Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

EC Number: **214-881-6**

CAS Number: **1205-17-0**

Index Number:

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3.5.1.1 Anonymous, 2005

Study reference

Anonymous, 2005. Information retrived from the publicly available REACH registration dossier.

Detailed study summary and results

An OECD 429 Local Lymph Node Assay (LLNA) study in mice was conducted under GLP conditions. The concentration levels of the test substance, helional (CAS no. 1205-17-0), were 2.5, 5, 10, 25 and 50 % (w/v) in 1:3 Ethanol:Diethylphtalate (EtOH:DEP).

Concurrent positive control (PC) groups were included in the study to assess intra-, and inter-laboratory reproducibility and comparability. The PC groups were exposed to hexyl cinnamic aldehyde (CAS no. 101-86-0) (HCA) in doses of 5, 10 and 25 % (w/v) with 4:1 acetone:oliveoil as a vehicle. In addition, a separate vehicle control-group (VC) for the concurrent PCs was included due to different vehicles in the PC group and dose groups.

Approximately 25 µL of the preparation was applied to the dorsal surfaces of both ears. The procedure was repeated on three consecutive days (day 1-3). On day 6, three days after the last application, the mice were humanely killed. One animal in group 4 died during thymidine dosing and was excluded from the study.

The available study summary includes no information of a pre-screen test nor scientific justification for the selection of the concentration series used. Further the choice of EtOH:DEP as a vehicle is a deviation from the OECD Guideline 429, of which a justification is not included in the summary.

The study method is summarized in Table 1.

Table 1: Study method

Test type	Local lymph node assay (LLNA): OECD Guideline 429 (Skin Sensitisation: Local Lymph Node Assay) Performed 2005
Test substance	Test substance: α-methyl-1,3-benzodioxole-5-propionaldehyde (Helional) CAS no. 1205-17-0. EC no. 214-881-6 Vehicle: 1:3 EtOH:DEP [1] PC [2]: Substance: Hexyl cinnamic aldehyde (CAS no. 101-86-0) Conc.: 5, 10 and 25 % (w/v) preparation in acetone:olive oil (4:1)
Test animals	Mice, female (young adults) Strain: CBA/Ca Animal no. per dose: 4 [3] Weight (day 1): 16.4-20.1 g.
Administration exposure	Dose-groups: 0, 2.5, 5, 10, 25 and 50 % w/v in 1:3 EtOH:DEP [1] Control-groups: One VC[4] group and three PC[2] groups Exposure: 25 µL of the preparation was applied to the dorsal surface of the ear on day 1-3

[1] Ethanol:Diethylphthalate. [2] Positive control. [3] Animal no. 59 in group 4 died during thymidine dosing and was hence excluded from the study. [4] Vehicle control.

Results

Table 2 summarizes the result for each VC-, dose- and PC-groups in the study. The available study summary is sparse and includes no further parameters to monitor the local skin response (optional in the OECD 429).

Table 2: Study results

Test concentration (% w/v)	No. lymph nodes assays	Disintegrations per Minute	DPM per lymph node	Stimulation Index (SI)	Result
Vehicle treated control (VC)					
0 (Vehicle)	8	6458	807	N/A	-
Dose					
2.5	8	6518	815	1.0	Negative (SI < 3)
5	8	17482	2185	2.7	Negative (SI < 3)
10	8	15285	1911	2.4	Negative (SI < 3)
25	6[1]	18159	3027	3.8	Positive (SI ≥ 3)
50	8	53752	6719	8.3	Positive (SI ≥ 3)

Positive control (PC)					
Vehicle (VC(PC))	8	4397	550	N/A	-
HCA 5	8	6402	800	1.5	Negative (SI < 3)
HCA 10	8	9771	1221	2.2	Negative (SI < 3)
HCA 25	8	28921	3615	6.6	Positive (SI ≥ 3)

[1]Animal no. 59 in group 4 died during thymidine dosing and was hence excluded from the study. The Registrant evaluates the integrity of the study not to be affected by the loss of one animal in a group and the Dossier Submitter agrees with this evaluation.

All control-groups confirmed the local laboratory performance and the validity of the protocol: The VC- and the VC(PC)-groups had negative results and the PC 25 % (w/v) was positive.

The test substance caused skin sensitisation when applied in 25 and 50 % (w/v) preparations, with Stimulation Index (SI) of 3.8 and 8.3, respectively.

The Estimated Concentration needed to produce a SI of 3 (EC3) was calculated to be 16.4 % w/v (4100 µg/cm^2).

Discussion

The Registrant evaluates the study reliable without restrictions – Klimish 1 (Klimish et al. 1997).

EtOH:DEP was used as a vehicle in the dose-groups, which is a deviation from the OECD Guideline 429. Vehicles not recommended in the Guideline can be used if sufficient scientific rationale is provided. A rationale was not available in the available study summary or in the study report.

The use of EtOH:DEP as a vehicle in a LLNA assay have been discussed in relation to previous CLH proposals, e.g. citral (CAS no. 5392-40-5). In the RAC Opinion proposing harmonised classification and labelling of citral (ECHA, 2018) the use of EtOH:DEP was discussed and the vehicle was concluded to be acceptable in the conducted LLNA (ECHA, 2018). For these reasons, the Dossier Submitter evaluates the vehicle as suitable.

Conclusion

Helional was shown to be sensitising with an EC3 of 16.4 %.

3.5.2 Human data

3.5.2.1 Bennike et al., 2019

Study reference

Bennike, N.H., Zachariae, C., Johansen, J.D. Optimal patch test concentrations for three widely used sensitizing fragrance substances without mandatory labelling in cosmetics. Contact Dermatitis, 2019, 80, 325-327.

Detailed study summary and results

The objective of the study was identification of an optimal patch test concentration for three widely used sensitising fragrances including helional (CAS no. 1205-17-0), purity ≥ 98%. The study was conducted according to a protocol published by the European Society of Contact Dermatitis (ESCD).

484 consecutive dermatitis patients, aged ≥18 years, were referred to the department of Dermatology and Allergy, Copenhagen University Hospital Herlev and Gentofte (Hellerup, Denmark) and tested in five different dose groups (n ≈ 100). Exclusion criteria and scoring of patch test results were conducted according to the ESCD '*Guideline for diagnostic patch testing – recommendations on best practice*'.

A starting concentration of 3.0 % (w/w) was used for patch testing helional followed by concentrations of 4.5 %, 6.8 %, 10.1 % and 15.2 %, with an occlusion time of two days. Reading was performed on day 2-5 and day 7. Interim evaluations of the patch test results were performed to assess the individual concentrations before increasing (by 50 %) or decreasing (by 33 %) in the next dose group as described in the ESCD Guideline. To record induced contact allergy (skin sensitisation) patients were told to contact the department if reactions occurred after final visit. In all no contact allergy (skin sensitisation) was discovered to be induced in the study and no more than a few irritant reactions were registered, which lead to an increase in all the following doses.

The patch tests were conducted by applying 20 mg of helional suspended in petrolatum (pet.) to the upper back in Finn Chambers (8mm; SmartPractice, Phoenix, Arizona), with an occlusion time of two days. Reading was performed on day 2-5 and day 7.

Table 3: Study results

Concentration (% (w/w))	Total number of patients	Positive reactions (no.)	Doubtfull reactions (no.)	Irritant reaction (no.)
3.0 %	100	0	0	0
4.5 %	104	2	0	0
6.8 %	103	1	0	0
10.1 %	100	0	0	1
15.2 % [1]	87	1	1	0

[1] Maximum allowed patch test concentration

Of the 494 patch tests performed four (0.8%, 95% confidence interval: 0.3-2.1%) had a positive reaction to helional. The authors of the study reports of clearly allergic positive patch test reactions to helional. The study resulted in recommendations of patch testing helional at 7.5 % (w/w) pet (3.0 mg/cm2).

3.6 Germ cell mutagenicity

Hazard class not assessed in this dossier

3.7 Carcinogenicity

Hazard class not assessed in this dossier

3.8 Reproductive toxicity

Hazard class not assessed in this dossier

3.9 Specific target organ toxicity – single exposure

Hazard class not assessed in this dossier

3.10 Specific target organ toxicity – repeated exposure

Hazard class not assessed in this dossier

3.11 Aspiration hazard

Hazard class not assessed in this dossier

4 ENVIRONMENTAL HAZARDS

Hazard class not assessed in this dossier

5 REFERENCES

Anonymous, 2005. Information retrived from the publicly available REACH registration dossier.

Bennike, N.H., Zachariae, C., Johansen, J.D. (2019). Optimal patch testconcentration for three widely used sensitizing fragrance substances without mandatory labelling in cosmetics. Contact Dermatitis, 2019, 80, 325-327.

ECHA (2018). RAC Opinion proposing harmonised classification and labelling at EU level of citral; 3,7-dimethylocta-2,6-dienal. Adopted 14 September 2018.