# CLH report

# Proposal for Harmonised Classification and Labelling

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

# International Chemical Identification:

## 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**

**Index Number:** **015-192-00-1**

**Contact details for dossier submitter:** **UK Competent Authority**
**Chemicals Regulation Directorate**
**Health and Safety Executive**
**United Kingdom**

**CLH report prepared by CS Regulatory Ltd. in accordance with Article 37(6) of CLP**

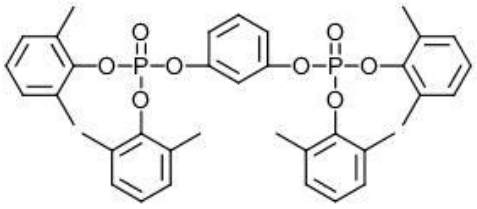
**Version number: 2** **Date: August 2018**

# CONTENTS

# 1 IDENTITY OF THE SUBSTANCE

## 1.1 Name and other identifiers of the substance

**Table 1: Substance identity and information related to molecular and structural formula of the substance**

| | |
|---|---|
| **Name(s) in the IUPAC nomenclature or other international chemical name(s)** | Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate |
| **Other names (usual name, trade name, abbreviation)** | PX-200 |
| **ISO common name (if available and appropriate)** | - |
| **EC number (if available and appropriate)** | 432-770-2 |
| **EC name (if available and appropriate)** | Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate |
| **CAS number (if available)** | 139189-30-3 |
| **Other identity code (if available)** | |
| **Molecular formula** | C38 H40 O8 P2 |
| **Structural formula** |  |
| **SMILES notation (if available)** | Cc5cccc(C)c5OP(=O)(Oc1c(C)cccc1C)Oc2cccc(c2)OP(=O)(Oc3c(C)cccc3C)Oc4c(C)cccc4C |
| **Molecular weight or molecular weight range** | 687.0 |
| **Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)** | *None* |
| **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)** | 95 ≤ C ≤ 99.9% (w/w) |

## 1.2 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 CLH in Annex VI Table 3.1 (CLP) | Current self-classification and labelling (CLP) |
|---|---|---|---|
| Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate<br><br>EC no.: 432-770-2 | 95 ≤ C ≤ 99.9% (w/w) | Skin Sens. 1; H317 | Skin Sens. 1; H317<br><br>(nb. in addition to the harmonised classification, the REACH registrants have also included a self-classification of 'not classified' in the registration dossier to reflect the current proposal) |

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

Information on impurities is confidential - none of the impurities are relevant for the classification.

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

Not applicable – substance does not have any additives.

**Table 5: Test substances (non-confidential information) (this table is optional)**

This information is provided within the study summary tables throughout the dossier.

# 2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

## 2.1 Proposed harmonised classification and labelling according to the CLP criteria

**Table 6:**

| | 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 | n/a | n/a | n/a |
| 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 | n/a | n/a | n/a |
| Resulting Annex VI entry if agreed by RAC and COM | - | Not Classified | | | | | | | | | |

**Table 7: Reason for not proposing harmonised classification and status under public consultation**

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

# 3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The substance was originally notified under the NONS notification scheme (EC Directive 92/69/EEC adapting Directive 67/548/EEC). Harmonised classification was assigned under this scheme as R43, R53 on the basis of the available data. Under Regulation (EC) 1272/2008 (hereafter referred as CLP or CLP Regulation, the corresponding harmonised classification of tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate was Skin Sens. 1 (H317) and Aquatic Chronic 4 (H413) in CLP Annex VI. This adopted classification was revised by CLP ATP 6 with removal of the H413 classification on the basis of additional data available to assess the chronic environmental toxicity effects. The H317 classification was not challenged at that time due to a lack of adequate data to justifiably re-assess the endpoint; data available at that time were a guinea pig maximisation test (positive) and a Buehler sensitisation test (negative).

The Skin Sens. 1 (H317) harmonised classification is now being revisited due to the development and adoption of additional test methods, not least the *in chemico* assessment models developed by ECVAM and adopted by the EU and OECD, plus additional data from a LLNA and human study which has enabled a much broader review and consideration of the effects.

# 4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Justification that action is needed at Community level is required.

Reason for a need for action at Community level:

*Change in existing entry due to new data*
*Change in existing entry due to new interpretation/evaluation of existing data*

Further detail on need for action at Community level
Additional study data have recently been developed which allow further assessment of the classification of the substance. The REACH registration dossier has been updated to include this new information, and a self-classification of 'Not Classified' in addition to the current harmonised classification of H317. It is therefore appropriate to consider all of the available data and review the existing classification.

This dossier has been prepared by CS Regulatory Ltd., but submitted by the UK MSCA in accordance with Article 37(6) of CLP.

# 5 IDENTIFIED USES

The substance is used as a flame retardant in electronic products, such as circuit boards and is a direct replacement for halogenated flame retardants. The neat substance is produced outside the EU but may be used by industry in the EU predominantly in processing of polymers in, for example, pellet form, for production of final articles. The substance is bound into a solid matrix and not subject to wide dispersive use. Where the neat substance is available in the EU it is predominantly processed in closed conditions to avoid exposure to workers. The substance is never available to professional workers or consumers.

The substance is registered under REACH at 10 – 100 tonnes per year.

# 6 DATA SOURCES

All data referred to for consideration of the classification are study data prepared by or on behalf of the substance manufacturer and submitted in support of the REACH registration.

**For convenience, the substance will be referred to as PX-200 throughout the rest of the dossier**.

# 7 PHYSICOCHEMICAL PROPERTIES

## Table 8: Summary of physicochemical properties

| Property | Value | Reference | Comment (e.g. measured or estimated) |
|---|---|---|---|
| **Physical state at 20°C and 101,3 kPa** | white solid at stp | Hogg, A.S., Report No. 519/005 | By visible assessment of the substance |
| **Melting/freezing point** | 95°C (398K) | Hogg, A.S., Report No. 519/005 | Measured by means of the EC A1 test method |
| **Boiling point** | decomposed from approximately 174°C (472K) | Hogg, A.S., Report No. 519/005 | Measured by means of the EC A2 test method |
| **Relative density** | 1.24 at 20°C (+/- 0.5°C) | Hogg, A.S., Report No. 519/005 | Measured by means of the EC A3 test method |
| **Vapour pressure** | 4.0E-04 Pa at 25°C | Tremain, S.P., Report No. 519/007 | Measured by means of the EC A4 test method (vapour pressure balance) |
| **Surface tension** | Not measured due to low water solubility | - | - |
| **Water solubility** | 0.101 mg/l at 20°C +/- 0.5°C | Hogg, A.S., Report No. 519/005 | Measured by means of the EC A6 test method (column elution method) |
| **Partition coefficient n-octanol/water** | log10 Pow > 6.2<br>(QSAR estimate = 11.79) | Hogg, A.S., Report No. 519/005 | Measured by means of the EC A8 test method (HPLC method)<br>(QSAR prepared using EPI KOCWIN Program (v2.00)) |
| **Flash point** | Not available | | |
| **Flammability** | Not flammable (failed to ignite) | Tremain, S.P., report No. 519/006 | Measured by means of the EC A10 test method |
| **Explosive properties** | Not explosive by impact, friction or heating | Tremain, S.P., report No. 519/006 | Measured by means of the EC A14 test method |
| **Self-ignition temperature** | >=400°C | Tremain, S.P., report No. 519/006 | Measured by means of the EC A15 test method |
| **Oxidising properties** | Not oxidising | Tremain, S.P., report No. 519/006 | Measured by means of the EC A17 test method |
| **Granulometry** | 10.1% with particle size <100 µm | Hogg, A.S., Report No. 519/005 | Particle Size Distribution, Fibre Length and diameter Distribution, June 1996 European Commission technical guidance document. (sieve method) |
| **Stability in organic solvents and identity of relevant degradation products** | Not available | - | - |
| **Dissociation constant** | Not available | - | - |
| **Viscosity** | Not available | - | - |

# 8 EVALUATION OF PHYSICAL HAZARDS

Physical hazards have not been assessed in this dossier.

# 9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

## 9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

No specific data are available to assess toxicokinetics of the substance; the summary below is based on a review of the data available for the EU NONS notification and REACH registration of the substance.

The substance is an aromatic organo-phosphorus ester of molecular weight that does not preclude absorption. No specific predictions about toxicokinetic behaviour can be made from the chemical structure. The structure suggests potential for cholinesterase inhibition, but this was specifically investigated in a repeated dose oral toxicity study with no effect identified. The substance is a non-volatile powder of non-respirable particle size, so inhalation exposure is not anticipated. Non-enzymatic hydrolysis is unlikely so exposure to degradants is not applicable.

Absorption:

The substance has very high log P value, which may suggest ready diffusion across membranes and hence absorption. In view of the extremely low water solubility and calculated log P, however, this may not be a true representation of lipophilicity. Evidence of absorption by the oral route was observed in a 28 day repeated dose study in rats (macroscopic changes in the liver in 2/5 males at the top dose).

Distribution:

There is no experimental evidence to indicate distribution except, perhaps, to the liver in the repeated dose oral toxicity study. The extremely high Pow values obtained by testing and QSAR may be suggestive of potential for accumulation, but bioaccumulation potential tends to decrease as Pow becomes increasingly high, becoming more an effect of low water solubility rather than accumulation. This observation is further borne out by the data available from fish bioaccumulation and QSAR estimations of BCF.

Metabolism:

The studies conducted provide no information about potential metabolism, but from the chemical structure, biotransformation of any absorbed substance would be expected. Ester hydrolysis by hydrolase enzymes could occur together with oxidative metabolism by the microsomal mixed function oxidase system and subsequent conjugation reactions.

Excretion:

There is no experimental evidence to indicate a route of excretion but the parent substance is not sufficiently water-soluble for elimination in its unchanged form in urine or bile, but may be eliminated in faecal matter. Biotransformation of any absorbed substance is, however, anticipated and the resulting metabolites could be eliminated either in urine, bile or faeces. The parent substance is non-volatile and could not be eliminated via the lungs in expired air.

# 10 EVALUATION OF HEALTH HAZARDS

## Acute toxicity

### 10.1 Acute toxicity - oral route

Hazard class not assessed in this dossier.

### 10.2 Acute toxicity - dermal route

Hazard class not assessed in this dossier.

### 10.3 Acute toxicity - inhalation route

Hazard class not assessed in this dossier.

### 10.4 Skin corrosion/irritation

**Table 9: Summary table of animal studies on skin corrosion/irritation**

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance</th><th>Dose levels duration of exposure</th><th>Results</th></tr>
<tr>
<td>OECD Guideline 404 (Acute Dermal Irritation / Corrosion); EU Method B.4 (Acute Toxicity: Dermal Irritation / Corrosion)<br><br>GLP Compliant<br><br>No deviations reported<br><br>Anonymous (1995)</td>
<td>Rabbit (New Zealand White), 3 females/dose</td>
<td>PX-200<br><br>Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate<br><br>EC no.: 432-770-2<br><br>CAS: 139189-30-3<br><br>Purity: 98.42%</td>
<td>100% moistened with distilled water<br><br>Area of exposure: 2.5 x 2.5 cm<br><br>Semi-occluded for a period of four hours.<br><br>Test substance removed by gentle swabbing with cotton wool in distilled water</td>
<td>Not irritating
<table>
<tr><th>Effect</th><th>Rabbit #</th><th>Mean Score at 24, 48 and 72 hours</th></tr>
<tr><td rowspan="3">Erythema</td><td>108</td><td>0</td></tr>
<tr><td>114</td><td>0</td></tr>
<tr><td>91</td><td>0</td></tr>
<tr><td rowspan="3">Oedema</td><td>108</td><td>0</td></tr>
<tr><td>114</td><td>0</td></tr>
<tr><td>91</td><td>0</td></tr>
</table>
</td>
</tr>
</table>

**Table 10: Summary table of human data on skin corrosion/irritation**

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations |
|---|---|---|---|
| Basic Study for Standardisation of patch test, Japanese Dermatological Association News, 80, 301- | PX-200<br><br>Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate | Coverage: occlusive<br>Vehicle: unchanged (no vehicle)<br>20 human volunteers (19 male/ 1 female aged between 19 and 31 yrs) | **Not irritating**<br><br>No skin reactions were observed by any test subject to the test material or control.<br><br>No pain reactions.<br><br>No clinical observations. |

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations |
|---|---|---|---|
| 314 (1970)<br>No deviations reported.<br>Yukio Yanagimoto (2002) | EC no.: 432-770-2<br>CAS: 139189-30-3<br>Purity: 95.3% | 0.1g of neat sample exposed to upper arm.<br>Initial pain responses recorded.<br>48-hour exposure, site occluded with circular cloth area of the adhesive tape (small amount of petroleum jelly applied to the cloth to adhere test substance)<br>Patch removed after 48 hours and exposure site assessed.<br>Concurrent control of circular cloth area of the adhesive tape on upper inner arm. | |

**Table 11: Summary table of other studies relevant for skin corrosion/irritation**

No other data are available.

### 10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

In a standard study in rabbits, no skin responses were observed 24, 48 or 72 hours after exposure to undiluted test substance. Furthermore, no skin responses were reported in the 14 day observation period which followed the study. Similarly, no skin responses or signs of irritation were observed in a human patch test.

### 10.4.2 Comparison with the CLP criteria

For animal data, classification is triggered where a mean value of ≥ 2.3 - ≤ 4.0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours is observed. Since no evidence of an irritant effect was observed in the available study, and no evidence of an irritant effect was observed in a study using human volunteers, the criteria for classification are not met.

### 10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

| Not classified – data conclusive but not sufficient for classification |
|---|

## 10.5 Serious eye damage/eye irritation

Hazard class not assessed in this dossier.

## 10.6 Respiratory sensitisation

Hazard class not assessed in this dossier.

## 10.7 Skin sensitisation

The skin sensitisation potential of PX-200 has been investigated in three standard *in vivo* studies (see Table 12 and 13), three standard *in chemico/in vitro* studies (see Table 14), and a human volunteer study (see Table 15).

### 10.7.1 *In vivo* studies

**Table 12: Summary table of the guinea pig maximisation test (GPMT) on which the current harmonised classification is based**

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance</th><th>Dose levels, duration of exposure</th><th>Results</th></tr>
<tr>
<td>M&K Maximisation<br><br>OECD Guideline 406 (EU Method B.6)<br><br>GLP Compliant<br><br>Anonymous (1999)<br><br>Klimisch score = 1</td>
<td>Guinea pig (Dunkin-Hartley)<br><br>females<br><br>10 tested +<br>5 controls</td>
<td>PX-200<br><br>Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate<br><br>EC no.: 432-770-2<br><br>CAS: 139189-30-3<br><br>Purity: 98.4%</td>
<td>Intradermal injection (Day 1):<br><br>3 injections in a row of 0.1 ml each of<br><br>- Freund’s Complete Adjuvant plus distilled water (1:1)<br><br>- 5% w/v in arachis oil BP<br><br>- 5% w/v in a mixture of Freund’s Complete Adjuvant plus distilled water (1:1)<br><br>Topical Induction (Day 7): Over area used for injections<br><br>75% w/w in arachis oil BP<br><br>Topical Challenge (Day 21): Over area used for injections<br><br>75% and 50% w/w in arachis oil BP<br><br>Control animals treated in an identical manner</td>
<td>Positive: 40% (4/10) sensitisation rate<br><br>
<table>
<tr><th rowspan="2">Challenge dose</th><th colspan="2">No. of animals with positive skin responses</th></tr>
<tr><th>24 hours</th><th>48 hours</th></tr>
<tr><td>50% PX-200</td><td>4/10</td><td>3/10</td></tr>
<tr><td>75% PX-200</td><td>3/10</td><td>2/10</td></tr>
</table>
</td>
</tr>
</table>

| Method, guideline, deviations if any | Species, strain, sex, no/group | Test substance | Dose levels, duration of exposure | Results |
|---|---|---|---|---|
| | | | except for an absence of test substance. | |

***In vivo Magnusson & Kligman Maximisation Study in the Guinea Pig, Anonymous (1999)***

This guideline study was conducted to assess the contact sensitisation potential of PX-200 in the albino guinea pig.

Ten test and five control animals (all female) were used for the main study. The concentrations of test material for the induction and challenge phases were selected based on the results of sighting tests.

**Induction of the Test Animals:** Shortly before treatment on Day 0 the hair was removed from an area approximately 40 mm x 60 mm on the shoulder region of each animal with veterinary clippers. A row of three injections (0.1 ml each) was made on each side of the mid-line. The injections were:

a) Freund's Complete Adjuvant plus distilled water in the ratio 1:1

b) a 5% w/v formulation of PX-200 in arachis oil BP (highest volume that could be intradermally injected)

c) a 5% w/v formulation of PX-200 in a 1:1 preparation of Freund's Complete Adjuvant plus distilled water.

One week later (Day 7), the same area on the shoulder region used previously for intradermal injections was clipped again and treated with a topical application of the test material formulation (75% w/w of PX-200 in arachis oil BP – this was the highest concentration to cause skin effects at 24 hours but no skin effects after 48 hours in a sighting test).

**Challenge:** On Day 21, animals were subject to a challenge dose of 50% or 75% PX-200 w/w in arachis oil BP (concentrations which caused no skin effects in a sighting test). A semi-occlusive dressing was applied after the topical applications, and skin reactions were assessed 24 and 48 hours after challenge.

**Results**: Following the intradermal injection, patchy to intense erythema was observed in test animals, whereas patchy to moderate erythema was observed in control animals. Following the topical induction, patchy erythema was observed in 6 test animals after 1 hour, and no erythema was observed in test animals after 24 hours. In the control group, patchy erythema was observed in 2 animals after 1 hour, and no erythema was observed in any animal after 24 hours.

Following the topical challenge of 50% w/w in arachis oil BP, positive skin responses (erythema with or without oedema) were observed in 4 test animals at 24 hours and 3 animals at 48 hours. No skin responses were observed in control animals. Following the topical challenge of 75% w/w in arachis oil BP, positive skin responses (erythema with or without oedema) was observed in 3 test animals at 24 hours and 2 test animals at 48 hours. It is not clear why a greater number of animals responded to the 50% challenge dose compared to the 75% challenge dose.

Overall, it was concluded that PX-200 produced a 40% (4/10) sensitisation rate, and this forms the basis of the current harmonised classification as Skin Sens. 1 (H317).

**Table 13: Summary table of *in vivo* studies carried out since PX-200 was classified as a skin sensitiser.**

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance</th><th>Dose levels<br>duration of exposure</th><th>Results</th></tr>
<tr>
<td>Buehler test (3 applications)<br>OECD 406<br>Deviations from guideline: fewer test and control animals used<br>Not GLP<br>Anonymous (2008)<br>Klimisch score = 2</td>
<td>Guinea pig (Hartley)<br>Female<br>10 Test +<br>5 control</td>
<td>PX-200<br>Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate<br>EC no.: 432-770-2<br>CAS: 139189-30-3<br>Purity: 96.4%</td>
<td>Induction Treatment:<br>Applications on days 1, 8 and 15 of 50% w/v PX-200 in propylene glycol<br>(Control animals were treated with propylene glycol only)<br>Challenge Treatment:<br>Application on day 29 of 25% w/v PX-200 in propylene glycol<br>(to both test and control animals)<br>Skin reactions assessed 24 and 48 hours after challenge.</td>
<td>Not sensitising: 0% sensitisation rate<br>Induction Treatment:<br>No skin responses observed in test or control animals<br>Challenge Treatment:<br>No skin responses observed in test or control animals</td>
</tr>
<tr>
<td>Local Lymph Node Assay (BrdU-ELISA)<br>OECD Guideline 442B<br>GLP Compliant (no deviations)<br>Anonymous (2017)<br>Klimisch score = 1</td>
<td>Mice (CBA/J (SPF, 7 weeks old))<br>Female<br>12 test animals (3 dose groups of 4 test animals),<br>4 vehicle control animals,<br>4 positive control animals</td>
<td>PX-200<br>Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate<br>EC no.: 432-770-2<br>CAS: 139189-30-3<br>Purity: 99.6%</td>
<td>Test groups:<br>0% w/v PX-200 in AOO (vehicle control)<br>10% w/v PX-200 in AOO<br>25% w/v PX-200 in AOO<br>50% w/v PX-200 in AOO (maximum attainable concentration)<br>AOO = Acetone/ olive oil (4:1 v/v)<br>Positive control:<br>HCA (α-hexyl cinnamaldehyde) 25% w/v in AOO<br>Topical application (25µl) of each dose group, vehicle</td>
<td>Not Sensitising<br>
<table>
<tr><th>Concentration of PX-200</th><th>Stimulation Index (mean)</th></tr>
<tr><td>0% ( vehicle control)</td><td>-</td></tr>
<tr><td>10%</td><td>1.0</td></tr>
<tr><td>25%</td><td>1.0</td></tr>
<tr><td>50%</td><td>0.9</td></tr>
<tr><td>Positive control</td><td>2.6</td></tr>
</table>
Test criteria: SI ≥ 2.0 = sensitising; SI between 1.6-1.9 = statistical analysis required; SI ≤ 1.6 = non sensitising</td>
</tr>
</table>

| Method, guideline, deviations if any | Species, strain, sex, no/group | Test substance | Dose levels duration of exposure | Results |
|---|---|---|---|---|
| | | | control or positive control on days 1, 2 and 3.<br><br>Intraperitoneal injection of 0.5mL of BrdU solution (10mg/mL of 5-bromo-2'-deoxyuridine in physiological saline) on day 5<br><br>Collection and weights measurement of auricular lymph nodes on Day 6 | |

***In vivo Buehler Test (Anonymous, 2008)***

A Buehler skin sensitisation study was conducted on female Hartley guinea pigs according to OECD 406; however 10 test and 5 control animals were used instead of the 20 test and 10 control animals specified in the guideline. During a preliminary study, slight skin reactions were observed at 50% w/v in propylene glycol (the maximum concentration practicable) but not at 25% w/v. These concentrations were chosen for the induction and challenge doses respectively.

On days 1, 8 and 15, induction doses of PX-200 were applied to the skin of one flank of the test animals. The test substance was held in place by an occlusive patch for 6 hours after application. Control animals were similarly treated, but with propylene glycol only. On day 29, a challenge dose was applied to the contralateral flank of both test and control animals; again, the test substance was held in place with an occlusive patch for 6 hours. Skin reactions were assessed 24 and 48 hours after the challenge dose.

After the challenge treatment, no skin reactions were observed in animals in the group applied with PX-200 during the induction phase (test substance treatment group). Similarly, no skin reactions were observed in control animals. Therefore, under the conditions of this study, it was concluded that PX-200 was not a skin sensitiser.

Although there was no claim of compliance with GLP, the study appears to have been well designed, conducted and fully reported.

***Local Lymph Node Assay: BrdU-ELISA (Anonymous 2017)***

A standard local lymph node assay was performed using female CBA/J mice (SPF). The study followed OECD 442B, except that the mice were 7 weeks old at the beginning of the study, compared to the 8-12 weeks recommended in the guideline. This is not thought to have affected the validity of the study, particularly as the positive controls behaved as expected.

A pre-screen test was conducted with 2.5, 5.0, 10.0 and 25.0 % w/v of PX-200 in acetone:olive oil (4:1 v/v, AOO), applied to mice daily for three consecutive days (one animal per dose level), and clinical

observations, body weights measurements and ear thickness measurements were conducted. There were no changes which suggested excessive irritation or systemic toxicity.

The main study was conducted with doses of 0, 10.0, 25.0 and 50.0% w/v of PX-200 in AOO; a-hexylcinnamaldehyde at 25% w/v was used as a positive control. Four animals per group were treated for three days (25 µl applied to the dorsum of each ear); approximately 48 hours after the final sensitisation, 5-bromo-2'deoxyuridine (BrdU) was administered. Approximately 24 hours later, auricular lymph nodes were collected and their BrdU uptake quantities were measured to calculate the Stimulation Indices.

*Further detail on the results of the LLNA Assay*

| Parameter measured | Concentration of PX-200 | | | | HCA (positive control) |
|---|---|---|---|---|---|
| | 0% (vehicle control) | 10% | 25% | 50% | |
| **Weight of auricular lymph nodes (mean) (mg)** | 4.0 | 5.8 | 4.7 | 4.8 | 9.8 |
| **BrdU labelling index (mean)** | 0.194 | 0.196 | 0.192 | 0.168 | 0.507 |
| **Stimulation Index (mean)** | - | 1.0 | 1.0 | 0.9 | 2.6 |

Test criteria: SI $\geq$ 2.0 = sensitising; SI between 1.6-1.9 = statistical analysis required; SI $\leq$ 1.6 = non sensitising

No changes indicative of excessive irritation or systemic toxicity were noted. The Stimulation Indices were 1.0, 1.0 and 0.9 for the 10.0, 25.0 and 50.0% w/v concentrations respectively. The positive control behaved as expected. Under the conditions of the test, PX-200 was considered to be non-sensitising.

***Summary of the available in vivo data***

A standard GPMT is available which indicated that PX-200 was a sensitiser under the conditions of the test. 40% of animals were sensitised following an intradermal induction of 5%, a topical induction of 75% and a challenge dose of 50% PX-200 w/v in arachis oil BP. In the same study, a higher challenge dose (75%) resulted in fewer sensitised animals (30%); the reason for this is not clear. According to the test guideline (OECD 406), a response of at least 30% in an adjuvant test should be expected for mild to moderate sensitisers.

A Buehler test is also available, which was conducted according to OECD 406 but with fewer animals than specified in the guideline. In this study, no skin responses were observed in test animals exposed to an induction dose of 50% w/v PX-200 and a challenge dose of 25% w/v PX-200 in propylene glycol. Although fewer animals were used in this study than required by the guideline, the fact that no reactions were observed provides some reassurance that this is not a false negative caused by the reduced animal numbers. This study is therefore considered adequate for inclusion in a weight of evidence assessment, and supports no classification.

Most recently, a standard LLNA BrdU-ELISA was conducted. In this study, PX-200 was found to be not sensitising up to a dose of 50% w/v in AAO (acetone/olive oil vehicle). The LLNA study was not conducted specifically for application to EU regulatory assessment, so the test guideline used was the OECD 442B rather than the OECD 429 which is the preferred method for assessment of sensitising potency in the EU.

Consequently, the data do not allow direct comparison to the CLP criteria, but the ECHA Guidance[1] does recognise that an SI value ≥ 1.6 is regarded as sensitising leading to an understanding that a SI value <1.6 can generally be regarded as non-sensitising. This is further supported by the results obtained from the study which demonstrate results for the test item to be comparable to the vehicle control, and well below the results obtained for the positive control. Furthermore, there was no dose-related increase in the SI. The study is therefore considered adequate for classification as part of the weight of evidence approach and the substance does not meet the criteria for classification under the conditions of the study.

### 10.7.2 *In chemico* and *in vitro* studies addressing key events leading to skin sensitisation

The skin sensitisation potential of PX-200 has been investigated *in chemico* in a direct peptide reactivity assay (DPRA), and *in vitro* in an ARE-Nrf2 Luciferase test (KeratinoSens™) and a human cell line activation test (h-CLAT). The results of these studies are provided in Table 14.

Each of these tests investigates a different stage in the Adverse Outcome Pathway (AOP) which has been developed for skin sensitisation caused by organic chemicals (OECD 2012). The DPRA assesses the molecular initiating event of the AOP – namely protein reactivity – by quantifying the reactivity of test chemicals towards model synthetic peptides. The second key event in the AOP takes place in the keratinocytes, and includes inflammatory responses as well as gene expression associated with specific cell signalling pathways such as the antioxidant/electrophile response element (ARE)-dependent pathways. The test method described in Test Guideline 442D (ARE-Nrf2 luciferase test method) addresses this second key event. The third key event in the AOP is the activation of dendritic cells, typically assessed by expression of specific cell surface markers, chemokines and cytokines. The h-CLAT (Test Guideline OECD 442E) addresses this stage of the AOP.

As each test only looks at one step in the pathway, information from 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 assessment.

**Table 14: Summary table of *in chemico* and *in vitro* studies carried out since PX-200 was classified as a skin sensitiser.**

| Method, guideline, deviations if any | Species, strain, sex, no/group | Test substance | Dose levels duration of exposure | Results |
|---|---|---|---|---|
| Direct Peptide Reactivity Assay (DPRA)<br><br>OECD guideline No. 442C | Cysteine peptide<br>Peptide sequence: Ac-RFAACAA-COOH<br><br>Lysine | PX-200<br>Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate | Test item concentration: 100mM PX-200 in acetonitrile (soluble after 1 minute of sonication)<br><br>Reference control concentration:<br>0.5mM peptide solution | **Negative**<br><br>Depletion rate of test item (mean): 0.36%<br>(= no reactivity/ minimal reactivity) |

[1] ECHA (2017b) ECHA Guidance on Information Requirements and Chemical Safety Assessment under REACH, Chapter R.7a: Endpoint specific guidance (Version 6.0 July 2017), pp 293; ECHA (2017a) ECHA Guidance on the Application of the CLP Criteria (Version 5.0 July 2017) (pp. 341-343)

| Method, guideline, deviations if any | Species, strain, sex, no/group | Test substance | Dose levels duration of exposure | Results |
|---|---|---|---|---|
| GLP Compliant<br><br>Chevallier (2017a)<br><br>Klimisch score = 2 | peptide<br>Peptide sequence: Ac-RFAAKAA-COOH | EC no.: 432-770-2<br>CAS: 139189-30-3<br><br>Purity: 99.6% | (cysteine or lysine) in acetonitrile<br><br>Positive control concentration:<br>100mM cinnamaldehyde in acetonitrile | Depletion rate of positive control (mean): 63.18%<br>(= high reactivity) |
| KeratinoSens™ Test<br><br>OECD guideline No. 442D<br><br>GLP Compliant<br>Chevallier ( 2017b)<br><br>Klimisch score = 2 | HaCaT keratinocytes, immortalized cell line | PX-200<br>Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate<br><br>EC no.: 432-770-2<br>CAS: 139189-30-3<br><br>Purity: 99.6% | Test item concentration: 0.98, 1.95, 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250, 500, 1000 and 2000 µM in culture medium containing 1% DMSO<br><br>Vehicle and negative control: DMSO, applied to cells at 1% in culture medium<br><br>Positive Control:<br>200 mM Cinnamic Aldehyde in DMSO<br><br>Treatment medium: treatment medium: DMEM with 1% FCS without G-418<br><br>Test item was found to be not soluble in water and treatment culture medium at 200 mM even after 5 minutes of sonication and 40 minutes of heating at 80°C. It was found soluble in DMSO at 200 mM after 5 minutes of sonication and 40 minutes of heating at 80°C. | **Negative**<br><br>No potential to activate the Nrf2 transcription factor<br><br>Test item:<br>Cell viability > 70% (Therefore no $IC_{30}$ or $IC_{50}$ was calculated)<br>$I_{max}$ value (mean) was < 1.5 (no statistically significant gene-fold induction above the threshold of 1.5 in comparison to the negative control)<br><br>Slight to strong precipitate at the end of the 48-hour treatment at concentrations ≥ 125 µM<br><br>Positive control:<br>$I_{max}$ value (mean) was 8.11<br>EC1.5 (geometric mean): 10.53µM |
| Human-Cell Line Activation Test (H-Clat) Screening Assay<br><br>OECD guideline | THP-1 cell line<br>(an immortalized human | PX-200<br>Tetrakis(2,6-dimethylphenyl)-m- | Test item:<br>144.68, 173.61, 208.33 and 250 µg/mL in DMSO | **Negative**<br><br>Test item: no effect to THP-1 cells indicating no DC activation effect to T- |

| Method, guideline, deviations if any | Species, strain, sex, no/group | Test substance | Dose levels duration of exposure | Results |
|---|---|---|---|---|
| No. 442E (with deviations – see text)<br><br>Conducted by GLP laboratory to GLP standard, but with no GLP compliance claimed (the study protocol achieved GLP accreditation a few weeks after completion of the study)<br>Gerbeix (2017)<br><br>Klimisch score = 3 | monocytic leukaemia cell line) | phenylene biphosphate<br><br>EC no.: 432-770-2<br>CAS: 139189-30-3<br><br>Purity: 99.6% | Vehicle/ negative control: DMSO (applied to cells at a concentration of 1% in culture medium)<br><br>Positive control: 4 µg/mL 2,4-Dinitrochlorobenzene (DNCB) in DMSO | cell priming.<br><br>No precipitation in test model.<br><br>Positive and vehicle/ negative controls responded as expected and the test is considered valid. |

***In Chemico Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA) (Chevalier, 2017a)***

This GLP compliant study design was based on the OECD guideline No. 442C: *in chemico* skin sensitisation: Direct Peptide Reactivity Assay (DPRA). The objective of this study was to evaluate the reactivity of the test item to synthetic cysteine and lysine peptides, *in chemico* by monitoring peptide depletion following a 24-hour contact between the test item and synthetic cysteine and lysine peptides. The method consisted of the incubation of a diluted solution of cysteine or lysine with the test item (dissolved at 100 mM in acetonitrile) for 24 hours. At the end of the incubation, the concentrations of residual peptides were evaluated by HPLC with Ultra-Violet detection at 220 nm. Peptide reactivity was reported as percent depletion based on the peptide peak area of the replicate injection and the mean peptide peak area in the three relevant reference control C samples (in the appropriate solvent).

DPRA % Depletion calculation formula:

$$\%\,depletion = \left[1 - \frac{Peptide\,Peak\,Area\,in\,Replicate\,Injection}{Mean\,Peptide\,Peak\,Area\,in\,relevant\,Reference\,Control\,C\,samples}\right] \times 100$$

Precipitate and/or phase separation (micelles) were observed in the test item, positive control and reference control samples incubated with the cysteine, lysine peptides and in co-elution samples prepared with the lysine or cysteine dilution buffer. Vials were centrifuged at 400*g* for 5 minutes at room temperature to force precipitate to the bottom of the vial. Only supernatants were injected into the HPLC/UV system.

Analysis of the chromatograms of the co-elution samples indicated that the test item did not co-elute with either the lysine or the cysteine peptides. As a result, the mean percent depletion values were calculated for each peptide using the formula above. For the cysteine peptide, the mean depletion value was 0.59%; for the lysine peptide, the mean depletion value was 0.13%. The mean of the percent cysteine and percent lysine depletions was equal to 0.36%. According to the criteria in the test guideline, the test item was considered to have no/minimal peptide reactivity. Therefore, the DPRA prediction is considered to be negative, and no potential to cause skin sensitisation was demonstrated. The acceptance criteria for the calibration curve samples, the reference and positive controls as well as for the study samples were satisfied.

According to the test guideline, if a precipitate or phase separation is observed, samples may be centrifuged at low speed (100 – 400*g*) to force the precipitates to the bottom of the vial as a precaution (large amounts of precipitate can clog the HPLC tubing or columns). If precipitation or phase separation is observed after the incubation period, as it was in this study, 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.

However, a precipitate was also formed in the positive control, and even after centrifuging a very high depletion rate was observed (63.18%). Furthermore, the mean depletion value calculated for PX-200 was very low (mean 0.36%). The cut-off for a positive result in this test is 6.38%. In other words, this is not a borderline result. This gives us confidence that the centrifuging step, which was a necessary part of this study, did not create a false negative result.

In conclusion, under the experimental conditions of this study PX-200 was considered to have no/minimal peptide reactivity, though with limitations due to test item precipitation or phase separation.

***KeratinoSens™ Test an In Vitro Skin Sensitisation Assay (Chevallier, 2017b)***

The objective of this study was to evaluate the potential of PX-200 to activate the Nrf2 transcription factor. The test used the KeratinoSens™ cell line, an immortalized and genetically modified Human adherent HaCaT keratinocyte cell line. The KeratinoSens™ cell line is stably transfected with a plasmid containing a luciferase gene under the transcriptional control of the SV40 origin of replication promoter. This promoter is fused with an ARE sequence. Sensitisers with electrophilic properties provoke the dissociation of Keap-1 from the transcription factor Nrf2. The free Nrf2 binds to the ARE sequence contained in the plasmid and therefore induces transcription of firefly luciferase.

The KeratinoSens™ cells were first plated on 96-well plates and grown for 24 hours at 37°C. Then the medium was removed and the cells were exposed to the vehicle control or to different concentrations of test item and of positive controls. The treated plates were then incubated for 48 hours at 37°C. At the end of the treatment, cells were washed and the luciferase production was measured by flash luminescence. In parallel, the cytotoxicity was measured by a MTT reduction test and was taken into consideration in the interpretation of the sensitisation results. Two independent runs were performed.

All acceptance criteria were met for the positive and negative controls in each run, both runs were therefore considered as validated.

Both runs were performed using the following concentrations 0.98, 1.95, 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250, 500, 1000 and 2000 µM in culture medium containing 1% DMSO. At these tested concentrations:

- a slight to strong precipitate was observed in treated wells at the end of the 48-hour treatment at concentrations ≥ 125 µM, in both runs,
- no noteworthy decrease in cell viability was noted in either run (i.e. cell viability > 70% in both runs), therefore no geometric mean IC30 or IC50 was calculated,
- no statistically significant gene-fold induction above the threshold of 1.5 was noted in comparison to the negative control at any tested concentrations, in either run. Moreover, the Imax values were < 1.5.

The evaluation criteria for a negative response were met in both runs, the final outcome is therefore negative.

Since precipitate was observed in the test item-treated wells at the end of the 48-hour treatment period, the luciferase activity may be underestimated. Therefore, the conclusion on the lack of activity cannot be drawn with sufficient confidence. Furthermore, according to the test guideline, the test has been validated on test substances with a log P of up to 5. Extremely hydrophobic substances with a log P above 7 are outside the known applicability of the test method, and only limited information is available for substances with a log P value of between 5 and 7. PX-200 has a log P of > 6.2, therefore it is not clear whether PX-200 can be reliably investigated using this method.

In conclusion, under the conditions of this study, PX-200 was negative and no potential to activate the Nrf2 transcription factor was demonstrated. The study was limited by precipitation issues, and the high log P of the substance, which may mean it is unsuitable for testing via this method.

***Human-Cell Line Activation Test (h-CLAT) Screening Assay (Gerbeix, 2017)***

The study was performed in a Test facility certified by the French National Authorities for Good Laboratory Practice but GLP status was not claimed. The study followed established practices and standard operating procedures of CiToxLAB France.

The objective of the study was to determine the ability of PX-200 to induce an increase in cell surface markers expression in THP-1 cells using the h-CLAT test method. The study was conducted according to OECD guideline 442E except that only one dose-range finding assay was performed and only 4 concentrations were tested. No further information on controls/positive controls is available.

The study was divided into two successive phases. First, a dose-range finding assay (DRF) was performed to assess test item toxicity and, if applicable, determine the CV75 i.e. the test item concentration that results in 75% cell viability compared to the vehicle control. Secondly, based on cytotoxicity data obtained from the DRF, a concentration series was tested in a minimum of two runs in the main tests to identify potential CD86 and CD54 upregulations.

Summary results of all runs and conclusion

Study No. 44584 EP

| Test item Name | Conc. (µg/mL) | RFI for CD86 | | RFI for CD54 | | Viability (%) | | Run conclusion | | General conclusion |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | A | B | A | B | A | B | |
| **PX-200** | 144.68 | 75 | 82 | 122 | 73 | 95.5 | 94.8 | N | N | **Negative** |
| | 173.61 | 63 | 93 | 117 | 82 | 95.3 | 95.6 | | | |
| | 208.33 | 72 | 81 | 131 | 67 | 95.0 | 95.2 | | | |
| | 250.00 | 67 | 86 | 117 | 64 | 94.5 | 95.7 | | | |

N = run with negative outcome
S = run with positive outcome
I = Invalidated run
Inc = Inconclusive run
Conc. = concentration
RFI = Relative Fluorescence Index

No precipitate/emulsion was noted in the wells following treatment.

Under the experimental conditions of this study, the test item PX-200 was negative in the h-CLAT assay. The results must, though, be considered with some limitation due to the log P of the substance which has been measured as >6.2 and predicted by QSAR to be 11.92. According to the OECD test guideline, test chemicals with a log P greater than 3.5 tend to produce false negatives. Therefore negative results with test chemicals with a log P greater than 3.5 should not be considered.

***Summary of the available in chemico and in vitro data***

The skin sensitisation potential of PX-200 has been investigated *in chemico* in a direct peptide reactivity assay (DPRA), and *in vitro* in an ARE-Nrf2 Luciferase test (KeratinoSens™) and a human cell line activation test (h-CLAT). Each of these tests investigates a different stage in the Adverse Outcome Pathway which has been developed for skin sensitisation. As each test only looks at one step in the pathway, information from 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 assessment.

All three studies were negative, and no evidence of a skin sensitising potential was demonstrated in any test. However, all three studies had limitations. Indeed, the substance has a very low water solubility (1.01E-04 g/l) and very high log P (measured >6.2 and EPIWIN calculation 11.79), which makes the substance difficult to test in *in vitro* test systems.

In the DPRA study, precipitation occurred which meant that it was necessary to centrifuge the samples prior to analysis. This can lead to an underestimation of reactivity, and result in a false negative; however, given that a strong result (high reactivity) was seen in the positive control (which also had precipitate), and the reactivity seen with PX-200 was negligible (i.e., it was not close to the cut-off for a positive result), it seems unlikely that this is a false negative. This is consistent with the chemical structure of PX-200, which is unlikely to react with proteins.

In the KeratinoSens™ study, precipitation occurred which may mean that the luciferase activity was underestimated (i.e., resulting in a false negative). Furthermore, the test has only been validated on test substances with a log P $\leq$ 5, whereas PX-200 has a log P of > 6.2. It is therefore not clear whether it is appropriate to test PX-200 in this assay.

In the h-CLAT study, no precipitation occurred, however the test is only intended for substances with a log P of $\leq$3.5. Therefore, this study is not informative for the assessment of PX-200.

Overall, only the DPRA and the KeratinoSens™ study can potentially provide information about the sensitising properties of PX-200. Given the limitations of these studies, it cannot be concluded that PX-200 is non-sensitising, based on these results. However, the studies certainly 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 LLNA studies.

### 10.7.3 Human data

**Table 15: Summary table of human data on skin sensitisation**

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations |
|---|---|---|---|
| Assessment of The Skin Sensitisation Potential of a Product to Be Applied to The Skin, Under Controlled and Maximized Conditions<br><br>Conducted according to the Resolution CNS no. 466/2012, and in the spirit of Good Clinical Practices | PX-200<br>Purity: 99.4% | 58 subjects, male and female, aged between 18 and 67 with skin types graded using the Fitzpatrick scale for phototypes:<br>II - The skin gets easily sunburned, tans slightly (11 subjects)<br>III - The skin gets moderately sunburned, tans gradually (30 subjects)<br>IV - The skin gets minimally sunburned, tans well (17 subjects)<br>V - The skin rarely gets sunburned, gets very tanned. (0 subjects)<br>No deeply pigmented subjects were | Negative: no sensitisation observed<br><br>During the study, no subjects presented skin clinical signs related to the product.<br><br>PX-200 did not induce skin sensitisation in the |

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations |
|---|---|---|---|
| Deviations: 3 subjects (all female) demonstrated irritation resulting from exposure to the semi-occlusive tape (sticking plaster) and were removed from the study.<br><br>Pessoto Rosa (2017) | | included in the test.<br><br>Patch test methodology (Kligman & Wooding, 1967), also known as contact test or epicutaneous test<br><br>Exposure area: back (scapular area)<br><br>Test product: 0.05g/cm² on a patch test filter paper disc (disc size: 1cm$^2$)<br><br>Control: 0.9% sterile physiological solution | study group. |

***Assessment of the Skin Sensitisation Potential of PX-200 (human volunteer study) Pesotto Rosa (2017)***
The substance, PX-200, is produced and used at high volumes outside the EU for use as a flame retardant in plastics applied to a range of products. The neat substance is principally handled outside the EU by industrial workers. The substance manufacturer has received extensive concern principally from commercial entities based in jurisdictions outside the EU and a requirement to clarify the effects to human since contradictory results were obtained from accepted animal models used for regulatory compliance.

Whilst the substance manufacturer has continued to develop data according to the accepted regulatory strategy of the EU jurisdiction for compliance with the requirements of CLP, additional data to address direct correlation to exposure to humans was considered appropriate to address concerns outside the EU jurisdiction due to production volumes.

While the data are not necessarily developed specifically for the purposes of CLP classification, it is considered appropriate to include available data relevant to the endpoint for the registration of the substance and to assist with consideration of the classification.

The objective of this study was to investigate the skin sensitisation potential of PX-200 in human subjects when applied to the skin, under maximized conditions, supervised by a dermatologist. This study was conducted in conformance with the Declaration of Helsinki principles, setting the ethical principles for medical research involving human subjects, including Resolution CNS no. 466/12, and in spirit of the Good Clinical Practices (Document of the Americas and ICH E6: Good Clinical Practice).

The study was initiated with 70 subjects, being 63 female and 7 male subjects, aged from 18 to 67 years. The study was completed with 58 subjects; 9 subjects withdrew from the study due to personal reasons unrelated to the test product, while a further 3 subjects were removed from the study after presenting with signs of irritation due to exposure of the semi-occlusive tape (sticking plaster). There were 9 applications in the 3 first weeks (induction period) and 1 application in the last week (challenge period). The methodology applied for the test (i.e. induction period and challenge) were based on the principles applied for investigation of repeat insult tests in humans (Kligman & Wooding, 1967; Marzulli & Maibach, 1975), and are considered adequate to assess the sensitising effect of a substance in humans.

Both the test substance (PX-200, 0.05g/cm²) and control (sterile physiological solution) were applied to patch test filter paper discs (1cm$^2$) and then applied to the right or left back (scapular area) of the study

subjects. The applications were performed on Mondays, Wednesdays and Fridays, during 3 consecutive weeks. Forty-eight hours (48h) after the application, the patch test was removed by trained technicians and, approximately 30 minutes after the patch test removal, the site was assessed in order to check the presence of possible clinical signs.

After this induction period, there was a 10 day-period (minimum) when no patch was applied to the study subjects' back (rest period). After the rest period, for the challenge phase, a patch with the test product and control was applied to the right or left back of the subjects on a virgin area, that is, where no patches had been applied before. The patch was removed by the investigators after approximately 48 hours of contact with the skin. The assessments (readings) were performed approximately 30 minutes (48h reading) and 24 hours (72h reading) after patch test removal. The subjects were assessed at the end of the study by a dermatologist and supervised during the study.

During the study, no subjects presented skin clinical signs related to treatment with PX-200. It was concluded that the substance did not induce skin sensitisation in the study group.

**Table 16: Summary table of other studies relevant for skin sensitisation**

No other data are available.

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

The initial data assessment of PX-200 was devised under the NONS scheme (EC Directive 92/69/EEC adapting Directive 67/548/EEC). At that time, the only available study regarding skin sensitisation was a guinea pig maximisation test (GPMT). The study was deemed to be positive, with a sensitisation rate of up to 40%. It was therefore classified as Xi; R43 (May cause sensitisation by skin contact) under DSD which was directly translated to Skin Sens. 1 (H317) under CLP.

Since then, a number of additional investigations have been conducted using PX-200; three *in chemico/in vitro* studies intended to investigate the Adverse Outcome Pathway for skin sensitisation (OECD, 2012), a LLNA, a Buehler test and a human volunteer study. Valid results from all 3 *in chemico/in vitro* studies are needed to conclude on skin sensitisation potential. The key events in the Adverse Outcome Pathway, and a brief summary of the available studies, is provided in Table 17.

**Table 17: Key events in the Adverse Outcome Pathway for skin sensitisation (organic chemicals, taken from OECD 2012) and short summary of the relevant available studies.**

| Key Event in Skin Sensitisation AOP | Relevant study | Result | Comments |
|---|---|---|---|
| Key Event 1: covalent binding at cysteine and/or lysine | Direct Peptide Reactivity Assay (DPRA)<br>(OECD 442C) | **Negative** | Precipitate and/or phase separation were observed with the test item, positive control and reference control samples. According to the test guideline, this may cause peptide depletion to be underestimated and a conclusion on the lack of reactivity cannot be drawn in the case of a negative result. |
| Key Event 2: keratinocyte | KeratinoSens™ Test | **Negative** | Precipitate was observed with the test item, which may mean luciferase activity was underestimated. The high log Pow of |

| Key Event in Skin Sensitisation AOP | Relevant study | Result | Comments |
|---|---|---|---|
| inflammatory response | (OECD 442D) | | PX-200 may mean it is unsuitable for testing via this method. |
| Key Event 3: activation of dendritic cells | Human Cell Line Activation Test (H-Clat)<br>(OECD 442E) | **Negative** | According to the test guideline, test substances with log P >3.5 tend to produce false negatives. |
| Key Event 4: T-cell proliferation | LLNA<br>(OECD 442B) | **Negative** | Well conducted guideline study. |
| Adverse outcome (contact dermatitis/ hypersensitivity) | Guinea pig maximisation test (OECD 406) | **Positive** (4/10 sensitisation rate) | In at least one animal at each challenge concentration, the severity of the response decreased between 24 and 48 hours (as indicated by a reduction in the total number of animals responding). The nature of the response in these animals is more characteristic of irritation than it is of sensitisation (ECETOC, 2000). |
| | Buehler test (OECD 406, with deviations) | **Negative** | 10 test and 5 control animals used (20 test and 10 control animals are required by the guideline). |
| | Human volunteer study (patch test) | **Negative** | No sensitisation was observed in 58 subjects (treated with 9 applications of 0.05g PX-200, followed by a challenge dose of 0.05g) |

In addition to the key events outlined in Table 17, 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 and EPIWIN calculation 11.79), very low water solubility (1.01E-04 g/l) and a high molecular weight (687.0), which suggests it does not easily penetrate to the viable epidermis.

### 10.7.5 Comparison with the CLP criteria

According to the CLP criteria, substances shall be classified as skin sensitisers (Category 1) if:

a) there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons; or
b) there are positive results from an appropriate animal test.

The current harmonised classification of Skin Sens. 1 (H317) was based on the positive result seen in a guideline Guinea pig maximisation study (GPMT). Overall, it was concluded that PX-200 produced a 40% (4/10) sensitisation rate. According to the CLP criteria, a substance is classified as Skin Sens. 1 if at least 30% of animals respond in an adjuvant type test.

In the GPMT, 40% of animals were sensitised following an intradermal induction dose of 5%, a topical induction dose of 75% and a challenge dose of 50% PX-200 w/v in arachis oil BP. In the same study, a higher challenge dose (75%) resulted in fewer sensitised animals (30%); the reason for this is not clear.

According to the test guideline (OECD 406), a response of at least 30% in an adjuvant test should be expected for mild to moderate sensitisers.

A Buehler test has been conducted since the initial classification. The Buehler test was conducted according to OECD 406 but with fewer animals than specified in the guideline. In this study, no skin responses were observed in animals exposed to an induction dose of 50% w/v PX-200 and a challenge dose of 25% w/v PX-200 in propylene glycol. Although fewer animals were used in this study than required by the guideline, the fact that no reactions were observed at all provides some reassurance that this is not a false negative caused by the reduced animal numbers. The study is considered adequate for inclusion in the weight of evidence assessment, supporting no classification.

Most recently, a standard LLNA BrdU-ELISA was conducted. In this study, PX-200 was found to be not sensitising up to a dose of 50% w/v in AAO (acetone/olive oil vehicle). The LLNA study was not conducted specifically for application to EU regulatory assessment, so the test guideline used was the OECD 442B rather than the OECD 429, which is the preferred method for assessment of sensitising potency in the EU. Consequently, the data do not allow direct comparison to the CLP criteria, but the ECHA Guidance[2][3] does recognise that an SI value $\geq$ 1.6 is regarded as sensitising, leading to an understanding that a SI value <1.6 can generally be regarded as non-sensitising. This is further supported by the results obtained from the study which demonstrate results for the test item to be comparable to the vehicle control, and well below the results obtained for the positive control. Furthermore, there was no dose-related increase in the SI. The study is therefore considered adequate for classification as part of the weight of evidence approach, and PX-200 does not meet the criteria for classification under the conditions of the study.

It is not clear why the GPMT was positive, whereas the Buehler and the LLNA were negative. The differences in the results are unlikely to be species-related, as the GPMT and the Buehler were both carried out in guinea pigs. It could be related to the different vehicles used in each study, or it could be due to differences in the sensitivity of the tests.

The GPMT is known to be a particularly sensitive test, as it utilises intradermal induction doses, and the animals are dosed with adjuvant in addition to the test material. In the Buehler and LLNA assay, on the other hand, topical inductions are used in the absence of an adjuvant. According to the ECHA guidance (ECHA 2017b), the use of adjuvant in the GPMT may lower the threshold for irritation and so lead to false positive reactions (see section R.7.3.6.1, p296 of the guidance). The study report for the GPMT does not provide individual observation data of the various injection sites, therefore it is not possible to assess whether the reactions at the sites with adjuvant were greater than those at the sites injected with PX-200 only. Furthermore, the study report does not provide any information on the severity of the reactions at the two time points (24 and 48 hours). What is clear, however, is that in at least one animal at each challenge concentration, the severity of the response decreased between 24 and 48 hours (as indicated by a reduction in the total number of animals responding). The nature of the response in these animals (i.e., fading at the later time point) is more characteristic of irritation than it is of sensitisation (ECETOC, 2000).

PX-200 tested negative in all 3 *in chemico/in vitro* studies. However, the h-CLAT study is deemed to be not valid, due to the log P value of PX-200 falling outside the range specified in the test guideline, and there are similar concerns regarding the KeratinoSens™ assay.

In the DPRA, a precipitate was formed in both test and control samples, which necessitated a centrifuging step which has the potential to lead to false negatives. It is noted that even after centrifugation, a very high depletion rate was observed (63.18%) in the positive control. Furthermore, the mean depletion value calculated for PX-200 was very low (mean 0.36%). The cut-off for a positive result in this test is 6.38%. This

[2] ECHA Guidance on the Application of the CLP Criteria (Version 5.0 July 2017) (pp. 341-343) (please refer to ECHA, 2017a in the list of references)

[3] ECHA Guidance on Information Requirements and Chemical Safety Assessment under REACH, Chapter R.7a: Endpoint specific guidance (Version 6.0 July 2017) (R.7.3.5.1, pp. 293) (see ECHA, 2017b in the list of references)

gives some confidence that the centrifugation step, which was a necessary part of this study, did not create a false negative result.

PX-200 tested negative in a LLNA, a Buehler test, and no positive skin reactions were observed in 58 subjects exposed to a high dose of PX-200 in the human volunteer study. The human volunteer study is limited by the small number of volunteers included in the study, however, it appears to have been well conducted. The study is therefore considered suitable for inclusion in the weight of evidence assessment, supporting no classification.

Taken together, these more modern studies present an internally consistent picture of the skin sensitisation potential of PX-200:

- Given the methodological limitations of the h-CLAT and KeratinoSens™ studies it is not possible to conclude on skin sensitisation potential using the *in chemico/in vitro* studies. However, the DPRA indicates that PX-200 does not have any intrinsic protein reactivity.

- The negative LLNA indicates that PX-200 does not induce lymphocyte proliferation in the mouse auricular lymph node. This is consistent with the negative DPRA, as protein reactivity is a necessary first step in the induction of skin sensitisation.

- Though limited, the negative Buehler indicates that PX-200 does not have the capacity to elicit a skin sensitisation reaction in guinea pigs (consistent with the negative DPRA and LLNA).

- Similarly, the human volunteer study indicates that PX-200 does not have the capacity to elicit a skin sensitisation reaction in humans (consistent with the negative DPRA, LLNA and Buehler)

The positive GPMT conflicts with these more recent studies, and there is no obvious explanation for the clear differences. As discussed above, it is possible that the GPMT was a false positive result, although there is no way of knowing for sure. However, the apparent lack of protein reactivity, the lack of induction potential in the LLNA and the lack of positive responses in a Buehler test and human volunteer study strongly suggests that PX-200 does not have skin sensitisation potential. This is consistent with the physico-chemical properties of the substance (high log P, very low water solubility and high molecular weight), which suggest that PX-200 is unlikely to penetrate to the viable epidermis of the skin.

Overall, based on weight of evidence, no classification is proposed.

### 10.7.6 Conclusion on classification and labelling for skin sensitisation

| |
|---|
| *Not classified – data conclusive but not sufficient for classification* |

## 10.8 Germ cell mutagenicity

Hazard class not assessed in this dossier.

## 10.9 Carcinogenicity

Hazard class not assessed in this dossier.

## 10.10 Reproductive toxicity

Hazard class not assessed in this dossier.

### 10.11 Specific target organ toxicity-single exposure

Hazard class not assessed in this dossier.

### 10.12 Specific target organ toxicity-repeated exposure

Hazard class not assessed in this dossier.

### 10.13 Aspiration hazard

Hazard class not assessed in this dossier.

## 11 EVALUATION OF ENVIRONMENTAL HAZARDS

Environmental hazards have not been assessed in this dossier.

## 12 EVALUATION OF ADDITIONAL HAZARDS

Additional hazards have not been assessed in this dossier.

## 13 ADDITIONAL LABELLING

No additional labelling is relevant for this substance

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## 15 ANNEXES (SEPARATE DOCUMENTS)

ANNEX I: DETAILED STUDY SUMMARIES

ANNEX II: CONFIDENTIAL REFERENCES