review of the Cosmetic Ingredient Review Expert Panel (CIR, 2005) one case report is described: A 38-year old woman (non-atopic) had been working installing car rear-view mirrors on a production line for the past 6 years. The glue used was found (by GC-MS) to contain ethylene glycol dimethacrylate (0.4%), 2-hydroxyethyl methacrylate (24.6%), and tetrahydrofurfuryl methacrylate (% not stated). The major component was isobornyl acrylate (61.9%). For 2 years she had been experiencing a dry and fissured dermatitis on both hands. The dermatitis spread to her arms, chest, neck, and face and she developed rhinitis and tenderness of the mucous membranes of the nose. She also had paresthesia of the fingertips but her dermatitis cleared while she was away from work. The patient was patch tested using several acrylates (at a concentration of 2%), showing positive reactions (+2 and +3) for ethylene glycol dimethacrylate, 2-hydroxyethyl methacrylate, hydroxypropyl methacrylate, THFMA and triethylene glycol dimethacrylate but not for Di-HEMA trimethylhexyl dicarbamate and isopropylidenediphenyl bisglycidyl methacrylate (Kanerva et al. 1995 as cited in CIR, 2005).

DFG (2001) cites several studies and reports documenting positive patch-test results with THFMA, however, like for most of the studies presented above, no direct link between THFMA-exposure and positive test results can be established and cross-reactions cannot be excluded or even seem to be highly relevant. Three studies were indicated as most relevant: Peiler (2000) presents results from patch-tests with dental technicians (from 1995 to 1999), where 3/126 were positive for THFMA (2% in vaseline) (one with clinical relevance). Vilaplana (2000) reports results from 520 patients with possible reactions to the composition of dental prostheses. 3/520 were tested positive for THFMA (2% in vaseline). DFG (2001) also presents an evaluation of data recorded by the IVDK[6] between 1992 and 2000. 5/298 patients were tested positive for THFMA (2% in vaseline) and evaluated as relevant (no further information given).

In a combination of three *in chemico*/*in vitro* methods the key steps for skin sensitisation were addressed (AOP). The presented test were conducted according to knowledge at that time but are all similar to current OECD guidelines. The reports provide information on chemical identity, test procedure, test results and cytotoxicity but they do not provide any information on cell culture conditions. The main results are presented in the table below.

Table 13: Summary table of *in vitro* studies with THFMA relevant for skin sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Direct peptide reactivity assay (DPRA)	THFMA, 98.7% 100mM THFMA in acetonitrile	Incubation with synthetic proteins for 24h at room temperature	Peptide depletion [%]: Cysteine-containing peptide Neg. control 0.0 % THFMA 49.3 %	Anonymous, 2013a

[6] Informationsverbund Dermatologischer Kliniken (ivdk.org)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Non-GLP Similar to OECD 442C 2 (reliable with restrictions)	Neg control: acetonitirile Pos. control: ethylene glycol dimethacrylate (CAS 97-90-5), 50mM in acetonitirile	HPLC-UV (220nm)	Pos control 50.3 % <u>Lysine-containing peptide</u> Neg. control 0.0 % THFMA 10.7 % Pos control 11.9 % <u>Mean of cysteine and lysine % depletion</u> THFMA 30.0 % Pos control 31.1 %	
Keratinocyte Activation Assay, LuSens Non-GLP Similar to OECD 442D 2 (reliable with restrictions)	THFMA, 98.7% Conc: 128.38, 154.05, 184.86, 221.83, 266.20, 319.44, 383.33 µg/mL Vehicle DMSO Neg control: DL-lactic acid (CAS 50-21-5), 450 µg/ml Pos. control: ethylene glycol dimethacrylate (CAS 97-90-5), 18 µg/ml	LuSense cell line (modified keratinocytes) 48h incubation two independent experiments with 3 replicates each Luminescence MTT assay for cytotoxicity	Cytotoxicity: CV75 = 266.20 µg/ml >1.5 fold induction at concentrations not reducing cell viability below 70% on two independent experiments	Anonymous, 2013b
Dendritic cell line activation assay, myeloid U937 skin sensitization test (MUSST) Non-GLP similar to OECD 442E 2 (reliable with restrictions)	THFMA, 98.7% 79.18, 158 .36, 316.72, 633.44, 1266.87 µg/mL Vehicle: culture medium Neg control: lactic acid, 200 µg/ml Pos. control: ethylene diamine (EDA) 70µg/ml	U937 cells Flow cytometry : FITC-labelled anti-CD86 propidium iodide staining for cytotoxicity	Cytotoxicity: CV75 = 633.44 µg/ml induction of the expression of CD86 above 1.2-fold was observed at sufficiently non-cytotoxic concentration (viability ≥70%) in two independent experiments	Anonymous, 2013c

The **DPRA** addresses the first key event (KE1) of the AOP for skin sensitisation (OECD, 2014), namely the covalent binding to proteins by quantifying the reactivity of the test chemical towards model synthetic peptides containing either lysine or cysteine. The relevant guideline OECD 442C has been published 2021.

The provided DPRA for THFMA (Anonymous, 2013a) is similar to the current OECD 442C guideline (OECD, 2021). The reactivity of THFMA towards synthetic cysteine- or lysine-containing peptides has been determined, following 24h exposure at room temperature, by HPLC with gradient elution and UV detection at 220 nm. THFMA was solved at a 100 mM concentration in acetonitrile. Three samples of the test substance were incubated with each peptide in ratios of 1:10 (for cysteine-peptide) or 1:50 (for lysine-peptide). Additionally triplicates of the concurrent vehicle control acetonitirile were incubated with the peptides. Ethylene glycol dimethacrylate (EGDMA, CAS 97-90-5) was used as positive control instead of cinnamic aldehyde, which is recommended in OECD 442C. According to the guideline other suitable positive controls providing mid-range depletion values may be used if historical data are available to derive comparable run acceptance criteria. EGDMA provides mid-range depletion values, however, the mean depletion values do not fulfill the acceptance criteria of the guideline (between 60.8% and 100% for the cysteine peptide and between 40.2% and 69.0% for the lysine peptide) and historical control data are not presented. Further, a co-elution control was performed (samples consisted of the test substance, vehicle and the respective peptide buffer but without peptide) in order to detect possible interference of the test substance with the peptides.
Results: Visual observation after the 24-hour incubation time did not reveal precipitates in any samples of the test substance with both peptides. No co-elution occurred. Calibration curves for cysteine and lysine peptides showed a correlation of > 0.99. The peptide depletions are presented in Table 14 and Table 15.

Table 14: Reaction of THFMA with cysteine-peptide (Anonymous, 2013a).

	Peak area at 220 nm (AUC) [mAU*s]			Peptide concentration [mM]				Peptide depletion [%]			
Samples	1	2	3	1	2	3	Mean SD	1	2	3	Mean SD
Neg. control acetonitirile	714.9	733.7	722.0	0.460	0.472	0.464	0.465 0.006	1.2	-1.4	0.2	**0.0** **1.3**
THFMA	392.5	366.3	333.7	0.254	0.237	0.216	0.236 0.019	45.4	49.0	53.5	**49.3** **4.0**
Pos. control EGDMA	389.4	356.7	325.6	0.252	0.231	0.211	0.231 0.020	45.9	50.4	54.6	**50.3** **4.4**

Table 15: Reaction of THFMA with lysine-peptide (Anonymous, 2013a).

	Peak area at 220 nm (AUC) [mAU*s]			Peptide concentration [mM]				Peptide depletion [%]			
Samples	1	2	3	1	2	3	Mean SD	1	2	3	Mean SD
Neg. control acetonitirile	685.8	683.5	686.0	0.496	0.495	0.497	0.496 0.001	-0.1	0.2	-0.1	**0.0** **0.2**
THFMA	618.4	609.1	605.9	0.448	0.441	0.439	0.443 0.005	9.7	11.0	11.5	**10.7** **0.9**
Pos. control	612.0	602.0	596.2	0.443	0.436	0.432	0.437	10.6	12.1	12.9	**11.9**

EGDMA							0.006				**1.2**

The test results were evaluated using the prediction model by Gerberick (2007), which is the same as the cysteine 1:10/lysine 1:50 prediction model in OECD 442C. Mean peptide depletion was calculated as shown in Table 16.

Table 16: Mean depletion according Gerberick (2007) (Anonymous, 2013a).

	Cysteine Peptide		Lysine-Peptide		Mean of both depletions [%]
	Mean depletion [%]	SD	Mean depletion [%]	SD	
Pos. control EGDMA	50.3	4.4	11.9	1.2	**31.1**
THFMA	49.3	4.0	10.7	0.9	**30.0**

The prediction model (OECD, 2021) is as follows:

Mean of cysteine and lysine % depletion	Reactivity Class	DPRA prediction
0% ≤ mean % depletion ≤ 6.38%	No or minimal reactivity	Negative
6.38% < mean % depletion ≤ 22.62%	Low reactivity	Positive
22.62% < mean % depletion ≤ 42.47%	Moderate reactivity	
42.47% < mean % depletion ≤ 100%	High reactivity	

Based on this model THFMA has a mean peptide depletion of 30.0% resulting in a positive DPRA prediction and a moderate reactivity under the described conditions.

To evaluate the keratinocyte activating potential (KE2) of THFMA a **LuSens assay** was conducted (Anonymous, 2013b). The provided *in vitro* test is similar to OECD 442D (OECD, 2018a). In genetically modified keratinocytes (luciferase reporter cell line) the activation of the antioxidant response element (ARE) is investigated. As an indicator for activation of the Leap1/Nrf2/ARE signalling pathway the upregulation of the luciferase activity is measured.

In a preliminary experiment the cytotoxicity of THFMA (in DMSO) was assessed. The results are presented in the table below. The CV75 (concentration at which cell viability is reduced to 75%) of 266.20 µg/ml was then used as a basis for determining the concentrations to be tested in the main luciferase test and the parallel cytotoxicity test (CV75x1.2², CV75x1.2; CV75, CV75/1.2, CV75/1.2², CV75/1.2³, CV75/1.24) as recommended in OECD 442D. According to the guideline DL-Lactic acid (CAS 50-21-5) was used as negative control at a concentration of 450 µg/ml and ethylene glycol dimethacrylate (CAS 97-90-5) as positive control at a concentration of 18 µg/ml. In addition for positive and negative control historic control data are available.

Table 17: Preliminary cytotoxicity assessment (Anonymous, 2013b).

THFMA concentration [µg/mL]	Mean viability (3 replicates)	Rel. viability [%]

Vehicle control	0.539	100.00
0.5	0.530	98.39
1.0	0.553	102.66
5.0	0.547	101.49
10.0	0.559	103.84
50.0	0.591	109.78
100.0	0.557	103.47
500.0	0.188	34.95
1000.0	0.003	0.54
2000.0	0.001	0.23

The main test consisted of two independent experiments with 3 replicates each. After a 48h exposure time cells were lysed and luciferase induction was evaluated by measuring luminescence signal after substrate addition. In parallel a MTT assay was performed. The results are presented in Table 18.

Table 18: LuSens, main experiment results (Anonymous, 2013b).

THFMA concentration [µg/mL]	1st experiment		2nd experiment	
	fold induction	Rel. viability [%]	fold induction	Rel. viability [%]
Vehicle control	1	100	1	100
128.38	n.d.	n.d.	25.41	116.0
154.05	25.29	100.2	29.79	103.2
184.86	28.49	89.5	37.30	97.6
221.83	33.70	82.8	41.86	98.2
266.20	39.89	60.0	48.35	89.7
319.44	37.19	55.4	65.03	65.6
383.33	42.50	39.8	n.d.	n.d.
Pos control: EGDMA	7.17	111.1	7.46	117.2
Neg control: DL-Lactic acid	0.98	100.5	0.91	103.6

According to OECD 442D a test chemical is considered positive in the LuSens test method if it induces a statistically significant induction of the luciferase activity above a given threshold (i.e. ≥1.5 fold, or 50% increase) in at least two consecutive concentrations which do not significantly affect cell viability (i.e. at which the cellular viability is above 70%). The substance shows an induction of more than 1.5 fold at concentrations that did not reduce cell viability below 70%. The acceptance criteria according OECD 442D are fulfilled. It can be concluded that THFMA has a keratinocyte activating potential.

In a myeloid **U937 Skin Sensitisation Test (MUSST)** the key event “activation of dendritic cells” (KE3) of the AOP for skin sensitization is addressed (Anonymous, 2013c). The provided test is similar to OECD 442E (OECD, 2018b) and quantifies the change in the expression of the cell surface marker CD86. CD86 is known to be a co-stimulatory molecule that may mimic monocytic activation, which plays a critical role in T-cell priming. The changes of CD86 cell surface marker expression are measured by flow cytometry following cell staining with fluorescein isothiocyanate (FITC)-labelled antibodies. Cytotoxicity is measured in parallel via propidium iodide staining. Lactic acid (200 µg/ml) was used as negative control, ethylene diamine (70µg/ml) as positive control. OECD 442E recommends TNBS (picrylsulfonic acid, CAS 2508-19-2) as

positive control, however, the level of expression in this study was within the range of the historical negative and positive control data and the acceptance criteria according OECD 442E are fulfilled.

The cytotoxicity of THFMA on U937 cells after 48h of exposure was evaluated in a pre-experiment by flow cytometry using propidium iodide (PI) staining (Table 19). The CV75 was determined to be 633.44 µg/ml. In the main test the following final concentrations were used: CV75x2, CV75, CV75/2, CV75/4, CV75/8.

Table 19: Preliminary assessment of cytotoxicity on U937 cells (Anonymous, 2013c).

Concentration [µg/mL]	%PI negative cells experiment 1	%PI negative cells experiment 2	%PI negative cells mean	Rel.viability mean
Vehicle control	98.48	99.37	98.925	100.00
0.5	99.48	99.49	99.49	100.57
1	99.31	99.43	99.37	100.45
5	99.30	99.47	99.39	100.46
10	99.41	99.38	99.40	100.48
50	99.21	99.36	99.29	100.36
100	99.00	98.74	98.87	99.94
500	94.39	94.83	94.61	95.64
1000	8.52	27.69	18.11	18.30
2000	n.d.	n.d.	n.d.	n.d.

n.d. no viable cells detected

After 48h of exposure to THFMA concentrations of 79.18, 158 .36, 316.72, 633.44, 1266.87 µg/mL (in culture medium) U937 cells were stained with FITC labelled anti-human-CD86 antibody and propidium iodide. Fluorescence was analysed by flow cytometry. Two independent experiments were performed. Cell viability was decreased below 70% at 1266.87 µg/mL (experiment 1) and 316.72 µg/mL (experiment 2). In experiments 1 and 2 an induction of the expression of CD86 was observed at sufficiently non-cytotoxic concentrations (see Table 20).

Table 20: Cell surface marker expression (CD86) and cell viability (Anonymous, 2013c).

Concentration [µg/mL]	1st experiment		2nd experiment	
	CD86 fold induction	Rel. viability [%]	CD86 fold induction	Rel. viability [%]
Vehicle control	1.00	100	1.00	100
79.18	1.18*	99.8*	1.46	99.0
158 .36	1.63	98.9	1.87	92.5
316.72	1.74	92.6	2.29	69.4
633.44	1.87	71.0	0.60	24.4
1266.87	2.89	23.7	**	**
Neg control	0.96	99.9	1.11	99.8
Pos control	2.21	95.4	2.32	93.9

* value of only one sample; ** no viable cells detected

In the study a test substance was predicted to have a dendritic cell activating potential, when the marker expression exceeded the threshold of 1.2 with respect to vehicle treated cells at any tested sufficiently non-cytotoxic (cell viability ≥ 70%) concentration in two independent experiments. For THFMA it has been shown that after 48 hours of exposure CD86 expression was induced (up to 1.87 fold) in U937 cells at concentrations affording at least 70% viability. From this it has to be concluded that THFMA does induce

dendritic cell activation. According to OECD 442E a stimulation index of CD86 higher or equal to 150% has to be considered as positive and the prediction is considered positive if at least two independent runs are positive. THFMA also fulfills the OECD 442E criteria for a positive response.

Further derivations from the current OECD guideline (which did not influence the result of the study): an EC150 value (concentration at which the test chemical induced a CD86 stimulation index of 150) is not given. Instead of a CV70 a CV75 value is reported.

To get a prediction on a possible skin sensitizing potential for THFMA the three *in vitro* studies have been evaluated by registrants based on a publication by Bauch (2012) in a weight of evidence approach (two of three tests determine the overall result). Evaluation criteria and individual results are presented in Table 21. The substance is predicted to be a skin sensitizer.

Table 21: Evaluation of *in vitro* test results according Bauch (2012).

Test method	Endpoint	Evaluation criteria	Test result	Test evaluation
Direct Peptide Reactivity assay (DPRA)	Peptide delpletion	Positive if ≥6.38% mean depletion	30.0% mean peptide delpletion (49.3% cysteine and 10.7% lysine peptide depletion)	**Positive**
Keratinocyte Activation Assay (LuSens)	ARE-dependent luciferase activity	Positive if ≥1.5-fold luciferase activity when viability is >70% of the vehicle control.	In at least two independent experiments ARE-dependent luciferase activity induction above 1.5-fold at THFMA concentrations that did not reduce cell viability below 70% was observed.	**Positive**
Dendritic Cell Line Activation Assay (MUSST)	CD86 expression	Positive if ≥1.2fold of CD86 when viability is >70% of the control	In at least two independent experiments an induction of the expression of CD86 above 1.2-fold was observed at sufficiently non-cytotoxic concentration (viability ≥70%)	**Positive**

The OECD guideline 497 on defined approaches (DA) for skin sensitisation has been published by OECD in 2021. Results from multiple information sources can be used together in DAs to achieve an equivalent or better predictive capacity than that of the animal tests to predict responses in humans. A DA consists of a fixed data interpretation procedure applied to data generated with a defined set of information sources (DPRA, KeratinoSens™, h-CLAT), to derive a prediction without the need for expert judgment. In the available *in vitro* dataset for THFMA the key events of (1) covalent binding to proteins, (2) activation of the antioxidant response element (ARE) and (3) activation of dendritic cells have been tackled by using the methods (1) DPRA, (2) LuSens assay and (3) MUSST. Due to the deviation of methods compared to the ones defined in OECD 497 expert judgment is needed to derive a prediction: Non of the used methods is considered sufficient as stand-alone method to conclude on the skin sensitisation potential of THFMA, however three different key events have been investigated with positive results for each of them. The studies were conducted similar to current OECD test guidelines:

- The *in chemico* assay DPRA is described in the OECD test guideline 442C and data generated with this method is proposed to be used within Integrated Approaches to Testing and Assessment (IATA) together with other relevant complementary information. DPRA is part of OECD 497 on DA for skin sensitisation.
- The LuSens test method was considered scientifically valid to be used as part of an IATA, to support the discrimination between skin sensitisers and non-sensitisers for the purpose of hazard identification and was taken up into the OECD test guideline 442D. ESAC concludes that the LuSens test method, like the KeratinoSens™ test method, is ready to be considered for regulatory use (ESAC, 2016) .

- The MUSST is similar to the U937 cell line activation test (U-SENS™) described in the OECD test guideline 442E. The U-SENS™ was recommended by EURL ECVAM (2017) to be used as part of an IATA to support the discrimination between sensitisers and non-sensitisers for the purpose of hazard classification and labelling.

All methods used are defined as valid information sources to be used within IATA for skin sensitization by OECD (2017).

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

Several retrospective studies investigated filed patch-tests conducted in beauticians, dental staff and patients as well as persons with occupational exposure to acrylic glues. It can be assumed that they were exposed to different methacrylates and most of them also had positive patch-test results for more than one methacrylate. Cross reactions can be supposed.

- In patients with history of (meth)acrylate exposure 5/147 (3.4%) showed positive reactions to THFMA (Tucker, 1999).
- 7/258 (2.7%) patients filed at FIOH and working in dentistry were positive for THFMA (Aalto-Korte, 2007)
- From 10 patients filed at FIOH with occupational allergic contact dermatitis from methacrylates in glues 7 were tested positive for THFMA (Aalto-Korte, 2008).
- From 39 patients with allergic contact dermatitis caused by (meth)acrylates in long-lasting nail 31 were tested positive for THFMA (Gatica-Ortega, 2017).
- Patch-test with dental technicians (Peiler, 2000) or dental patients (Vilaplana, 2000) gave positive results in 3/126 and 3/520, respectively.

Investigation of dental staff or patients via patch-testing was done by Lyapina (2014 and 2016). THFMA was identified as component in crowns and bridged and in the formulation of uv-light-curable adhesives, coatings, paints. 26 – 48% of dental patients were tested positive for THFMA while about 13% of dental professionals show positive reactions. The calculated dose of THFMA for patch testing was 70 µg/cm^2.

After exposure to (meth)acrylates in general symptoms like contact dermatitis, paraesthesia or upper respiratory tract symptoms are described.

The *in chemico/in vitro* assays (DPRA, LuSens, MUSST) gave positive results for three key events (KE1, KE2, KE3) defined in the AOP for skin sensitizers. Tests have been conducted according to the knowledge at that time, and no substantial deviations from the currents OECD guidelines influencing the outcome of the studies could be identified. Used methods are defined as valid information sources to be used within IATA for skin sensitization by OECD (2017).

No standard animal test to evaluate a possible skin sensitisation property is available. The only available guinea pig study was rated as not reliable and cannot be used for classification purpose.

10.7.2 Comparison with the CLP criteria

Category	Criteria
Category 1	Substances shall be classified as skin sensitisers (Category 1) where data are not sufficient for sub- categorisation in accordance with the following criteria: (a) if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons; or (b) if there are positive results from an appropriate animal test
Subcategory 1A:	Substances showing a high frequency of occurrence in humans and/or a high potency in animals can be presumed to have the potential to produce significant sensitisation in

	humans. Severity of reaction may also be considered.
Subcategory 1B:	Substances showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals can be presumed to have the potential to produce sensitisation in humans. Severity of reaction may also be considered.

Further details on sub-categorisation based on human data are given in CLP, Annex I, 3.4.2.2.2.1. and 3.4.2.2.2.2.

Human evidence for sub-category 1A can include	(a) positive responses at ≤ 500 μg/cm^2 (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.
Human evidence for sub-category 1B can include	a) positive responses at > 500 μg/cm^2 (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.

No reliable animal test is available to evaluate the sensitizing property of THFMA.

Evaluating the human evidence the following conclusion can be drawn:

- Patch tests with a calculated dose of 70 μg THFMA/cm^2 resulted in 26 – 48% of dental patients tested positive for THFMA while about 13% of dental professionals show positive reactions (elicitation). No HRIPT (Human Repeat Insult Patch Test) or HMT (Human Maximization Tests) are available to determine an induction threshold. Usually the dose required for induction is higher than for elicitation.
- Retrospective studies focused on beauticians, dental staff / patients and persons with occupational exposure to (meth)acrylates. No measured exposure information on THFMA or other (meth)acrylates is available, only general assumptions based on questionnaires and general product information (dental material, nail products, glues) have been made. However, at least for dental staff and beauticians a relatively high frequency of exposure (≥ once/daily; ≥100 exposures) can be assumed (CLP guidance, 2017).
  Evaluation of filed patch test series gave positive results with THFMA for 3,4% (in patients with a history of exposure to acrylates), 2,7% (in dentists, dental nurses, and dental technicians with allergic reaction), 70% (in patients with occupational exposure to acrylic glues) and 80% (in beauticians with allergic reactions). All together a high frequency of occurrence can be determined and cross sensitization seems to be highly relevant.

Available *in chemico/in vitro* assays (DPRA, LuSens, MUSST) with THFMA gave clear positive results for the key events KE1, KE2 and KE3 investigated. The *in vitro* data was evaluated to be relevant, reliable and sufficient for the regulatory purpose. All used methods are recommended to be used for IATA for skin sensitization. According to CLP guidance (ECHA, 2014) these data can be used in a weight of evidence approach for classification. Based on these positive *in chemico/in vitro* data it can be concluded that THFMA is a skin sensitizer, however methods are not suitable to give information on the potency of THFMA.

All together the data clearly demonstrate the sensitizing property of THFMA. For human data qualitative exposure information is missing, however, considering the high frequency of occurence combined with the low dose (70 μg THFMA/cm^2) needed for elicitation a classification as Skin Sens. 1A is indicated.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Based on the documented human evidence (high frequency of occurrence, low dose needed for elicitation) a classification as Skin Sens. 1A, H317 is proposed. These results are supported in a weight of evidence approach by positive results for three key events in the AOP for skin sensitization demonstrated in the available *in chemico/in vitro* assays (DPRA, LuSens, MUSST).

The setting of SCL is not possible as no robust data on potency of THFMA is available.

Additional remark:

The registered substance THFMA contains an impurity with sensitizing properties (boundary composition). Methyl methacrylate, has a harmonized classification as Skin Sens. 1, H317 and is included in the composition in concentrations up to the generic concentration limit (GCL) of ≥1% (Table 3.4.2, CLP). According to CLP, Art 10 GCLs indicating a threshold at or above which the presence of that substance in another substance or in a mixture as an identified impurity, additive or individual constituent leads to the classification of the substance or mixture as hazardous. However, as the substance THFMA itself shows clear sensitising properties *in vitro* and in humans the impurity has not been considered further.

10.8 Germ cell mutagenicity

Not addressed in this dossier.

10.9 Carcinogenicity

Not addressed in this dossier.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

For the evaluation of adverse effects on sexual function and fertility a Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test (OECD 422) with THFMA is available.

Table 22: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
OECD 422 Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test	THFMA (99.0% purity) 0, 50, 120, 300 mg/kg bw/d Oral, gavage (5ml/kg bw/d) Vehicle: corn	$NOAEL_{(systemic, male)}$ = 300 mg/kg bw/d $NOAEL_{(systemic female)}$ = 120 mg/kg bw/d $NOAEL_{(fertility, males)}$ = 300 mg/kg bw/d $NOAEL_{(fertility, females)}$ = 120 mg/kg bw/d 300 mg/kg bw/d: body weight ↓ (f), food consumption ↓ (f), absolute thymus weight ↓ (m), absolute adrenals weights ↓ (f), relative thymus weight ↓ (m,f),	Anonymous, 2015

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
GLP 1 (reliable without restriction) Rat, Sprague Dawley SD N=10/sex/dose	oil, suspension Daily, 7d/week M: 29d F: ~43d	relative adrenals weight ↓ (f), relative uterus weight ↑ (f); thrombocytopenia slight/moderate (m), leucopenia slight/moderate (m, f), Red blood cell count ↑ (f); reticulopenia (f), prothrombin time ↑ (m,f) slight increase in mean pre-coital interval; gestation length ↑ (24 d); total resorptions in 3/10 f; pre-birth loss of ~66%, total litter loss in 7/10 f; 120 mg/kg bw/d: Gestation length slightly ↑ (23 d); Cumulative loss (13.48 %) 50 mg/kg bw/d: Gestation length slightly ↑ (23 d), Cumulative loss (5.36 %) No microscopic observations in testes	

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

In an OECD 422 study (Anonymous, 2015) THFMA in concentrations of 0, 50, 120 or 300 mg/kg bw/d was administered (oral, gavage; vehicle: corn oil) to male and female Sprague Dawley rats (n=10/sex/dose). Control group received the vehicle alone. Males were dosed for 29 days (two weeks prior to pairing and continuously thereafter, up to the day before necropsy) and females throughout the study for ~43 days (two weeks prior to pairing and thereafter during pairing and gestation until day 3 or 4 post partum). Doses were selected based on information from previous studies (no further information given). Doses were verified analytically and the final results for all levels were well within acceptable limits for concentration (85-115%) and homogeneity (CV<10%). No satellite groups were included. The studie was rated as Klimisch 1.

Males were killed after mating of all females. Females with live pups were killed on day 4 post partum; females with total litter loss were killed on the day of occurance of total litter loss. The females which did not give birth 25 days after positive identification of mating were sacrificed on days 26, 27 or 28 post coitum. In parental animals following parameters were evaluated: body weight, clinical signs (including neurotoxicity assessment, motor activity and sensory reaction to stimuli), food consumption, oestrous cycle, mating performance, clinical pathology investigations (haematology and clinical chemistry), litter data, macroscopic observations, organ weights and histopathological examination (including staging of spermatogenic cycle). For determination of oestrus cyclicity in females vaginal smears were taken daily in the morning starting two weeks before pairing until a positive identification of copulation was made.

The following parameters were examined in F1 offsprings: number and sex of pups, stillbirths, live births, postnatal mortality, presence of gross anomalies, body weight on days 1 and 4 post partum.

Statistical analysis were done, depending on the homogeneity of the data, by Dunnett's test or a modified t test.

In adult rats no **mortality** occurred during the study and no significant **clinical signs**/observations (neurotoxicity assessment) were observed. Motor activity was unaffected by treatment. Variations at the end of treatment period were considered of no toxiclogical significance since they were low, inconsistent and without dose-response-relationship.

In males no relevant differences in **body weight and body weight gain** were documented as well as in females up to day 14 of the post coitum period. On day 20 in females of the highest dose a decrease in bodyweight and body weight gain was reported (see Table 23).

In high dosed females also a decrease in **food consumption** was seen when compared with controls during the post coitum and post partum periods with statistical significance on days 7 and 14 post coitum and 4 post partum (see Table 24). In lower dosed females as well as males no differences were observed.

Table 23: Female body weight [g] development (Anonymous, 2015)

N Mean body weight [g] SD	0 mg/kg bw/d	50 mg/kg bw/d	120 mg/kg bw/d	300 mg/kg bw/d
Day 1 of pretest phase	10 194.17 9.72	10 194.42 9.87	10 194.22 8.00	10 193.75 6.97
Day 1 of premating phase	10 225.26 13.70	10 218.42 9.75	10 218.71 11.25	10 223.93 15.69
Day 8 of premating phase	10 240.31 11.81	10 231.00 9.17	10 228.71 12.53	10 237.88 20.83
Day 15 (start of pairing)	10 247.23 15.98	10 248.57 13.17	10 244.11 12.43	10 253.03 20.13
Day 0 (gestation phase)	6 260.63 16.10	10 255.23 11.26	9 255.57 20.88	10 261.60 24.19
Day 7 (gestation phase)	6 296.99 11.51	10 297.91 12.75	9 293.38 15.33	10 298.36 21.97
Day 14 (gestation phase)	6 333.95 13.52	10 339.13 13.45	9 332.18 14.99	10 334.66 21.54
Day 20 (gestation phase)	6 422.35 21.54	10 425.96 25.29	9 417.96 17.68	10 **384.66*** 38.81

Day 1 postpartum phase	6 329.77 17.74	10 335.76 17.20	9 327.78 14.48	6§ 316.83 16.38
Day 4 postpartum phase	6 313.51 28.71	10 323.03 21.13	9 319.85 23.30	6 299.06 16.50

* statistically significant at p<0.05%; Dunnett´s test
§ One high dose female was sacrificed on day 0 post partum due to total litter loss. Three females with total resorption.

Table 24: Food consumption of females [g/female/day], whole study period (Anonymous, 2015)

N Mean food cons (g/female/d] SD	0 mg/kg bw/d	50 mg/kg bw/d	120 mg/kg bw/d	300 mg/kg bw/d
Day 8 of premating phase (no SD reported)	2 17.14	2 16.50	2 15.06	2 16.15
Day 15 (start of pairing) (no SD reported)	2 17.45	2 17.67	2 16.88	2 16.39
Day 7 (gestation phase)	6 24.70 1.44	10 24.61 1.95	9 23.03 1.85	10 **22.45*** 1.18
Day 14 (gestation phase)	6 24.65 2.08	10 25.20 3.66	9 23.94 2.23	10 **22.31**# 1.00
Day 20 (gestation phase)	6 25.81 4.03	10 28.42 3.57	9 26.73 3.06	10 24.07 1.83
Day 4 postpartum phase	6 27.11 9.44	10 32.23 9.76	9 29.26 7.00	6 **13.33*** 4.44

* significant at p< 0.05; ** at p< 0.01; Dunnett´s test, data domogeneous
# significant at p<0.05; ## at p< 0.01; modified t-test, data inhomogeneous
§ One high dose female was sacrificed on day 0 post partum due to total litter loss. Three females with total resorption.

Terminal **body weights** were unaffected by treatment. **Organ weights** show some variance (see Table 25 and Table 26). Absolute and relative thymus weights were slightly reduced in mid and high dose males compared to controls. High dose females (300 mg/kg bw/day) showed a slight reduction in absolute and relative adrenals weights and in relative thymus weights.

Table 25: Mean terminal body weights and mean absolute organ weights (selected) in males and females (Anonymous, 2015)

Mean weight [g] SD	0 mg/kg bw/d N=10	50 mg/kg bw/d N=10	120 mg/kg bw/d N=10	300 mg/kg bw/d N=10
Males				
Terminal bodyweight	429.14 30.30	423.17 28.86	422.33 40.21	432.96 32.57
Adrenals	0.0591 0.0068	0.0570 0.0089	0.0548 0.0086	0.0555 0.0091
Epididymides	1.1692 0.1249	1.1785 0.0784	1.2016 0.1282	1.1905 0.1077
Testes	3.3293 0.1215	3.3832 0.2510	3.3195 0.2738	3.4358 0.2966
Thymus	0.5640 0.1476	0.5124 0.0598	**0.4440*** 0.0814	**0.3873**** 0.1132
Thyroid	0.0240 0.0031	0.0242 0.0032	0.0234 0.0035	0.0245 0.0037
Females				
Terminal bodyweight	298.41 30.47	320.72 21.49	316.90 20.44	306.58 24.96
Adrenals	0.0743 0.0100	0.0769 0.0120	0.0694 0.0058	**0.0618*** 0.0103
Ovaries	0.1260 0.0254	0.1385 0.0234	0.1442 0.0246	0.1467 0.0110
Thymus	0.3497 0.0892	0.3147 0.0602	0.3072 0.0607	0.2821 0.0586
Thyroid	0.0227 0.0027	**0.0274**** 0.0048	0.0242 0.0027	0.0229 0.0024
Uterus	0.6434 0.1428	**0.8203**# 0.1013	**0.8643**# 0.2635	2.0144 1.9880

* significant at p< 0.05; ** at p< 0.01; Dunnett´s test, data domogeneous
# significant at p<0.05; modified t-test, data inhomogeneous

Table 26: Relative organ weights (selected) in males and females (Anonymous, 2015)

Mean rel. weight [g] SD	0 mg/kg bw/d N=10	50 mg/kg bw/d N=10	120 mg/kg bw/d N=10	300 mg/kg bw/d N=10
Males				
Adrenals	0.0138 0.0019	0.0135 0.0022	0.0131 0.0027	0.0129 0.0023

Epididymides	0.2742 0.0396	0.2789 0.0145	0.2858 0.0305	0.2752 0.0184
Testes	0.7798 0.0673	0.8012 0.0581	0.7899 0.0706	0.7948 0.0570
Thymus	0.1306 0.0296	0.1209 0.0087	**0.1046**[#] 0.0111	**0.0891**[##] 0.0244
Thyroid	0.0056 0.0008	0.0057 0.0008	0.0056 0.0011	0.0057 0.0007
Females				
Adrenals	0.0251 0.0039	0.0242 0.0051	0.0220 0.0026	**0.0201*** 0.0023
Ovaries	0.0426 0.0101	0.0433 0.0075	0.0456 0.0084	0.0481 0.0056
Thymus	0.1176 0.0298	0.0982 0.0183	0.0965 0.0151	**0.0927*** 0.0214
Thyroid	0.0077 0.0011	0.0086 0.0016	0.0077 0.0011	0.0075 0.0012
Uterus	0.2171 0.0478	0.2575 0.0435	0.2724 0.0788	**0.6265**[#] 0.5301

* significant at p< 0.05; ** at p< 0.01; Dunnett´s test, data domogeneous
# significant at p<0.05; ## at p< 0.01; modified t-test, data inhomogeneous

Macroscopic observations like cervical nodes with abnormal colour or areas, kidneys with abnormal area/colour or pelvic dilatation are documented for individual animals in some groups, however, they were not considered treatment related.
Microscopic observations are documented for cervical nodes, heart, kidneys, liver, lungs, mesenteric nodes and thymus in males and females as well as prostate in males. However, they were considered to be sporadic/incidental and not treatment related as they were reported in control and treated animals and/or without dose response (see Table 27). No effects were seen when evaluating seminiferous tubules with respect to their stage in the spermatogenic cycle and to the integrity of the various cell types within the different stages; regular layering in the germinal epithelium was noted.

Table 27: Incidence of microscopic findings [incidence of finding/number of tissues examined] (Anonymous, 2015)

	males				females			
Tissues with findings	0 mg/kg bw/d	50 mg/kg bw/d	120 mg/kg bw/d	300 mg/kg bw/d	0 mg/kg bw/d	50 mg/kg bw/d	120 mg/kg bw/d	300 mg/kg bw/d
Cervical nodes: o Congestion/Haemorrhage	2/7	1/2	1/1	0/7	1/5	0/0	0/0	0/5
Heart: o Inflammatory cell foci	0/5	0/0	0/0	0/5	1/5	0/0	0/0	0/5
Kidneys:								

o Nephropathy	1/5	1/2	0/0	3/6	0/5	1/2	0/0	0/6
o Hydronephrosis	0/5	1/2	0/0	1/6	0/5	1/2	0/0	0/6
Liver:								
o Inflammatory cell foci	7/10	10/10	8/10	10/10	8/10	6/10	8/10	7/10
o Periportal hepatocytic vacuolation	1/10	3/10	4/10	1/10	1/10	5/10	1/10	4/10
o Fibrosis	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10
o Granulomatour reaction	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10
o Single cell apoptosis/necrosis	0/10	1/10	1/10	0/10	0/10	0/10	0/10	0/10
o Extramedullary haemopoiesis	0/10	0/10	0/10	0/10	0/10	0/10	2/10	3/10
Lungs:								
o Inflammatory cell foci	2/5	0/0	0/0	0/6	0/5	0/0	0/0	1/5
o Aggreg. of alveolar macrophages	1/5	0/0	0/0	0/6	0/5	0/0	0/0	0/5
Mesenteric nodes								
o Congestion/haemorrhage	0/5	0/0	1/2	1/6	0/5	1/1	0/0	0/6
Thymus								
o Congestions/Haemorrhage	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10
o Lymphoid depletion	0/10	0/10	0/10	0/10	0/10	0/10	0/10	2/10
Prostate								
o Inflammatory cell foci	1/5	0/0	0/0	0/5	-	-	-	-

For **haematology** 5/sex/group were investigated. When compared with controls, a number of treated males showed slight to moderate thrombocytopenia and leucopenia, with no dose-relation. In particular, platelets were decreased in low dose (15%) and high dose males (23%) and leucocytes (mainly neutrophils, lymphocytes and basophils) were decreased by 21%, 40% and 25% in males receiving 50, 120 and 300 mg/kg bw/day, respectively. Leucopenia was also recorded in females dosed with 300 mg/kg bw/day (19%). However, the decrement comprised mainly neutrophils and eosinophils. In addition, females dosed with 120 mg/kg bw/day and 300 mg/kg bw/day showed slight increase of erythrocytes, haemoglobin and haematocrit (6% to 16%) associated with reticulopenia (55%) and slight decrease of mean corpuscular haemoglobin concentration (4%) in females dosed at 300 mg/kg bw/day. A statistically significant increase of prothrombin time was recorded in animals dosed with 300 mg/kg bw/day (7% in males, 17% in females).

Table 28: Haematology (selected parameters) (Anonymous, 2015)

Mean SD	0 mg/kg bw/d n=5	50 mg/kg bw/d n=5	120 mg/kg bw/d n=5	300 mg/kg bw/d n=5
Males				
White blood cell count [10^3/µl]	12.372 3.205	9.740 3.623	**7.452*** 2.545	9.310 1.770
Neutrophils [10^3/µl]	1.134 0.251	1.018 0.316	1.082 0.521	0.862 0.161
Lymphocytes [10^3/µl]	10.590 2.997	8.166 3.401	**5.856*** 1.959	7.788 1.933
Basophils	0.106	0.076	**0.048***	0.070

[10^3/µl]	0.036	0.041	0.025	0.019
Platelets	1157.0	979.4	1102.8	**894.8***
[10^3/µl]	156.4	149.6	92.8	133.0
Prothrombin time	24.06	23.44	25.50	**25.76***
[sec]	0.55	1.39	0.78	1.01
Females				
Red blood cell count	6.174	6.544	6.738	**7.172***
[10^6/µl]	0.274	0.130	0.840	0.712
Haemoglobin	12.40	12.66	13.10	13.56
[g/dl]	023	0.39	0.90	0.98
Haematocrit	37.26	38.96	**40.46***	42.56
[%]	0.92	1.11	2.30	4.2
Mean corpusc HB conc	33.30	32.52	32.42	**31.92***
[g/dl]	0.57	0.67	0.72	1.24
Reticulocytes	6.548	5.306	6.374	**2.662***
[%]	1.079	1.491	3.820	1.875
Reticulocytes	402.56	346.06	407.14	**182.76***
[10^6/L]	52.65	92.15	202.35	113.07
Neutrophils	0.996	1.146	0.968	**0.378***
[10^3/µl]	0.294	0.502	0.407	0.083
Eosinophils	0.064	0.036	0.038	0.036
[10^3/µl]	0.043	0.026	0.013	0.021
Neutrophils	16.14	20.38	17.90	**7.82***
[%]	3.64	4.34	5.06	1.79
Monocytes	1.52	1.90	2.94	**5.30****
[%]	0.31	0.32	1.58	1.43
Platelets	1703.0	1896.0	1569.8	**1386.8***
[10^3/µl]	180.3	581.7	268.6	104.2
Prothrombin time	23.86	24.76	24.40	**27.96****
[sec]	2.06	1.94	0.85	1.67

* significant at $p< 0.05$; ** at $p< 0.01$;

Clinical chemistry (5/sex/group investigated) showed an increase of phosphorus in high dose males (14%). Due to the absence of other related findings, this change was considered of no toxicological importance. Females receiving 300 mg/kg bw/day showed decrease of alanine aminotransferase (57%), aspartate aminotransferase (29%), urea (39%) and sodium (7%) and increase of glucose (48%).

Five females were found not pregnant at necropsy (four in the control group and one in the mid dose group – see Table 30). Unilateral implantation was observed in one low dose female. At 300 mg/kg bw/day three females had total resorption and seven females had total litter loss within 1 day of parturition. The number of females with live pups in each dose group is presented in Table 30. In the high dose group no live pups were recorded.

Table 29: Reproductive indices of females (Anonymous, 2015)

	0 mg/kg bw/d	50 mg/kg bw/d	120 mg/kg bw/d	300 mg/kg bw/d
Copulatory index [%]	100.0%	100.0%	100.0%	100.0%
Fertility index [%]	60.0%	100.0%	90.0%	100.0%

Corpulation Index [%] = No. of animals mated / No. of animals paired x 100
Fertility Index [%] = No. of pregnant females / No. of females paired x 100

Table 30: Female group parameters after treatment with THFMA (Anonymous, 2015)

	0 mg/kg bw/d	50 mg/kg bw/d	120 mg/kg bw/d	300 mg/kg bw/d
Initial group size (n)	10	10	10	10
Pre-coital interval (mean, SD, n=10)	2.9 1.2	2.7 1.34	3.2# 3.99	4.2 2.25
Not pregnant	4	0	1	0
Unilateral implantation	0	1	0	0
Uterus abnormal size	0	0	0	2
Total litter loss	0	0	0	7
Total resorption	0	0	0	3
With live pups on day 4 post partum	6	10	9	0

one female mated 14 days after pairing

Oestrous cycle as well as copulatory and **fertility indices** were unaffected by treatment (Table 29). A slight increase in mean pre-coital interval was observed in the mid- (120 mg/kg bw/day) and high dose (300 mg/kg bw/day) animals compared to controls. The increase in the mid-dose group was related to one female which mated 14 days after pairing (Table 30).

Gestation length of the low (50 mg/kg bw/day) and mid-dose (120 mg/kg bw/day) groups was slightly higher than of the control group in which the majority of dams gave birth on day 22 of gestation. Most of low and mid-dose females gave birth on day 23. High dose females had more prolonged gestation length, statistically significant, compared to controls. In particular, four females gave birth on day 25 post coitum, two gave birth on day 24 post coitum and one on day 22 post coitum.

Pre-implantation loss was not effected by treatment with THFMA. The **pre-birth loss** was significantly increased in high dose females (~66 %) compared to controls. According to the study authors this increase could be attributable to the prolonged gestation period which caused most probably offspring suffering and the death during or shortly after the birth. No information on possible dystocia is given in the report. For detailed information see Table 31. Individual animal data are shown in Table 32.

Table 31: Mean group data for implantations, losses and gestation length after treatment with THFMA (Anonymous, 2015)

		0 mg/kg bw/day	50 mg/kg bw/day	120 mg/kg bw/day	300 mg/kg bw/day

Number of females pregnant	N	6	10	9	10
Number of litters	N	6	10	9	7
Corpora lutea	Total	95	163	147	153
	mean	15.83	16.30	16.33	15.43
	Std.Dev	1.6	2.87	2.12	2.15
	N	6	10	9	10
Implantations	Total	93	157	145	149
	mean	15.50	15.70	16.11	15.00
	Std.Dev	1.38	2.54	1.96	3.06
	N	6	10	9	10
Pre-implantation loss [%]	Litters affected	2	4	2	2
	mean	1.97	3.29	1.26	3.57
	Std.Dev	3.05	5.15	2.51	9.45
	N	6	10	9	10
Total Litter size at birth	Total	87	142	123	39
	mean	14.50	14.20	13.67	**5.57***
	Std.Dev	1.64	2.44	2.55	5.13
	N	6	10	9	7
Pre-birth loss [%]	Litters affected	4	7	7	7
	mean	6.52	9.34	14.64	**65.87***
	Std.Dev	5.66	8.53	15.00	28.08
	N	6	10	9	7
Gestation length [days]	mean	22.17	22.60	22.78	**24.29***
	Std.Dev	0.41	0.52	0.44	1.11
	N	6	10	9	7

* mean value is significantly different from control $p<0.05$

Data from females with total resorption or non-pregnant and from dams without live pups were excluded from group mean calculations.

Table 32: Individual animal data on pre-implantation and pre-birth losses and gestation length (Anonymous, 2015)

Dose	Animal #	Corpora lutea	Implantations	Pre-implantation loss [%]	Total litter size at birth	Pre-birth loss [%]	Gestation length [d]

0 mg/kg bw/d	1	15	15	0.0	13	13.3	23
	2	17	17	0.0	15	11.8	22
	3	17	16	5.9	16	0.0	22
	4	17	16	5.9	15	6.3	22
	5	13	13	0.0	12	7.7	22
	6	16	16	0.0	16	0.0	22
50 mg/kg bw/d	1	16	16	0.0	16	0.0	22
	2	15	15	0.0	15	0.0	23
	3	19	16	15.8	14	12.5	23
	4	15	15	0.0	14	6.7	23
	5	15	15	0.0	14	6.7	23
	6	17	16	5.9	12	25.0	23
	7	10	10	0.0	9	10.0	22
	8	19	18	5.3	18	0.0	23
	9	17	16	5.9	14	12.5	22
	10	20	20	0.0	16	20.0	22
120 mg/kg bw/d	1	15	15	0.0	15	0.0	23
	2	19	19	0.0	10	47.4	23
	3	17	17	0.0	15	11.8	23
	4	20	19	5.0	17	10.5	22
	5	15	15	0.0	13	13.3	23
	6	16	15	6.3	15	0.0	23
	7	14	14	0.0	10	28.6	23
	8	14	14	0.0	12	14.3	23
	9	17	17	0.0	16	5.9	22
300 mg/kg bw/d	1	13	13	0.0	1	92.3	25
	2	16	16	0.0	4	75.0	24
	3	16	16	0.0	8	50.0	25
	4	18	18	0.0	15	16.7	22
	5	16	16	0.0	-	-	-
	6	16	16	0.0	8	50.0	24
	7	15	14	6.7	-	-	-
	8	17	17	0.0	2	88.2	25
	9	12	9	25.0	1	88.9	25
	10	14	14	0.0	-	-	-

For effects on **female** fertility a NOAEL of 120 mg/kg bw/d (increased pre-birth loss, prolonged gestation, slight increase in mean pre-coital interval) can be derived. Reprotoxic effects were seen in the absence of other toxic effects. For general toxicity in females a NOAEL of 120 mg/kg bw/d can be derived based on reduced body weight (-9% on day 20 of gestation), food consumption (-9% on day 7 and 14 of gestation as well as -50% on day 4 post partum) and some effects on haematology parameters at 300 mg/kg bw/d.

In **male** rats no relevant changes were recorded during the study and at the post mortem examinations at any dose level investigated. No effects on body weight and body weight gain were seen. Some variations on organ weights and haematology were recorded. A qualitative examination of the testes was performed in five control and high dosed males. Seminiferous tubules were evaluated with respect to their stage in the spermatogenic cycle and to the integrity of the various cell types within the different stages; no alterations were noted. The NOAEL for general toxicity and impairment of fertility in male rats is 300 mg/kg bw/d.

10.10.3 Comparison with the CLP criteria

Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).

- The classification of a substance in Category 1A is largely based on evidence from humans.
- The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.

No human data is available to justify classification in Category 1A.

In a guideline conform animal study (rat) exposure to THFMA resulted in clear evidence of an adverse effects on female fertility. The mean pre-coital interval was slightly increased and pre-birth loss was increased (dose-dependant) by ~66% in the 300 mg/kg bw/d group. Gestation length was prolonged from 22.17 days in the control group to 24.29 days in the 300 mg/kg bw/d group. A NOAEL of 120 mg/kg bw/d for fertility bw can be set. The effects were considered not to be secondary non-specific consequences of other toxic effects, as only slight effects of general toxicity have been seen (slightly reduced bodyweight and food consumption, hematology). For males no effects on fertility are documented. Category 1B is indicated.

Mechanistic data to doubt the relevance for humans is not available.

10.10.4 Adverse effects on development

For the evaluation of adverse effects on development a Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test (OECD 422) with THFMA is available. A short description of the study is given in Table 33. For further details see Chapter 10.10.2.

Table 33: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
OECD 422 Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test GLP 1 (reliable without restriction) Rat, Sprague Dawley SD N=10/sex/dose	THFMA (99.0% purity) 0, 50, 120, 300 mg/kg bw/d Oral, gavage (5 ml/kg bw/d) Vehicle: corn oil, suspension Daily, 7d/week M: 29d F: ~43d	NOAEL $_{(systemic\ female)}$ = 120 mg/kg bw/d NOAEL $_{(F1\ dev\ tox)}$ = 120 mg/kg bw/d 300 mg/kg bw/d: Total resorptions 3/10; total litter loss 7/10 120 mg/kg bw/d: Increased cumulative loss (13.48%) 50 mg/kg bw/d: Increased cumulative loss (5.36%)	Anonymous, 2015

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

In an OECD 422 study (Anonymous, 2015) THFMA in concentrations of 0, 50, 120 or 300 mg/kg bw/d was administered (oral, gavage; vehicle: corn oil) to male and female Sprague Dawley rats (n=10/sex/dose). Control group received the vehicle alone. Males were dosed for 29 days (two weeks prior to pairing and continuously thereafter, up to the day before necropsy) and females throughout the study for ~43 days (two weeks prior to pairing and thereafter during pairing and gestation until day 3 or 4 post partum).

Evaluated parameters in parental animals are presented in Chapter 10.10.2. For F1 offsprings the following parameters were examined: number and sex of pups, stillbirths, live births, postnatal mortality, presence of gross anomalies, body weight on days 1 and 4 post partum.

For the high dose group in 3/10 females total resorption is documented (Table 32). Mean litter data are presented in Table 34. A total litter loss is reported in 7/10 females of the high dose group. Decreases in litter weights, seen in low and mid-dose groups (not statistically significant), were due to the lower number of pups in treated groups respect to control. Mean pup weights show no differences. Cumulative loss (post natal death in %[7]) on day four is documented in the low dose group for 5/10 females (individual data: 26.7%, 7.1%, 7.1%, 5.6%, 7.1%), in the mid dose group in 4/9 females (53.3%, 30.0%, 6.7%, 31.3%) compared to control in 0/6 females.

[7] Calculated as: (total litter size at birth – live litter size at day 4) x 100 / total litter size at birth

Table 34: Group mean litter data at birth and days 1-4 post patum (Anonymous, 2015). Highest dose group showed total litter loss.

			0 mg/kg/day	50 mg/kg/day	120 mg/kg/day	300 mg/kg/day
At birth	Total litter size	mean	14.50	14.20	13.67	Total litter loss
		Std.Dev	1.64	2.44	2.55	
		N	6	10	9	
	Live litter size	mean	14.50	14.10	12.89	
		Std.Dev	1.64	2.47	3.06	
		N	6	10	9	
	Pup loss [%]	mean	0.00	0.71	5.56	
		Std.Dev	0.00	2.25	13.33	
		N	6	10	9	
Day 1 post partum	Litter weight [g]	Mean	111.03	103.41	92.39	
		Std.Dev	7.72	18.39	25.01	
		N	6	10	9	
	Mean pup weight [g]	mean	7.72	7.56	7.38	
		Std.Dev	0.60	0.73	1.00	
		N	6	10	9	
Day 4 post partum	Live litter size	mean	14.50	13.40	11.56	
		Std.Dev	1.64	2.46	3.24	
		N	6	10	9	
	Cumulative loss [%]	mean	0.00	5.36	13.48	
		Std.Dev	0.00	8.22	19.78	
		N	6	10	9	
	Litter weight [g]	mean	151.6	141.15	117.22	
		Std.Dev	14.33	24.61	37.25	
		N	6	10	9	
	Mean pup weight [g]	mean	10.58	10.60	10.08	
		Std.Dev	1.61	1.22	1.74	
		N	6	10	9	

Clinical signs, terminal body weight and organ weights were not affected in pups. At necropsy no treatment-related findings were noted in pups which died or in pups sacrificed on day 4 post partum. No structural abnormalities, altered growth or functional deficiencies are reported. Unscheduled deaths are documented

with the remark “organs autolysed”. No difference in sex ratios was noted between the control and treated groups (low and mid-dose).

For F1-pups a NOAEL of 120 mg/kg bw/d can be derived based on total resorptions in 3/10 and total litter loss in 7/10 females in the 300 mg/kg bw/d group. Cumulative loss (% postnatal death) has been observed in a dose dependant manner in the low and mid dose but without statistic significance.

10.10.6 Comparison with the CLP criteria

Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B). Adverse effects on development

- The classification of a substance in Category 1A is largely based on evidence from humans.
- The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.

No human data is available to justify classification in Category 1A.

In the OCED 422 guideline animal study (rat) clear evidence of an adverse effect on development is documented. Total resorptions in 3/10 females as well total litter loss in 7/10 females at exposure to 300 mg/kg bw/d THFMA are described. Cumulative loss was slightly increased in the low (5.36 %) and high dose (13.48 %) groups . A NOAEL of 120 mg/kg bw/d for developmental effects on pups can be derived. The effects were considered not to be secondary consequences of other toxic effects. For general toxicity in females a NOAEL of 120 mg/kg bw/d can be derived based on minimal effects on body weight, food consumption and haematology parameters at 300 mg/kg bw/d.

Mechanistic data to doubt the relevance for humans is not available.

10.10.7 Adverse effects on or via lactation

Not relevant.

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

Not relevant.

10.10.9 Comparison with the CLP criteria

Not relevant.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Reproductive Toxicity has been reported in an OECD 422 guideline study in rats. Effects on female fertility (slightly increased mean pre-coital interval, increased pre-birth loss, prolonged gestation length) in the absence of other toxic effects are described with a NOAEL of 120 mg/kg bw/d. Developmental toxicity of THFMA (total resorptions, total litter loss) with a NOAEL of 120 mg/kg bw/d is documented.

Based on these animal data a classification as Repr. 1B, H360FD is proposed.

10.11 Specific target organ toxicity-single exposure

Not addressed in this dossier.

10.12 Specific target organ toxicity-repeated exposure

For evaluation of the repeated dose toxicity of THFMA only a OECD 422 study is available, which is described in detail in Chapter 10.10.

Table 35: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
OECD 422 Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test GLP 1 (reliable without restriction) Rat, Sprague Dawley SD N=10/sex/dose	THFMA (99.0% purity) 0, 50, 120, 300 mg/kg bw/d Oral, gavage (5ml/kg bw/d) Vehicle: corn oil, suspension Daily, 7d/week M: 29d F: ~43d	NOAEL $_{(systemic, male)}$ = 300 mg/kg bw/d NOAEL $_{(systemic female)}$ = 120 mg/kg bw/d 300 mg/kg bw/d: body weight ↓ (f), food consumption ↓ (f), absolute thymus weight ↓ (m), absolute adrenals weights ↓ (f), relative thymus weight ↓ (m,f), relative adrenals weight ↓ (f), relative uterus weight ↑ (f); thrombocytopenia slight/moderate (m), leucopenia slight/moderate (m, f), Red blood cell count ↑ (f); reticulopenia (f), prothrombin time ↑ (m,f) slight increase in mean pre-coital interval; gestation length ↑ (24 d); total resorptions in 3/10 f; pre-birth loss of ~66%, total litter loss in 7/10 f; 120 mg/kg bw/d: Gestation length slightly ↑ (23 d); Cumulative loss (13.48 %) 50 mg/kg bw/d: Gestation length slightly ↑ (23 d), Cumulative loss (5.36 %)	Anonymous, 2015

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		No microscopic observations in testes	

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

The Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test is described in detail in Chapter 10.10 (Anonymous, 2015). For male rats after oral administration of 0, 50, 120, 300 mg THFMA/kg bw/d for 29 days no effects on body weight and body weight gain were documented as well as in females up to day 14 of the post coitum period. On day 20 in females of the highest dose a significant decrease (-9%) in bodyweight and body weight gain was reported (see Table 23). Terminal body weights were unaffected by treatment. In high dosed females also a decrease in food consumption was seen when compared with controls during the post coitum and post partum periods with statistical significance on days 7 and 14 post coitum and 4 post partum (see Table 24). In lower dosed females as well as males no differences were observed.

Organ weights show some variance (see Table 25 and Table 26). In males the absolute thymus weights were significantly reduced at 120 mg/kg bw/d (-21%) and 300 mg/kg bw/d (-31%) as well as the relative thymus weight at 120mg/kw (-20%) and 300 mg/kg bw/d (-32%). High dose females (300 mg/kg bw/d) showed a slight reduction in absolute (-17%) and relative adrenals weights (-20%) and in relative thymus weights (-21%). Absolute uterus weights were increased in all dosed females; relative uterus weight was increased about 3-fold in the 300 mg/kg bw/d group. However, it has to be noted that females have been sacrificed on different points in time (females with live pups were killed on day 4 post partum; females with total litter loss were killed on the day of occurance of total litter loss).

Macroscopic observations like cervical nodes with abnormal colour or areas, kidneys with abnormal area/colour or pelvic dilatation are documented for individual animals in some groups, however, they were not considered treatment related. Microscopic observations were considered to be sporadic/incidental and not treatment related as they were reported in control and treated animals and/or without dose response (see Table 27).

Clinical chemistry (5/sex/group) showed an increase of phosphorus in high dose males (14%). Due to the absence of other related findings, this change was considered of no toxicological importance. Females receiving 300 mg/kg bw/d showed decrease of alanine aminotransferase (57%), aspartate aminotransferase (29%), urea (39%) and sodium (7%) and increase of glucose (48%). A liver injury cannot be conculded based on these results.

For haematology 5/sex/group were investigated (Table 28). When compared with controls, a number of treated males showed slight to moderate thrombocytopenia and leucopenia, with no dose-relation. In particular, platelets were decreased in low dose (15%) and high dose males (23%) and leucocytes (mainly neutrophils, lymphocytes and basophils) were decreased by 21%, 40% and 25% in males receiving 50, 120 and 300 mg/kg bw/d, respectively. Leucopenia was also recorded in females dosed with 300 mg/kg bw/d (19%). However, the decrement comprised mainly neutrophils and eosinophils. In addition, females dosed with 120 mg/kg bw/d and 300 mg/kg bw/d showed slight increase of erythrocytes, haemoglobin and haematocrit (6% to 16%) associated with reticulopenia (55%) and slight decrease of mean corpuscular haemoglobin concentration (4%) in females dosed at 300 mg/kg bw/d. A statistically significant increase of prothrombin time was recorded in animals dosed with 300 mg/kg bw/d (7% in males, 17% in females).

10.12.2 Comparison with the CLP criteria

A substance is classified with STOT RE under CLP when it has produced or has been shown to have the potential to produce significant toxicity to humans or be harmful to human health following repeated exposure by the oral, dermal or inhalation routes. This can be on the basis of human data or evidence from

studies in animals that cause such effects at or below given Guidance Values. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed are included under this classification.

Category 1	Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: • reliable and good quality evidence from human cases or epidemiological studies; or • observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Guidance dose/concentration values are provided below (see 3.9.2.9), to be used as part of a weight-of- evidence evaluation.
Category 2	Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values are provided in the CLP regulation in order to help in classification. In exceptional cases human evidence can also be used to place a substance in Category 2

For THFMA no information on repeated dose toxicity in humans is available.

The available OECD 422 guideline study shows some effects on body weight, organ weights, haematology and clinical chemistry; in general females seems to be more susceptible. The NOAEL for systemic toxicity for males is 300 mg/kg bw/d and for females 120 mg/kg bw/d. However, no clear dose response relationship can be established and target organs could not be identified based on these data.

10.12.3 Conclusion on classification and labelling for STOT RE

No relevant adverse effect with a dose-response could be identified in rats dosed orally with THFMA in concentrations up to 300 mg/kg bw/d. No classification for STOT RE is proposed.

10.13 Aspiration hazard

Not addressed in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not addressed in this dossier.

12 EVALUATION OF ADDITIONAL HAZARDS

Not addressed in this dossier.

13 ADDITIONAL LABELLING

Not relevant.

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15 ANNEX I - CONFIDENTIAL