Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

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

EC Number: 211-979-0
CAS Number: 729-43-1

CLH-O-0000007030-90-01/F

Adopted

16 September 2021

P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

16 September 2021

CLH-O-0000007030-90-01/F

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

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

Chemical name: **1-phenylethan-1-one (1-phenylethylidene)hydrazone**

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

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

The proposal was submitted by **France** and received by RAC on **6 July 2020.**

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

PROCESS FOR ADOPTION OF THE OPINION

France has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **24 August 2020**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **23 October 2020**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Ivan Dobrev**

Co-Rapporteur, appointed by RAC: **Gerlienke Schuur**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **16 September 2021** by **consensus**.

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

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	1-phenylethan-1-one (1-phenylethylidene)hydrazone	211-979-0	729-43-1	Skin Sens. 1	H317	GHS07 Wng	H317			
RAC opinion	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 COM	TBD	1-phenylethan-1-one (1-phenylethylidene)hydrazone	211-979-0	729-43-1	Skin Sens. 1	H317	GHS07 Wng	H317			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

1-Phenylethan-1-one (1-phenylethylidene)hydrazone, or acetophenone azine, is not registered under the RAECH regulation. Nevertheless, the substance is present in consumer products such as sports equipment and footwear containing the foam elastomer ethyl vinyl acetate (EVA). According to the Dossier Submitter (DS), its presence might be explained by the use as a synthetic intermediate, or it may result from the reaction of hydrazine (a blowing agent for polymer foam) with acetophenone (a plasticizing agent and polymerization catalyst). Both substances are also considered plausible degradation products of acetophenone azine (as indicated in the CLH report). Another hypothesis is that it might be generated *in-situ*; acetophenone from the degradation of the initiator dicumylperoxide and hydrazine from degradation of the foaming agent azodicarbonamide (Raison-Peyron *et al.* 2017).

In a study on the stability of acetophenone azine in artificial sweat, 95% of the test substance was converted within 72h to the main degradation product acetophenone. Though hydrazine was not detected due to the poor reported detection limit for this molecule, the authors considered that its presence could not be excluded (Anonymous, 2017). Acetophenone has no current classification for skin sensitisation, while hydrazine has a harmonized classification as Skin Sens. 1 H317.

ANSES (2018) reported that 14% of sampled footwear contained acetophenone azine. Most recently, the American Contact Dermatitis Society chose acetophenone azine as the 2021 Allergen of the Year (Reeder & Atwater, 2021).

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The CLH proposal to classify acetophenone azine as a skin sensitiser is based on several recent case reports of children and adults showing, partly severe, allergic skin reactions from wearing sports equipment such as shin pads and shoes. Additional support is provided by two positive *in vitro* tests for key events in the adverse outcome pathway for skin sensitisation, and by alerts for skin sensitisation potential from QSAR modelling. The dossier also includes the results from a negative LLNA test in mice, including a discussion on its significance in the scope of overall evidence assessment.

Human data

The CLH dossier includes four human case reports of dermal allergy associated with the use of sports equipment containing acetophenone azine.

The first case of severe allergic contact dermatitis caused by acetophenone azine after contact with shin pads has been reported in a young football player from France (Raison-Peyron *et al.*, 2016). Subsequently, two additional cases of boys with severe allergic contact dermatitis caused by acetophenone azine present in shin pads, flip-flops, and sneakers were published (Raison-Peyron *et al.*, 2017). A study by De Fré *et al.* (2017) described the first case of an adult male hockey player with dermatitis on both legs, which had commenced shortly after wearing a new

pair of shin pads, lined with a grey foam. Strong positive reactions were observed in patch tests with pieces of his shin pads and with solutions of acetophenone azine in acetone.

Two additional clinical cases were published after finalisation of the CLH report and were discussed during the standard consultation. The DS provided a brief summary of these studies in their response to a comment by a MSCA in the consultation on the CLH report.

Koumaki *et al.* (2019) reported on the case of a 17-year-old hockey player with allergic contact dermatitis of the shins caused by acetophenone azine present in his shin pads. Besner Morin *et al.* (2020) described a new case of acetophenone azine-induced shin pad and sports shoe dermatitis in a 6-year-old soccer player from North America. The child reacted positively to acetophenone azine in a petrolatum vehicle at concentrations of 1% and 0.1%.

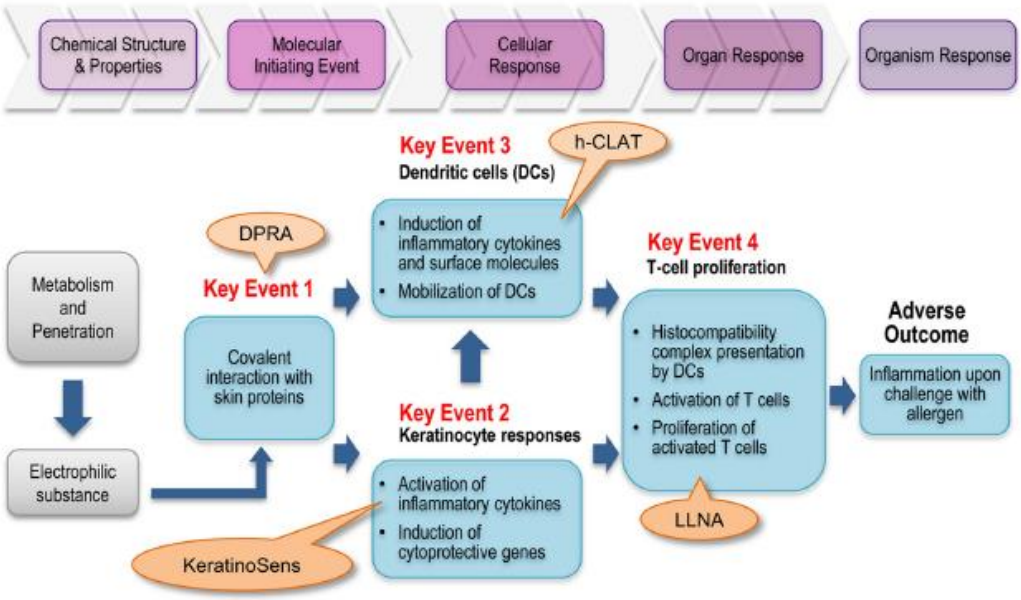
All these reports describe a typical pattern of reactions: first, localized eczema on the skin in close contact with EVA foam; and second, a severe and diffuse eczematous rash involving the whole body. The DS concluded that acetophenone azine has clearly shown to be a skin sensitiser in child and adult. With regard to the limited number of human cases, the DS noted that incidences of sensitisation are likely to be underestimated because of underdiagnoses, underreporting and lack of registration for milder cases of dermatitis.

Structure-activity relationship (SAR)

Two different (quantitative) structure-activity relationship [(Q)SAR] modelling tools were used. DEREK Nexus 5.0.2. software identified a structural alert for skin sensitisation (hydrazine or precursors) with a plausible reliability. CAESAR 2.1.6 also identified a structural alert for skin sensitisation with a weak reliability. The DS concluded that, in line with the human test results, the (Q)SAR software tools DEREK and CAESAR indicated a skin sensitiser potential for acetophenone azine.

Adverse outcome pathway (AOP)

The AOP for skin sensitisation developed by the OECD in 2012 (see Figure below; Strickland *et al.*, 2016) was applied by the DS to select experimental tests addressing some of the key events leading to skin sensitisation.



The AOP includes four key events with well-accepted biological significance: 1) initial binding of haptens to endogenous proteins in the skin, 2) keratinocyte activation, 3) dendritic cell activation, and 4) proliferation of antigen-specific T cells. The following tests were chosen to investigate key events for this AOP:

- *in vitro* ARE-Nrf2 Luciferase Test Method (KeratinoSens™)
- *in vitro* Human Cell Line Activation Test (h-CLAT)
- Local lymph Node Assays (LLNA)

Experimental data

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

The second key event is an inflammatory reaction as well as the expression of genes associated with the cell activation pathways in keratinocytes. The assay measures the luciferase expression in a human keratinocyte cell line, harbouring the antioxidant response element (ARE) and is designed to evaluates the capacity of substances to induce cytoprotective gene expression in keratinocytes based on activation of the Keap1-Nrf2 pathway.

The assay was performed twice, with inclusion of positive and negative controls, and using 12 concentrations. An apparent dose response relationship was noted, followed by a decrease in induction related to the appearance of cytotoxicity (from the 8^{th} dose and up). The substance resulted in a positive result according to the evaluation criteria, and therefore is considered to activate the Nrf2 transcription factor.

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

The third key event is the activation of dendritric cells. The method evaluates the ability of substances to mobilize and activate dendritic cells in the dermis by quantifying the expression of cell surface markers (CD86 and CD54) in human monocytic leukemia cell line (THP-1 cells) by flow cytometry after a 24 h exposure to the test substance.

The result with acetophenone azine was positive, the substance was considered to activate dendritic cells.

Both *in vitro* tests were found positive with acetophenone azine, and the DS concluded that the results from these tests point to a skin sensitising potential of the substance.

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

The fourth key event is activation and proliferation of antigen-specific T cells. In a recent LLNA test according to OECD TG 429, acetophenone azine formulated in dimethylformamide (DMF) was applied on 20 female CBA/CaOlaHsd mice (4/group) at dose levels of 5, 2.5 and 1% (w/v) (Anonymous, 2018c). No mortality or signs of systemic toxicity were observed during the study. SI values of 0.7, 0.4 and 0.5 were reported at concentrations of 5, 2.5 and 1% (w/v), respectively. Under the conditions of this assay, acetophenone azine did not show a sensitisation potential (SI = 0.7 at the maximum concentration tested).

The DS concluded, with regard to evidence in humans, that positive serious reactions allocated to acetophenone azine are reported. The limited number of cases (3 children and 1 adult wearing sport equipment) could be explained either by the fact that it is a relatively new substance, or by the type of consumer product (sports clothes). It is noted that incidences of sensitisation are likely to be underestimated. With regard to severity, the reported dermatitis in one of the human cases was so severe that a boy had to be hospitalized, while in the adult the dermatitis was generalized to trunk and arms, not limited to the exposed legs.

Further information considered was the positive QSAR predictions, positive results in *in vitro* tests performed with acetophenone azine in Keratinosens® assay and in h-Clat assay. However, a negative result was obtained in the LLNA at concentrations up to 5%.

Considering the overall data, including the severe human cases of allergic contact dermatitis and *in vitro* results, supported also by the presence of structure alerts for skin sensitisation in the chemical structure of the molecule, the DS concluded that a classification for skin sensitisation of acetophenone azine is warranted. In addition, concerns about the rather low dose selection in LLNA and somewhat conflicting results from both the negative and positive control groups may indicate a false negative result. The DS concluded that acetophenone azine fulfils the CLP criteria for classification as Skin Sens. 1. Due to the limited data available, no subcategorization nor SCL was proposed.

Comments received during consultation

Comments were received from two Member States and one individual.

Both MSs supported the classification as Skin Sens. 1, based on the case reports and noted that more have been published recently (see table below). One MS asked for a recommendation of the GCL or SCL. The other MS noted the supporting information from two positive *in vitro* tests from key events in the AOP for skin sensitisation. These are included in the "2 out of 3" Defined Approach, not yet accepted by the OECD, but indicative for sensitisation potential. Moreover, some support is provided by alerts for skin sensitisation potential by QSAR modelling. With regard to the negative LLNA test, the MS asked for some more elaboration on the rationale behind the dose selection.

The one commenting individual presented a case of severe allergic contact dermatitis in a 10-year old boy, caused by sports equipment.

The DS thanked the contributors for the new case studies and summarized them. With regard to the SCL, the DS reacted that SCLs are generally set based on results from animal testing. However, the LLNA test is negative. Based on the human data, the substance gave strong reactions with positive result until 0.001%. However, further data would be needed to allow subcategorization or to set a limit concentration. With regard to the dose setting in the LLNA test, this was based on a preliminary irritation/toxicity test using four doses (0,005, 005, 0.5 and 5%). Based on the results of this study, 5% was selected as top dose for the main test.

Assessment and comparison with the classification criteria

Human data

Several recent human cases have been published in the literature describing the occurrence of severe allergic contact dermatitis secondary to the use of specific sports equipment such as shin pads or footwear. Subsequent analysis identified acetophenone azine as the allergen in shin pads and footwear containing the foam elastomer EVA. Initially, the CLH dossier included 4 cases of allergic contact dermatitis described in France (publications from 2016 and 2017; see table below). After completion of the CLH report, 8 further cases from Canada, UK, Belgium and France were published in 3 additional publications and are therefore included in this opinion (2019-2020). Key information on these studies is summarised in the table below.

***Table**: Summary of the human case reports on skin sensitisation to acetophenone azine*

Study	Test substance	Study details	Observations
Raison-Peyron *et al.,* 2016 Patch test on a 13-year-old boy with no history of atopy or contact dermatitis France	Acetophenone azine (AA) 0.1% 0.01% 0.001% 0.0001% in acetone and water (w/v) 2% hydrazine sulphate in petrolatum	Patch tests over several sessions with numerous standardized series: the European baseline series, the plastics/glues and rubber series, the dyes and preservative series Large pieces of the black shin pad foam in close contact with the skin tested 'as is', simply moisturized with acetone, water, and ethanol.	Negative results from tests with the standardized series. Positive reactions to AA dilutions in acetone at 1%, 0.1%, 0.01%, and 0.001%, and to aqueous solutions of AA at 1% and 0.1%. Strong positive reactions to pieces of the shin pads, whereas tests with acetophenone and hydrazine sulphate were negative. Twenty control subjects were negative for 0.01% AA in acetone.
Raison-Peyron *et al.,* 2017 Patch test on a 11-year-old boy France	Acetophenone azine (AA) 0.1% and 0.01% in acetone (w/v) 1% hydrazine sulphate in petrolatum	Patch tests on 11-year-old non-atopic football player after recovery from eczematous eruption linked to close contact with football shin pads 2-3 times a week, for 3 months. Patch tests with pieces of shin pads and flip-flop soles moistened with acetone, ethanol, and water. In addition, the European baseline series, the plastic and glues series, and the rubber series were tested.	Patch tests with commercial allergens were all negative. Strong reactions (++/++, D2 and D3) that persisted for 12 days were reported with pieces of shin pads and flip-flop soles. Patch tests with 0.1% and 0.01% AA in acetone were positive (++/++, D2 and D3), while results were negative for hydrazine sulphate 1% pet. HPLC analysis of shin pads inner foam and flip-flops sole identified AA at 69 and 21 µg/g, respectively.
Raison-Peyron *et al.,* 2017 Patch test on a 12-year-old atopic boy France	Acetophenone azine (AA) 0.1% and 0.01% in acetone (w/v) 1% hydrazine sulphate in petrolatum	A case of 12-year-old non-atopic boy with acute itchy, vesicular dermatitis of both soles soon after wearing new sneakers. Patch testing performed 3 months later with the European baseline series and a shoe series. Patch tests with pieces of the soles of the sneakers in water, ethanol and acetone were performed. Detection of AA by HPLC in two sports brands.	Patch tests with commercial allergens were all negative. Patch tests with pieces of the sneaker soles were positive in water and acetone (++ and +, resp.), while samples in ethanol were negative. Strong positive reactions (++ on D2 and D3) to AA, whereas test with 1% hydrazine sulphate was negative. AA was detected in both brands at 15 µg/g and <0.5 µg/g, respectively.
De Fré *et al.*, 2017	Acetophenone azine(AA) 0.1% and	A 29-year-old non-atopic male hockey player referred for the evaluation of dermatitis on both legs, which had commenced shortly	Patch tests with pieces of the grey foam from the shin pads and from the soles of the sport shoes were

Study	Test substance	Study details	Observations
Patch test in 29-year-old hockey player France	0.01% in acetone.	after the wearing of a new pair of shin pads, lined with a grey foam. Patch testing performed with the Belgian baseline series including additional series (cosmetics, rubbers, plastics and glues, shoe allergens, and textile colorants). Patch tests with pieces of the internal grey foam of shin pads and sport shoe insoles, were performed 'as is', moistened with acetone.	positive (+ and ++ on D2 and D4, respectively). Patch tests with 0.1% and 0.01% AA were positive ++ and + on D2 and D4, respectively. No later-occurring reactions were observed.
Koumaki *et al.,* 2019 Patch test in 17-year-old hockey player London, UK	Acetophenone azine (AA) 0.1%, 0.01%, 0.001%, 0.0001%, 0.00001% in acetone.	A case of 17-year-old non-atopic male hockey player with a 12-month history of an erythematous pruritic and vesicular eruption localized bilaterally to both shins and ankles. This has coincided with wearing of a new pair of shin pads twice per week. Patch tests with pieces of the foam of shin pads moistened with water. Patch testing with an extended Society of Cutaneous Allergy baseline series, thiourea, phthalates, and 2 blue textile dyes.	Patch tests with the foam of shin pads were positive (++ and + on D2 and D4). Strong positive reactions to AA at 0.1% (++/++, on D2 and D4), and positive reactions to AA at 0.01% and 0.001% (+/-, on D2 and D4). HPLC analysis of the inner foam identified AA at 25 µg/g.
Darrigade *et al.*, 2020 Patch tests in 6 boys (7-14 years of age) France and Belgium	0.1% in petrolatum and/or acetone	Six boys (mean age 11.8 years; range 7-14) presented shin dermatitis related to wearing of shin pads. Patch tests were performed according to published guidelines. AA was patch-tested at 0.1% in petrolatum and/or acetone, as well as inner foam parts of the shin pads or shoes (as is, and moistened with action, water and/or ethanol).	Positive reactions were observed (in all 6 patients) to AA and to the foam pieces on D3 and D4. One patient also tested positive to limonene and linalool.
Besner Morin *et al.*, 2020 Patch test in 6-year-old soccer player Canada	Acetophenone azine (AA) 1% and 0.1% in petrolatum.	A case of a 6-year-old boy with eczematous dermatitis on the anterior of his legs at the site of contact with the EVA core of his shin pads. Later, a pruritic dermatitis appeared on the soles of both feet linked to wearing soccer shoes. Initial patch testing included a 34-allergen paediatric series and a shoe series, as well as 2×2 cm piece of black EVA from the shin pad, moistened with water. A second patch test was carried out with a glues and plastics series, pieces of the insole of the soccer shoe, and AA diluted to 1% and 0.1% in petrolatum.	Initial patch: the only positive + reaction was to the piece of EVA. Second patch: Positive + reactions seen to the insole from soccer cleats and AA, both being close together, merging into a single large reaction. Positive + reactions to AA at 1% and 0.1% (+/+, on D2 and D4). HPLC analysis did not identify AA in the pieces of EVA or shoes insole.

In the first reported case, a 13-year-old football player with no history of atopy or contact dermatitis presented acute, vesicular dermatitis on his shins after wearing shin pads for playing football (Raison-Peyron *et al.*, 2016). Patch tests gave strong positive reactions to pieces of the shin pads and to acetophenone azine down to dilutions of 0.001% in acetone, whereas tests with acetophenone and hydrazine sulphate were both negative.

Two further cases of severe allergic contact dermatitis caused by acetophenone azine present in shin pads, flip-flops, and sneakers were reported in young boys of age 11 and 12 (Raison-Peyron *et al.* 2017).

An 11-year-old non-atopic football player experienced an itchy, erythematous and vesicular eruption localized to both shins in close contact with football shin pads. Patch tests with pieces of shin pads and flip-flop soles moistened with acetone, ethanol, and water gave strong positive reactions (++/++, D2 and D3) that persisted for an additional 12 days. A 12-year-old non-atopic boy presented with acute itchy, vesicular dermatitis of both soles soon after wearing new sneakers. Patch tests 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 results were negative when the sample was moistened with ethanol. Acetophenone azine at concentrations of 0.1% and 0.01% w/v in acetone gave a strong reaction (++ on D2 and D3), whereas hydrazine sulphate 1% in petrolatum gave a negative result.

De Fré *et al.* (2017) reported the first adult case of allergic contact dermatitis on the legs, caused by acetophenone azine present in shin pads and sport shoes. Dermatitis started on his shins, and rapidly spread to his trunk and arms. 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.

Koumaki *et al.* (2019) reported on the case of a 17-year-old British hockey player with a 12-month history of an erythematous pruritic and vesicular eruption localized to the anterior aspect of both shins and ankles bilaterally. This has coincided within a couple of months after the wearing of a new pair of shin pads twice per week. His eczema flared up 2 days after each exposure to the shin pads. The localization of the dermatitis closely matched the areas of skin in contact with the blue foam backing of the pads. The eczema only resolved after discontinuing wearing them and applying moderately potent topical corticosteroids, leaving residual depigmentation.

HPLC analysis of samples from the foam lining of the shin pads identified the presence of acetophenone azine at 25 µg/g. Patch testing was performed with an extended Society of Cutaneous Allergy baseline series, thiourea, phthalates, 2 blue textile dyes using Finn Chambers on Scanpor tape. Acetophenone azine was tested at concentrations of 0.1, 0.01, 0.001, 0.0001, and 0.00001% in acetone. Strongly positive reactions were reported only to the pieces of the shin pads and to acetophenone azine down to a concentration of 0.001%.

Besner Morin *et al.* (2020) reported a new case of acetophenone azine-induced shin pad and sports shoe dermatitis in a 6-year-old soccer player from North America. During the summer of 2017, the boy began to play soccer and developed progressively an eczematous dermatitis on the anterior of his legs at the site of contact with the EVA core of his shin pads. A pruritic dermatitis later appeared on the soles of both feet. Discarding the soccer shoes resulted in resolution of the dermatitis, however, a relapse occurred when he wore a different brand. Initial patch testing included a 34-allergen paediatric series and a shoe series, as well as a 2×2 cm piece of black EVA from the shin pad, moistened with water. The only positive reaction was to the piece of EVA. A second patch test was carried out in January 2020 with glues and plastics series, pieces of the insole of the shoe, and acetophenone azine at concentrations of 1% and 0.1% in petrolatum. Positive reactions were seen to the insole and acetophenone azine.

According to the authors, the concentration of acetophenone azine is higher in shin pads than in shoes, explaining why patients, primarily sensitised by the former, later react to their shoes.

In addition to the previously described cases, Darrigade *et al.* (2020) published a case series of six boys with ages between 7–14 years, all non-atopic except for one, observed in France or Belgium between January 2018 and July 2019. All patients presented long-standing shin dermatitis related to the wearing of shin pads. Four patients also had secondary episodes of plantar vesicular and/or hyperkeratotic, fissured dermatitis, related to the shoes they were wearing. Extension of the dermatitis frequently occurred beyond the contact sites, for example to the legs, trunk, face and ears, and even generalized dermatitis occasionally developed.

Patch tests were performed according to published guidelines with a baseline and additional series (not further specified). Acetophenone azine was patch-tested at 0.1% in petrolatum and/or acetone. Pieces (2x2 cm) of the inner foam parts of the shin pads and/or shoes were patch-tested 'as is' and moistened with acetone, water and/or ethanol. Positive reactions were always observed to acetophenone azine and to the foam pieces on day 3 or 4.

Most recently, Raison-Peyron and Sasseville (2021) published a summary of the above dermatitis cases and the results from the associated patch testing (Table below).

***Table**: Summary of all published cases on allergic contact dermatitis to acetophenone azine (reviewed in Raison-Peyron and Sasseville, 2021).*

Reference	Country of origin	Age	Sex	Source	Test material	Concentration (%)	Vehicle	Test results	
								D2	D3/D4
Raison-Peyron *et al.*, 2016	France	13	M	Shin pads	Shin pads	100	Aqua	++	+++
					Shin pads	100	Acetone	++	+++
					Shin pads	100	Ethanol	++	+++
					AA	1	Aqua	++	++
					AA	0.1	Aqua	+	+
					AA	0.01	Aqua	–	–
					AA	0.001	Aqua	–	–
					AA	0.0001	Aqua	–	–
					AA	1	Acetone	++	++
					AA	0.1	Acetone	++	++
					AA	0.01	Acetone	+?	+
					AA	0.001	Acetone	–	+?
					AA	0.0001	Acetone	–	–
Raison-Peyron *et al.*, 2017	France	11	M	Shin pads	Shin pads	100	Aqua	++	++
					Shin pads	100	Acetone	++	++
					Shin pads	100	Ethanol	++	++
					Flip-flops	100	Aqua	++	++
				Flip-flops	Flip-flops	100	Acetone	++	++
					Flip-flops	100	Ethanol	++	++
					AA	0.1	Acetone	++	++
					AA	0.01	Acetone	++	++
		12	M	Sneakers	Sneakers	100	Aqua	++	++
					Sneakers	100	Acetone	+	+
					Sneakers	100	Ethanol	–	–
					AA	0.1	Acetone	++	++
					AA	0.01	Acetone	++	++
De Fré *et al.*, 2017	Belgium	29	M	Shin pads	Shin pads	100	Acetone	+	++
				Sports shoes	Sports shoes	100	Acetone	+	++
					AA	0.1	Acetone	++	++
					AA	0.01	Acetone	+	+

Reference	Country of origin	Age	Sex	Source	Test material	Concentration (%)	Vehicle	Test results	
								D2	D3/D4
Koumaki *et al.*, 2019	United Kingdom	17	M	Shin pads	Shin pads	100	Aqua	++	+
					AA	0.1	Acetone	++	++
					AA	0.01	Acetone	+	–
					AA	0.001	Acetone	+	–
					AA	0.0001	Acetone	–	–
					AA	0.00001	Acetone	–	–
Darrigade *et al.*, 2020	France and Belgium	7	M	Shin pads	Shin pads	100	As is	–	+
					AA	0.1	Petrolatum	–	+
		12	M	Shin pads	Shin pads	100	As is	++	++
				Flip-flops	Flip-flops	100	As is	++	++
					AA	0.1	Petrolatum	++	++
		12	M	Shin pads	Shin pads	100	As is	++	++
				Sneakers	Sneakers	100	As is	++	++
					AA	0.1	Petrolatum	++	++
		14	M	Shin pads	Shin pads	100	As is	?	++
				Sneakers	Sneakers	100	As is	?	++
				Flip-flops	Flip-flops	100	As is	?	++
					AA	0.1	Petrolatum	–	++
		13	M	Shin pads	Shin pads	100	As is	+	+
					AA	0.1	Petrolatum	–	+
		13	M	Shin pads Nike	Shin pads Puma	100	Aqua	++	++
				Shin pads Puma	Shin pads Nike	100	Aqua	++	++
				Sneakers	Sneakers	100	Aqua	?	+
				Sneakers	Sneakers	100	Aqua	+	++
					AA	0.1	Petrolatum	+++	+++
Besner Morin *et al.*, 2020	Canada	6	M	Shin pads	Shin pads	100	Aqua	+	+
				Sneakers	Adidas cleats	100	Aqua	–	+
				Soccer cleats	AA	1	Petrolatum	+	+
					AA	0.1	Petrolatum	+	+

Before application for patch testing, some pieces of shin pads, sneakers, flip-flops, or cleats were moistened with either water (aqua), acetone, or ethanol.
AA; D2, day 2; D3/D4, day 3/day 4; M, male.

Of the 12 reported cases of allergic contact dermatitis to acetophenone azine, 11 have been in children and adolescents. The clinical picture comprises similar effects starting with localized eczema on the skin in close contact with EVA foam followed by a severe and diffuse eczematous rash on the whole body, including the face (Raison-Peyron *et al.*, 2017). Some authors speculate that the concentration of acetophenone azine is higher in shin pads than in shoes, explaining why patients, primarily sensitised by the former, later react to their shoes (Besner Morin *et al.*, 2020). When secondary to footwear, the dermatitis presented either as dyshidrosiform vesiculobullous eczema, sometimes accompanied by palmar lesions, or as plantar hyperkeratotic dermatitis. Widespread dissemination was also often seen in these cases. Some of the patients healed with scarring and marked post-inflammatory hypopigmentation (Raison-Peyron and Sasseville, 2021).

In vivo skin sensitisation test: Local Lymph Node Assay (OECD TG 429)

In a recent OECD TG 429 compliant study, female mice (CBA/CaOlaHsd, 4/group) were treated topically with acetophenone azine (5, 2.5 or 1%), vehicle control (dimethylformamide, DMF) or positive control (α-hexylcinnamaldehyde, HCA). In a preliminary study, DMF was selected as the best vehicle considering the test item characteristics, and the highest achievable concentration was established at 5% (w/v). There was no mortality, marked body weight loss, or signs of systemic toxicity observed during the study. Treatment with acetophenone azine resulted in Stimulation Indices (SI) of 0.7, 0.4 and 0.5 at concentrations of 5, 2.5 and 1%, respectively. A positive response (SI: 3.7) was observed in animals that received the positive control. Under the conditions of the study, acetophenone azine did not show a sensitisation potential.

***Table**: Summary of the LLNA test on skin sensitisation*

Study	**Species**	**Test substance**	**Dose levels**	**Results**
Anonymous 2018c LLNA OECD TG 429, GLP Klimisch 1	CBA/CaOlaHsd mice, female (n=20) 4/group	Acetophenone azine (AA) Purity 97.2%	5, 2.5 and 1% (w/v) in dimethylformamide (DMF) Positive control: 25% α-hexylcinnamaldehyde (HCA) in DMF	No mortality, no signs of systemic toxicity, nor marked BW losses (≥5%) observed. Normal appearance of the lymph nodes in the negative control and treated groups, enlarged in the positive control group. The SI values for AA were 0.7, 0.4 and 0.5 at concentrations of 5, 2.5 and 1%, respectively. SI=3.7 for HCA (positive control) **No skin sensitisation potential**

The DS addressed several questions regarding contradictory results from both the negative and positive control data. For the positive control HCA, the historical data from the performing laboratory indicate a low range for SI of 4.7, while a SI of 3.7 was measured in the current study. In addition, the disintegrations per node (DPN) value of 463.6 in negative control samples was rather high for DMF (HCD range 62.0-649.6, with average of 256.1), whereas the response to the positive control in the same vehicle is clearly below the range of HCD. The DS concluded that the possibility for obtaining a false negative result could not be completely excluded.

RAC considers that the LLNA study is properly documented and compliant with the current OECD guideline. A major limitation of the test however is the low maximum dose treatment of up to only 5%, which is linked to the poor solubility of the test substance in the chosen solvent. No firm conclusion can be drawn with regard to the possibility of a false negative result due to the rather high DPN readings from the negative control samples and a positive control response out the historical control data range. Nevertheless, the above limitations lower the weight of this negative LLNA test in the overall assessment of acetophenone azine.

In vitro studies on skin sensitisation: Human Cell Line Activation Test (h-CLAT) and ARE-Nrf2 Luciferase Test Method (KeratinoSens™)

Key parameters and main results from the *in vitro* KeratinoSens™ and h-CLAT assays are discussed in detail in the CLH report. Acetophenone azine was found positive in both assays under the conditions tested, and therefore considered to activate both the dendric cells and the Nrf2 transcription factor. Such type of data can be used to support the discrimination between skin sensitisers and non-sensitisers in the context of an Integrated Approach to Testing and Assessment (IATA). In the present assessment, these positive results do not contradict the human case reports and provide additional support for classification of acetophenone azine as skin sensitiser.

Comparison with the CLP criteria

Acetophenone azine was shown to be a skin sensitiser in twelve documented case reports on partly severe allergic contact dermatitis in children and adults from Europe (11) and North America (1). Further information supporting classification includes positive QSAR predictions and positive results from *in vitro* tests performed with Keratinosens® and h-Clat assays. A negative result was obtained in the LLNA at concentrations up to 5% acetophenone azine.

RAC agrees with the conclusion of DS that there is sufficient information to evaluate the skin sensitisation potential of acetophenone azine, including evidence from human cases and results from an appropriate animal or *in vitro/in chemico* tests. The limited number of human cases can be due to the recent discovery of the substance as an allergen, and/or to the less frequent use of this type of consumer products (sport equipment) compared to classic clothes. Importantly, incidences of sensitisation are likely to be underestimated because of underdiagnoses, underreporting and lack of registration for milder cases of dermatitis. It is also plausible that cases of allergic contact dermatitis would have been missed and labelled irritant contact dermatitis or dyshidrosis (Raison-Peyron and Sasseville, 2021).

For newly identified skin sensitisers, additional elements such as (1) Hospitalisation due to acute skin reaction, 2) Chronic dermatitis (lasting >6 months), and (3) Generalised (systemic/whole body) dermatitis can be taken into consideration to support classification (Guidance on the Application of the CLP Criteria, 3.4.2.2.2; 2017). Hospitalization after exposure to acetophenone azine of boys wearing shin pads was reported by Raison-Peyron *et al.* (2016) and Darrigade *et al.* (2020), and a dermatitis generalized to the trunk and arms, and not just limited to the exposed parts of the body (i.e., the legs) was observed in one adult hockey player (De Fré *et al.*, 2017). Frequent extension of the dermatitis beyond the contact sites, for example to the legs, trunk, face and ears, and occasionally even generalized dermatitis was also reported in Darrigade *et al.* (2020). These cases are considered to clearly fulfil the above recommendations of the guidance.

Additional support is provided by several *in vitro* tests and *in silico* approaches. Acetophenone azine was positive in the KeratinoSens assay (OECD TG 442D) and in the h-CLAT assay (OECD TG 442E). With a Log Kow value of 3.7 (i.e., slightly above 3.5), acetophenone azine is likely to activate both keratinocytes and dendritic cells in human cells. (Q)SAR modelling using the DEREK and CAESAR software packages predicted skin sensitising potential for acetophenone azine.

However, a negative result was obtained from a recent (2018) GLP and guideline-conforming LLNA test with acetophenone azine. A major limitation of the test is the low maximum dose treatment of up to only 5%. Some further observations, such as the weak response in the positive control group lying outside of HCD, and the rather strong DPN readings from the vehicle control DMF, might at least partly provide an explanation for the negative test outcome.

Overall, considering the whole data available, and specifically the severity of the reactions in humans, RAC concludes that a classification for skin sensitisation of acetophenone azine is warranted. In view of the low exposure required to be sensitised and the severity of the responses,

acetophenone azine fulfils criteria for classification as Skin Sens. 1 according to the CLP regulation. However, the limited data (low number of cases reported until now) available do not allow for a sub-categorisation. RAC notes that according to the CLP guidance (3.4.2.2.2), the severity/strength of diagnostic patch test reactions normally cannot be used for this purpose. Further, SCLs shall be set when there is adequate and reliable information available (data from e.g. workplace studies where the exposure is defined) showing that the specific hazard is evident below the GCL. **Since such data is lacking, a SCL is not proposed.**

Given the severity of some responses, RAC recommends that this substance should be carefully monitored/investigated in the future.

Additional references

Besner Morin C, Stanciu M, Miedzybrodzki B, Sasseville D (2020). Allergic contact dermatitis from acetophenone azine in a Canadian child. Contact Dermatitis 83: 41–42.

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Koumaki D, Bergendorff O, Bruze M, Orton D (2019). Allergic contact dermatitis to shin pads in a hockey player: acetophenone is an emerging allergen. Dermatitis 30: 162–163.

Raison-Peyron N, Sasseville D. (2021). Acetophenone Azine. Dermatitis 32(1):5-9.

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ANNEXES:

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

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