Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

tetrakis(2,6-dimethylphenyl)-*m*-phenylene biphosphate; tetrakis(2,6-dimethylphenyl) 1,3-phenylene bis(phosphate)

EC Number: 432-770-2
CAS Number: 139189-30-3

CLH-O-0000001412-86-291/F

Adopted

13 June 2019

Annankatu 18, P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

13 June 2019

CLH-O-0000001412-86-291/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: **tetrakis(2,6-dimethylphenyl)-*m*-phenylene biphosphate; tetrakis(2,6-dimethylphenyl) 1,3-phenylene bis(phosphate)**

EC Number: **432-770-2**

CAS Number: **139189-30-3**

The proposal was submitted by **the United Kingdom** and received by RAC on **30 August 2018.**

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

PROCESS FOR ADOPTION OF THE OPINION

The United Kingdom 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 **8 October 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **7 December 2018**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Ralf Stahlmann**

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 **13 June 2019** by **consensus**.

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

	Index No	International Chemical Identification	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 Annex VI entry	015-192-00-1	tetrakis(2,6-dimethylphenyl)-*m*-phenylene biphosphate	432-770-2	139189-30-3	Skin Sens. 1	H317	Wng	H317			
Dossier submitters proposal	015-192-00-1	tetrakis(2,6-dimethylphenyl)-*m*-phenylene biphosphate; tetrakis(2,6-dimethylphenyl) 1,3-phenylene bis(phosphate)	432-770-2	139189-30-3	**Remove** Skin Sens. 1	**Remove** H317	**Remove** Wng	**Remove** H317			
RAC opinion	015-192-00-1	tetrakis(2,6-dimethylphenyl)-*m*-phenylene biphosphate; tetrakis(2,6-dimethylphenyl) 1,3-phenylene bis(phosphate)	432-770-2	139189-30-3	**Remove** Skin Sens. 1	**Remove** H317	**Remove** Wng	**Remove** H317			
Resulting Annex VI entry if agreed by RAC and COM	No entry in Annex VI of CLP										

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

The harmonised classification of tetrakis(2,6-dimethylphenyl)-*m*-phenylene biphosphate (elsewhere in this document referred to as: PX-200) was translated from the Dangerous Substance Directive (DSD) to Regulation (EC) 1272/2008 (CLP) as Skin Sens. 1 (H317) and Aquatic Chronic 4 (H413). The classification for aquatic chronic toxicity (Aquatic Chronic 4; H413) was removed from Annex VI of CLP following the RAC opinion adopted on 30/11/2012, based on additional data. The harmonised classification for skin sensitisation was retained due to a lack of adequate data to re-assess this hazard class.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) summarised a standard OECD Test Guideline (TG) 404 (GLP-compliant) study in rabbits (Anonymous, 1995) and a human patch test study using 20 volunteers (Yanagimoto, 2002) in the CLH report. The DS proposed no classification for skin irritation in the absence of any evidence for skin reaction in rabbits and human volunteers.

Comments received during public consultation

One individual and two Member States Competent Authorities (MSCAs) commented and agreed with the proposal from the DS that PX-200 does not warrant classification as a skin irritant according to CLP.

Assessment and comparison with the classification criteria

Human Data

PX-200 was tested in 20 Japanese human volunteers (19 males, 1 female) in an occlusive patch test for 48 hours using 0.1 g of neat substance under a circular cloth fixed with adhesive tape. A small amount of petrolatum jelly was used to adhere the test substance. The same conditions, but without PX-200, were used for the individuals serving as controls. No skin reactions were reported in either the exposed areas or the control areas.

Animal Data

In a guideline and GLP-compliant acute dermal irritation/corrosion assay in 3 female New Zealand White rabbits, 100 % of PX-200 moistened with water produced no observable skin reactions after semi-occlusive exposure for 4 hours. All mean scores after 24, 48 and 72 hours were 0.

According to the CLP criteria, classification for skin irritation is triggered when mean scores of $\geqslant$ 2.3 - $\leqslant$ 4.0 for erythema/eschar or for oedema in at least 2 out of 3 tested animals from gradings at 24, 48 and 72 hours are observed. This was not the case with PX-200. Additionally, no irritative effects were observed in humans after exposure to PX-200 for 48 hours. Further evidence that classification is not justified is provided by the fact that no skin reactions were observed in the

human patch test described in the skin sensitisation section (see below). Therefore, RAC concurs with the DS that **classification of PX-200 for skin irritation is not justified**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS summarised three standard *in vivo* studies, three *in chemico*/*in vitro* studies, and a human volunteer study in the CLH report.

In chemico/in vitro studies

The skin sensitisation potential of PX-200 was investigated *in chemico* in a direct peptide reactivity assay (DPRA) and two *in vitro* tests, i.e. an ARE-Nrf2 Luciferase test (KeratinoSens™) and a human cell line activation test (h-CLAT). Each of these tests investigated a different key event in the Adverse Outcome Pathway (AOP) for skin sensitisation (organic chemicals, OECD 2012). As each test only addressed one event in the pathway, the DS informed that a single test is not sufficient to conclude on the skin sensitisation potential of a chemical. However, data generated via the tests can be used as part of an integrated approach, or can be considered alongside other available data in a weight of evidence approach.

According to the DS, all three *in chemico/in vitro* studies were negative, and no evidence of skin sensitising potential was demonstrated in any test. However, the DS raised concerns that the h-CLAT study may not have been valid, due to the log Po/w value (see further below) of PX-200 falling outside the range specified in the test guideline, and there were similar concerns regarding the KeratinoSensTM assay. The DS nevertheless concluded that the studies do not provide any evidence for a sensitising potential of PX-200, and the negative results are consistent with the negative results obtained in the *in vivo* Buehler and Local lymph node assay (LLNA) studies.

In vivo animal studies

The DS summarised an *in vivo* Magnusson & Kligman Maximisation Study in Guinea pigs (GPMT) (Anonymous, 1999) conducted according to OECD TG 406 (GLP-compliant) which was the basis for the current harmonised classification as Skin Sens. 1 (H317). Intradermal injection (day 1) was conducted with or without Freund's Complete Adjuvant at 5% w/v PX-200. Topical induction (day 7) was done with 75% w/w PX-200 and topical challenge (day 21) with 75% and 50% w/w PX-200. The results showed that PX-200 induced a 40% (4/10) sensitisation rate at 50% w/w PX-200 (24-h reading) and a 30% (3/10) sensitisation rate at 75% w/w PX-200 (24-h reading). The sensitisation rate was reduced by 10% at both concentrations at the 48-h reading. According to the test guideline (OECD TG 406), a response of at least 30% in an adjuvant test should be expected for mild to moderate sensitisers. The DS concluded that a substance should be classified as Skin Sens. 1 if at least 30% of animals respond in an adjuvant type test, confirming the existing classification.

The DS further assessed a non-GLP Buehler test (3 applications) (Anonymous, 2008) as well as a recent GLP-compliant LLNA (BrdU-ELISA, OECD TG 442B) (Anonymous, 2017). The DS considered both tests negative while recognising that the LLNA assay, although reliable, did not allow a direct comparison with the CLP criteria, unlike an LLNA conducted according to OECD TG 429. The DS used the Guidance on the Application of the CLP Criteria (CLP Guidance; ECHA, 2017) and the stimulation index (SI) value of < 1.6 to conclude that PX-200 was non-sensitising in the LLNA conducted.

Human study

The skin sensitisation potential of PX-200 was assessed in 58 volunteers (males and females) according to the Resolution CNS no. 466/2012, and in the spirit of Good Clinical Practices (Pessoto Rosa, 2017). There were 9 applications in the first three weeks (induction period) and one application in the last week (challenge period) at a dose of 0.05 g/cm² PX-200 (1 cm² disk). During the study, no subjects presented clinical signs on the skin related to treatment with PX-200 and at the end of the challenge phase, no positive skin reactions were observed. The DS considered that the study was well conducted and suitable for inclusion in the weight of evidence assessment. It was concluded by the DS that the substance did not induce skin sensitisation in human volunteers, thus supporting no classification.

In addition to providing an analysis the key events of the AOP (OECD, 2012), the DS argued that in order for a substance to cause sensitisation it must be bioavailable, i.e., it must penetrate the stratum corneum of the skin (OECD, 2012). Although no data on dermal absorption are available, PX-200 has a very high log P (measured >6.2), very low water solubility (1.01E-04 g/L) and a high molecular weight (687.0), which suggests it does not easily penetrate the viable epidermis.

Overall, the DS considered that the substance does not meet the criteria for classification under the conditions of the *in vivo* tests (Buehler and LLNA) and the human volunteer study and proposed no classification for skin sensitisation using a weight of evidence approach.

Comments received during public consultation

One individual and one MSCA commented and agreed with the proposal from the DS that PX-200 should not be classified as a skin sensitiser, based on a weight of evidence assessment. Another MSCA questioned the sensitivity of the human study and the Buehler test to detect weak sensitisers and the low, non-irritant concentration (50%) tested in the LLNA study, which contradicted the well-conducted (positive) GPMT. The DS replied that the GPMT was not conducted with the preferred vehicle and that the reliable LLNA assay was conducted at concentrations in accordance with the test guideline as well as an independent peer review evaluation of the assay (ICCVAM, 1999). The highest concentration should maximise exposure while avoiding systemic toxicity and/or excessive local skin irritation. The DS considered that "there is no specific 'aim' in the LLNA to induce a certain level of irritation", and in the case of PX-200 (a solid), 50% was the maximum concentration that could be achieved in acetone-olive oil.

Assessment and comparison with the classification criteria

Human Data

In an epicutaneous test in 58 volunteers, no clinical signs related to the test substance were observed. The test was conducted according to the principles applied for the HRIPT with 9 induction applications of 0.05 g PX-200/cm^2, and one challenge application for 48 hours after at least 10 days of a rest period. Although the test cohort was small, RAC notes that the tested dose of 0.05 g/cm^2 (i.e. 50 000 µg/cm^2) was relatively high in comparison to the threshold of 500 µg/cm^2, mentioned in the CLP Guidance to discriminate between sub-categories 1A and 1B in such tests. It seems reasonable to conclude that PX-200 is at least not a sensitiser with high potency. On the other hand, due to its chemical properties and given that the substance was applied undissolved, no or very limited dermal absorption may have taken place.

Animal studies

In a non-GLP compliant Buehler assay, no sensitisation was observed in any tested animal at an induction concentration of 50 % w/v PX-200 in propylene glycol (PG) and a challenge concentration of 25 % w/v PX-200 in PG. However, only 10 test animals and 5 controls were used. The OECD TG 406 states: "*When fewer than 20 test and 10 control guinea pigs have been used, and it is not possible to conclude that the test substance is a sensitiser*, *testing in additional animals to give a total of at least 20 test and 10 control animals is strongly recommended*". Thus the small number of animals used lowers the reliability of the results obtained in this study. Furthermore, the Buehler assay is in general less sensitive than a GPMT or a LLNA assay. Therefore, results from this assay are regarded as less relevant for classification purposes.

In a recent BrdU-LLNA which had no deviations from the guideline and was performed under GLP conditions with up to of 50 % w/v PX-200 in acetone:olive oil (AOO), the SI were 1.0, 1.0, and 0.9 for 10, 20, and 50 % PX-200, respectively. These are clearly negative results. After consulting industry, the DS confirmed that 50 % PX-200 was indeed the maximum attainable concentration in AOO. Concerning the choice of vehicle there is some evidence from the literature that AOO actually tends to produce false positive skin sensitisation results (Montelius, 1996).

In the guideline and GLP-compliant GPMT on which the current classification is based, 4 out of 10 animals showed positive reactions after a challenge dose of 50 % w/w PX-200 in arachis oil, but only 3 out of 10 animals reacted to a challenge dose of 75 % w/w PX-200. This is considered as a borderline positive result (relatively high induction concentration of 5 %, but relatively low incidence at high challenge concentration of 75 %). RAC notes that while in the LLNA concentrations were given as % w/v, in the GPMT study concentrations were reported as % w/w. Thus, translated to w/v concentrations using the relative density of arachis oil, positive reactions in the GPMT were observed at 46 % and 69 %, respectively (for details see supplemental information section in the Background Document).

Reactions were reversible in at least one animal in each dose group, which in RAC's opinion may indicate an irritative rather than a sensitising response. Furthermore, there are indications that the injection of Freund's complete adjuvant may cause unspecific hypersensitivity reactions to common vehicles (Buehler, 1996). Taking this into account and in light of negative results in a guideline compliant LLNA and a human patch test, RAC places less weight on the results obtained in this GMPT.

In chemico/in vitro studies

None of the *in chemico/in vitro* assays described in the Annex XV report were suitable for detecting potential sensitising properties of PX-200.

In the presented DPRA, precipitation and/or phase separation was observed after the incubation period in samples and controls. The test guideline states that if precipitation and/or phase separation occurs after incubation with peptides, peptide depletion may be underestimated and a conclusion on the lack of reactivity cannot be drawn with sufficient confidence in case of a negative result.

Precipitation was also observed in the KeratinoSens™ assay, leading to a potential underestimation of the sensitising properties of the test substance. Furthermore, this assay is not validated for substances with a logP above 5, and it is not applicable for substances with a logP of above 7. The measured logP of PX-200 is above 6.2, and the calculated logP equals 11.8.

According to the OECD test guideline, test chemicals with a log P greater than 3.5 tend to produce false negatives in the h-CLAT assay. Negative results with test chemicals with a logP greater than 3.5 should not be considered. The logP of PX-200 clearly exceeds this value.

RAC notes that generally, *in vitro* testing in aqueous media is not suitable for substances with a very high lipophilicity and poor water solubility.

Therefore, RAC considers the results from all three alternative methods for this substance as not reliable for classification purposes.

Overall, RAC concludes that apart from the previously considered GPMT, none of the animal or human test methods presented showed any sensitising potential for PX-200. However, all of the presented methods have some limitations, inherent with substances with a low (water) solubility. RAC considers the guideline compliant negative LLNA to be the key study. Negative results from human patch testing and the Buehler assay are considered supportive, although no firm conclusions can be drawn from these results on their own. The only positive results (from the GPMT) showed no clear dose-response relationship and were partially reversible, lowering their reliability. RAC also notes that PX-200 is a large molecule (molecular weight of 687 g/mol) with an extremely low water solubility (0.1 mg/L at 20 °C) and very high measured logP (6.2). All of these properties decrease absorption through human skin, thus lowering the concern for a human health hazard via this route of exposure. Furthermore, PX-200 has no structural features that would indicate a sensitising potential. Therefore, using a weight of evidence approach, RAC concluded that **the existing classification for PX-200 as skin sensitiser should be removed, leading to 'no classification' based on new data**.

Additional references

Buehler, EV. (1996) Contact Dermatitis (34):111-14.

Montelius, J; Boman, A; Wahlkvis, H; Wahlberg, JE. (1996) Contact Dermatitis (34):428-29.

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).