CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Thixatrol MAX

EC Number: 432-430-3

CAS Number: Not assigned

Index Number: 616-200-00-1

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Thixatrol MAX
EC number:	432-430-3
CAS number:	*Not assigned*
Annex VI Index number:	616-200-00-1
Degree of purity:	>95%
Impurities:	*CONFIDENTIAL*

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	Skin Sens. 1 H317: May cause an allergic skin reaction Aquatic Chronic. 4 H413: May cause long lasting harmful effects to aquatic life	Xi: R43: May cause sensitisation by skin contact R53: May cause long-term adverse effects in the aquatic environment .
Current proposal for consideration by RAC	Propose to remove Classification: Skin Sens. 1: H317	Propose to remove Xi: R43: May cause sensitisation

		by skin contact
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Aquatic Chronic. 4 H413: May cause long lasting harmful effects to aquatic	R53: May cause long-term adverse effects in the aquatic environment

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

This proposal addresses the removal of the harmonised classification for skin sensitisation and soes not address the other classifications in Annex VI. In this context, the 2nd ATP to CLP published on the 30th March 2011 has been considered and classification of this substances is proposed as follows:

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
4.1.	Hazardous to the aquatic environment	Aquatic chronic 4			

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: No signal word is used
Hazard statements: H413
Precautionary statements: P273 (Prevention), P501 (Disposal)

Proposed notes assigned to an entry:

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Environment	**R53, S61**	**None**	**R53**	

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Indication of danger:
R-phrases: R53
S-phrases: S61

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Thixatrol MAX was notified in the UK under the Notification of New Substances (NONS) Regulation (00-06-1340) in 2000. The existing classification is based upon read-across to a structural analogue (Thixatrol Plus, EC# 430-050-2, reaction product of decanoic acid, 12-hydroxystearic acid and 1,2-ethandiamine (mol1:1:1)). Based on the results of a GPMT study on Thixatrol Plus, Thixatrol MAX was classified as a skin sensitiser.

Read across was considered to be appropriate at the time based on the similarities in structure of the two materials, plus their uses. The composition of both substances is considered to be confidential and is not provided in this document, further details are included in the IUCLID dossier.

However, sensitisation is an intrinsic property of the substance itself and, hence, it was considered justified to test the substance in mice for evaluation of safe use of the substance. This does not affect the integrity of the original read across argument as sensitisation is an intrinsic property of an individual substance.

2.2 Short summary of the scientific justification for the CLH proposal

The results of the GPMTs on Thixatrol Plus were not conclusive, although in the absence of valid data from other predictive tests the test item was precautiously classified as a skin sensitiser based on these studies. A subsequent LLNA conducted on Thixatrol Max clearly demonstrated that this substance has no need to be classified as a skin sensitiser. The LLNA can be considered to be valid since it was conducted under GLP conditions in general accordance with OECD test guideline 429, using a vehicle demonstrated to be suitable for use in the LLNA (1% Pluronic L92 in distilled water) and at the maximum practical concentration for application to the mouse ears (25%). The known moderate skin sensitiser α-HCA produced a clear positive result at the same test facility when formulated at 25% in the same vehicle. The result of the adequately conducted LLNA on Thixatrol Max must be assigned greater reliability than the result from a guinea pig skin sensitisation study conducted on the structurally related, but not identical substance, Thixatrol Plus, for which the result must be considered to be of limited reliability.

Skin sensitisation is an immune delayed-type (T-cell mediated) hypersensitivity response to a small molecule (hapten). The induction of a sensitisation response requires absorption of the hapten into the skin, binding with a proteins, recognition and internalisation by Langerhans' cells, and transport to the draining lymph node where further processing results in proliferation of a population of memory T-cells. Elicitation of the skin sensitisation response occurs following subsequent exposure of the skin to the hapten resulting in a localised inflammatory response. The skin sensitisation potential of a substance will be dependent upon its ability to covalently bind to proteins, and its specific antigenicity. It therefore follows that slight differences in the structure of the molecule, and the presence of different functional groups can result in differences in skin sensitisation potential. This potential can be further modulated by physico-chemical properties of the substance that influence solubility and uptake into the skin. It is also feasible that differences in impurity profiles of a set of similar substances could result in differences in skin sensitisation potential. It is considered that if a substance has demonstrated clear potential to induce a skin sensitisation response which can be attributed to a specific structural characteristic of the molecule, then read-across of the data to a substance containing the same structural characteristic may be possible, although the degree of skin sensitising potential (i.e. potency) of the substances could vary.

However, if the evidence for skin sensitisation potential of a substance is not convincing, it is not recommended to read across to a similar substance of the same chemical category. Owing to the specificity of the antigenicity of chemical substances, although it may not be possible to read-across from skin sensitisation data for one chemical to that of a related chemical, this would not necessarily invalidate read-across for other endpoints such as acute or repeated dose toxicity, reproductive toxicity or genotoxicity since these endpoints will be dependent upon mechanisms unrelated to those required to induce skin sensitisation.

Due to the results of studies which were conducted upon the substance itself it has been concluded that the substance does not meet the criteria for classification as a skin sensitiser. This conclusion is based upon the key study (Sanders, 2009) and the weight of evidence of the supporting studies (Driscoll, 2009 & Aitchison, 2003).

It is therefore considered justified that the substance does not meet the criteria for classification and labelling as a sensitizer under Directive 67/548/EEC and Regulation (EC) No. 1272/2008.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Classification: Skin Sens. Cat. 1

H317

Aquatic Chronic Cat. 1

H413

Labelling: GSH07 Wng

H317

H413

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

Classification: R43

R53

Labelling: R45-53

2.4 Current self-classification **and labelling**

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Classification and labelling is per harmonised classification and labelling according to Annex VI of CLP Regulation

2.4.2 Current self-classification and labelling based on DSD criteria

Classification and labelling is per harmonised classification and labelling according to Annex VI of CLP.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

The existing classification is based upon read-across to an analogue with a similar chemical structure.

Sensitisation is an intrinsic property of a substance and hence, test data on the substance itself should take precedence over data from structural analogues. Studies conducted in mice (LLNA) clearly show that the substance does not induce sensitisation response in skin. Hence, to allow accurate communication of safe use of the substance, Thixatrol MAX should not be considered to be a potential skin sensitiser.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	432-430-3
EC name:	Reaction mass of: N,N'-ethane-1,2-diylbis(hexanamide);12-hydroxy-N-[2-[(1-oxyhexyl)amino]ethyl]octadecanamide;N,N'-ethane-1,2-diylbis(12-hydroxyoctadecanamide)
CAS number (EC inventory):	Not available
CAS number:	Not assigned
CAS name:	Not applicable
IUPAC name:	Reaction mass of N,N'-ethane-1,2-diylbis(hexanamide) and 12-hydroxy-N-[2-[(1-oxyhexyl)amino]ethyl]octadecanamide and N,N'-ethane-1,2-diylbis(12-hydroxyoctadecanamide)
CLP Annex VI Index number:	616-200-00-1
Molecular formula:	CONFIDENTIAL
Molecular weight range:	CONFIDENTIAL

Structural formula: *CONFIDENTIAL*

1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
CONFIDENTIAL	> 95 %	≥ 90% and < 100%	

Current Annex VI entry: none that are attributable to any single constituent

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
CONFIDENTIAL			

Current Annex VI entry: None that are attributable to the known impurities

Information on the full composition and impurity profile is provided in the IUCLID dossier.

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

Current Annex VI entry: Not applicable

1.2.1 Composition of test material

As described above and in the technical dossier

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Explosiveness		conclusive but not sufficient for classification	
Oxidising properties		conclusive but not sufficient for classification	

Flammability		conclusive but not sufficient for classification	
Thermal stability		conclusive but not sufficient for classification	

2 MANUFACTURE AND USES

2.1 Manufacture

The substance is manufactured outside the EU.

2.2 Identified uses

The substance is imported neat and as part of a solution for formulation into paints, varnishes and coatings

Identified use	**Process category (PROC)**	**Preparation Category (PC)**	**Sector of Use (SU)**	**Article category (AC)**
Used in paints, varnishes and coatings (SU10)	Charging (PROC8b) of mixing vessels and mixing (PROC5).	Viscosity control agent (PC9a)	C20.3 - manufacturing: manufacture of paints, varnishes and similar coatings, printing ink and mastics	

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

3.1 Conclusions on classification and labelling:

The classification for physico-chemical properties is not considered in this dossier.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

The substance has a low vapour pressure, however, based on typical particle size distribution may contain particles of inhalable size.

The substance is unlikely to hydrolyse and hence exposure to degradation products is unlikely.

It is unlikely to be absorbed systemically and no single route of excretion is favoured over another.

4.2 Acute toxicity

Not covered in this proposal

4.3 Irritation

Not covered in this proposal

4.3.1 Skin irritation

Not covered in this proposal

4.3.2 Eye irritation

Not covered in this proposal

4.3.3 Respiratory tract irritation

Not covered in this proposal

4.4 Corrosivity

Not covered in this proposal

4.5 Sensitisation

4.5.1 Skin sensitisation

Table 15: Summary table of relevant skin sensitisation studies

<table>
<tr><th>Method</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr>
<td>Mouse (CBA/Ca (CBA/CaOlaHsd)) female

Local lymph node assay

OECD Guideline 429 (Skin Sensitisation: Local Lymph Node Assay)

EU Method B.42 (Skin Sensitisation: Local Lymph Node Assay)</td>
<td>Not sensitising

Stimulation index: A stimulation index of less than 3 was recorded for the test material at concentrations of 25%, 10% and 5% w/w in 1% Pluronic L92 in distilled water.

Results:
<table>
<tr><th>Test Item</th><th>Concentration in 1% Pluronic L92 in distilled water</th><th>Stimulation Index</th><th>Result</th></tr>
<tr><td rowspan="3">Thixatrol Max</td><td>5 % w/w</td><td>0.94</td><td>Negative</td></tr>
<tr><td>10 % w/w</td><td>0.91</td><td>Negative</td></tr>
<tr><td>25 % w/w</td><td>0.82</td><td>Negative</td></tr>
<tr><td rowspan="3">α-HCA</td><td>5 % v/v</td><td>1.30</td><td>Negative</td></tr>
<tr><td>10 % v/v</td><td>2.37</td><td>Negative</td></tr>
<tr><td>25 % v/v</td><td>8.14</td><td>Positive</td></tr>
</table>
</td>
<td>1 (reliable without restriction)

key study

experimental result

Valid positive control study</td>
<td>Sanders A (2009)</td>
</tr>
<tr>
<td>mouse (CBA/Ca (CBA/CaOlaHsd)) female

Local lymph node assay

OECD Guideline 429 (Skin Sensitisation: Local Lymph Node Assay)

EU Method B.42 (Skin Sensitisation: Local Lymph Node Assay)</td>
<td>Not sensitising

Stimulation index: A stimulation index of less than 3 was recorded for the test material at concentrations of 25%, 10% and 5% w/w in Corn oil.

Results:
<table>
<tr><th>Test Item</th><th>Concentration (in corn oil)</th><th>Stimulation Index</th><th>Result</th></tr>
<tr><td rowspan="3">Thixatrol Max</td><td>5% w/w</td><td>0.56</td><td>Negative</td></tr>
<tr><td>10% w/w</td><td>0.37</td><td>Negative</td></tr>
<tr><td>25% w/w</td><td>0.31</td><td>Negative</td></tr>
<tr><td rowspan="3">α-HCA</td><td>5% v/v</td><td>1.42</td><td>Negative</td></tr>
<tr><td>10% v/v</td><td>1.04</td><td>Negative</td></tr>
<tr><td>25% v/v</td><td>1.28</td><td>Negative</td></tr>
</table>
</td>
<td>3 (not reliable)

supporting study

experimental result

non-valid positive control study</td>
<td>Driscoll R (2009)</td>
</tr>
</table>

mouse (CBA/Ca (CBA/CaOlaHsd)) female

Local lymph node assay

OECD Guideline 429 (Skin Sensitisation: Local Lymph Node Assay)

EU Method B.42 (Skin Sensitisation: Local Lymph Node Assay)

Not sensitising

Stimulation index: A Stimulation Index of less than 3 was recorded for the test material at concentrations of 10%, 5% and 2.5% w/w in propylene glycol.

Results:

Concentration (% w/w) in propylene glycol	Stimulation Index (SI)	Result
2.5	1.26	Negative
5	1.89	Negative
10	1.55	Negative

Propylene glycol was the only OECD recommended vehicle (OECD 429) that was suitable for administration of Thixatrol Max to the ears of the mice.

The maximum concentration of Thixatrol Max suitable for administration to the ears of the mice was 10% (w/w) in propylene glycol.

Positive Control: No positive control study using propylene glycol was available at the time of testing.

Supporting information:

Subsequent to the above study, a group of five animals was treated with 50 µl (25 µl per ear) of the moderate skin sensitiser Phenylacetaldehyde (90%) as a solution in propylene glycol at a concentration of 2.5% v/v. A further group of five animals was treated with propylene glycol alone.

2 (reliable with restrictions)

supporting study

experimental result

Aitchison G (2003)

	The Stimulation Index expressed as the mean radioactive incorporation of the treatment group divided by the mean radioactive incorporation of the vehicle control group was as follows: Concentration % v/v in propylene glycol: 2.5 Stimulation Index: 8.00 Result: Positive A clear and satisfactory sensitisation response was therefore obtained in the LLNA for the moderate skin sensitiser Phenylacetaldehyde (90%), at a test concentration of 2.5% in propylene glycol.		
guinea pig (Dunkin-Hartley) female Guinea pig maximisation test Induction: intradermal and epicutaneous Challenge: epicutaneous, occlusive OECD Guideline 406 (Skin Sensitisation) equivalent or similar to EU Method B.6 (Skin Sensitisation)	Ambiguous No. with positive reactions: 1st reading: 0 out of 10 (test group); 24 h after chall.; dose: 71% w/v 2nd reading: 0 out of 10 (test group); 48 h after chall.; dose: 71% 1st reading: 2 out of 10 (test group); 24 h after chall.; dose: 50% w/v 2nd reading: 2 out of 10 (test group); 48 h after chall.; dose: 50% w/v 1st reading: 0 out of 5 (negative control); 24 h after chall.; dose: 71% w/v 2nd reading: 0 out of 5 (negative control); 48 h after chall.; dose: 71% w/v 1st reading: 0 out of 5 (negative control); 24 h after chall.; dose: 50% w/v 2nd reading: 0 out of 5 (negative control); 48 h after chall.; dose: 50% w/v	3 (not reliable) disregarded study experimental result Test material Thixatrol Plus	Lees D (1998a)
guinea pig (Dunkin-Hartley)	Ambiguous No. with positive reactions:	3 (not reliable)	Lees D (1998b)

female Guinea pig maximisation test Induction: intradermal and epicutaneous Challenge: epicutaneous, occlusive OECD Guideline 406 (Skin Sensitisation) equivalent or similar to EU Method B.6 (Skin Sensitisation)	1st reading: 3 out of 10 (test group); 24 h after chall.; dose: 71% w/v 2nd reading: 1 out of 10 (test group); 48 h after chall.; dose: 71% w/v 1st reading: 9 out of 10 (test group); 24 h after chall.; dose: 50% w/v 2nd reading: 4 out of 10 (test group); 48 h after chall.; dose: 50% w/v rechallenge: 3 out of 5 (test group); 24 h after chall.; dose: 50% w/v rechallenge: 3 out of 5 (test group); 48 h after chall.; dose: 50% w/v rechallenge: 1 out of 5 (test group); 24 h after chall.; dose: 25% w/v rechallenge: 4 out of 5 (test group); 48 h after chall.; dose: 25% w/v 1st reading: 0 out of 5 (negative control); 24 h after chall.; dose: 71% w/v 2nd reading: 0 out of 5 (negative control); 48 h after chall.; dose: 71% w/v 1st reading: 3 out of 5 (negative control); 24 h after chall.; dose: 50% w/v 2nd reading: 2 out of 5 (negative control); 48 h after chall.; dose: 50% w/v rechallenge: 0 out of 5 (negative control); 24 h after chall.; dose: 50%w/v rechallenge: 0 out of 5 (negative control); 48 h after chall.; dose: 50% w/v rechallenge: 0 out of 5 (negative control); 24 h after chall.; dose: 25% w/v rechallenge: 0 out of 5 (negative control); 48 h after chall.; dose: 25% w/v	disregarded study experimental result Test material Thixatrol Plus	

4.5.1.1 Non-human information

4.5.1.2 Human information

4.5.1.3 Summary and discussion of skin sensitisation

Thixatrol Plus is a substance structurally related to Thixatrol Max. Two GPMTs were conducted on Thixatrol Plus at the Central Toxicology Laboratory (CTL), Alderley Park UK. The first was conducted between 6 August 1997 and 16 September 1997 and the second between 13 July and 6 September 1998. At this time, the GPMT was considered to be the method of choice for determination of the skin sensitisation potential of chemicals. The GPMT and another test involving the use of guinea pigs, the Buehler test, were described in OECD test guideline 406. An OECD test guideline for the mouse local lymph node assay had not been adopted at this time. In the reports of the two studies conducted at CTL, the test item is identified as EA-2525, an alternative name for Thixatrol Plus.

First GPMT on Thixatrol Plus. CTL Report No. CTL/P/5748 (Lees, 1998)

This test was conducted at the Central Toxicology Laboratory, Alderley Park, UK between 6 August and 16 September 1997. The study was based on the method described by Magnusson and Kligman (1970) and in accordance with OECD 406, 1992. Ordinarily, this study design would consist of an induction phase (intradermal administration followed seven days later by a 48-hour topical application) and a challenge phase (single 24-hour topical application approximately two weeks after the topical induction). In order to determine the skin sensitisation potential of the test item, the skin responses produced in previously-induced animals after topical challenge are compared to the responses in control animals not previously exposed to the test item. On some occasions, for instance where the results of the first challenge are equivocal, a second challenge (re-challenge) is considered appropriate. This was the case for this test on EA-2525 (Thixatrol Plus).

The concentrations of test item used at each phase of the test were as follows:

Induction Phase

Intradermal injection: 0.3% w/v in corn oil

Topical application: 71% w/v in corn oil

Challenge Phase

First topical challenge: 71% and 50% w/v in corn oil

Second topical challenge (re-challenge): 50% and 25% w/v in corn oil

Grade 1 or 2 skin responses were produced in nine of the ten previously-induced animals following the first challenge with a 50% formulation of the test item in corn oil, but grade 1 skin responses were also produced in three of the five non-induced control animals by the same formulation. The occurrence of skin reactions in the control animals following challenge often creates difficulty in interpretation of the study results and this was commented upon in the study report. Grade 1 skin responses were produced in three of the ten previously-induced animals following challenge with a 71% formulation of the test item in corn oil but no skin reactions were seen in any of the five non-induced control animals by the same formulation. It was noted that the 50% formulation had adhered to the skin of all ten previously-induced test animals and four of the non-induced control animals, and that this had not prevented evaluation of erythema. However, it is not known if adherence of the test item to the skin had induced local skin irritation and so this possibility cannot be eliminated.

Since it was considered that interpretation of the results of the challenge was complicated by the presence of skin responses in the control animals, a second challenge ('re-challenge') was conducted nine days after the initial challenge by exposing the skin of the previously-induced animals and a new group of five non-induced control animals to the test item formulated at concentration of 50% w/v and also at the lower concentration of 25% w/v in corn oil. A 71% w/v formulation of the test item was not investigated. Grade 1 skin responses were produced in three previously-induced/challenged test animals by the 50% and 25% formulations of test item in corn oil and a grade 2 skin response was produced in one other previously-induced/challenged test animal by these formulations. It was noted that the 50% and 25% formulations had adhered to the skin of the majority of the previously-induced and challenged test animals and new non-induced control animals, and that this had not prevented evaluation of erythema. However, it is not known if adherence of the test item to the skin had induced local skin irritation in the previously induced test animals and so this possibility cannot be eliminated. No skin responses were produced in any of the new non-induced control animals by either the 50% w/v or 25% w/v formulations of test item in corn oil. It was concluded that there was a net skin sensitisation response of 40%.

The presence of skin responses in previously-induced test animals and the non-induced control animals after the first challenge, plus the evidence that the 50% w/v formulation had adhered to the skin effectively invalidated this set of results in terms of interpretation of the skin sensitisation potential of the test item. Hence it was necessary to conduct a re-challenge using the same previously-induced test animals and a new set of five non-induced control animals. This was conducted using 50% w/v and 25% w/v formulations of the test item in corn oil but not 71% w/v formulation in corn oil. No explanation is given why a 71% w/v formulation. The presence of skin responses in four previously-induced/challenged test animals and absence of skin responses in the control animals following re-challenge was used to conclude that the test item is a skin sensitiser. However, this conclusion did not take into account the following considerations:

- No explanation was provided why a 71% w/v formulation of test item in corn oil was not investigated at re-challenge.
- The presence of skin reactions in control animals following the first challenge indicated that some irritation of the skin had certainly been caused by the 50% w/v formulation of test item.
- An impact assessment upon the outcome of the study was not provided with respect to the adherence of the 50% w/v and 25% w/v formulations of the test item to the skin of the test and control animals. It is possible that adherence of the formulations had contributed to the presence of skin reactions.

- It is likely that the presence of skin reactions in the previously-induced test animals after the first challenge, together with the necessary repeated procedures for bandaging, clipping of hair from the skin, application of patches and attempted removal of test item would have reduced the integrity of the skin, possibly resulting in a lowering of the threshold of irritation prior to the re-challenge phase i.e. some of the animals could have been rendered hyper-reactive to non-specific stimuli.
- The pattern of skin responses in the previously induced test animals did not provide conclusive evidence that the responses were due to skin sensitisation. For instance:

  - After the first challenge with the 71% w/v formulation of test item, a skin response persisted in only one previously-induced test animal 48 hours after challenge. For true skin sensitisation responses, the incidence and/or the intensity of reactions at the 48-hour challenge observation is often the same or higher than at the 24-hour challenge observation, as demonstrated by the data provided in the study report for the positive control study on hexylcinnamaldehyde..
  - One of the previously induced animals that had shown a skin response to the 71% w/v and 50% w/v formulations after the first challenge (Number 77), showed no skin responses to 50% w/v or 25% w/v formulations of test item after the re-challenge.
  - Another previously induced test animal that had shown a skin response to the 71% w/v and 50% w/v formulations after the first challenge (Number 73) showed no response to the 25% w/v formulation after-rechallenge.
  - Five other previously-induced test animals that had shown a skin response to the 50% w/v formulation after the first challenge (Numbers 72,76,77,79 and 80), showed no skin response to the 25 % w/v formulation after the re-challenge.

With the lack of clarity regarding the results of the initial challenge due to the presence of skin reactions in test and control animals, the adherence of the test item formulations to the skin, plus the other considerations presented above, the conclusion of the study report that the test item is a moderate skin sensitiser must be viewed as being without clear evidence and of limited reliability.

It is considered that this was the reason why it was considered justified to perform a second GPMT on EA-2525 (Thixatrol Plus).

Second GPMT on Thixatrol Plus. CTL Report No. CTL/P/6058 (Lees, 1998)

This test was conducted at the Central Toxicology Laboratory (CTL), Alderley Park, UK between 13 July and 6 September 1998. The study was based on the method described by Magnusson and Kligman (1970) and in accordance with OECD 406, 1992.

The concentrations of test item used at each phase of the test were as follows:

Induction Phase

Intradermal injection: 10% w/v in corn oil

Topical application: 71% w/v in corn oil

Challenge Phase

Topical challenge: 71% and 50% w/v in corn oil

A second challenge (re-challenge) was not conducted.

Following topical challenge no evidence of adherence of the test item to skin was recorded for any test or control animal (but was recorded in the first GPMT after topical challenge). No skin responses were produced in any of the previously-induced test animals or non-induced control animals following challenge with the 71% w/v formulation of EA-2525 (Thixatrol Plus) in corn oil. At the observation conducted 24-hours after topical challenge, Grade 1 skin responses were seen at the skin sites of two previously-induced test animals that had been exposed to the 50% w/v formulation (animal numbers 254 and 258). The grade 1 skin reaction persisted in only one of these animals at the 48-hour observation (animal number 258), whilst the skin of the other animal (number 254) appeared normal at this time. A grade 1 skin response was seen at the 50% w/v formulation-treated skin site of one other animal (number 256) at the 48-hour observation . No skin responses were seen at the skin sites of any non-induced control animal exposed to the 50% w/v formulation, at either the 24 or 48-hour observations.

It was concluded in the study report that the net skin sensitisation response was 30%. This was based on the occurrence of grade 1 skin reactions at the 50% w/v formulation-treated sites of three previously-induced test animals following topical challenge, and an absence of skin responses at the corresponding skin sites of the non-induced control animals.

No explanation was offered in the study report why there was an absence of skin responses at the skin sites of the previously-induced test animals exposed to the 71% w/v formulation of EA-2525 (Thixatrol Plus) at topical challenge. Furthermore, the grade 1 skin response that was noted in two previously-induced test animals 24-hours after topical challenge persisted in only one of these animals at the 48-hour observation, although a grade 1 skin response was seen at the 50% w/v formulation treated skin site of another animal at the 48-hour observation. Taking into consideration the fact that that a total of only 30% (3/10) animals showed a weak skin response after topical challenge, that skin responses were only produced by the 50% w/v formulation and not by the 71% w/v formulation, and that the skin response had disappeared in one animal at the 48-hour observation, it is considered that the skin responses in all three animals cannot be regarded as clear skin sensitisation responses. Given that the criterion for classification of a substance as skin sensitiser in an adjuvant-type test such as the GPMT, is the occurrence of clear skin sensitisation responses in 30% of the previously-induced test animals, it is considered that a second challenge (re-challenge) should have been conducted to confirm whether the three animals were truly sensitised to the EA-2525 (Thixatrol Plus).

As some doubt existed regarding the outcome of the test on EA-2525 (Thixatrol Plus), it was considered both necessary and justified to investigate the skin sensitisation potential of the substance, Thixatrol MAX, itself.

Local Lymph Node Assays (LLNAs) on Thixatrol Max

Choice of concentration of test item for investigation in the LLNA

In order to ensure that the skin sensitisation potential of a test item is adequately assessed in the LLNA, exposure of the skin covering the dorsum of the mouse ears should be maximised. This can usually be achieved by applying the test item to the ears at the maximum achievable concentration in a suitable vehicle. For solid test items this will require formulation of the test item in a suitable vehicle, such as one of those listed in paragraph 11 of OECD test guideline 429, i.e. acetone: olive oil (4:1), dimethyl formamide, methyl ethyl ketone, propylene glycol or dimethyl sulphoxide. The formulated test item should be stable and homogeneous, and it should also allow accurate administration and adherence of the required dose volume to the mouse ears. It is preferable to administer the test item to the ears as a solution, but if this is not possible it may be acceptable to administer as an emulsion or a fine homogeneous suspension. In order to permit an assessment of skin sensitising potency of the test item, and in accordance with the requirements of paragraph 10 of OECD 429, it is necessary to investigate at least two lower concentrations of the test item.

Difficulties of formulation of Thixatrol Max

The test item, Thixatrol Max, is a solid rheological additive intended for incorporation into various products such as paints and other surface coatings. It has the property that when mixed with some organic solvents the individual particles soften and swell and are not dissolved. It is not possible to produce a solution of the test item in such solvents and it is very difficult to produce a homogeneous suspension suitable for application to the ears of the mouse.

First LLNA conducted on Thixatrol Max (Aitchison G, 2003)

In a LLNA conducted at Safepharm Laboratories Ltd in 2003 (Project Number 1176/004), the skin sensitisation potential of the test item, Thixatrol Max, was investigated by administration to the ears of groups of four mice at concentrations of 2.5%, 5% and 10% in propylene glycol. Formulation of the test item had been attempted in the other vehicles listed in OECD 429 (cited above), and also at higher concentrations than 10% in propylene glycol, but none of these formulations were considered suitable for application to the ears in the LLNA. The Stimulation Index (SI) produced by each of the three formulations of test item in propylene glycol (2.5%, 5% and 10%) were all below 3, the threshold for classification as a skin sensitiser, and there was also no indication of a clear dose response (SIs of 1.26, 1.89 and 1.55 respectively). Despite the absence of evidence of skin sensitisation potential, it was considered that the maximum concentration of test item investigated in the study (10%) might be insufficient to conclude that the test item is not potentially a skin sensitiser. This was because some moderate skin sensitisers may not produce a SI of 3 or greater in the LLNA when tested at a concentration of 10% (it should be noted however, that in a LLNA conducted at Harlan Laboratories Ltd on the known moderate skin sensitiser phenyl acetaldehyde, a 2.5% dilution of this substance in propylene glycol produced a SI of 8.0, i.e. a clear positive response. This demonstrated the laboratory's ability to identify a substance with moderate skin sensitisation potential in the LLNA at a concentration of less than 10% in propylene glycol). It was decided that a further investigation of skin sensitisation potential using a higher concentration of test item would be necessary in order to confirm the negative result obtained in the initial LLNA.

Second LLNA conducted on Thixatrol Max (Driscoll R, 2009)

In the second LLNA conducted at Safepharm Laboratories Ltd in July 2009, the test item was formulated in corn oil (the vehicle used to formulate the structural analogue, EA-2525 9Thixatrol Plus) in the guinea pig maximisation test conducted at the Central Toxicology Laboratory, UK in 1997). This vehicle does not appear in the recommended list of vehicles in OECD 429, but the test guideline does permit the use of other vehicles if sufficient scientific rationale is supplied. It was considered that corn oil might be an appropriate choice of vehicle on the basis that it has been extensively used in skin sensitisation studies conducted in guinea pigs without any reported major concerns. Formulation trials conducted at Safepharm Laboratories demonstrated that the test item could be formulated in corn oil but that the maximum concentration considered suitable for administration to the ears of the mouse in a LLNA was 25% w/v. Higher concentrations of the test item in corn oil were considered unsuitable for application to the ears of mice. In the earlier guinea pig maximisation test the test item was formulated and applied to the skin at a maximum concentration of 71%, but it can be reasonably assumed that this was possible because the formulation would have been retained in place on the skin under a closed patch, thereby minimising loss of test item from the skin at such a high concentration. Retention of formulated test item to the skin in the LLNA using a patch system is not possible. The LLNA was conducted on the test item formulated in corn oil at concentrations of 25%, 10% and 5% w/v.

In order to demonstrate the suitability of corn oil as a vehicle for use in the LLNA, it was considered necessary to conduct a concurrent positive control study using a known moderate[†] skin sensitiser under the same test conditions. The moderate skin sensitiser that was chosen was α-Hexylcinnamaldehyde, Tech., 85% (α-HCA) and it was also tested at concentrations of 25%, 10% and 5% in corn oil. Whilst SIs of less than 3 were produced by the test item at each of the three test concentrations, with no evidence of a clear dose response, the known moderate skin sensitiser α-HCA also produced SIs of less than 3 with no evidence of a clear dose response. The corn oil alone produced high DPM values in the LLNA negative (vehicle) control animals. It was concluded that despite the absence of skin sensitisation responses to the test item, the failure to demonstrate a satisfactory skin sensitisation response to the known moderate skin sensitiser α-HCA formulated in corn oil, would not allow the conclusion to be made that the test item is not potentially a skin sensitiser, so whilst none of the two LLNAs conducted on the test item had provided any evidence of skin sensitisation potential, without additional investigations this could not be verified.

([†] moderate category assigned according to both the ECB and ECETOC schemes as discussed in ECETOC Document No. 43 “Contact Sensitisation: Classification According to Potency. A Commentary. ECETOC. ,Brussels, July 2003” and ECETOC Technical Report No. 87. “Contact Sensitisation: Classification According to Potency.” ECETOC. Brussels. April 2003. EC3’s of α-Hexylcinnamaldehyde given as 8.0.

LLNA conducted using a known moderate skin sensitiser formulated in 1% Pluronic L92 in distilled water

Subsequent to completion of the second LLNA on the test item, it became known that a 1% solution of Pluronic L92 in distilled water had been demonstrated to be a suitable vehicle for use in the LLNA, primarily for water soluble substances (Ryan et al 2003; Boverhof et al, 2008).

In order to confirm the validity of using this vehicle at Harlan Laboratories Ltd, a LLNA was conducted in July 2009 in accordance with OECD test guideline 429 using the moderate skin sensitiser α-HCA. The α-HCA was formulated and administered at concentrations of 25%, 10% and 5% v/v in the 1% Pluronic L92 in distilled water. The SIs obtained were 8.14, 2.37, and 1.3 respectively. The SI obtained at the α-HCA concentration of 25% was well above the threshold for classification as a skin sensitiser (SI of 3), whereas the SIs obtained at α-HCA concentrations of 10% and 5% were below the threshold for classification as a skin sensitiser. The EC3 was calculated to be 11.6, which is not dissimilar to the mean EC3 of 10.4 obtained at the test facility for α-HCA formulated in acetone:olive oil (4:1), the preferred vehicle for use in LLNAs (as specified by OECD 429).

As the previous LLNA conducted using concentrations of test item up to 25% was considered invalid owing to the inability to obtain a satisfactory response to the known moderate skin sensitiser α-HCA when formulated in corn oil, it was considered appropriate to investigate the possibility of conducting a LLNA on the test item at a concentration of 25% or higher in 1% Pluronic L92 in distilled water.

Third LLNA conducted on Thixatrol Max (Sanders A, 2009)

A LLNA was conducted on Thixatrol Max at Harlan Laboratories in September 2009 (Project Number 1843/0010) in accordance with OECD 429 using a 1% solution of Pluronic L92 in water as vehicle. The maximum concentration of the test item considered suitable for application to the ears of the mice using this vehicle was 25%. Higher concentrations of the test item in a 1% solution of Pluronic L92 in distilled water were considered unsuitable for application to the ears of mice. Two lower concentrations of the test item in a 1% solution of Pluronic L92 in water were also investigated in the study. The SI obtained at concentrations of 25%, 10% and 5% were 0.82, 0.91 and 0.94 respectively, all well below the threshold for classification as a skin sensitiser (SI of 3). The results of the study were clearly negative and provided the strongest evidence to date that the test item should not be regarded as a skin sensitiser.

This study using 1% Pluronic L92 in distilled water as vehicle was considered valid since it had been shown that a satisfactory sensitisation response to the known moderate skin sensitizer α-HCA could be obtained in the LLNA when administered at a concentration of 25%.

Conclusion

Sanders (2009) has been selected as the key study; reliability 1 due to the concentration of samples tested, the choice vehicle and the methodology employed.

A stimulation index of less than 3 was recorded for the test material at concentrations of 25%, 10% and 5% w/w in 1% Pluronic L92 in distilled water.

Furthermore, satisfactory sensitization response (Stimulation Index greater than 3.0) was achieved at Harlan Laboratories Ltd. with the moderate[1] skin sensitizer α-Hexylcinnamaldehyde, Tech., 85%, when prepared and administered in a number of vehicles recommended by OECD 429 (2002), and also when prepared and administered in 1% Pluronic L92 in distilled water. Further evidence that 1% Pluronic L92 in distilled water is a suitable aqueous based vehicle for use in the LLNA has been provided by Ryan et al (2002) and Boverhof

It is therefore considered that due to the conclusions of the key study and the weight of evidence of the supporting data that the substance is not a skin sensitiser.

4.5.1.4 Comparison with criteria

Study	Guinea pig maximisation test: A response from at least 30% of animals is considered positive	Local lymph node assay: A stimulation index of 3 or above is considered to be a positive response
Sanders (2009)	Not applicable	Reliability of 1 due to the concentration of samples tested, the choice vehicle and the methodology employed. A stimulation index of less than 3 was recorded for the test material at concentrations of 25%, 10% and 5% w/w in 1% Pluronic L92 in distilled water. Therefore not classified according to CLP Regulations
Driscoll (2009)	Not applicable	Study not reliable due to high DPM values in the LLNA negative (vehicle) control animals and a positive response was not obtained with the moderate[1] skin sensitiser α-Hexylcinnamaldehyde in this vehicle at concentrations of 5%,

[1] Moderate category assigned according to both the ECB and ECETOC schemes as discussed in ECETOC, (2003[a]) and ECETOC (2003[b]) EC3 (%) of α-Hexylcinnamaldehyde and phenylacetaldehyde are given as 8.0 and 4.7 respectively.

		10% and 25% v/v).
Aitchison (2003)	Not applicable	Criteria not met because the study used lower concentrations than would normally be expected (maximum of 10%), and was not accompanied by a contemporaneous validated positive control using the selected solvent (propylene glycol). For these reasons this study has been allocated a reliability ranking of 2 (Klimisch, 1996).
Lees D (1998a)	Test conducted on structural analogue, Thixatrol Plus (EA2525). With the lack of clarity regarding the results of the initial challenge due to the presence of skin reactions in test and control animals and the adherence of the test item formulations to the skin, the conclusion of the study report that the test item is a moderate skin sensitiser must be viewed as being without clear evidence and of limited reliability.	Not applicable
Lees D (1998b)	Study conducted on structurally related analogue, Thixatrol Plus. Inconsistent signs of sensitisation were observed, which did not permit interpretation as a clear sensitiser. Substance did not induce a clear sensitisation response at all dose levels. Substance was observed to have adhered to skin in two animals during the sighting study at a concentration of 50% w/v. The positive skin responses observed during challenge phase at this concentration (but not at the higher concentration of 71% w/v) may have been due to the adherence of the test substance to the animal's skin (causing irritation) and hence interpretation as a clear skin sensitiser was inconclusive.	Not applicable

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4.5.1.5 Conclusions on classification and labelling

With a stimulation index of less than 3 (Sanders 2009) at a maximum test concentration of 25% w/w, the substance is considered not to be a skin sensitiser according to Directive 67/548/EEC (Dangerous Substances Directive; DSD) or the CLP Regulation.

4.5.2 Respiratory sensitisation

Not covered in this proposal

4.6 Repeated dose toxicity

Not covered in this proposal

4.7 Germ cell mutagenicity (Mutagenicity)

Not covered in this proposal

4.8 Carcinogenicity

Not covered in this proposal

4.9 Toxicity for reproduction

Not covered in this proposal.

4.10 Other effects

Not covered in this proposal.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not covered in this proposal.

6 OTHER INFORMATION

Not covered in this proposal.

7 REFERENCES

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8 ANNEXES