CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

1-phenylethan-1-one (1-phenylethylidene)hydrazone

EC Number: **211-979-0**

CAS Number: **729-43-1**

Index Number: **-**

Contact details for dossier submitter:

ANSES (on behalf of the French MSCA)
14 rue Pierre Marie Curie
F-94701 Maisons-Alfort Cedex
classification.clp@anses.fr

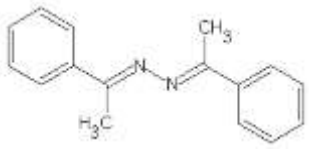
Version number: 1 **Date: October 2019**

CONTENTS

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)	1-phenylethan-1-one (1-phenylethylidene)hydrazone
Other names (usual name, trade name, abbreviation)	bis(1-phenylethylidene)hydrazine, 1-phenyl-N-[(E)-1-phenylethylideneamino]ethanimine, acetophenone azine[1]
ISO common name (if available and appropriate)	/
EC number (if available and appropriate)	211-979-0
EC name (if available and appropriate)	Acetophenone azine
CAS number (if available)	729-43-1
Other identity code (if available)	/
Molecular formula	$C_{16}H_{16}N_2$
Structural formula	
SMILES notation (if available)	CC(c1ccccc1)=NN=C(C)c1ccccc1
Molecular weight or molecular weight range	236.318 g/mol
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)	96% - 99%

[1] the name *acetophenone azine* is used throughout the document to identify the proposed substance.

1.2 Composition of the substance

There is no data on composition as the substance is not yet registered.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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 existing Annex VI entry										
Dossier submitters proposal	tbd	1-phenylethan-1-one (1-phenylethylidene)hydrazone	211-979-0	729-43-1	Skin Sens. 1	H317	GHS07 Wng	H317			
Resulting Annex VI entry if agreed by RAC and COM	tbd	1-phenylethan-1-one (1-phenylethylidene)hydrazone	211-979-0	729-43-1	Skin Sens. 1	H317	GHS07 Wng	H317			

Tbd: to be determined

Table 2: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public 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	***harmonised classification proposed***	**Yes**
Germ cell mutagenicity	*hazard class not assessed in this dossier*	No
Carcinogenicity	*hazard class not assessed in this dossier*	No
Reproductive toxicity	*hazard class not assessed in this dossier*	No
Specific target organ toxicity-single exposure	*hazard class not assessed in this dossier*	No

Hazard class	Reason for no classification	Within the scope of public consultation
Specific target organ toxicity-repeated exposure	*hazard class not assessed in this dossier*	No
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

There is currently no harmonised classification and labelling for acetophenone azine.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Reason for a need for action at Community level:

Change in existing entry due to new data
Disagreement by DS with current self-classification

Several cases of skin allergies and/or irritations a priori relating to textile clothing or footwear have been reported in France in recent years. The French Agency for food environmental and occupational health safety (ANSES) was mandated to assess the risks linked to the presence of substances in textile and shoes. The report of the work was published on 4th July 2018 (https://www.anses.fr/en/system/files/CONSO2014SA0237RaEN.pdf)

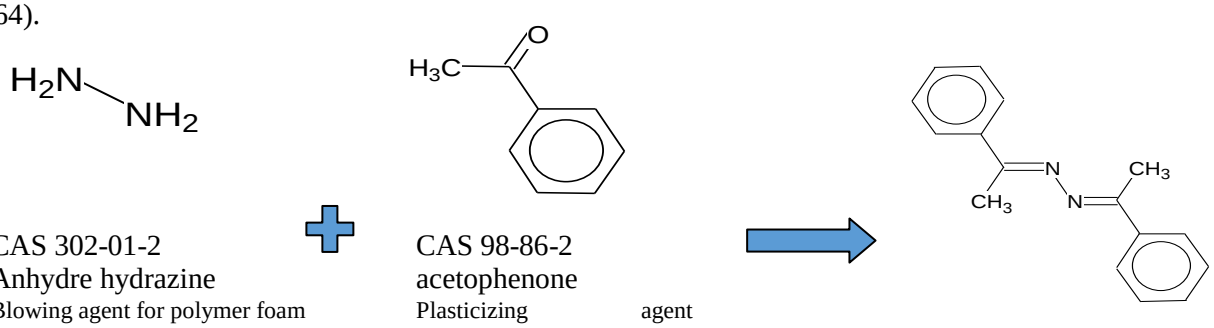
In order to answer the request, a study of the scientific literature, supplemented by tests on a sampling of new clothes taken from several points of sale and shoes that led to complaints from customers was performed to find the presence of skin irritant or allergic substances. These investigations on footwear and garments led to identify a new substance named acetophenone azine (CAS No 729-43-1). Regarding the analyzed results of the fourteen footwear articles tested, acetophenone azine as a new emerging substance was found in 14% of the articles.

In 2016 and 2017, cases of severe contact dermatitis in children and adult involving acetophenone azine were published (see section 9.1.1). *In vivo* metabolism of acetophenone azine to hydrazine and acetophenone is possible based on expert judgement and QSAR. If acetophenone azine is not self classified for its skin sensitizing properties, hydrazine (EC n°206-114-9 CAS n°302-01-2) is classified for skin sensitisation under CLP Regulation 1272/2008 EC. Some recommendations following the identification of substance of concern on textile and footwear were issued by the French Agency, among them classification of sensitising compounds under CLP. As a consequence of a harmonised classification as Skin Sens., the substance, as all classified sensitisers, would be included in the scope of the FR/SE Restriction on skin sensitising substances in textile, leather, hide and fur articles which has recently been submitted to ECHA.

Therefore, considering the new data available as well as the fact that no skin sens. is indicated in the current self-classification of acetophenone azine, a CLH report is considered justified for acetophenone azine.

5 IDENTIFIED USES

There is few information available on the uses of acetophenone azine as the substance is not yet registered. Acetophenone azine may be used as a synthetic intermediate in the chemical industry. In addition, this substance may result from the reaction of hydrazine (EC n°206-114-9 CAS n°302-01-2) with acetophenone (EC n°202-708-7, CAS n°98-86-2) (production of acetophenone azine - US Patent 3153089A publication 1964).



CAS 302-01-2
Anhydre hydrazine
Blowing agent for polymer foam (PE, PVC, EPA, rubber)

CAS 98-86-2
acetophenone
Plasticizing agent
Polymerization catalyst

CAS 729-43-1
Acetophenone azine

Acetophenone azine is present in products of consumers such as sport equipment (Raison-Peyron *et al.*, 2016; 2017a and b). Acetophenone azine was measured in the equipment wearing by the football players (Raison-Peyron *et al.*, in 2016 and 2017) and the concentrations are indicated in the following table 3:

Table 3: Concentrations of acetophenone azine found in sport equipment ((Raison-Peyron *et al.*, in 2016 and 2017)

Type of sport equipment	Concentrations measured	Publication
Shin pad sample of 1st case in a 13-year-old football player containing foam based on EVA (consisted of copolymer of ethylene and vinyl acetate)	~20 µg/g	Raison-Peyron *et al.*, 2016,
Inner foam of the shin pads sample (based on EVA) of 2nd case in a 11 year old boy	69 µg/g	Raison-Peyron *et al.*, 2017a
Foam of the sneaker sole of the flip-flops	21 µg/g	Raison-Peyron *et al.*, 2017a
the first brand in the foam of sneaker soles from both sports brands in the 12 year old boy	15 µg/g	Raison-Peyron *et al.*, 2017b
the second brand in the foam of sneaker soles from both sports brands in the 12	<0.5 µg/g	Raison-Peyron *et al.*, 2017b

year old boy		

The substance was identified causing skin allergy in these children wearing a shin pad containing foam based on EVA (consisted of copolymer of ethylene and vinyl acetate) (Raison-Peyron et al., 2016; 2017 and b). According to information from the Joint Laboratory Service (SCL) in Massy (France), acetophenone azine was measured in socks, sneakers, children's leather shoes, walking shoes, shin pad, acrylic fur at concentrations between 20 ppm (sneakers) and 70 ppm (children's shoes). No data is available on the presence of acetophenone azine in other products.

6 PHYSICOCHEMICAL PROPERTIES

Given the lack of literature data available, there is very limited data on the physical and chemical properties of acetophenone azine.

Table 4: Summary of available physicochemical properties of acetophenone azine

Property	Result	Source
Physical form	White to yellow solid	PubChem, 2016 : Information on acetophenone azine https://chem.nlm.nih.gov/chemidplus/rn/729-43-1
Molecular mass	236,318 g/mol	PubChem, 2016 : Information on acetophenone azine https://chem.nlm.nih.gov/chemidplus/rn/729-43-1
Partition coefficient	Log P = 3.7	confirmed by the lab Sponsor Representative in exchanges by emails January 2018 (h-CLAT report)
Melting range	120-124°C	MSDS

7 EVALUATION OF PHYSICAL HAZARDS

Not assessed

8 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

8.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Dermal absorption is an important element to be considered. Prediction of the physicochemical properties and behavior of the substance when in contact with the skin is essential for assessing potential skin sensitiser of a substance. Indeed, to induce sensitising effects, the substance must first cross the skin barrier. The most important factors in the dermal bioavailability of a substance are the molecular weight and lipophilicity that can respectively be evaluated by the molar mass (MM) and the partition coefficient between octanol and water (Log P). Other factors may also influence bioavailability such as volatility, melting point, contact time at the level of the skin and the total exposure dose.

The European Food Safety Agency (EFSA) estimates that a substance having a molar mass greater than 500 $g.mol^{-1}$ and a log P < -1 or > 4 has a low dermal absorption (about 10%) (EFSA, 2017). The ability of the substance to induce sensitising effects will be therefore limited. However, it is important to note that low exposure may still induce sensitising effects.

Regarding the molecular mass of 236 $g.mol^{-1}$ and log P = 3.7, acetophenone azine has dermal absorption potential and can have the ability to induce sensitising to skin (EFSA, 2017).

An hydrolysis study was first performed to determine the hydrolysis rate and the degradation products of acetophenone azine.

The aim was to investigate the possibility of hydrolysis of the substance by sweat leading to the formation of urea and hydrazine.

A study was therefore conducted to determine the degradation products from hydrolysis and to determine the hydrolysis rate of acetophenone azine (Anonymous, 2017).

Description of the hydrolysis test protocol:

The stability of acetophenone-azine was examined in artificial sweat for 5 days at 37°C. Two detection modes were: UV-photometry at 245 nm and mass spectrometry with APCI ionization. In the first 8 hours no major changes were detected. After 24 hours 30-40% of the initial acetophenone-azine amount was hydrolysed. After 72 hours approximately 95% of the test item has reacted and after 120 hours only traces can be detected in the 2nd and the 3rd sample while in sample 1 no more acetophenone-azine is present. Based on this it can be stated that the test item completely hydrolyses within 5 days. The hydrolysis product is identified as acetophenone.

Hydrazine was not detected, but may have been present. As described in the study, the detection level for this small molecule was not good. The lab did not make an evaluation of where the LOQ was for hydrazine. A study was not performed with hydrazine.

9 EVALUATION OF HEALTH HAZARDS

9.1 Skin sensitisation

9.1.1 Human data

The available clinical cases are indicated in the following summary table 5:

Table 5: Summary table of human data on skin sensitisation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Clinical case 1 Patch test on a 13 year old boy	Acetophenone azine 1.0% wt/vol stock solutions in acetone and water, Dilutions 0.1%, 0.01%, 0.001% and 0.0001% wt/vol	A 13-year-old boy with no history of atopy or contact dermatitis Patch tests over several sessions: -first, with the European baseline series (Trolab, Stallergènes, Antony, France) and plastics/glues and rubber series (Chemotechnique, Vellinge, Sweden), and -at a second time with dyes and preservative series (Chemotechnique), with dimethylfumarate 0.1% and 0.01% wt/wt in petrolatum and with all of the topical medicaments used. Large pieces of the black shin pad foam in close contact with the skin tested 'as is', simply moisturized with acetone, water, and ethanol.	At the first patch test session,: - **all patch tests gave negative results,** except for a positive reaction to abitol (1+ on D2 and D3) with no apparent relevance. - strong reactions to pieces of the black foam moisturized with ethanol, acetone, and water (++ on D2; +++ on D3). - Testing with acetophenone azine resulted **in positive reactions to acetone dilutions at 1%, 0.1%, 0.01%, and 0.001%, and to aqueous solutions at 1% and 0.1%**. All other tests based on acetone and water solutions gave negative results. - HPLC identified acetophenone azine at concentrations of approximately 20 µg/g of shin pad samples. -Patch tests gave strongly positive reactions to pieces of shin pads and to acetophenone azine down to 0.001% in acetone, whereas acetophenone and hydrazine sulfate were both negative. -Twenty controls were negative for acetophenone azine 0.01% in acetone.	Nadia Raison-Peyron *et al.*, 2016
Clinical Case 2 Pacth test on a 11	Acetophenone azine 0.1% and 0.01% wt/vol in acetone Hydrazine sulfate	An 11-year-old non-atopic football player experienced an itchy, erythematous and vesicular eruption,	<u>*- Patch tests results:*</u> -with commercial allergens: all gave negative results on day D2 and D4. - with pieces of shin pads and flip-flop	Nadia Raison-Peyron *et al.*, 2017a

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
year old boy	1% pet	initially localized to both shins, in close contact with football shin pads, after having used these two or three times a week during a 3-month period. After the patient had recovered from the eczematous eruption, patch testing with IQ Ultra® chambers (Chemotechnique, Vellinge, Sweden) was performed on the back with the European baseline series, a plastic and glues series, and a rubber series (Chemotechnique). The patches were removed from the back after 48 h. Patch tests with pieces of shin pads and flip-flop soles moistened with acetone, ethanol, and water were performed. Analysis of samples by high-performance liquid chromatography (HPLC) coupled with a diode array detector.	soles moistened with acetone, ethanol, and water: strong reactions (++ on D2 and ++ on D3) that persisted 12 days later. - Patch tests with acetophenone azine (0.1% and 0.01% wt/vol in acetone gave positive results ++ on D2 and ++ on D3), while results were negative for hydrazine sulfate 1% pet. (Chemotechnique). - *HPLC analysis:* identification of acetophenone azine, at 69 and 21 µg/g, respectively, in the two samples of the inner foam of the shin pads and of the sole of the flip-flops.	
Clinical Case 3 Patch test on a 12 year old boy	Acetophenone azine 0.1% and 0.01% wt/vol in acetone Hydrazine sulfate 1% pet	A 12-year-old non-atopic boy Patch testing performed 3 months later with the European baseline series and a shoe series (Chemotechnique) Patch tests with pieces of the soles of the sneakers in water, ethanol and acetone were performed Detection of acetophenone azine by HPLC in both sports brands.	*Patch tests results:* - with commercial allergens : negative results on D2 and D3. - with pieces of the soles of the sneakers in water, ethanol and acetone gave ++ positive reactions to the samples in water on D2 and D3, and + positive reactions to the samples in acetone on D2 and D3, but negative results with the material moistened with ethanol. - with dilutions of Acetophenone azine: a strong reaction (++ on D2 and D3), whereas hydrazine sulfate 1% pet. gave a negative result. *HPLC analysis:* Acetophenone azine was detected by HPLC in the foam of sneaker soles	Nadia Raison-Peyron *et al.*, 2017b

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			from both sports brands: 15 µg/g for the first sport brand, and <0.5 µg/g for the second sport brand.	
Clinical case 4 Patch test in 29 year old adult hockey player	Acetophenone azine 0.1% and 0.01% in acetone.	A 29-year-old non-atopic male hockey player referred for the evaluation of dermatitis on both legs, which had commenced shortly after thewearing of a new pair of shin pads, lined with a grey foam. Patch test: All tests were removed on D2 and read on D2, D4, and D7, according to ESCD guidelines[1] Patch testing performed with the Belgian baseline series including additional series (cosmetics, rubbers, plastics and glues, shoe allergens, and textile colourants), all from Chemotechnique (Vellinge, Sweden), mounted on Allergeaze® patch test chambers (SmartPractice, Calgary, Canada), and occluded for 2 days with Fixomull® stretch (BSN Medical, Hamburg, Germany). Both patch tests with pieces of the internal grey foam of the patient's newest shin pads, and of the similar grey foam of the sport shoe insoles, were performed 'as is', moistened with acetone. The older shin pads were not brought in by the patient, and could therefore not be patch tested separately.	*Patch results:* -Positive reactions to pieces of the grey foam, contained in the shin pads and in the soles of the sport shoes, were seen on D2 and on D4 (+ and ++, respectively). - positive ++ and + reactions observed to acetophenone azine 0.1% and 0.01%, respectively, on D2 and D4. No later-occurring reactions were observed.	De Fré Charlotte *et al.*, 2017

Several human cases have been published including 3 children and 1 adult with test patchs.

[1] Johansen J D, Aalto-Korte K, Agner T et al. European Society of Contact Dermatitis guideline for diagnostic patch testing – recommendations on best practice. *Contact Dermatitis* 2015: **73**: 195–221.

The first case of severe allergic contact dermatitis caused by acetophenone azine from shin pads was reported in a 13-year-old football player with no history of atopy or contact dermatitis (Raison-Peyron *et al.*, 2016). The young presented acute, vesicular dermatitis on his shins 1 month after wearing shin pads for playing football as a goalkeeper. This eruption became generalized 1 week later, and resulted in hospitalization. Hypereosinophilia was noted (1120/mm^3; normal: <700/mm^3). A skin biopsy confirmed the diagnosis of eczema. The patient was patch tested. Testing with acetophenone azine resulted in positive reactions to acetone dilutions at 1%, 0.1%, 0.01%, and 0.001%, and to aqueous solutions at 1% and 0.1%. All other tests based on acetone and water solutions gave negative results. HPLC analysis identified acetophenone azine at concentrations of approximately 20 μg/g of shin pad samples. Patch tests gave strongly positive reactions to pieces of shin pads and to acetophenone azine down to 0.001% in acetone, whereas acetophenone and hydrazine sulfate were both negative. Twenty controls were negative for acetophenone azine 0.01% in acetone. **In conclusion, according to the authors, acetophenone azine is a skin sensitiser.**

Acetophenone azine 0.1% and 0.01% wt/vol in acetone was patched tested in 2 boys (11 and 12 year old) (Raison-Peyron *et al.*, 2017a and 2017b). For the 11 year old boy, patch tests with acetophenone azine at both concentrations gave positive results (++/++, D2 and D3), while results were negative for hydrazine sulfate 1% pet. (Chemotechnique commercial allergens tested). Analysis of samples of the inner foam of the shin pads and of the sole of the flip-flops by HPLC coupled with a diode array detector, identified acetophenone azine, at 69 and 21 μg/g, respectively, in the two samples. In the following 2 months, the eruption spread all over the body, including the face, when he continued to play football with a jersey garment under the shin pads. He also had erythematous, vesicular and scaly lesions on both soles 3 days after starting to wear new flip-flops without socks, 8 months after the beginning of the dermatitis on the shins. The eczematous eruption resolved slowly with residual depigmentation under treatment with a corticosteroid creamFor the 12 year old boy, acetophenone azine diluted as above gave a strong reaction (++ on D2 and D3) at both concentrations tested, whereas hydrazine sulfate 1% pet. gave a negative result. Acetophenone azine was detected by HPLC in the foam of sneaker soles from both sports brands: 15 μg/g for the first brand, and <0.5 μg/g for the second brand. Acute itchy, vesicular dermatitis of both soles appeared soon after wearing new sneakers. Four months later, the boy also showed a severe and diffuse eczematous eruption with secondary depigmentation, mainly on his back and upper limbs, and also involving the cheeks. The dermatitis of the soles relapsed when he bought and used sneakers of another sports brand. **These 2 cases of severe allergic contact dermatitis caused by acetophenone azine in young boys confirm that this substance is a skin sensitiser.**

De Fré Charlotte *et al.*, 2017 reported the first adult case with allergic contact dermatitis of the legs, caused by acetophenone azine present in shin pads, inwhom, additionally, AA-containing sport shoes was reported

and was shown to be the cause of recalcitrant foot dermatitis. A 29-year-old non-atopic male hockey player was referred for the evaluation of dermatitis on both legs, which started shortly after the wearing of a new pair of shin pads, lined with a grey foam. Dermatitis started on his shins, and rapidly spread to his trunk and arms. Previously, dermatitis had also occurred after the wearing of another (older) brand of shin pads, with a similar, blue inner foam. More recently, the patient experienced severe dermatitis on the soles of both feet, which he related to the wearing of new sports shoes with a grey foam insole. Occasionally, generalized skin lesions would appear on top of the foot dermatitis. Acetophenone azine 0.1% and 0.01% wt/vol in acetone was patched tested. Positive reactions to pieces of the grey foam, contained in the shin pads and in the soles of the sport shoes, were seen on D2 and on D4 (+ and ++, respectively). Moreover, ++ and + positive reactions were observed to acetophenone azine at 0.1% and 0.01%, respectively, on D2 and D4. No later-occurring reactions were observed. **This case of severe allergic contact dermatitis caused by acetophenone azine confirm that this substance is also a skin sensitiser in adult.**

In conclusion, acetophenone azine has shown to be a skin sensitiser in 4 case reports in child and adult.

Few number of cases are reported with acetophenone azine. However, it is important to note that incidences of sensitisation are likely to be underestimated because of underdiagnosis, underreporting and lack of registration for milder cases of dermatitis.

It is however difficult to estimate the prevalence of allergic textile dermatitis in the general population in the EU based on available data. The risk of skin sensitisation of the general population related to textile and leather articles such as clothing and footwear is of increasing concern in Europe (Lisi *et al.,* 2014, Seidenari *et al.*, 2002). According to ANSES and KEMI in the scope of FR/SE Restriction on skin sensitising substances in textile, leather, hide and fur articles, the number of people sensitized to chemicals in textiles and leather is estimated at around 4 to 5 million people in Europe, which corresponds to 0.8% -1% of the population of the European Economic Area 31 (EEA 31). Between 45 000 and 180 000 new cases per year of sensitisation (incidence) are estimated, corresponding to 0.01% - 0.04% of the population of the EEA.

9.1.2 QSAR modelling

Regarding human cases, a QSAR modelling was performed to emphasize patch test results. Moreover, there is few information on physical and chemical properties and toxicological information on acetophenone azine.

Several *in silico* tools are available to evaluate the sensitising potential of a substance. The tools *in silico* hereafter allow to predict the aptitude of the substances to induce a link with the proteins of the skin at the molecular level (initiator event, see below in section 9.2.1 figure 1) and thus induce skin sensitisation. The QSAR toolbox can also predict the sensitising potential skin of a substance for

the 2nd and 3rd key event of the adverse outcome pathway AOP of the skin sensitisation (described below in section 9.2.1)).

The VEGA platform is an open access tool developed by a community of international scientists from the public or private sector (https://www.vegahub.eu). In particular, it makes it possible to predict the potential of sensitising for skin and associating the result obtained with a confidence index (weak, moderate or good). A low degree of confidence indicates that the compound is outside the field of applicability and that the prediction is not reliable. For the skin sensitisation, this platform uses the CAESAR model (the model CAESAR is also open access, http://www.caesar-project.eu/). DEREK Nexus is a commercial software developed by Lhasa Limited (http://www.lhasalimited.org). It allows to predict the sensitising potential of a substance for the skin with an associated degree of confidence: unlikely, equivocal or plausible.

Therefore, a QSAR modeling was performed internally using two software packages, the DEREK Nexus 5.0.2 software and the VEGA 2.1.9 platform (including CAESAR 2.1.6 software) to predict alerts on skin sensitisation for acetophenone azine. The QSAR modeling makes it possible to predict the potential effects related to acetophenone azine by structure analogy. DEREK Nexus 5.0.2 software has been used to highlight alerts on the potential for sensitisation of the substance. As shown in the table 6 below the prediction results by DEREK software shows that acetophenone azine is plausibly sensitive to the skin.

Table 6 : Predicted alerts of acetophenone azine obtained from DEREK Nexus software

Alerts	Reliability	Comparison
Skin Sensitisation	Plausible	Hydrazine and hydrazine precursors

The DEREK Nexus software has also the advantage of predicting the EC3 of the Local Lymph Node Assay. For acetophenone azine, EC3 is predicted at 0.15%, thus classifying the substance for strong sensitisation.

CAESAR 2.1.6 software used in the VEGA platform 2.1.9 has also been used to highlight alerts on skin sensitisation potential effects of the substance. As shown in the table 7 below the prediction results by VEGA software shows that acetophenone azine is a weak sensitiser to the skin.

Table 7 : Predicted alerts of acetophenone azine obtained from VEGA 2.1.9 platform

Alerts	Reliability	Model
Skin sensitisation	Weak	Skin Sensitization model (CAESAR 2.1.6)

Therefore, QSAR modelling using DEREK and CAESAR softwares predict skin sensitiser potential for acetophenone azine, which is in line with patch test on human. According to these results, an AOP

for skin sensitisation was searched in order to know which experimental tests may confirm the skin sensitisation potential.

9.1.3 Adverse outcome pathway (AOP)

An AOP for skin sensitisation was built by the OECD 2012 and is synthesized in Figure 1 below.

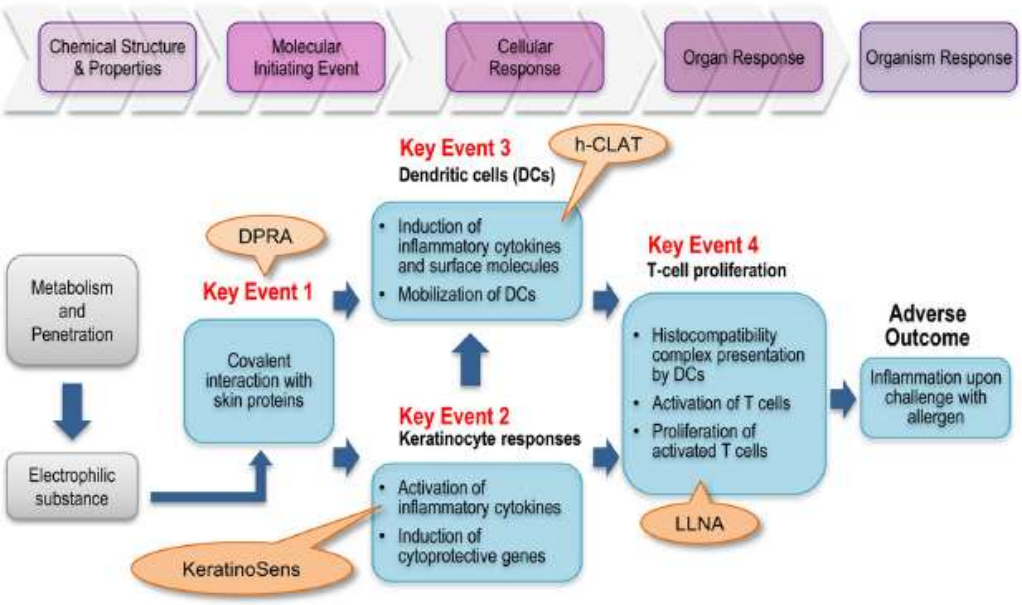


Figure 1: From Strickland et al., 2016

In the AOP presented above, the *in silico* tools available make it possible to evaluate skin sensitising potential of substances at different levels:

- molecular level: the ability of substances to induce binding (usually covalent) with the proteins of the skin. This binding leads to training of a hapten-protein complex that is responsible for the reactions immune and inflammatory at the cellular level. This mechanism corresponds to the first key event of the AOP (initiating event) and can to be evaluated experimentally *in chemico* by the OECD TG 442C (test direct binding on peptide reactivity, DPRA).
- cellular level:
    - inflammatory reaction in keratinocytes linked to pathways specific cell signaling such as pathways dependent on the element of antioxidant / electrophilic response (ARE, Antioxidant Response Element). This mechanism corresponds to the second key event of the AOP and can be evaluated experimentally *in vitro* thanks to the OECD TG 442D (test method ARE-Nrf2 luciferase, KeratinoSens®).

- activation of dendritic cells via the expression of markers of surface-specific chemokines and cytokines. This mechanism corresponds to the third key event of the AOP and can be evaluated experimentally in vitro using the OECD TG 442E (test of *in vitro* skin sensitisation on the key event related to activation of dendritic cells in the toxicological pathway involved in adverse effects for skin sensitisation, h-CLAT).

- Organ level :
  - T-cell proliferation *via* activation of T cells, histocompatibility complex presentation by DCs. This mechanism corresponds to the fourth key event of the AOP and can be evaluated experimentally *in vivo* using the OECD TG 429 (Local Lymph Node assays (LLNA)).

In order to predict the skin sensitising potential of substances, the tools *in silico* combine the use of the physicochemical and structural properties of the substance to identify functional groups or areas of reactivity involved in the mechanisms that would be likely to induce effects sensitisers.

For the prediction of protein binding, different mechanisms exist and are integrated in the *in silico* tools to determine, according to the structure of the substance, if protein binding is likely to ocurr.

The following skin sensitisation tests may be used to investigate this AOP and refered to the four key events of AOP (method described above (Figure 1 Strickland *et al.*, 2016, INERIS 2012). The testings were retained based on expert judgement regarding results of hydrolysis assay and regarding the prediction of alerts using QSAR modeling. Considering also that results of hydrolysis showed that acetophenone azine was not only hydrolyzed in hydrazine but also in acetophenone, a classified substance. The conditional assays (whom principle are described below) following combination of 3 skin sensitisation tests were performed according AOP:

a) *in vitro* ARE-Nrf2 Luciferase Test Method (KeratinoSens™) (OCDE TG 442D) ;

b) *in vitro* Human Cell Line Activation Test (h-CLAT) (OCDE TG 442E)

c) Local lymph Node Assays (LLNA) (OCDE TG 429);

9.1.4 Experimental data

a) *In vitro* Skin Sensitisation: ARE-Nrf2 Luciferase Test Method (KeratinoSens™) (OECD 442D)

The ARE-Nrf2 luciferase test method according OECD TG 442D was used to investigate the key event 2 of the skin sensitisation pathway involved in adverse effects, that is to say the inflammatory response as well as the expression of the genes associated with the cell activation pathway of the keratinocytes.

At present, the only *in vitro* ARE-Nrf2 luciferase assay method covered by OECD TG 442D is the KeratinoSens™ method. The KeratinoSens™ test method was considered scientifically valid to be used as part of an Integrated Approach to Testing and Assessment (IATA), to support the discrimination between skin sensitisers and non-sensitisers for the purpose of hazard classification and labelling.

b) *In vitro* skin sensitisation: Human Cell Line Activation Test (h-CLAT) (OECD TG 442E)

The h-CLAT test according OECD TG 442E allows to investigate the key event 3 of the skin sensitisation pathway by quantifying changes in the expression of cell surface markers associated with the process of activation of monocytes and dendritic cells (i.e. CD86 and CD54), The measured expression levels of CD86 and CD54 cell surface markers are then used for supporting the discrimination between skin sensitisers and non-sensitisers. However, it may also potentially contribute to the assessment of sensitising potency when used in integrated approaches such as IATA.

c) *In vivo* Skin sensitisation: Local Lymph Node Assay (OECD TG 429)

The Local Lymph Node Assay (LLNA) test is the first-choice method for *in vivo* testing as given information on potency and dose-response.

The pre-screen test is conducted under conditions identical to the main LLNA study, except there is no assessment of lymph node proliferation and fewer animals per dose group can be used. Consecutive doses are normally selected from an appropriate concentration series such as 100%, 50%, 25%, 10%, 5%, 2.5%, 1%, 0.5%,

In the main study, the treatments are applied on Day 1 (pre-dose), Day 3 (approximately 48 hours after the first dose), and Day 6 on the back of each ear of the animal: 25 µl of a suitable dilution of the test substance, of the vehicle alone or of the positive control. Depending of the results of the pre-screened test, at least 3 concentrations will be used to observe a dose-resposne.

The proliferation indices are compared between the mean proliferation of each test group and the mean proliferation of the control group treated with the vehicle. The results obtained for each treatment group are expressed by an average stimulation index (SI). This SI is obtained by dividing the average BrdU score of each group by the average BrdU score of the solvent-treated control group. The decision process regards a result as positive when $SI \geq 3$.

Clinical signs and irritation at the site of application should also be observed and reported as they may indicate systemic toxicity.

The skin sensitisation results *in vitro* and animal testings performed with acetophenone azine are indicated in the following summary tables 6 and 7 and summarized in the text below:

***In vitro* human data**

Table 6: Summary table of *in vitro* human studies on skin sensitisation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
OECD TG 442D *In vitro* Skin Sensitisation: ARE-Nrf2 Luciferase Test Method KeratinoSens® assay	Acetophenone azine 97% DMSO, water or treatment culture medium). test items with a log P ≤5.	cell line stably transfected with a modified plasmid which contains an ARE sequence from the AKR1C2 gene and a SV40 promotor which are inserted upstream of a luciferase gene. The resulting plasmid was transfected into HaCaT keratinocytes and clones with a stable insertion selected in the presence of Geneticin / G-418. Induction of luciferase gene is the endpoint evaluated and reflects the activation by the test item of the Nrf2 transcription factor in this test. Concentrations tested: 0.49, 0.98, 1.95, 3.91, 7.81, 15.6, 31.3, 62.5, 125, 250, 500 and 1000 µM in culture medium Negative and positive control in each run - first plated on 96-well plates and grown for 24 hours at 37°C. - cells exposed to the vehicle control or to different concentrations of test item and of	Both runs validated - slight to strong test item precipitate observed in treated wells at concentrations ≥ 62.5 µM in the first run and ≥ 31.3 in the second run, - high decrease in cell viability (i.e. cell viability < 70%) noted at concentrations ≥ 125 µM in the first run and ≥ 250 µM in the second run, - corresponding IC30 and IC50 calculated to be 97.68 and 163.11µM and 152.77 and 238.11 µM, in the first and second runs respectively, - statistically significant gene-fold inductions above the threshold of 1.5 noted in comparison to the negative control at several successive concentrations in both runs (from 0.98 to 15.6 µM in the first run and from 0.49 to 31.3 µM in the second run). - apparent dose response relationship noted, followed by a decrease of induction related to the appearance of cytotoxicity (i.e. from 62.5 µM in both runs), - the Imax values = 2.14 and 3.31 and the calculated EC1.5 = 0.63 and	Anonymous 2018a

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		positive controls. The treated plates then incubated for 48 hours at 37°C. - luciferase production measured by flash luminescence. - cytotoxicity measured by a MTT reduction test - Two independent validated runs performed	estimated < 0.49 µM in the first and second runs, respectively. IC30 and IC50 : 122.16 and 197.07 µM, for the first and second runs, respectively. **Positive**	
OECD TG 442E *In vitro* skin sensitisation: human Cell Line Activation Test (h-CLAT)	Acetophenone azine 97%	THP-1 is an immortalized human monocytic leukemia cell line derived from an acute monocytic leukemia patient. final concentrations: 139.54, 167.45, 200.94, 241.13, 289.35, 347.22, 416.67 and 500 µg/mL. 24 hours expression CD86 and CD54 was analyzed by flow cytometry	*Solubility assessment* Test item found soluble in DMSO at 250 mg/mL. Positive controls: 2,4-Dinitrochlorobenzene (DNCB) and Nickel Sulfate ($NiSO_4$) *Dose-Range Finding (DRF)* -During both DRF assays, no decrease in cell viability (*i.e.* cell viability < 75%) was noted in test item treated wells. No mean CV75 value calculated, and the highest tested concentration retained for the main test = 500 µg/mL. - Log K_{ow} value of the test item slightly > 3.5 (*i.e.* 3.7). However, this slightly high Log K_{ow} value is not considered to be a limitation for the applicability of this test since the positive outcome obtained in two validated runs guaranted the test system exposure to the test item. -DPN values with DPN (disintegrations per node) = DPM (disintigrations per minute) divided by the number of lymphatic nodes) are within the historical control data. - DPN value for negative control = 463.6 (> DPN value of DMF ((HC range : 62,0-649,6, average : 256,1) that contributes to the lower SI of positive control. The size of lymphatic nodes found are coherent with the conclusions despite the risk of false negative cannot be excluded. **Positive**	Anonymous 2018b

Experimental data was generated for acetophenone azine. The substance was tested by the *in vitro* ARE-Nrf2 Luciferase Test Method (Keratinosens®) and in the *in vitro* human Cell Line Activation Test (h-CLAT).

In **the Keratinosens® assay**, the test item, **Acetophenone azine** was tested at concentrations: 0.49, 0.98, 1.95, 3.91, 7.81, 15.6, 31.3, 62.5, 125, 250, 500 and 1000 µM**.** The KeratinoSens cells were first plated on 96-well plates and grown for 24 hours at 37°C. Then the medium was removed and the cells were exposed to the vehicle control or to different concentrations of test item and of positive controls. The treated plates were then incubated for 48 hours at 37°C. At the end of the treatment, cells were washed and the luciferase production was measured by flash luminescence. In parallel, the cytotoxicity was measured by a MTT reduction test and was taken into consideration in the interpretation of the sensitisation results. Two independent validated runs were performed as part of this study. All acceptance criteria were met for the positive and negative controls in each run; both runs performed using the following concentrations: 0.49, 0.98, 1.95, 3.91, 7.81, 15.6, 31.3, 62.5, 125, 250, 500 and 1000 µM in culture medium containing 1% DMSO were therefore considered as validated. At these tested concentrations: slight to strong test item precipitate were observed in treated wells at concentrations ≥ 62.5 µM in the first run and ≥ 31.3 in the second run, a high decrease in cell viability (*i.e.* cell viability < 70%) was noted at concentrations ≥ 125 µM in the first run and ≥ 250 µM in the second run, the corresponding IC_{30} and IC_{50} were calculated to be 97.68 and 163.11µM and 152.77 and 238.11 µM, in the first and second runs respectively, statistically significant gene-fold inductions above the threshold of 1.5 were noted in comparison to the negative control at several successive concentrations in both runs (from 0.98 to 15.6 µM in the first run and from 0.49 to 31.3 µM in the second run). Moreover, an apparent dose response relationship was also noted. Then a decrease of induction related to the appearance of cytotoxicity (*i.e.* from 62.5 µM in both runs) was observed. The I_{max} values were 2.14 and 3.31 and the calculated $EC_{1.5}$ were 0.63 and estimated < 0.49 µM in the first and second runs, respectively. The geometric means IC_{30} and IC_{50} of the two validated runs were calculated to be 122.16 and 197.07 µM, for the first and second runs, respectively. The evaluation criteria for a positive response are met in both runs, the final outcome is therefore positive. This positive result can be used to support the discrimination between skin sensitisers and non-sensitisers in the context of an IATA. **Under the experimental conditions of this study, the test item, Acetophenone azine, was positive in the KeratinoSens assay and therefore was considered to activate the Nrf2 transcription factor.**

In **the h-CLAT assay**, **Acetophenone azine**, was tested at concentrations: 139.54, 167.45, 200.94, 241.13, 289.35, 347.22, 416.67 and 500 µg/mL.

Following the solubility assays, the cytotoxic potential was assessed in a Dose-Range Finding assay in order to select sub-toxic concentrations for testing in the main test. The skin sensitising potential of the test item was then evaluated in the main test, in three validated runs (Runs A, C and D). During the main test, treatments were performed at the following final concentrations: 139.54, 167.45, 200.94, 241.13, 289.35, 347.22, 416.67 and 500 µg/mL. In each run, the test item formulations were applied to THP-1 cells and

cultured in a 24-well plate for 24h ± 30 minutes at 37°C, 5% CO2 in a humidified incubator. A set of control wells was also added in each plate to guarantee the validity of each run. At the end of the incubation period, cells from each well were distributed to three wells of 96-well plate: the first well was labeled with IgG1-FITC antibodies, the second one was labeled with CD86-FITC antibodies and the third one was labelled with CD54-FITC antibodies. Then, just before flow cytometry analysis of CD86 and CD54 expression, all cells were dyed with Propidium Iodide for viability discrimination. For each run, the Mean Fluorescence Intensity (MFI) obtained for each test sample was corrected by the isotype control IgG1 MFI value to obtain the corrected MFI. Corrected MFI value from the corresponding vehicle control was set to 100% CD54 and CD86 expression by default. Then, corrected MFI values from each test sample were compared to the corresponding vehicle control to obtain the Relative Fluorescence Index for CD86 and CD54 expression for each tested concentration (RFI CD86 and RFI CD54). The test item was found soluble in DMSO at 250 mg/mL. During both DRF assays, no decrease in cell viability (i.e. cell viability < 75%) was noted in test item treated wells. No mean CV75 value was therefore calculated, and the highest tested concentration retained for the main test was 500 µg/mL. The results showed that all acceptance criteria were reached in each run except for the Run B, where the cell viability of the positive control NiSO4 was < 50% (i.e. 45.3%). Therefore, this run was invalidated. For Run A, RFI CD86 and RFI CD54 did not exceed the positivity thresholds at any tested concentration. The run A was therefore considered negative. For Run C, moderate to strong test item precipitate was noted in treated wells from the lowest concentration of 139.54 µg/mL, RFI CD86 did not exceed the positivity thresholds at any tested concentration. RFI CD54 exceeded the positivity threshold from 139.54 µg/mL to 241.13 µg/mL. The run C was therefore considered positive for RFI CD54. For Run D, moderate to strong test item precipitate was noted in treated wells from the lowest concentration of 139.54 µg/mL, RFI CD86 did not exceed the positivity thresholds at any tested concentration. RFI CD54 reached or exceeded the positivity threshold at the concentrations of 167.45; 241.13; 289.35; 347.22 and 500.00 µg/mL (i.e. 210; 200; 214; 200 and 241, respectively). The run D was therefore considered positive for RFI CD54.

In this assay, it was observed that the Log Kow value of the test item is slightly > 3.5 (i.e. 3.7). However, this slightly high Log Kow value is not considered to be a limitation for the applicability of this test since the positive outcome obtained in two validated runs guaranted the test system exposure to the test item. The positive control 25% α-hexylcinnamaldehyde (HCA) in DMF is relatively low (SI = 3,7 for a threshold of 3) and would question about the high risk of false negative. From this, it can be concluded that test substance **acetophenone azine was considered to activate dendritic cells under the test conditions chosen**.

Both ***in vitro*** **human Cell Line Activation Test (h-CLAT) method and** ***in vitro*** **ARE-Nrf2 Luciferase Test Method (Keratinosens®) were found positive with acetophenone azine**. Based on the prediction model for *in vitro* skin sensitisation testing, two out of three tests have to be congruent in order to arrive at a conclusion regarding the skin sensitisation potential of a given test substance (INERIS 2018). Since congruent results were observed in Keratinosens® assay and h-CLAT assay, testing the substance in the

DPRA test detecting the covalent binding of the molecule to 2 nucleophilic peptides was considered not necessary. In accordance with the prediction model, the substance is considered to have a skin sensitising potential.

Animal data

Table 7: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
LLNA OECD TG 429 GLP	20 female CBA/CaOlaHsd mice 4/group	Acetophenone azine Purity 97.2 %	5, 2.5 and 1% (w/v) formulated in Dimethylformamide (DMF) Positive control 25% α-Hexylcinnamaldehyde (HCA) in DMF	*Clinical observation* No mortality or signs of systemic toxicity observed during the study. No test item residue was noted on the ears of the animals in any groups. *Body weight measurement* No marked body weight losses (≥5%) were observed in any groups. Individual and mean body weights are given in annex. *Proliferation assay* The appearance of the lymph nodes was normal in the negative control group and in the 5, 2.5 and 1% (w/v) test item treated dose groups. The SI values were 0.7, 0.4 and 0.5 at concentrations of 5, 2.5 and 1% (w/v), respectively. Larger than normal lymph nodes were observed in the positive control group. DPN values observed for the vehicle and positive control substance in this experiment were in within the historical control range **No skin sensitisation potential**	Anonymous 2018c Klimisch score = 1

One LLNA study was available to assess skin sensitisation property of acetophenone azine. Acetophenone azine was applied at 5, 2.5 and 1% (w/v) formulated in Dimethylformamide (DMF) on 20 female CBA/CaOlaHsd mice. A Positive control 25% HCA in DMF was used. Each treated and control

group included 4 animals. The test item was powder, which was formulated in DMF. No mortality or signs of systemic toxicity was observed during the study. No test item residue was noted on the ears of the animals in any groups. No marked body weight losses (≥5%) were observed in any groups. The results showed the lymph nodes were normal in the negative control group and in the 5, 2.5 and 1% (w/v) test item treated dose groups. The SI values were 0.7, 0.4 and 0.5 at concentrations of 5, 2.5 and 1% (w/v), respectively. Larger than normal lymph nodes were observed in the positive control group. The result of the positive control substance HCA dissolved in the same vehicle was used to demonstrate the appropriate performance of the assay. The positive control substance was examined at a concentration of 25 % (w/v) in the relevant vehicle (DMF) using CBA/CaOlaHsd mice. No mortality, cutaneous reactions or signs of toxicity were observed for the positive control substance in the study. A lymphoproliferative response in line with historical positive control data (SI value of 3.7) was noted for HCA in the Main Assay. This value was considered to confirm the appropriate performance of the assay. Furthermore, the DPN values observed for the vehicle and positive control substance in this experiment were in within the historical control range. Since there were no confounding effects of irritation or systemic toxicity at the applied concentrations, the proliferation values obtained are considered to reflect the real potential of acetopheneon azine to cause lymphoproliferation in the LLNA. The resulting stimulation indices observed under these test conditions was considered to be evidence that Acetophenone azine is a non-sensitiser in this specific study design. The size of lymph nodes was in good correlation with this conclusion. **In conclusion, under the conditions of the present assay, Acetophenone azine, tested in N,N-dimethylformamide, did not show a sensitisation potential (non-sensitiser) in the LLNA.**

In the LLNA acetophenone azine, **applied at 5, 2.5 and 1% (w/v)**, is **negative** under the experimental conditions.

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

In summary, acetophenone azine has shown to be **a skin sensitiser in 4 case reports in child and adult.**

Under the conditions of the **Local Lymph Node Assay (OECD TG 429), Acetophenone azine**, tested at 5, 2.5 and 1% (w/v) formulated in N,N-dimethylformamide, **did not show a sensitisation potential (non-sensitiser) in mice**.

Under the experimental conditions of ***in vitro*** **Skin Sensitisation: ARE-Nrf2 Luciferase Test Method study (OECD TG 442D)**, the test item, Acetophenone azine, tested at concentrations: 0.49, 0.98, 1.95, 3.91, 7.81, 15.6, 31.3, 62.5, 125, 250, 500 and 1000 µM, **was positive in the KeratinoSens assay and therefore was considered to activate the Nrf2 transcription factor.**

Under the experimental conditions of **"*In vitro* skin sensitisation: human Cell Line Activation Test (h-CLAT)" (OECD TG 442E), the Log Kow value of the test item soluble in DMSO at 250 mg/mL, is slightly up to 3.5 (i.e. 3.7). Under the experimental conditions of this study, the test item, Acetophenone azine, was concluded to be positive in the h-CLAT.**

The LLNA is the preferred and regulatory *in vivo* test required under REACH. The test is based on the incorporation of 3H thymidine into the lymph nodes and consists to know how many times the proliferation is increased, and this is expressed in SI. From regulatory view, a test is positive when the proliferation of lymph node numbers in the mouse is increased by 3 or more compared to that of the control. Under the experimental conditions the LLNA test with acetophenone azine was negative. However some questions raised regarding results of negative and positive controls. First, in respect with OECD TG 429, the positive control which was used was α-HCA. The DPN values of positive control and negative control are within historical data. The laboratory had positive control historical data with a low SI = 4.7. However, in the experimental conditions, the SI was 3.7, which is low for a positive control even if it higher than 3. Second, it seems doubtfull to find a quiet high negative control whereas in the vehicle used DMF the positive control is weak (DPN* negative control = 463,6 >>> DMF (HC range : 62,0-649,6, average: 256,1; *DPN (disintegrations per node) = DPM (disintigrations per minute) divided by the number of lymph nodes). Therefore, it turns out that there could be a risk of false negatives. The concentration range used is quite limited and does not go beyond the 5% concentration to be tested.

From a regulatory point of view, the test conditions meet the OECD TG 429 criteria. The test is correct with a SI less than 3. The positive control is barely positive and out of the historical data. Lymphocyte proliferation increases with dose. Five percent is a relatively low concentration for defining sensitisation classification thresholds. It is estimated that up to 10%, a substance is a mild sensitiser. The choice of concentrations was dependent on the solubility of the molecule. **It can be stated that the test on the mouse including some deviations should not outweigh the two positive *in vitro* alternative tests on human cells. Based on two *in vitro* human cell tests (both positive), QSAR predictions and the available human cases (4 case reports in child and adult), acetophenone azine is considered to be a skin sensitiser.**

9.1.6 Comparison with the CLP criteria

The decision logic for classification of substance described in the CLP guidance on application of the CLP criteria, version 5.0 (July 2017) (hereafter referred to as "the guidance") has been followed:

" *Are there data and/or information to evaluate skin sensitisation?"*

Yes: there are both experimental studies and human data assessing skin sensitisation properties of acetophenone azine

a) *Is there evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons*

Yes: positive serious reactions clearly allocated to acetophenone azine were reported in human case reports. However, there is a limited number of human cases (3 children and 1 adult wearing sport equipment) , which can be explained either by the fact that acetophenone azine is a relatively new substance, and by the type of consumer products where it can be found (sport clothes), a type of clothes not worn as frequently as classic clothes. It is important to note that incidences of sensitisation are likely to be underestimated because of underdiagnosis, underreporting and lack of registration for milder cases of dermatitis. It is however difficult to estimate the prevalence of allergic textile dermatitis in the general population in the EU based on available data. The risk of skin sensitisation of the general population related to textile and leather articles such as clothing and footwear is of increasing concern in Europe (Lisi *et al*., 2014, Seidenari *et al.*, 2002). According to ANSES and KEMI in the scope of FR/SE Restriction on skin sensitising substances in textile, leather, hide and fur articles, the number of people sensitized to chemicals in textiles and leather is estimated at around 4 to 5 million people in Europe, which corresponds to 0.8% -1% of the population of the European Economic Area 31 (EEA 31). Between 45 000 and 180 000 new cases per year of sensitisation (incidence) are estimated, corresponding to 0.01% - 0.04% of the population of the EEA.

b) *Are there positive results from an appropriate animal test or in vitro / in chemico test?*

Yes: positive results were obtained in *in vitro* human OECD testings performed with acetophenone azine **in Keratinosens® assay and in h-Clat assay**. Acetophenone azine, tested at concentrations: 0.49, 0.98, 1.95, 3.91, 7.81, 15.6, 31.3, 62.5, 125, 250, 500 and 1000 µM, **was positive in the KeratinoSens assay (OECD TG 442D).** Acetophenone azine, was concluded to **be positive in the h-CLAT assay (OECD TG 442E), the Log Kow value of the test item soluble in DMSO at 250 mg/mL, is slightly up to 3.5 (i.e. 3.7).** That means that Acetophenone azine is able to activate keratinocytes and to activate dendritic cells on human lines.

However, negative result was obtained in **LLNA** at concentration up to 5%. Some deviations described previously (SI =3.7 low for the positive control even if higher than 3; a quiet high negative control whereas in the vehicle used DMF the positive control is weak (DPN negative control = 463,6 >>> DMF (HC range : 62,0-649,6, average : 256,1) with a risk of false negatives) were however highlight, possibly explaining this negative result.

Another element can be taken into consideration to support a classification, according to the guidance, which states that severity may be considered for a newly substance:

"For a newly identified skin sensitiser, which might also be a substance newly introduced onto the market, or a substance not included in the baseline diagnostic patch test series, the high severity of responses might be used as an indication that classification as Category 1A is appropriate. For example, where the substance has caused:

- *Hospitalisation due to acute skin reaction*
- *Chronic dermatitis (lasting > 6 months)*
- *Generalised (systemic/whole body) dermatitis"*

In human cases reported, in one of the boy wearing skin pads, the dermatitis was so severe that he had to be hospitalized after exposure to acetophenone azine (Raison-Peyron *et al.*, 2016), and in the adult hockey player (De Fré *et al.*, 2017), the dermatitis was generalized to the trunk and arms, and not just limited to the legs, the exposed part of the body. These two cases completely fulfills the recommendations of the guidance.

Therefore, considering the whole data available, including not only human cases and *in vitro* results, but also positive QSAR predictions and severity of reactions in human, it is concluded that acetophenone azine warrants a classification for skin sensitisation.

9.1.7 Conclusion on classification and labelling for skin sensitisation

Based on human data, particularly the low exposure required to be sensitized and the severity of responses, but also *in vitro* assays and QSAR, acetophenone azine fulfills criteria for classification Skin Sens. 1 according to the CLP regulation. However, data available (only 4 human cases, negative LLNA, *in vitro* assays, QSAR), do not allow a sub-categorisation.

10 ADDITIONAL LABELLING

-

11 REFERENCES

Anonymous 2017. STUDY OF ACETOPHENONE AZINE HYDROLYSIS IN ARTIFICAL SWEAT (non GLP). Date: 14. August 2017

Anonymous 2018a. Keratinosens TEST AN IN VITRO SKIN SENSITISATION ASSAY.

Anonymous 2018b. ASSESSMENT OF THE SKIN SENSITIZATION POTENTIAL USING THE HUMAN-CELL LINE ACTIVATION TEST.

Anonymous 2018c. Acetophenone azine: Skin Sensitization Test (Local Lymph Node Assay). Date: 24 August 2018.

ChemIDplus, Michael. 2016. "ChemIDplus - 729-43-1 - MOKMQSIJAHPSQX-JTFWXBGUSA-N - Ethanone, 1-phenyl-, 2-(1-phenylethylidene)hydrazone - Similar Structures Search, Synonyms, Formulas, Resource Links, and Other Chemical Information." https://chem.nlm.nih.gov/chemidplus/rn/729-43-1.

OECD 4 May 2012. ENVIRONMENT DIRECTORATE. JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins. Part 1: Scientific Evidence. Series on Testing and Assessment No.168. ENV/JM/MONO(2012)10/PART1. https://dx.doi.org/10.1787/9789264221444-en

De Fré C, Bergendorff O, Raison-Peyron N, van de Voorde K, Romaen E, Lambert J, Persson C, Aerts O. Acetophenone azine: a new shoe allergen causing severe foot dermatitis. Contact Dermatitis. 2017 Dec;77(6):416-417. doi: 10.1111/cod.12843.

ECHA. 2020. "1-phenylethan-1-one (1-phenylethylidene)hydrazone - Substance Information." https://echa.europa.eu/fr/substance-information/-/substanceinfo/100.010.891.

EFSA 2017. Guidance on dermal absorption. Revision of guidance issued in 2012 from EFSA PPR panel. Adopted : 24 may 2017. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2017.4873

INERIS 2018. Published report N° DRC-18-170857-03926A- 21/01/2019 état de l'art des outils *in silico* de sensibilisation cutanée [EAT DRC 49 2018 Enrico MOMBELLI, Adrien TROISE, Direction des Risques Chroniques (DRC) Pôle Dangers et Impact sur le Vivant (VIVA) Unité « Expertise en Toxicologie/Ecotoxicologie des Substances Chimiques (ETES).

Lisi, P., L. Stingeni, A. Cristaudo, C. Foti, P. Pigatto, M. Gola, D. Schena, M. Corazza, and L. Bianchi. 2014. "Clinical and epidemiological features of textile contact dermatitis: an Italian multicentre study. Contact Dermatitis. 2014 Jun;70(6):344-50. doi: 10.1111/cod.12179. Epub 2014 Jan 7.

Raison-Peyron N, Bergendorff O, Bourrain JL, Bruze M. Acetophenone azine: a new allergen responsible for severe contact dermatitis from shin pads. Contact Dermatitis. 2016 Aug;75(2):106-110. doi: 10.1111/cod.12579. Epub 2016 May 19.

Raison-Peyron N, Bergendorff O, Du-Thanh A, Bourrain JL, Bruze M. Two new cases of severe allergic contact dermatitis caused by acetophenone azine.Contact Dermatitis. 2017 Jun;76(6):380-381. doi: 10.1111/cod.12777.

Seidenari S., Giusti F., Massone F., Mantovani L. "Sensitization to disperse dyes in a patch test population over a five year period." American Journal of Contact Dermatitis 13 (3):101-107. doi: 10.1053/ajcd.2002.34570.

Strickland J, Zang Q, Kleinstreuer N, Paris M, Lehmann DM, Choksi N, Matheson J, Jacobs A, Lowit A, Allen D, Casey W. Integrated decision strategies for skin sensitization hazard.J Appl Toxicol. 2016 Feb 6.

12 ANNEXES

Detailed study summary for skin sens human, animal and *in vitro* studies.

See ANNEX I to the CLH report.