MCL Report for: Pigment Red 83

Proposal for mandatory classification and labelling (MCL) based on Annex VI, Part 2 of the retained CLP Regulation (EU) No. 1272/2008 as amended for Great Britain

EC Number: 200-782-5

CAS Number: 72-48-0

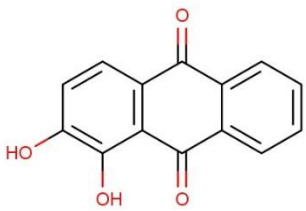
Date: November 2023

Contents

Identity of the substance

Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance (Source: ECHA, 2023)

Name(s) in the IUPAC nomenclature or other international chemical name(s)	1,2-dihydroxy-9,10-dihydroanthracene-9,10-dione
Other names (usual name, trade name, abbreviation)	1,2-dihydroxy-9,10-anthraquinone; 1,2-dihydroxyanthracene-9,10-dione 1,2-Dihydroxyanthraquinone Alizarin Mordant Red 11
ISO common name (if available and appropriate)	Pigment Red 83
EC number (if available and appropriate)	200-782-5
EC name (if available and appropriate)	N/A
CAS number (if available)	72-48-0
Other identity code (if available)	N/A
Molecular formula	$C_{14}H_8O_4$
Structural formula	
SMILES notation (if available)	OC1=C(O)C2=C(C=C1)C(=O)C1=CC=CC=C1C2=O

Molecular weight or molecular weight range	240.21 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	N/A
Description of the manufacturing process and identity of the source (for UVCB substances only)	N/A
Degree of purity (%) (if relevant for the entry in Annex VI)	N/A

1.1 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current MCL on GB MCL list (if applicable)
Pigment Red 83	100%	No current entry

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

No impurities relevant for classification

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current MCL on GB MCL list (if applicable)	The impurity contributes to the classification and labelling?
N/A	N/A	N/A	N/A

Table 4: Additives (non-confidential information) if relevant for the classification

No additives relevant for classification

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current MCL on GB MCL list (if applicable)	The additive contributes to the classification and labelling?
N/A	N/A	N/A	N/A	N/A

2. Proposed mandatory classification and labelling

Table 5: Proposed mandatory classification and labelling according to the GB CLP criteria

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current GB MCL list entry	No current GB MCL list entry.										
Proposed Classification	TBD	1,2-dihydroxy-9,10-dihydroanthracene-9,10-dione (Pigment Red 83)	200-782-5	72-48-0	Skin Sens. 1B	H317	GHS07 Wng	H317	N/A	N/A	N/A
Proposed entry on the MCL list	TBD	1,2-dihydroxy-9,10-dihydroanthracene-9,10-dione (Pigment Red 83)	200-782-5	72-48-0	Skin Sens. 1B	H317	GHS07 Wng	H317	N/A	N/A	N/A

Table 6: Reason for not proposing mandatory classification and status under public consultation

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

3. History of the classification and labelling

Pigment Red 83 does not have an existing entry on the GB MCL list and has not been considered in the GB MCL or EU CLH processes to date. In the EU, 73 notifiers have submitted self-classifications for this substance to ECHA's Classification and Labelling Inventory[1]. A breakdown of these notifications is provided below:

39 notifiers have classified as Acute Tox. 4 (H302).

19 notifiers have classified as Skin Irrit. 2 (H315); Eye Irrit. 2 (H319).

4 notifiers have classified as Eye Irrit. 2 (H319).

2 notifiers have classified as Acute Tox. 4 (H302) and Eye Irrit. 2 (H319).

1 notifier has classified as Skin Sens. 1 (H317); Eye Dam. 1 (H318) and Aquatic Chronic 1 (H410) with an M-factor of 1.

8 notifiers have not classified.

[1] Available at https://echa.europa.eu/home

4. Justification that action is needed

The Health and Safety Executive, in its capacity as the Agency for UK REACH, has prepared a proposal to restrict certain hazardous substances in inks used for tattooing and permanent makeup (PMU) in accordance with Article 69(1) of UK REACH[2].

The proposed restriction applies to substances that have mandatory classification for various hazards, including skin sensitisation (H317). It also applies to substances that are prohibited for use in cosmetic products under Regulation (EC) No 1223/2009 of the European Parliament and of the Council on cosmetic products (as amended) (hereafter referred to as the Cosmetic Products Regulation). HSE considered if certain substances, which are brought into scope because they are listed in Annex II of the Cosmetic Products Regulation, should be derogated from the restriction. Annex II of the Cosmetic Products Regulation prohibits the use of these substances in hair dyes. However, as they are also listed in Annex IV of the Cosmetic Products Regulation, they are permitted for use as colourants in some other cosmetic products. HSE has therefore reviewed the available hazard information on these substances and determined that for two of them (Pigment Red 83, CAS 72-48-0; and Solvent Violet 13, CAS 81-48-1) there is a potential concern for skin sensitisation. A mandatory classification for skin sensitisation (H317) would bring the substances into scope of the proposed restriction.

Therefore, HSE as the Agency for GB CLP (hereafter referred to as ‘the Agency’) has prepared this targeted report to propose the mandatory classification and labelling of Pigment Red 83 for skin sensitisation. The substance has not been included in the proposed restriction derogation.

[2] Restrictions - HSE

5. Identified uses

Pigment Red 83 is used in the textile industry for fabrics, textiles and apparel (ECHA, 2023).

6. Data sources

The data used for this report comes from the publicly available ECHA dissemination site (ECHA, 2023).

The Lhasa Derek Nexus skin sensitisation defined approach ITSv1 1.0 was used to conclude on sub-categorisation. This approach is outlined in OECD Guideline 497.

7. Physicochemical properties

Table 7: Summary of physicochemical properties (source: ECHA, 2023)

Property	Value	Reference	Comment (e.g., measured or estimated)
Physical state at 20°C and 101,3 kPa	Yellow solid substance	Anon (2017)	Visual inspection Observation made during water solubility test GLP
Melting/freezing point	289-290 °C	Lide, (1993)	No method/guideline specified Non GLP
Boiling point	430 °C	Lide, (1993)	No method/guideline specified Non GLP
Relative density	1.575 relative to the density of water at 4°C (0.999972 g/cm3). Measured at 20 °C	Anon (2017)	GLP Air comparison pycnometer (for solids) method
Vapour pressure	1.2×10^{-7} Pa at 25 °C Low volatility	Anon (1973)	OECD TG 104 (Vapour Pressure Curve) equivalent Effusion method: Knudsen cell Non GLP
Surface tension	N/A	N/A	Study not required due to structure. Surface activity is not expected or cannot be predicted
Water solubility	0.483 mg/L (20 °C, pH 4.5)	Anon (2017)	OECD TG 105 Council Reg (EC) No 440/2008 Method A.6 Column elution method GLP

Property	Value	Reference	Comment (e.g., measured or estimated)
Partition coefficient n-octanol/water	Log Pow at 20 °C was 2.91	Anon (2018)	OECD TG 117 HPLC method GLP
Flash point	N/A	N/A	N/A
Flammability	Not flammable	Anon (2017)	UN Manual of Tests and Criteria: Test N.1 GLP
Explosive properties	N/A There are no chemical groups present in the molecule which are associated with explosive properties	N/A	N/A
Self-ignition temperature	>400 °C	Anon (2018)	Guideline VDI 2261 GLP
Oxidising properties	N/A There are no chemical groups present in the molecule which are associated with oxidising properties.	N/A	N/A
Granulometry	The L10 D was 0.73 µm The L50 D was 2.12 µm The L90 D was 11.33 µm	Anon (2017)	Guideline CIPAC MT 187 GLP
Stability in organic solvents and identity of relevant degradation products	N/A	N/A	N/A
Dissociation constant	N/A	N/A	N/A
Viscosity	N/A	N/A	N/A

8. Evaluation of physical hazards

Not assessed.

9. Toxicokinetics (absorption, distribution, metabolism and excretion)

No experimental toxicokinetic data are available. The physicochemical properties of substances can inform on their potential to be absorbed dermally. Moderate octanol-water partition coefficient (log P_{ow}) values between -1 and 4 and relatively low molecular weights (MW) below 500 g/mol are favourable for absorption.

The substance's Log P_{ow} of 2.91 and MW of 240.21 g/mol indicate that dermal absorption is likely.

10. Evaluation of health hazards

10.1 Acute toxicity – oral route

Not assessed.

10.2 Acute toxicity – dermal route

Not assessed.

10.3 Acute toxicity – inhalation route

Not assessed.

10.4 Specific target organ toxicity – single exposure (STOT SE)

Not assessed.

10.5 Skin corrosion/irritation

Not assessed.

10.6 Serious eye damage/eye irritation

Not assessed.

10.7 Respiratory sensitisation

Not assessed.

10.8 Skin sensitisation

The skin sensitisation potential of Pigment Red 83 has been tested in two GLP-compliant mechanistic studies based on recently-developed and internationally validated test protocols: *in chemico*, direct peptide reactivity assay (DPRA; OECD TG 442C (OECD, 2023a); and *in vitro*, human cell line activation test (h-CLAT; OECD TG 442E (OECD, 2023b)).

The DPRA and h-CLAT each address a specific key molecular event (KE) defined in the adverse outcome Pathway (AOP) for skin sensitisation (AOP:40[3]; OECD, 2014), specifically, covalent binding to proteins (the molecular initiating event (MIE) / key event (KE) 1) and activation of dendritic cells (KE3).

Table 8: Summary of available studies on skin sensitisation (source: ECHA, 2023)

Method, guideline, deviations if any	Test system	Test substance	Concentration levels, duration of exposure	Results
DPRA Guideline: OECD TG 442C (2023a) Deviations: none GLP Reference: Anon, 2017	Reference control: undiluted (acetonitrile); Positive control (Cinnamic aldehyde ((2E)-3-phenylprop-2-enal)	Pigment Red 83 (1,2-dihydroxyanthraquinone) Purity: not reported Test material: solid Vehicle: dimethylformamide (DMF) Solubility in DMF: 100mM	100 mM Ratios of peptide / test item: 1:10 (cysteine) and 1:50 (lysine) 24 ± 2 h incubation in the dark at 25 ± 2.5°C Positive control: cinnamic aldehyde (100 mM)	**Positive indication of covalent binding to protein (KE1)** **Mean peptide depletion (MPD): 80.95% (cysteine)** Reported as having 'moderate' reactivity (23.09% < MPD (cysteine) ≤ 98.24%) The controls used satisfied the acceptance criteria.
h-CLAT Guideline: OECD TG 442E (2023b) Deviations: none GLP Reference: Anon, 2018	THP-1 cell line; cultured in RPMI medium suppl. with 10% FBS, 25 nM HEPES, 2 mM L-glutamine, 0.05 mM 2-mercaptoethanol and pen/strep mix @ 37 ± 1°C and 5% CO_2 Controls: medium control, solvent control (2% DMSO v/v in cell culture medium); positive control (4 µL/mL DNCB)	Pigment Red 83 (1,2-dihydroxyanthraquinone) Purity: not reported Test material (solid) dissolved in DMSO @ 250 mg/mL (highest soluble conc.); stock solutions prepared by serial dilution	Dose-finding assay: 500, 250, 125, 62.5, 31.25, 15.63, 7.81 and 3.91µg/mL Main experiment: 67.23, 55.83, 46.53, 38.77, 32.31, 26.93; 22.44 and 18.7 µg/mL Exposure conditions: 24 h @ 37 °C, 5% CO_2	**Positive indication of dendritic-cell activation** (CD86 & CD54 upregulated above threshold in 2/3 experimental repeats) Thresholds for a positive result = 1.5x upregulation of CD86 cell surface marker; 2x upregulation of CD54 cell surface marker (Results of individual experiments shown in Table 8 below) Positive & negative controls gave the expected/valid results.

[3] https://aopkb.org/aopwiki/index.php/Aop:40

Method, guideline, deviations if any	Test system	Test substance	Concentration levels, duration of exposure	Results
	8 doses tested	(7 x 1:2 dilution); test solutions prepared by diluting stock solutions 250-fold in cell culture medium		

Table 9: Results of the h-CLAT study (source: ECHA, 2023)

Experiment no.	Marker	Concentration (µg/mL)	Response (%)
1	CD86	NA	Negative
2	CD86	56.03	> 150
1	CD54	46.69	> 200
2	CD54	39.91	> 200

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

The skin sensitising potential of Pigment Red 83 was investigated in two modern GLP- and OECD-compliant *in chemico* and *in vitro* assays, the direct peptide reactivity assay (DPRA; OECD TG 442C) and the human cell line activation test (h-CLAT; OECD TG 442E), respectively. The purity of the test substance was not reported for either study, however the Agency still considers the studies to be suitable for classification purposes.

In the DPRA, the initial pre-experiment investigated the solubility of the substance and determined that dimethylformamide (DMF) was the most appropriate solvent to be used for the main study. The test substance was then incubated with either cysteine or lysine, in accordance with the relevant OECD test guideline (TG 442C), and subsequently analysed by HPLC to quantify the mean peptide depletion for each of the aforementioned two peptides.

Owing to the observed co-elution of the test substance with one of the peptide analytes, lysine, the authors used a prediction model based solely on cysteine depletion, as per OECD TG 442C. This showed a moderate peptide reactivity of 80.95%, defined as a positive result.

The *in vitro* h-CLAT study was performed in accordance with OECD TG 442E on the monocytic leukaemia cell line, THP-1. Cell cultures were exposed for 24 hours to 8 concentrations of the test substance.

An initial assay was conducted to identify suitable concentrations of Pigment Red 83 for the main test. A concentration range of 18.76 to 67.23 to µg/mL was subsequently used in the main experiment. The main experiment demonstrated significant upregulation of cell surface markers CD86 and CD54 above the defined thresholds of 150% and 200% for each biomarker, respectively, quantified by fluorescence-activated cell sorting (FACS) analysis in two independent experimental repeats.

CD54 was upregulated up to 446% and 1845% in each of the two repeats, respectively. The upregulation above the threshold (200%) was observed at concentrations starting at 46.69 µg/mL and 38.91 µg/mL in the first and second repeats, respectively. With regard to CD86, while the first repeat did not show significant upregulation of this marker, in the second repeat it was upregulated up to 352%; concentrations of 56.03 µg/mL and 46.69 µg/mL resulted in upregulation over the defined threshold (150%). The positive control, 2,4-dinitrochlorobenzene (DNCB), and solvent and cell culture-medium controls all performed within the acceptability range.

Positive observations of upregulation of CD54 in each of the two repeats, and a positive observation of upregulation of CD86 in the second repeat, jointly satisfy the criteria laid out in OECD TG 442E for the h-CLAT prediction to be considered positive.

Integration of information from the DPRA and h-CLAT enables hazard identification with respect to skin sensitisation, i.e., allows discrimination between skin sensitisers and non-sensitisers (GB CLP Cat 1 and no classification, respectively) by application of the so-called “2 out of 3” (2o3) defined approach (DA). However, this DA does not allow for a potency sub-categorisation and differentiation between Cat 1A and Cat 1B.

OECD Guideline 497 on Defined Approaches for Skin Sensitisation (June 2021) provides data interpretation procedures to interpret *in chemico* (KE1), *in vitro* (KE3) and *in silico* predictions, allowing a distinction between GB CLP skin sensitisation sub-categories 1A and 1B.Therefore, the Agency has used ITSv1 of this DA through use of the DPRA and h-CLAT results together with Lhasa Limited’s Derek Nexus Skin Sensitisation Defined Approach ITSv1 1.0[4]. This approach resulted in a prediction of Skin Sensitisation Category 1B (overall score of 5; see Annex I).

Combined, the DPRA and h-CLAT predictions indicate a hazard classification for Pigment Red 83 as a skin sensitiser. Additional application of the *in silico* prediction model Derek Nexus (i.e. the ITSv1 DA) allows determination of its potency.

[4] Lhasa Limited's Defined Approach

10.8.2 Comparison with the GB CLP criteria

Currently, the GB CLP criteria for skin sensitisation are based on animal and human data. However *in silico*, *in vitro and in chemico* data are still relevant, and can be used for classification as part of a weight-of-evidence assessment. The United Nations Globally Harmonised System of Classification and Labelling of Chemicals (UN GHS, on which GB CLP is based) is being revised to take account of the new approaches that can be used to predict skin sensitisation hazard and potency.

The fixed data interpretation procedure presented in OECD Guideline 497 (ITSv1) provides a defined approach to predict skin sensitisation hazard and potency from DPRA and h-CLAT experimental data together with a Derek Nexus prediction. This approach results in a prediction with high confidence of skin sensitisation Category 1B for Pigment Red 83, in accordance with Table A2.2 of OECD Guideline 497 (scenario 1: all of the information sources, i.e. *in chemico*/*in vitro* outcomes, are applicable and can be considered (as prescribed in each individual assay) and the *in silico* prediction is in domain).

10.8.3 Conclusion on classification and labelling for skin sensitisation

The available *in chemico* and *in vitro* experimental data integrated with *in silico* prediction using the ITSv1 DA indicates that **Pigment Red 83 should be classified as Skin Sens. 1B; H317 (May cause an allergic skin reaction).**

10.9 Specific target organ toxicity – repeated exposure (STOT RE)

Not assessed.

10.10 Germ cell mutagenicity

Not assessed.

10.11 Carcinogenicity

Not assessed.

10.12 Reproductive toxicity

Not assessed.

10.13 Aspiration hazard

Not assessed.

11. Evaluation of environmental hazards

11.1 Rapid degradability of organic substances

Not assessed.

11.2 Environmental transformation of metals or inorganic metals compounds

Not assessed.

11.3 Environmental fate and other relevant information

Not assessed.

11.4 Bioaccumulation

Not assessed.

11.5 Acute aquatic hazard

Not assessed.

11.6 Long-term aquatic hazard

Not assessed.

12. Evaluation of additional hazards

12.1 Hazardous to the ozone layer

Not assessed.

13. Additional labelling

No additional labelling is proposed.

14. References

ECHA (2017) Guidance on the application of the CLP criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, version 5.0, ref: ECHA-17-G-21-EN. Available at https://www.echa.europa.eu/

ECHA (2023) ECHA's dissemination website: http://echa.europa.eu/

Lide (1993) CRC Handbook of Chemistry and Physics, 73rd Edition. ISBN 0-8493-0473-3

OECD (2014), *The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins*, OECD Series on Testing and Assessment, No. 168, OECD Publishing, Paris. Available at OECD iLibrary (oecd-ilibrary.org)

OECD (2023a) Test Guideline No. 442C *In Chemico* Skin Sensitisation Assays addressing the Adverse Outcome Pathway key event on covalent binding to proteins. Available at OECD iLibrary (oecd-ilibrary.org)

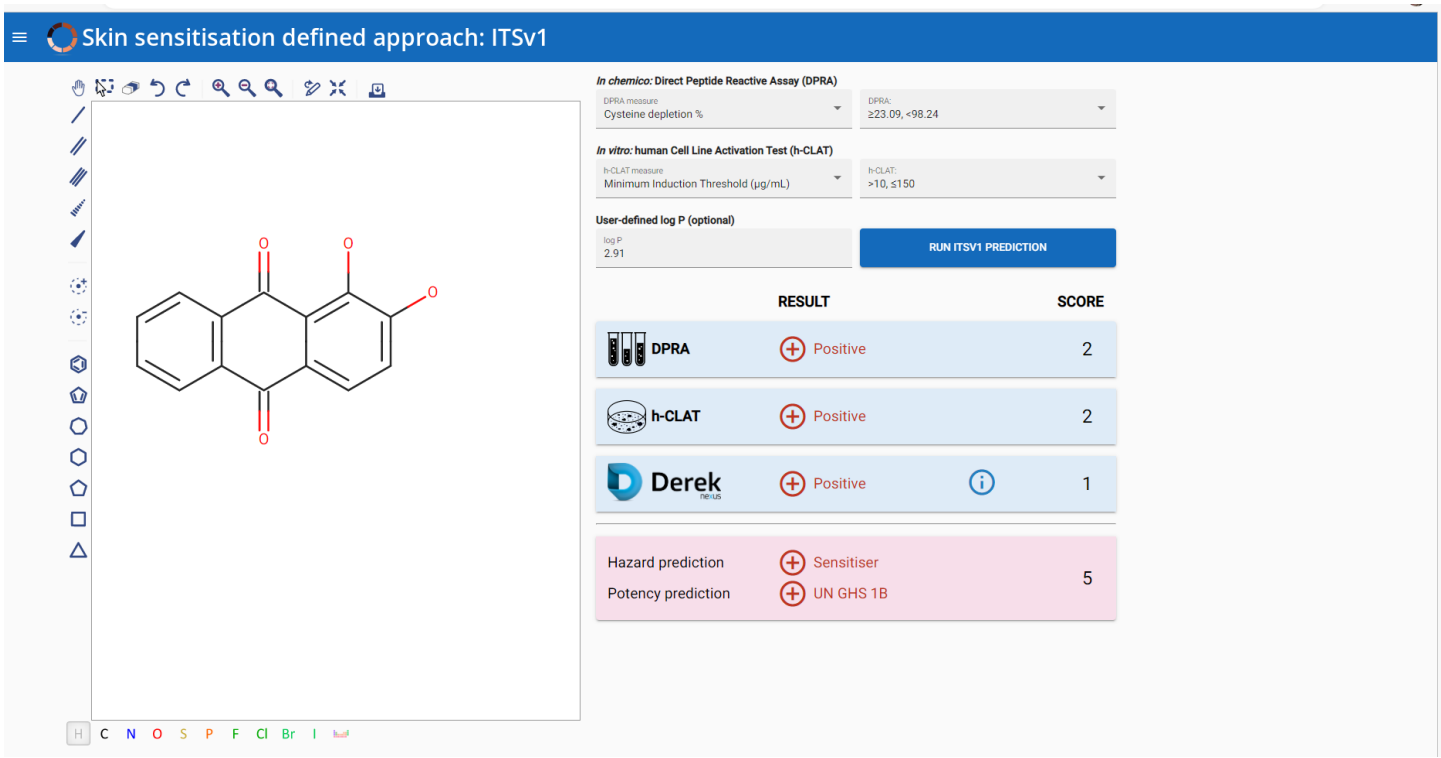
OECD (2023b) Test Guideline No. 442E *In Vitro* Skin Sensitisation - *In Vitro* Skin Sensitisation assays addressing the Key Event on activation of dendritic cells on the Adverse Outcome Pathway for Skin Sensitisation. Adopted: 30 June 2022 Corrected: 4 July 2023. Available at OECD iLibrary (oecd-ilibrary.org)

UK REACH Agency (2023) Agency opinion on the Annex 15 dossier proposing restrictions on Substances in tattoo ink and permanent make-up. Available at https://www.hse.gov.uk/reach/restrictions/tattoo-inks/opinion.pdf

15. Annexes

Annex I: Lhasa Derek Nexus output

Screenshot of Lhasa Derek Nexus skin sensitisation defined approach (ITSv1) prediction (1 March 2023)



Further information

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