baua:
Bundesanstalt für Arbeitsschutz
und Arbeitsmedizin
Federal Institute for Occupational
Safety and Health

SUBSTANCE EVALUATION CONCLUSION
as required by REACH Article 48
and
EVALUATION REPORT

for

1-(4-methyl-2-nitrophenylazo)-2-naphthol ("Pigment Red 3")
EC No 219-372-2; CAS No 2425-85-6

1-[(2-chloro-4-nitrophenyl)azo]-2-naphthol ("Pigment Red 4")
EC No 220-562-2; CAS No 2814-77-9

1-[(2,4-dinitrophenyl)azo]-2-naphthol ("Pigment Orange 5")
EC No 222-429-4; CAS No 3468-63-1

Evaluating Member State(s): Germany

Dated: 14 September 2020

Evaluating Member State Competent Authority

BAuA
Federal Institute for Occupational Safety and Health
Division 5 - Federal Office for Chemicals
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Year of evaluation in CoRAP: 2019

Member State concluded the evaluation without any further need to ask more information from the registrants under Article 46(1) decision. However, as a result of the substance evaluation, ECHA was requested to ask for standard information on the substances under Article 41(3) decisions.

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

The group of three azo pigments 1-(4-methyl-2-nitrophenylazo)-2-naphthol (EC 219-372-2, "Pigment Red 3", PR3), 1-[(2-chloro-4-nitrophenyl)azo]-2-naphthol (EC 220-562-2, "Pigment Red 4", PR4) and 1-[(2,4-dinitrophenyl)azo]-2-naphthol (EC 222-429-4, "Pigment Orange 5", PO5) was originally selected for substance evaluation in order to clarify concerns on:

- suspected CMR properties
- suspected PBT/vPvB properties
- wide dispersive use
- exposure of environment

During the evaluation, repeated dose toxicity was identified as an additional concern. For PO5 only, occupational exposure and risk assessment for workers were also included in the evaluation.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

For all three azo pigments, dossier evaluation processes have been conducted by ECHA resulting in additional requests to generate information to fulfil standard information requirements. For PR3, a decision has been issued by ECHA on 21 December 2017.[2] For PR4, a decision has been issued by ECHA on 29 March 2019.[3] For PO5, a decision has been issued by ECHA on 21 December 2017.[4]

In previous Dossier Evaluations, ECHA rejected the read-across hypothesis for the group of the three azo pigments (CCH-D-2114381690-46-01/F for PO5 and CCH-D-2114461479-37-01/F for PR4). The evaluating Member State Competent Authority (eMSCA) supports this decision. Study data to sufficiently support a group hypothesis is not available, therefore read-across between the three substances cannot be accepted to fill data gaps for specific toxicological endpoints and the three azo pigments are evaluated individually.

A further compliance check procedure has been opened by ECHA upon request of the eMSCA. The eMSCA considers standard information necessary to clarify the identified concerns.

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the eMSCA to the following conclusions, as summarised in the table below.

[2] ECHA dossier evaluation overview on PR3: https://echa.europa.eu/de/information-on-chemicals/dossier-evaluation-status/-/dislist/substance/100.017.612
[3] ECHA dossier evaluation overview on PR4: https://echa.europa.eu/de/information-on-chemicals/dossier-evaluation-status/-/dislist/substance/100.018.693
[4] ECHA dossier evaluation overview on PR5: https://echa.europa.eu/de/information-on-chemicals/dossier-evaluation-status/-/dislist/details/0b0236e1813ea44c

Table 1

Conclusion of substance evaluation	
Need for follow-up regulatory action at EU level	
Harmonised Classification and Labelling	
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	
No need for regulatory risk management follow-up action at EU level; a compliance check needed instead to clarify identified concerns.	x

Further information is necessary to inform on the concerns identified by the eMSCA. However, compliance check has been identified as the more expedient process in this case to require this information and therefore, the respective process has been triggered. Therefore, at this point in time, no conclusion on the concerns is possible as the information will be generated initially in a dossier evaluation step, potentially followed by another substance evaluation process in case non-standard information is necessary to clarify the concerns further.

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

The eventual need for regulatory follow-up action, e.g. harmonised classification and labelling or SVHC identification, e.g. based on the fulfilment of PBT/vPvB criteria, will be re-examined after the arrival of the standard information requested via compliance check.

4.1.1. Harmonised Classification and Labelling

N/A (see above).

4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

N/A (see above).

4.1.3. Restriction

N/A (see above).

4.1.4. Other EU-wide regulatory risk management measures

N/A (see above).

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1. No need for regulatory follow-up at EU level

N/A (see above).

5.2. Other actions

A compliance check for all three substances has been opened by ECHA to request additional standard information.

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

Indication of a tentative plan is not a formal commitment by the evaluating Member State. A commitment to prepare a REACH Annex XV dossier (SVHC, restrictions) and/or CLP Annex VI dossier should be made via the Registry of Intentions.

Table 2

FOLLOW-UP		
Follow-up action	**Date for intention**	**Actor**
Compliance Check	2020	ECHA
Subsequent Substance Evaluation	tbd	DE

The need for a re-opening of the Substance Evaluation process will be determined based on the outcome of the new information generated via the Compliance Check procedure.

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

A group of three azo pigments 1-(4-methyl-2-nitrophenylazo)-2-naphthol (EC 219-372-2, "Pigment Red 3", PR3), 1-[(2-chloro-4-nitrophenyl)azo]-2-naphthol (EC 220-562-2, "Pigment Red 4", PR4) and 1-[(2,4-dinitrophenyl)azo]-2-naphthol (EC 222-429-4, "Pigment Orange 5", PO5) was originally selected for substance evaluation in order to clarify concerns about:

- suspected CMR properties

- suspected PBT/vPvB properties

- wide dispersive use

- exposure of environment

During the evaluation, repeated dose toxicity was identified as an additional concern.

Table 3

Evaluated endpoints for Substance PR3	
Endpoint evaluated	**Outcome/conclusion**
Carcinogenicity	Limited evidence on carcinogenicity in animals; not sufficient for classification.
Mutagenicity	Concern based on incomplete standard information. Hand over to compliance check to request further studies.
Reproductive Toxicity	Conclusive, concern not substantiated.
Skin Sens.	Conclusive, no concern identified.
Repeated Dose toxicity	Conclusive, no concern identified.
P (Persistency)	Screening P/vP.
B (Bioaccumulation)	Missing information for screening B/vB. Available *in vivo* study on bioaccumulation considered not reliable by eMSCA. Hand over to compliance check to request further studies.
T ((Eco)Toxicity)	Potentially T.
Wide dispersive use	Identified uses for consumers were evaluated.

Table 4

Evaluated endpoints for Substance PR4	
Endpoint evaluated	**Outcome/conclusion**
Carcinogenicity	Limited evidence on carcinogenicity in animals; not sufficient for classification.
Mutagenicity	Concern based on incomplete standard information. Awaiting further studies requested under compliance check.
Skin Sens.	Conclusive, no concern identified.
Reproductive Toxicity	Awaiting studies requested under compliance check.
Repeated Dose toxicity	Concern based on incomplete standard information. Awaiting further studies requested under compliance check.
P (Persistency)	Missing information for screening P/vP. Hand over to compliance check to request further studies as read-across proposed by registrants is rejected by eMSCA.
B (Bioaccumulation)	Missing information for screening B/vB. Hand over to compliance check to request further studies as read-across proposed by registrants is rejected by eMSCA.
T ((Eco)Toxicity)	Potentially T.

Table 5

Evaluated endpoints for Substance PO5	
Endpoint evaluated	**Outcome/conclusion**
Carcinogenicity	Limited evidence on carcinogenicity in animals; not sufficient for classification.
Mutagenicity	Concern based on incomplete standard information. Hand over to compliance check to request further studies.
Reproductive Toxicity	Insufficient data. Hand over to compliance check to request further studies.
Skin Sens.	Concern confirmed (when minor constituent with skin sens. potential present), self-classification sufficient.
Repeated Dose toxicity	Concern based on incomplete standard information. Hand over to compliance check to request further studies.
P (Persistency)	Missing information for screening P/vP.
B (Bioaccumulation)	Missing information for screening B/vB.
T ((Eco)Toxicity)	Potentially T.
Wide dispersive use	An exposure assessment in the CSR is not required by REACH when the substance is not classified. However, based on first tier assessment the eMSCA identified a number of exposure situations where the risk characterisation ratios for PO5 are significantly above 1 (by using the DNEL based on repeated dose toxicity, i.e. potential haemolytic anaemia, derived by the eMSCA). The DNEL might be subject of change, when studies requested under compliance check become available.

7.2. Procedure

The substance PO5 was initially included in the Community Rolling Action Plan for substance evaluation (CoRAP) 2016-2018.[5] The planned evaluation of PO5 was postponed and aligned with the evaluation of structurally related substances PR3 and PR4, which were included in the subsequent CoRAP update 2019-2021 as new entries.[6] As documented in section 7.1, the set of initial concerns for the three substances was identical. Evaluation was started on 19 March 2019 following publication of the respective CoRAP update.

A PBT/vPvB assessment was conducted based on the available data from the registration dossiers of PO5, PR3 and PR4. QSAR calculations conducted by the eMSCA were used as supporting information.

7.3. Identity of the substance

Table 6

SUBSTANCE IDENTITY OF PR3	
Public name:	Pigment Red 3
EC number:	219-372-2
CAS number:	2425-85-6
Index number in Annex VI of the CLP Regulation:	
Molecular formula:	$C_{17}H_{13}N_3O_3$
Molecular weight range:	307.30 g/mol
Synonyms:	1-(4-methyl-2-nitrophenylazo)-2-naphthol 2-Naphthalenol, 1-[2-(4-methyl-2-nitrophenyl)diazenyl]- C.I. Pigment Red 3 C.I. 12120

Type of substance: Mono-constituent

[5] CoRAP update for 2016-2018: https://echa.europa.eu/documents/10162/13628/corap_list_2016-2018_en.pdf
[6] CoRAP update for 2019-2021: https://echa.europa.eu/documents/10162/13628/corap_update_2019-2021_en.pdf

Table 7

SUBSTANCE IDENTITY OF PR4	
Public name:	Pigment Red 4
EC number:	220-562-2
CAS number:	2814-77-9
Index number in Annex VI of the CLP Regulation:	
Molecular formula:	$C_{16}H_{10}ClN_3O_3$
Molecular weight range:	327.72 g/mol
Synonyms:	1-[(2-chloro-4-nitrophenyl)azo]-2-naphthol 1-[(E)-(2-Chloro-4-nitrophenyl)diazenyl]-2-naphthol 2-Naphthalenol, 1-[2-(2-chloro-4-nitrophenyl)diazenyl]- C.I. Pigment Red 4 C.I. 12085

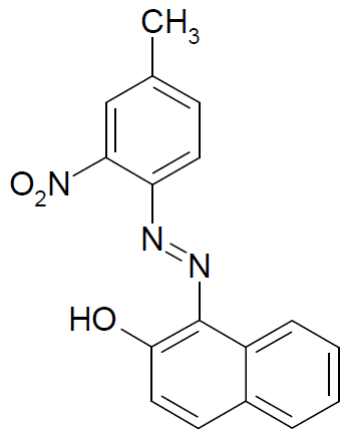
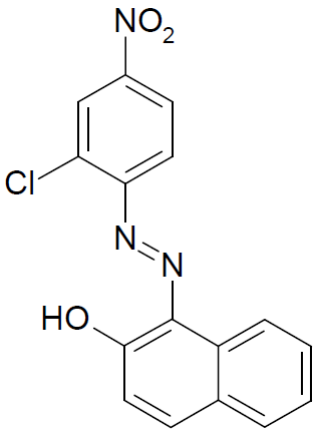
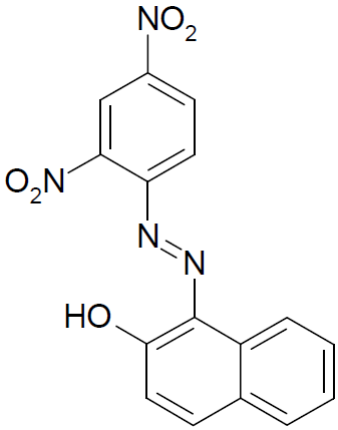
Type of substance: Mono-constituent

Table 8

SUBSTANCE IDENTITY OF PO5	
Public name:	Pigment Orange 5
EC number:	222-429-4
CAS number:	3468-63-1
Index number in Annex VI of the CLP Regulation:	
Molecular formula:	$C_{16}H_{10}N_4O_5$
Molecular weight range:	338.274 g/mol
Synonyms:	1-[(E)-(2,4-Dinitrophenyl)diazenyl]-2-naphthol 1-[(2,4-Dinitrophenyl)azo]-2-naphthol 1-[2-(2,4-Dinitrophenyl)diazenyl]-2-naphthalenol Dinitroaniline Orange Permanent Orange C.I. Pigment Orange 5 C.I. 12075

Type of substance: Mono-constituent

Structural formulas:

Pigment Red 3:	Pigment Red 4:	Pigment Orange 5:
		

7.4. Physico-chemical properties

Table 9

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES OF PR3	
Property	**Value**
Physical state at 20°C and 101.3 kPa	solid (red powder)
Vapour pressure	estimated by calculation (QSAR estimation): Modified Grain method (MPBPVP v1.43), EPI Suite, US EPA with a melting point of 279.5°C as an input parameter; result: 0 Pa at 25 °C
Water solubility	3.3 µg/L at 23-24°C (ca. pH 7) [insoluble (< 0.1 mg/L)]
Partition coefficient n-octanol/water (Log Kow)	Log Pow 3.7 at 23°C (ca. pH 7) (calculated from solubility in water and octanol)
Granulometry	volumetric distribution: D10 = 0.75 - 0.84 µm D50 = 2.69 - 8.75 µm D90 = 23.36 – 40.21 µm TEM images indicate, that the test substance mainly consists of aggregates and/or agglomerates while the measured BET surface area is 18.9 m^2/g (27.2 m^2/cm^3) (DIN 66132).

Table 10

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES OF PR4	
Property	**Value**
Physical state at 20°C and 101.3 kPa	solid (red powder)
Vapour pressure	estimated by calculation (QSAR estimation): Modified Grain method (MPBPVP v1.43), EPI Suite, US EPA with a melting point of 285°C as an input parameter; result: 0 Pa at 25 °C
Water solubility	3.3 µg/L at 23°C (pH not specified) [insoluble (< 0.1 mg/L)]
Partition coefficient n-octanol/water (Log Kow)	Log Pow 3.45 at 23°C (pH not specified) (calculated from solubility in water and octanol)
Granulometry	volumetric distribution: D10 = 0.503 µm D50 = 2.272 µm D90 = 11.227 µm TEM images indicate, that the test substance mainly consists of aggregates and/or agglomerates while the measured BET surface area is 12.2 m^2/g (19.5 m^2/cm^3) (DIN 66132).

Table 11

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES OF PO5	
Property	**Value**
Physical state at 20°C and 101.3 kPa	solid (orange powder)
Vapour pressure	Not relevant: Substance is a solid which melts above 300°C.
Water solubility	6.3 µg/L at 26°C (ca. pH 7) [insoluble (< 0.1 mg/L)]
Partition coefficient n-octanol/water (Log Kow)	Log Pow 2.45 at 26°C (ca. pH 7) (calculated from solubility in water and octanol)
Granulometry	D50 = 1.1 µm (volumetric distribution); TEM images indicate, that the test substance mainly consists of aggregates and/or agglomerates while the measured BET surface area is 11.7 m^2/g (18.4 m^2/cm^3) (DIN 66132).

7.5. Manufacture and uses

7.5.1. Quantities

Table 12

AGGREGATED TONNAGE (per year) for PR3				
☐ 1 – 10 t	☒ 10 – 100 t	☐ 100 – 1000 t	☐ 1000- 10,000 t	☐ 10,000-50,000 t
☐ 50,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☐ > 1000,000 t	☐ Confidential

Table 13

AGGREGATED TONNAGE (per year) for PR4				
☐ 1 – 10 t	☐ 10 – 100 t	☒ 100 – 1000 t	☐ 1000- 10,000 t	☐ 10,000-50,000 t
☐ 50,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☐ > 1000,000 t	☐ Confidential

Table 14

AGGREGATED TONNAGE (per year) for PO5				
☐ 1 – 10 t	☐ 10 – 100 t	☒ 100 – 1000 t	☐ 1000- 10,000 t	☐ 10,000-50,000 t
☐ 50,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☐ > 1000,000 t	☐ Confidential

7.5.2. Overview of uses

Table 15

USES OF PR3	
Manufacture	Industrial manufacture of pigments or pigment additives
Formulation	Formulation of pigment product Formulation of solid preparations containing pigment (including plastics) and non-solid preparations (including inks and paints) Coating, ink, plastic applications: manufacture of powder products
Uses at industrial sites	Formulation of solid preparations containing pigment (including plastics) and non-solid preparations (including inks and paints) Coating, ink, plastic applications: manufacture of powder products
Uses by professional workers	Indoor and outdoor use of pigmented articles with low release Widespread dispersive indoor and outdoor use resulting in inclusion into a matrix Removal of matrix, indoor and outdoor (e.g. abrasion)

	Coating, ink, plastics applications: professional
Consumer Uses	Indoor and outdoor use of pigmented articles with low release Widespread dispersive indoor and outdoor use resulting in inclusion into a matrix Removal of matrix, indoor and outdoor (e.g. abrasion) Coating, ink, plastic applications: consumer Cosmetics PC 9a: Coatings and paints, thinners, paint removes PC 18: Ink and toners PC 32: Polymer preparations and compounds
Article service life	Removal of matrix (e.g. abrasion), indoor and outdoor Indoor and outdoor use of coloured articles AC 1: Vehicles AC 7: Metal articles AC 8: Paper articles AC 11: Wood articles AC 13: Plastic articles AC 01: Other (non intended to be released): Painted articles

Table 16

USES OF PR4	
Manufacture	Industrial manufacture of pigments or pigment additives
Formulation	Formulation of solid preparations containing pigment (including plastics) and non-solid preparations (including inks and paints)
Uses at industrial sites	Manufacture of substance Manufacture of pigments or pigment additives
Uses by professional workers	Indoor and outdoor use of pigmented articles with low release Widespread dispersive indoor and outdoor use resulting in inclusion into a matrix Removal of matrix, indoor and outdoor (e.g. abrasion)
Consumer Uses	Indoor and outdoor use of pigmented articles with low release Widespread dispersive indoor and outdoor use resulting in inclusion into a matrix Removal of matrix, indoor and outdoor (e.g. abrasion) Cosmetics
Article service life	Removal of matrix (e.g. abrasion), indoor and outdoor Indoor and outdoor use of coloured articles

Table 17

USES OF PO5	
Manufacture	Industrial manufacture of pigments or pigment additives
Formulation	Industrial formulation of non-solid preparations containing pigment (including inks and paint) and solid preparations containing pigment (including plastics) Formulation of paints and inks Use in textile/leather/fishing Industrial manufacture of coatings and inks Use in plastic masterbatches

Uses at industrial sites	Manufacture of substance Manufacture of pigments or pigment additives Industrial application of coatings and inks Use in paints
Uses by professional workers	Indoor and outdoor use of pigmented articles with low release Widespread dispersive indoor and outdoor use resulting in inclusion into a matrix Removal of matrix, indoor and outdoor (e.g. abrasion) Professional application of coatings and inks
Consumer Uses	Indoor and outdoor use of pigmented articles with low release Widespread dispersive indoor and outdoor use resulting in inclusion into a matrix Removal of matrix, indoor and outdoor (e.g. abrasion) Consumer application of coatings Auxilliary activities in professional application of coating Consumer use in paints and inks Cleaning and maintenance products PC 1: Adhesives, sealants PC 9a: Coatings and paints, thinners, paint removes PC 9b: Fillers, putties, plasters, modelling clay PC 9c: Finger paints PC 12: Fertilisers PC 14: Metal surface treatment products PC 15: Non-metal-surface treatment products PC 18: Ink and toners PC 23: Leather treatment products PC 24: Lubricants, greases, release products PC 25: Metal working fluids PC 26: Paper and board treatment products PC 27: Plant protection products PC 31: Polishes and wax blends PC 32: Polymer preparations and compounds PC 34: Textile dyes, and impregnating products
Article service life	Removal of matrix (e.g. abrasion), indoor and outdoor Indoor and outdoor use of coloured articles AC 1: Vehicles AC 2: Machinery, mechanical appliances, electrical/electronic articles AC 3: Electrical batteries and accumulators AC 4: Stone, plaster, cement, glass and ceramic articles AC 5: Fabrics, textiles and apparel AC 7: Metal articles AC 8: Paper articles AC 10: Rubber articles AC 11: Wood articles AC 13: Plastic articles AC 01: Other (non intended to be released): Painted articles

Uses of these pigments in Canada have also been compiled by the Canadian Agencies (Health Canada, 2016)(Canada 2009 a, b, c). Uses outside the EU might be relevant if imported goods are considered. In addition to the uses listed in Table 15, Table 16 and Table 17, in the Canadian Assessments use in textiles has been reported for all three pigments, use in low volumes in cosmetics has been reported for PR4, and adhesive manufacture has been reported for PO5. It is stated, that PR3 has also uses in cosmetics in other countries and is allowed in Europe in cosmetic products with only short intended skin contact (Health Canada, 2016) (Canada 2009 a, b, c).

Pigment Orange 5 is mainly used in the consumer sector as a colouring agent for mixtures like inks, coatings and paints. Furthermore, it can be found in complex articles made of metal (e.g. cutlery, pots, toys, jewellery), wood (e.g. floors, furniture, toys), paper (e.g.

tissues, feminine hygiene products, nappies, books, magazines, wallpaper) and plastic (e.g. food packaging and storage, toys, mobile phones) providing the same technical function (CSR information, ECHA Dissemination Site).

In addition, several product registers were evaluated by the eMSCA to identify additional relevant uses of PO5. Particular attention was given to potential availability of PO5 containing mixtures and articles to the general public or other products not covered by the current registration.

According to the SPIN database (Substances in Preparations in Nordic Countries) and taking into account the years 2015-2017, PO5 was notified only for the already mentioned uses above.

According to the German product database GIFAS (Giftinformations- und Archivierungssystem) 308 mixtures containing PO5 were reported by German companies. It is, however, unclear if those products are still available on the market. Of those products 17 are not for industrial or professional use. The highest reported PO5 content here is 1.5% for paints and coatings and 1% for cleaning and maintenance products (shoe and leather maintenance) which is an additional use currently not reported in the CSR but also mentioned on the ECHA dissemination site.

As for PO5, in addition to the information on the ECHA dissemination site, product registers were evaluated for additional information on uses of PR3. According to the German GIFAS database 28 mixtures available for consumers (non-professional/industrial and non-biocide) were notified (it should be noted that usually only classified mixtures will be notified). The highest reported concentration was 10 % in paints or coatings (e.g. universal coatings, acrylic coatings). Furthermore additional products were notified that may be categorized as a type of lamp oil or similar product. According to the SPIN database, a number of preparations in the use categories "Paints, lacquers and varnishes" and "Colouring agents" are available in the Nordic countries (Data up to 2017 available).

PR3 (C.I. 12120) is allowed to be used as a colourant in rinse-off cosmetics (Cosmetics Regulation (EC) No 1223/2009, Annex IV, entry 10).

PR4 (C.I. 12085) is used as a colouring agent in cosmetics. Its use as a hair dye is prohibited (Cosmetics Regulation (EC) No 1223/2009, Annex II, entry 1345) PR4 can be used as a colorant in cosmetics up to a concentration of 3 % (Cosmetics Regulation (EC) No 1223/2009, Annex IV, entry 9).

The usage of PO5 (C.I. 12075) is prohibited in cosmetics.

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

For neither of the three azo pigments a harmonised classification entry in Annex VI of Regulation (EC) 1272/2008 (CLP Regulation) is available. No proposals for harmonised classification and labelling have been submitted.

7.6.2. Self-classification

PR3

- In the registration(s):

  Not classified

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory:

Aquatic Chronic 4	H413	Aquatic Acute 1	H400
Aquatic Chronic 1	H410	STOT SE 3	H335
Eye Dam. 1	H318		

PR4

- In the registration(s):

  Not classified

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory:

Eye Irrit. 2	H319	Acute Tox. 4	H302
Aquatic Chronic 4	H413	Skin Irrit. 2	H315

PO5

- In the registration(s):

  Not classified

  An additional notified classification exists which is affected by impurities/additives:

  Skin Sens. 1 H317

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory:

Eye Irrit. 2	H319	Flam. Sol. 2	H228
Muta. 2	H341	Expl. 1.1	H201
Carc. 2	H351		

7.7. Environmental fate properties

7.7.1. Degradation

7.7.1.1. Abiotic degradation

No data are available on abiotic degradation. Hydrolysis is not expected.

7.7.1.2. Estimated data

BIOWIN[7] estimations were conducted for the three substances.

PR3 is predicted not readily biodegradable by BIOWIN 1, BIOWIN 2, BIOWIN 5 and BIOWIN 6. BIOWIN 3 predicts an ultimate biodegradation timeframe of months (value < 2.25) and BIOWIN 4 predicts a primary biodegradation timeframe of weeks. PR3 is in the molecular weight range of the models and its structural fragments are represented in the training data set.

PR4 is predicted not readily biodegradable by BIOWIN 1, BIOWIN 2, BIOWIN 5 and BIOWIN 6. BIOWIN 3 predicts an ultimate biodegradation timeframe of months (value < 2.25) and BIOWIN 4 predicts a primary biodegradation timeframe of weeks. PR4 is in the molecular weight range of the models and its structural fragments are represented in the training data set.

PO5 is predicted not readily biodegradable by BIOWIN 1, BIOWIN 2, BIOWIN 5 and BIOWIN 6. BIOWIN 3 predicts an ultimate biodegradation timeframe of months (value < 2.25) and BIOWIN 4 predicts a primary biodegradation timeframe of weeks. PO5 is in the molecular weight range of the models and its structural fragments are represented in the training data set.

In summary, all three substances fulfil the BIOWIN based screening criterion for persistence.[8]

7.7.1.3. Screening tests

No biodegradation was observed in a screening test on ready biodegradability according to OECD Guideline 301C for PR3. For PR4 and PO5, no results from screening tests on ready biodegradability are available. The registrants use read-across and conclude that all three substances are not readily biodegradable (and very persistent).

The eMSCA acknowledges some structural similarities between the substances, but the read-across is not considered as robust enough to replace experimental data. For PR4 and PO5 therefore, testing for ready biodegradability has been requested following compliance check. [9]

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[7] 2010 U.S. Environmental Protection Agency. BIOWIN v4.10.

[8] ECHA 2017. Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.11: PBT/vPvB assessment. Version 3.0, p. 49. https://echa.europa.eu/documents/10162/13632/information_requirements_r11_en.pdf/a8cce23f-a65a-46d2-ac68-92fee1f9e54f (accessed 26 September 2019)

[9] The read-across approach was already rejected by ECHA in recent compliance check decisions: "ECHA considers that this grouping and read-across approach does not provide a reliable basis whereby the human health effects and environmental effects of the registered substance may be predicted from data for reference substance(s) within the group."
ECHA 2019, Decision CCH-D-2114461479-37-01/F, p. 6.

Table 18

Summary of screening test results for PR3			
Test method	**Results**	**Reliability**	**Reference**
OECD Guideline 301C	After 28 days: BOD: 0% HPLC: 1% elimination elimination = primary degradation or adsorption	2	(NITE - National Institute of Technology and Evaluation, 1998)[10]

7.7.1.4. Simulation tests (water and sediments)

Not available.

7.7.1.5. Summary and discussion of biodegradation in water and sediment

PR3 is considered to fulfil the screening criterion for persistence based on both an OECD Guideline 301C study and QSAR results.

PR4 and PO5 lack biodegradation data. However, the substances fulfil the screening criterion for persistence based on QSAR results.

7.7.1.6. Biodegradation in soil

No relevant information available.

7.7.1.7. Summary and discussion on degradation

In summary, the three substances are considered as potentially persistent based on the available screening data (QSAR predictions for all substances and experimental ready biodegradability data for PR3 only).

Further information on degradation is necessary. The choice of an adequate test system must consider the low water solubility and possible adsorption of the substances as this would affect substance accessibility and the data that has to give clear evidence of degradation processes.

The necessary information should be generated as standard information requirement. Therefore, degradation will not be addressed under this process but under compliance check instead.

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https://echa.europa.eu/de/information-on-chemicals/dossier-evaluation-status/-/dislist/substance/100.018.693 (10.10.2019)
"ECHA considers that this grouping and read-across approach does not provide a robust basis whereby the environmental effects and environmental fate may be predicted from data for reference substance within the group by interpolation to other substances in the group (read-across approach)."
ECHA 2017, Decision CCH-D-2114381690-46-01/F, p.5-6. https://echa.europa.eu/de/information-on-chemicals/dossier-evaluation-status/-/dislist/details/0b0236e1813ea44c (10.10.2019)

[10] https://www.nite.go.jp/chem/jcheck/template.action?ano=6628&mno=5-3209&cno=2425-85-6&request_locale=en, https://www.nite.go.jp/chem/jcheck/detail.action?request_locale=ja&cno=2425-85-6&mno=5-3209 (23.09.2019)

7.7.2. Environmental distribution

7.7.2.1. Adsorption/desorption

A study according to OECD 121 is available for PR3, yielding a log K_{OC} > 5.6. No data are available for PR4 and PO5. Further studies on this endpoint are requested via compliance check.

7.7.2.2. Volatilisation

No information on volatilisation is available for the three substances.

7.7.3. Bioaccumulation

7.7.3.1. Aquatic bioaccumulation

7.7.3.1.1. Screening information

Log K_{OW} values of 2.45 (PO 5), 3.45 (PR4) and 3.7 (PR3) are available. However, the available log K_{OW} values were not determined directly according to HPLC or Shake Flask Method, but were calculated from the respective solubilities in water and in n-octanol. Due to the poor solubility in both n-octanol and water the adequacy of the accuracy of the values is questionable. As consequence, the low accuracy of the solubilities-based log K_{OW} values renders the values unreliable.

In addition to the log K_{OW} values provided in the registration dossier, QSAR calculations applying KOWWIN[11], chemicalize[12] and COSMOtherm[13] were conducted by the eMSCA. The respective results are shown in table 10. There is reasonable agreement among the different QSAR methods, but the log K_{OW} values estimated from solubilities in n-octanol and water are distinctly lower than the QSAR results.

The applicability domain for chemicalize and COSMOtherm results was not checked as training data were not available. A check was conducted for KOWWIN results:

All three substances are within the molecular weight range of KOWWIN. However, they all share a common structural fragment called "Ring reaction OH ortho to azo" that is neither present in the training nor in the validation set. There is no possibility for automatic structure search in the KOWWIN training and validation sets. A cursory search of the sets was conducted. The substances share a (naphthalen-1-yl)(phenyl)diazene moiety that is substituted with several functional groups. No substances with this moiety were identified. However, azobenzene and some of its derivatives were identified in the data sets. These share the structural feature of an azo group connecting two aromatic ring systems.

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[11] 2010 U.S. Environmental Protection Agency. KOWWIN v1.68.
[12] ChemAxon, https://chemicalize.com/#/ (14.08.2018)
[13] COSMOtherm, C3.0, release 1601, COSMOlogic GmbH & Co KG, http://www.cosmologic.de
COSMOconf, 4.0, COSMOlogic GmbH & Co KG, http://www.cosmologic.de
TURBOMOLE 4.1.1 2015, a development of University of Karlsruhe and Forschungszentrum Karlsruhe GmbH, 1989-2007, TURBOMOLE GmbH ; available from http://www.turbomole.com
F. Eckert and A. Klamt, "Fast solvent screening via quantum chemistry: COSMO-RS approach," AIChE J., vol. 48, no. 2, pp. 369–385, 2002.
A. Klamt, "Conductor-like screening model for real solvents: a new approach to the quantitative calculation of solvation phenomena," The Journal of Physical Chemistry, vol. 99, no. 7, pp. 2224–2235, 1995.
A. Klamt, V. Jonas, T. Bürger, and J. C. Lohrenz, "Refinement and parametrization of COSMO-RS," The Journal of Physical Chemistry A, vol. 102, no. 26, pp. 5074–5085, 1998.

Predicted log K_{OW} values for these related structures were in reasonable agreement with experimental data (see Table 20).

In summary, both the QSAR results and the log K_{OW} value calculated from the solubilities are considered as relevant and generated according to sound scientific principles. However, the information generated is in both cases only valid with very clear restrictions as accuracy and reliability are obviously limited.

Considering all available log K_{OW} values together in a weight of evidence approach, no firm conclusion can be drawn on whether or not the log K_{OW} values of Pigment Red 3, Pigment Red 4 and Pigment Orange 5 are above the screening criterion for bioaccumulation of log Kow 4.5.[14] The MSCA therefore concludes that the screening information on bioaccumulation is inconclusive and new information needs to be generated.

Both, water solubility and octanol-water partitioning coefficient are standard information requirements and will not be addressed under this process but under compliance check instead.

Table 19

Octanol water partition coefficients (log Kow) of Azo Pigments				
	Pigment Orange 5 EC 222-429-4	**Pigment Red 4 EC 220-562-2**	**Pigment Red 3 EC 219-372-2**	**Rel.**
Octanol /water	2.45	3.45	3.7	3
KOWWIN	5.72	6.55	6.45	3
Chemicalize	4.94	5.61	5.52	3
COSMOtherm	3.97	4.49	4.06	3

Table 20

Experimental And KOWWIN Predicted log Kow values for related Substances[15]				
	Experimental log Kow	**Predicted log Kow**	**Estimation Error**	**Training /validation set**
Azobenzene (CAS 103-33-3)	3.82	4.11	0.29	Training set
Azo Dye N1 (CAS 68877-63-4)	5.40	5.34	0.06	Training set
4-(N,N-dimethylamino)azobenzene (CAS 60-11-7)	4.58	4.29	0.29	Training set
Azo Dye D5 (CAS 3-67-4)	4.44	5.04	0.60	Validation set
Azo Dye N5 (CAS 72828-64-9)	5.50	5.83	0.33	Validation set
Azo Dye N9 (CAS 6657-33-6)	4.00	3.87	0.13	Validation set
p-Phenylazoaniline (CAS 60-09-3)	3.41	3.19	0.22	Validation set

[14] ECHA 2017. Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.11: PBT/vPvB assessment. Version 3.0, p. 68. https://echa.europa.eu/documents/10162/13632/information_requirements_r11_en.pdf/a8cce23f-a65a-46d2-ac68-92fee1f9e54f (accessed 26 September 2019)

[15] Available at http://esc.syrres.com/interkow/KowwinData.htm (26.09.2019)

7.7.3.1.2. Bioaccumulation in fish

A bioaccumulation study according to OECD 305 C is available for PR3. Test concentrations were above the water solubility of the substance and hence, the study is not considered reliable. No data are available for PR4 and PO5. This information need is subject to the standard testing scheme of REACH and thus the generation of new information will not be requested under substance evaluation but in a compliance check.

Table 21

Bioconcentration factors (BCF) for PR3						
Organism	**Exposure [mg/L]**	**Exposure [weeks]**	**BCF whole body [l/kg]**	**Lipid content [%]**	**Rel.**	**Reference**
Cyprinus carpio	0.1	6	< 2.9	4.2	3	(NITE - National Institute of Technology and Evaluation, 1998)[16]
	1.0	6	0.3 - < 2.7			

7.7.3.1.3. Terrestrial bioaccumulation

There are no data available on terrestrial bioaccumulation.

7.7.3.2. Summary and discussion on bioaccumulation

Based on the available information, it is not possible to conclude whether or not the log K_{OW} values of the three substances are above the screening criterion for bioaccumulation.

The available study on bioaccumulation of PR3 is considered as not reliable.

In summary, the available data for bioaccumulation allow a conclusion neither on the definitive nor on the screening criterion.

7.8. Environmental hazard assessment

7.8.1. Aquatic compartment (including sediment)

7.8.1.1. Fish

Information on acute fish toxicity is available for Pigment Red 3. The test was conducted according to OECD TG 203 with *Oryzias latipes* but with a test duration of only 48 hours instead of 96 hours. It was a static limit test with the nominal test concentration of 400 mg/L, which is five orders of magnitude (one hundred thousand times) above the approximate water solubility of 3.3 µg/L. No effects occurred. No acute tests are available for Pigment Red 4 and Pigment Orange 5. However, as the substances are poorly water soluble in water they require longer time to be significantly taken up by the test organisms and so steady state conditions are likely not to be reached within the duration of a short-term toxicity test. Therefore, short-term tests may not give a true measure of toxicity for such substances and toxicity may actually not even occur at the water solubility limit of

[16] https://www.nite.go.jp/chem/jcheck/template.action?ano=28556&mno=5-3209&cno=2425-85-6&request_locale=en, https://www.nite.go.jp/chem/jcheck/detail.action?request_locale=ja&cno=2425-85-6&mno=5-3209 (23.09.2019)

the substance if the test duration is too short. For this reason, long-term toxicity needs to be investigated.

Information on long-term fish toxicity for the three substances is not available.

This information need is subject to the standard testing scheme of REACH and thus the generation of new information will not be requested under substance evaluation but in a compliance check.

7.8.1.2. Aquatic invertebrates

For PR3 and PO5, acute toxicity tests to aquatic invertebrates (*Daphnia magna*) are available. They were conducted according to OECD TG 202 as limit test under static conditions over 48 hours. No effect occurred at the limit concentration 100 mg/L (nominal; DOC= 2.6 mg/L). The test concentration was highly above the maximum water solubility of 3.3 µg/L. As already explained above, due to the poor solubility of these substances, long-term toxicity needs to be investigated.

For PR3 a 21-d long-term toxicity test on aquatic invertebrates (*Daphnia magna*) is available. No effects occurred up to the highest test concentration of 35 µg/L (mean measured). Five test concentrations were used (0.45, 1.4, 4.5, 14, and 45 µg/L nominal) in the semi-static test. The recovery rate was between 50 and 101% of the nominal concentrations. Therefore, the result was given as mean measured concentration. The test fulfils the validity criteria.

Information on long-term toxicity testing on aquatic invertebrates for PR3 and PO5 is not available.

This information need is subject to the standard testing scheme of REACH and thus the generation of new information will not be requested under substance evaluation but in a compliance check.

7.8.1.3. Algae and aquatic plants

In a 72 hour-toxicity test to the aquatic algae *Pseudokirchneriella subcapitata* with PR3 according to OECD TG 201 no effects occurred up to the highest test concentration of 6 µg/L (geometric mean measured). Five concentrations were used in the test additionally to the control. For test item preparation, a stock solution with a loading rate of 100 mg/L was prepared and continuously stirred at room temperature in the dark over 24 hours. Subsequently, the dispersion was filtered and dilutions were prepared. The test is considered valid by the eMSCA. Information on toxicity testing on aquatic algae for PR4 and PO5 is not available.

This information need is subject to the standard testing scheme of REACH and thus the generation of new information will not be requested under substance evaluation but in a compliance check.

7.9. Human Health hazard assessment

7.9.1. Toxicokinetics

Non-human information

For PO5 and PR4, no relevant non-human information on toxicokinetics is available. Relevant animal studies for PR3 related to the assessment of the endpoint toxicokinetics are documented in the Table 22 below.

Table 22

Relevant studies for PR3 related to the assessment of the endpoint toxicokinetics			
Methods	**Results**	**Remarks**	**Reference**
Toxicology and Carcinogenesis studies of PR3 in F344/N rats and B6C3F1 Mice (Feeding study)	Some evidence of absorption of PR3, metabolites, or impurities in the test substance was observed because toxic effects were found in blood samples from rats in sub-acute, sub-chronic and chronic studies in rats and mice.		(NTP, 1992b)
No guideline, no GLP Toxicokinetic study Species: Rats Strain: Fischer 344 Sex: male Age: 7-8 weeks weight: 146-180 g Group: 3 animals per time series group Absorbance detection of intact dye PR3 Purity 94.7 % Dosing: 11.8 mg/kg Route: oral gavage Sampling times: 1, 4, 24, 48 hours after dosing	Total recovery after 24 and 48 hours reduced, suggesting excretion of metabolite ("degraded by intestinal bacteria or complexed in an inextractable form during passage through the G.I. tract"). Dye not found in urine (< 1%) and only low amounts in tissues (discussed, rather adherence than absorption).	Method not sufficient for toxicokinetic analysis (metabolites, only intact dye, insensitive method) Not reliable	(El Dareer et al., 1984)

7.9.2. Acute toxicity and Corrosion/Irritation

7.9.2.1. Acute toxicity: oral, dermal and inhalation

All three azo pigments do not trigger concern for acute toxicity based on oral LD50 values > 10 000 mg/kg bw for PO5, PR3 and PR4. For PO5, additional dermal LD50 values above 2 000 mg/kg bw support this conclusion.

7.9.2.1.1. Conclusion from SEV

Overall, the eMSCA considers the available data as appropriate for an evaluation of the acute toxicity and no further study is necessary from the point of view of the eMSCA.

7.9.3. Corrosion/Irritation

From available data on all three azo pigments, no concern could be identified for skin and eye corrosion/irritation.

7.9.3.1. Skin Irritation/Corrosion

7.9.3.1.1. PR3

Skin irritation/corrosion was not an initial concern for PR3.

7.9.3.1.1.1. Non-human information

Animal data on irritation/corrosion after dermal exposure

Several relevant *in vivo* studies have been identified which addressed the dermal irritation/corrosion potential of PR3. The respective data is summarized in Table 23. PR3 has been tested in OECD TG 404 similar assays which gave no indication that the substance is a dermal irritant. The registrant concluded that PR3 is not a dermal irritant and based on the available data, the eMSCA can support this conclusion.

Table 23

Relevant studies related to the assessment of skin irritation/corrosion for PR3			
Methods	**Results**	**Remarks**	**Reference**
Acute skin irritation/corrosion tests in rabbits Species: Albino/Himalayan 6 animals Method: FDA protocol Substance: Hansa scharlach RNC PR3: purity not reported 500mg PR3 in 0,7 ml PEG 400 on 2,5 cm² skin areal (flank, clipped) + test on scarified skin 24 h treatment (dermal patch, occlusive) Assessment of skin at: 0h, 24h, 48h, 72h after application	Slightly irritant to skin Irritation score of 2,3 based on FDA Guideline scoring scheme Assessment at 24h and 72h after treatment Longer treatment (24h instead of 4h) Dermal damage of scarified skin also included in calculation No comment whether staining (colour) precluded judgment of erythema	Not reliable – OECD TG 404 comparable / similar (minor changes) ID/Purity of substance not reported with restriction (no examination at 72h after patch removal) No individual animal data reported (e.g. how many animals reacted how and when) No data on dermal response of the treated area	(Hoechst, 1980)

Relevant studies related to the assessment of skin irritation/corrosion for PR3			
Methods	**Results**	**Remarks**	**Reference**
		Ideally, solid should have been tested (OECD TG 404)	
Acute skin irritation/corrosion tests in rabbits Species: Albino/Himalayan 6 animals Method: FDA protocol Substance: Hansa scharlach rb Analytical comment in report: "The pigment is chemically identical to Hansa scharlach RNC and Hansa rot B, however due to the production process it is more yellow than Hansarot b and more blue than Hansa scharlach RNC" PR3: approx. 100 % technically pure 500 mg PR3 (powder) on gauze patch, tapped to skin, 2,5 cm² skin areal (flank, clipped), occlusive + test on scarified skin Patch removed after 24h Assessment of skin at: 0h, 24h and 48h after patch removal	Not a skin irritant No irritation observed after 24h, 48h and 72h of treatment Scoring based on FDA guideline Longer treatment (24h instead of 4h) Dermal damage of scarified skin also included in calculation	Reliable/Valid study – equivalent to OECD TG 404 (comparable, minor deviation) Purity of substance not documented just stated as approx. 100% technically pure No certificate No individual results (e.g. how many animals reacted how and when) No data on dermal responses of the treated area Induction of erythema (at 24h) could not be evaluated due to colour interference of PR3	(Hoechst, 1977a)
Acute skin irritation/corrosion tests in rabbits Species: Albino/Himalayan 6 animals Method: FDA protocol Substance: Hansa scharlach RNC PR3: approx. 100 % technically pure 500 mg PR3 (powder) on gauze patch, tapped to skin, 2,5 cm² skin areal (flank, clipped), occlusive + test on scarified skin Patch removed after 24h Assessment of skin at: 0h, 24h, 48h after patch removal	Not a skin irritant No irritation observed after 24h, 48h and 72h of treatment Scoring based on FDA guideline Longer treatment (24h instead of 4h) Dermal damage of scarified skin also included in calculation	Reliable/Valid study – equivalent to OECD TG 404 (comparable, minor deviation) Purity of substance not documented just stated as approx. 100% technically pure No individual animal data (e.g. how many animals reacted how and when) No data on dermal responses of the treated area Induction of erythema could not be evaluated at 24h due to colour interference of PR3	(Hoechst, 1976)

Relevant studies related to the assessment of skin irritation/corrosion for PR3			
Methods	**Results**	**Remarks**	**Reference**
Acute skin irritation/corrosion tests in rabbits Species: Albino/Himalayan 6 animals Method: FDA protocol Substance: Hansarot B PR3 purity: not reported 500 mg PR3 (powder) on gauze patch, taped to skin, 2,5 cm² skin areal (flank, clipped), occlusive + test on scarified skin Patch removed after 24h Assessment of skin at: 0h, 24h, 48h after patch removal	Not a skin irritant No irritation observed after 48h and 72h of treatment Scoring based on FDA guideline Longer treatment (24h instead of 4h) Dermal damage of scarified skin also included in calculation	Not reliable – equivalent to OECD TG 404 (comparable, minor deviation) Substance ID/ Purity not reported No individual animal data (e.g. how many animals reacted how and when) No data on dermal responses of the treated area No skin observation after 72h Potential induction of erythema at 24h could not be evaluated due to colour interference of PR3	(Hoechst, 1977b)

7.9.3.1.1.2. Human information

Human data addressing this endpoint was not available.

7.9.3.1.1.3. Conclusion

Data are conclusive, no trigger for harmonized classification was identified. No concern for study request(s) under SEv for PR3 regarding dermal irritation/corrosion was identified.

7.9.3.1.2. PR4

Skin irritation/corrosion was not an initial concern for PR4.

7.9.3.1.2.1. Non-human information

Animal data on irritation/corrosion after dermal exposure

Relevant and conclusive data of already performed animal studies have been identified and summarised in Table 24 which address the irritation/corrosion potential of PR4. In a reliable OECD TG 404 study topical application of 500mg of PR4 on 4cm² for 4h did not cause adverse skin reactions which would be indicative of irritation or corrosion (RCC-CCR, 2006b). The registrant concluded that PR4 is not a dermal irritant and based on the available data, the eMSCA can support this conclusion.

Table 24

Relevant studies related to the assessment of skin irritation/corrosion for PR4			
Methods	**Results**	**Remarks**	**Reference**
Acute skin irritation/corrosion tests in rabbits OECD TG 404 compliant (2002) Species: Albino/new Zealand 3 animals (1 male, 2 female) Substance: Hansa Rot R PR4: 98,2% Impurities reported 500mg /0,5 ml water 1 % (w/v) test solution, pH of 6,68 indicated good skin tolerance Treatment: 500 mg applied to 4 cm² of skin (left flank, clipped) 4h treatment (dermal patch, semi-occlusive) after removal of patch skin was flushed with warm water Assessment of treated skin: 1h, 24h, 48h, 72h and 7, 10, 14 days	Not a skin irritant Due to marked staining readings at 24h (2 animals) and at 72h (1 animal) not possible	Reliable/Valid study – key study OECD TG 404 compliant Purity/impurities reported Analysis certificate indicates quite a lot of impurities	(RCC-CCR, 2006b)
Acute skin irritation/corrosion tests in rabbits Method: subcutaneous/dermal testing according to Barail Species: Rabbit (not further specified), 3 animals C Red 41/Permanentrot R extra Plv purity not reported	Not a skin irritant	No reliability/not validity – limited value Not OECD TG 404 compliant No GLP Purity not reported	(Gewerbe- und Arzneimitteltoxikologie, 1962)

Relevant studies related to the assessment of skin irritation/corrosion for PR4			
Methods	**Results**	**Remarks**	**Reference**
Pigment dispersion in NaCl, filtrate was generated 0,5 ml of filtrate was applied subcutaneously and topically to clipped skin of flank		applied substance amount not reported treated area size not reported no detailed description of dermal responses no documentation of individual animal results	
Acute skin irritation/corrosion tests in rabbits Method: subcutaneous/dermal testing according to Barail Species: Rabbit (not further specified), 3 animals C Red 41/Permanentrot R extra Plv purity not reported Pigment dispersion in NaCl, filtrate was generated 0,5 ml of filtrate was applied subcutaneously and topically to clipped skin of flank	Not a skin irritant	No reliability/validity – limited value Not OECD TG 404 compliant No GLP Purity not reported applied substance amount not reported treated area size not reported no detailed description of dermal responses no documentation of individual animal results	(Gewerbe- und Arzneimitteltoxikologie, 1962)
Acute skin irritation/corrosion tests in rabbits Method: subcutaneous/dermal testing according to Barail Species: Rabbit (not further specified), 3 animals C Red 41/Permanentrot R extra Plv purity not reported Pigment dispersion in NaCl, filtrate was generated 0,5 ml of filtrate was applied subcutaneously and topically to clipped skin of flank	Not a skin irritant	No reliability/validity – limited value Not OECD TG 404 compliant No GLP Purity not reported applied substance amount not reported treated area size not reported no detailed description of dermal responses no documentation of individual animal results	(Gewerbetoxikologisches Laboratorium, 1959)

7.9.3.1.2.2. Human information

Human data addressing this endpoint was not available.

7.9.3.1.2.3. Conclusion

Overall, 626 classification and labelling notifications for PR4 have been submitted according to ECHA's dissemination website (last accessed on 02.10.2019). According to the aggregated self-classifications, PR4 is identified as Skin Irrit. 2 (H315). Data that would support such a conclusion is not available to the eMSCA.

Data are conclusive, no trigger for harmonized classification was identified. No concern for study request(s) under SEv for PR4 regarding dermal irritation/corrosion was identified.

7.9.3.1.3. PO5

Skin irritation/corrosion was not an initial concern for PO5.

7.9.3.1.3.1. Non-human information

Animal data on irritation/corrosion after dermal exposure

A reliable OECD TG 404 compliant study has not been identified that addresses whether PO5 is irritating or corrosive to skin. The study by (Hoechst, 1973c), see Table 25, is not reliable and of limited value as it lacks information i.e. on substance identity and purity. Additionally, the testing design and the respective experimental data is poorly recorded. Therefore, the eMSCA evaluated data of a valid and reliable dermal acute toxicity study performed in rats for its suitability to conclude on this endpoint (Harlan, 2012). In this study, a topical dose of 2000mg/kg bw over 24 h to clipped rat skin did not cause adverse skin responses meaning that a concentration of approx. 20 µg/cm² is non-irritating to rat skin. Whilst this amount is less than required by OECD TG 404, the time of exposure is more prolonged (24h). Additionally, higher animal numbers are been treated in an OECD TG 402 study. The registrant concluded that PO5 is not a dermal irritant and based on the available data, the eMSCA can support this conclusion.

Table 25

Relevant studies related to the assessment of skin irritation/corrosion for P05			
Methods	**Results**	**Remarks**	**Reference**
Acute skin irritation/corrosion tests in rabbits Method: subcutaneous/dermal testing according to Barail Species: Rabbit (Yellow/Silver), 6 animals Hansaorange RN 01: purity not reported Test concentrations: 0,001%; 0,01%, 0,1%, 1%, 5%, 10 % (w/v) in sesame oil Subcutaneuos injection 0,02 ml /each injected into clipped skin of the flank Topical treatment 0,5 ml, 5% and 10%(w/v) in sesame oil, daily for 5 days (clipped skin of flank)	Not irritating to skin Subcutaneous injection: Result: 10 % and 5 % caused local necrotic lesions around injection spot 1 % caused diverse skin reactions, e.g. (severe) redness, swelling, necrosis < 1 % - no skin reactions Considered to be irritating when much higher dilutions (10^{-3}) cause effects Treatment duration – not reported Dermal application Result: not irritating to skin -	Not valid /not reliable Not OECD TG conform Study is of limited value Purity of substance not reported No individual results reported (e.g. how many animals reacted how and when) No description of treated skin area (topical application) The solid should have been tested	(Hoechst, 1973b; Hoechst, 1973c)

7.9.3.1.3.2. Human information

Human data addressing this endpoint was not available.

7.9.3.1.3.3. Conclusion

Data are conclusive, no trigger for harmonized classification was identified. No concern for study request(s) under SEv for PO5 regarding dermal irritation/corrosion was identified.

7.9.3.2. Eye irritation/corrosion

7.9.3.2.1. PR3

Eye irritation/corrosion was not an initial concern for PR3.

7.9.3.2.1.1. Non-human information

Animal data on irritation/corrosion after ocular exposure

Several reliable and conclusive *in vivo* animal studies have been identified that assess the end point eye irritation/corrosion. Study data is summarised in Table 26. Instillation of 100mg of PR3 into the conjunctival sac of albino rabbits did not cause an irritative ocular response (Hoechst, 1976; Hoechst, 1977a; Hoechst, 1977b; Hoechst, 1980; Hoechst, 1983). The registrant concluded that PR3 is not an eye irritant and based on the available data, the eMSCA can support this conclusion.

Table 26

Relevant studies related to the assessment of eye irritation/corrosion for PR3			
Methods	**Results**	**Remarks**	**Reference**
Acute eye irritation/corrosion test OECD TG 405 (1982) Species: rabbit Albino/New Zealand 3 animals Hansa Scharlach RNC Purity not reported contains 0,4 % of un undisclosed additive Dose: 100mg in 0,1 ml of PEG 400 Instilled into the conjunctival sac of left eye 24h after application the eye was rinsed carefully with NaCl assessment of cornea, iris, conjunctiva with magnifying glass at 1h, 24h, 48h, 72h after application Assessment of damage to cornea and tissue with fluorescein under UV light at 24h and 48h post application	Not an eye irritant Slight swelling and reddening of conjunctivae 1h post application red discharge was observed according to expert judgement – both observations are considered insignificant at 24h only redness in 2 animals, at 48h animals cleared of effects	Valid study OECD TG 405 compliant /similar/ equivalent Purity of PR3 not reported Additive not disclosed Pigment + 0,4% additive	(Hoechst, 1983)

Relevant studies related to the assessment of eye irritation/corrosion for PR3			
Methods	**Results**	**Remarks**	**Reference**
Acute eye irritation/Corrosion Rabbit Albino/Himalayan 6 animals FDA Guideline protocol Hansa Scharlach RNC purity not reported 100mg of PR3 in 0,2 ml of PEG 400 1 x instillation into the conjunctival sac of left eye After 24h the eye was rinsed with NaCl Assessment of irritation by visual inspection of cornea, iris, conjunctiva with magnifying glass at time points: 1h, 7h, 24h, 48h, 72h after application Fluorescein (48h and 72h	Slightly irritant to the eye The highest irritancy score of 20 was observed at time point 7h According to the FDA guideline a score of 20 classifies a substance as slightly irritant to the eye	Not valid study with restrictions (?) no substance ID? OECD TG 405 comparable Substance ID/ Purity not reported magnifying glass/fluorescein No individual animal data reported No substance certificate	(Hoechst, 1980)
Acute eye irritation/corrosion test Species: rabbit Albino/Himalayan 6 animals FDA guideline protocol Hansa Scharlach RB The pigment is chemically identical to Hansa scharlach RNC and Hansa red B, however due to the production process it is more yellow than Hansarot b and more blue than Hansascharlach RNC Purity: approx. 100 % pigment Dose: 100mg instilled into the conjunctival sac of left eye 24h after application the eye was rinsed carefully with NaCl Assessment of irritation by visual inspection of cornea, iris, conjunctiva with magnifying glass at time points: 1h, 7h, 24h, 48h, 72h after application Assessment of damage to cornea and tissue with fluorescein at 48h and 72h post application	Non-irritant to the eye The highest irritancy score of 10 was observed at time point 7h According to the FDA guideline a score of 10 classifies a substance as not irritant to the eye	Valid study with minor restrictions OECD TG 405 comparable/similar/equivalent Purity: approx. 100 % pigment No analytical certificate No individual animal data reported	(Hoechst, 1977a)
Acute eye irritation/corrosion test Species: rabbit Albino/Himalayan	Not an eye irritant	Valid study with minor restrictions OECD TG 405 comparable	(Hoechst, 1976)

Relevant studies related to the assessment of eye irritation/corrosion for PR3			
Methods	**Results**	**Remarks**	**Reference**
6 animals FDA guideline protocol Hansa Scharlach RNC "Pigment is chemically identical to Hansa scharlach Rb and Hansa rot B but more yellow." Purity: approx. 100 % pigment Dose: 100mg instilled into the conjunctival sac of left eye 24h after application the eye was rinsed carefully with NaCl The assessment of cornea, iris, conjunctiva was performed with a magnifying glass at 1h, 24h, 48h, 72h post application Assessment of damage to cornea and tissue with fluorescein at 48h and 72h post application	Highest score (irritation index) of 2 after 1h Judgement based on FDA guideline protocol	Purity: approx. 100 % pigment No analytical certificate No individual animal data reported	
Acute eye irritation/corrosion test Species: rabbit Albino/Himalayan 6 animals FDA guideline protocol Hansarot B Purity: not reported Dose: 100mg instilled into the conjunctival sac of left eye 24h after application the eye was rinsed carefully with NaCl Assessment of irritation by visual inspection of cornea, iris, conjunctiva with magnifying glass at time points: 1h, 7h, 24h, 48h, 72h after application Assessment of damage to cornea and tissue with fluorescein at 48h and 72h post application	Not an eye irritant Highest score (irritation index) of 9 after 1h Judgement based on FDA guideline protocol	Not valid study OECD TG 405 comparable Purity not reported No analytical certificate No individual animal data reported	(Hoechst, 1977b)

7.9.3.2.1.2. Human information

Human data addressing this endpoint was not available.

7.9.3.2.1.3. Conclusion

On ECHAs dissemination website for PR3 the hazard class Eye Dam. 1 H318 is notified among the aggregated self-classifications in the C&L Inventory. Data that would support such a classification has not been identified by the eMSCA.

Data are conclusive, no trigger for harmonized classification was identified. No concern for study request(s) under SEv for PR3 regarding eye irritation/corrosion was identified.

7.9.3.2.2. PR4

Eye irritation/corrosion was not an initial concern for PR4.

7.9.3.2.2.1. Non-human information

Animal data on irritation/corrosion after ocular exposure

The eye irritation/corrosion potential of PR4 has been addressed in several animal studies which are summarised in Table 27. The endpoint has been investigated in a relevant und reliable OECD TG 405 study using albino rabbits (RCC-CCR, 2006a). The instillation of 100mg PR4 in the conjunctival sac of the eye resulted in mild, early-onset and reversible ocular changes that were judged not to be related to irritation or corrosive damage to the eye.

Table 27

Relevant studies related to the assessment of eye irritation/corrosion for PR4			
Methods	**Results**	**Remarks**	**Reference**
Acute eye irritation/corrosion test Method not specified Species: Rabbit 3 animals (not further specified) Substance: C Rot Nr. 41/Permanentrot R extra Plv: purity not reported Pigment dispersion in NaCl, filtrate was generated 0,25ml of filtrate was instilled into conjunctival sac of left eye	Reversible slight reddening / irritation of conjunctiva	Not reliable/not valid Study is not OECD TG 405 compliant SubstanceID/purity of C Rot Nr 41 not reported Treatment duration not reported Treatment concentration not reported No individual animal data reported No detailed assessment of adverse effects on eye tissue Solid should have been tested	(Gewerbe- und Arzneimitteltoxikologie, 1962)
Acute eye irritation/corrosion test Method not specified Species: Rabbit 3 animals (not further specified) Substance: C Rot Nr. 41/Permanentrot R extra Plv:	Reversible slight reddening of conjunctiva	Not reliable/not valid Study is not OECD TG 405 compliant Purity of C Rot Nr 41 not reported	(Gewerbe- und Arzneimitteltoxikologie, 1962)

Relevant studies related to the assessment of eye irritation/corrosion for PR4			
Methods	**Results**	**Remarks**	**Reference**
purity not reported Pigment dispersion in NaCl, filtrate was generated 0,25ml of filtrate was instilled into conjunctival sac of left eye		Treatment duration not reported Treatment concentration not reported No individual animal data reported No detailed assessment of adverse effects on eye tissue Solid should have been tested	
Eye irritation Acute eye irritation/Corrosion OECD Guideline 405 (2002) compliant Species: rabbit albino/ New Zealand 1 male, 2 females Hansa Rot R Purity: 98,2 % Impurity reported 100 mg of substance instilled into the conjunctival sac of left eye No rinse Ocular damage: 1h, 24h, 48h and 72h hours after instillation Eye examinations were made with a Varta Cliptrix diagnostic-lamp GLP compliant	Not irritating to the eye	Reliable/valid with restrictions Key study OECD TG405 (Acute Eye Irritation / Corrosion) 2002 compliant; however with restrictions (no fluorescein analysis of eye tissue – however Varta cliptrix lamp as diagnostic tool) No rinse Scoring across 3 scoring times Individual animal data Lots of impurities	(RCC-CCR, 2006a)
Eye irritation/corrosion testing Method not specified Species: Rabbit Permanent-rot R extra: purity not reported Pigment dispersion in NaCl, filtrate was generated 0,25 ml of filtrate instilled into the conjunctival sac of left eye	Not an eye irritant	Not valid /not reliable Study is not OECD TG 405 compliant Not GLP conform Purity of Permanent-rot R extra not reported Treatment duration not reported Treatment concentration not reported No individual animal data reported No detailed assessment of adverse effects on eye tissue Solid should have been used	(Gewerbetoxikologisches Laboratorium, 1959)

7.9.3.2.2.2. Human information

Human data addressing this endpoint was not available.

7.9.3.2.2.3. Conclusion

On ECHAs dissemination website for PR4 the hazard class Eye Irrit. 2 H319 is notified among the aggregated self-classifications in the C&L Inventory. Data that would support such a classification has not been identified by the eMSCA.

Data are conclusive, no trigger for harmonized classification was identified. No concern for study request(s) under SEv for PR4 regarding eye irritation/corrosion was identified.

7.9.3.2.3. PO5

Eye irritation/corrosion was not an initial concern for PO5.

7.9.3.2.3.1. Non-human information

Animal data on irritation/corrosion after ocular exposure

One *in vivo* study has been identified which investigated the irritation/corrosion potential of PO5 to the eye ((Hoechst, 1973c),Table 28). The report does not reveal any information on the purity of the substance and is therefore of limited value. No further relevant data has been identified that would support a conclusion on this endpoint by the eMSCA. Consequently, the eMSCA reserves judgement on the conclusion of the registrant regarding the potential of PO5 to cause ocular damage (registrant conclusion is non-irritating).

Table 28

Relevant studies related to the assessment of eye irritation/corrosion for P05			
Methods	**Results**	**Remarks**	**Reference**
Acute eye irritation/corrosion test Species: Rabbit (Yellow/silver) 6 animals Hansaorange RN 01: purity not reported 0,1 ml of 5% and 10% (w/v) suspension; instilled into the conjunctival sac Vehicle: sesame oil? Ocular damage: 1h, 3h, 7h, and 24h after instillation	Slightly irritant to the eye 10 % suspension caused in 3 of 6 rabbits slight reddening of conjunctiva for 3-7h – gone at 24h Remaining rabbits showed no sign of an adverse reaction	Not reliable/ not valid Study is not OECD TG 405 compliant Not GLP compliant Purity of Hansaorange RN 01 not reported Treatment duration not reported No individual animal data reported No detailed assessment of adverse effects on eye tissue	(Hoechst, 1973c)

7.9.3.2.3.2. Human information

Human data addressing this endpoint was not available.

7.9.3.2.3.3. Conclusion

Available data are not sufficient for a conclusion, however, no trigger for harmonized classification was identified. No concern for study request(s) under SEv for PO5 regarding eye irritation/corrosion was identified.

7.9.4. Sensitisation

7.9.4.1. PR3

Skin sensitisation was not an initial concern for PR3.

7.9.4.1.1. Non-human information

Animal data on the skin sensitising potential

A reliable and conclusive *in vivo* animal study has been identified that assessed the end point skin sensitisation. Study data is summarised in Table 29. In a Guinea Pig Maximisation Test (GPMT) according to OECD TG 406 PR3 did not induce an allergenic response in rats. 4 of 10 animals showed slight to well-defined erythema and oedema 48 hours after the first dermal challenge. In the second dermal challenge that was performed to verify these initial findings, only 1 of 10 animals of the treatment group showed slight to well defined erythema after 24/48h (Hoechst, 1992). The registrant concluded that PR3 is not a skin sensitiser and based on the available data the eMSCA can support this conclusion.

Table 29

Relevant studies related to the assessment of the sensitising potential of PR3			
Methods	**Results**	**Remarks**	**Reference**
Guinea pig maximisation test (GPMT) / Magnusson & Kligman OECD TG 406 study (1981) Guinea pig Pirbright-White, 15 females 10/test; 5/control Hansa Scharlach RNC 95,6 % Impurities not reported Pre-testing/dose finding Intradermal injection 2x 0.1ml of 0.2 % (w/w) PR3 in semi-liquid paraffin (DAB), 50 % FCA (Freund's Complete Adjuvants)	Not a skin sensitizer Mild/strong irritation conc.: intradermal **0.2 %** in semi-liquid parrafin Dermal: 5 % in petrolatum Non-irritating conc.: **1 %** No SDS treatment, as strong irritation was induced 1/10 animals showed positive skin response at both challenges 24/48h after 1st challenge Treatment group 2 animals – slight to well-defined erythema after 24h 4 animals – slight to well-defined erythema and slight oedema (48h)	Valid study – reliable/similar/equivalent Poor purity (95,6%) of substance/impurities not disclosed	(Hoechst, 1992)

Relevant studies related to the assessment of the sensitising potential of PR3			
Methods	**Results**	**Remarks**	**Reference**
Dermal induction 0.5 g of 5 % (w/w) PR3 in petrolatum (DAB), 2x4 cm² patch, occlusive Dermal challenge 0.5 g of 1 % (w/w) PR3 in petrolatum (DAB), 2x2 cm² patch, occlusive Repeated dermal challenge treatment 0.5g of 1 % (w/w) PR3 in petrolatum (DAB), 2x2 cm²occlusive	24/48h after 2nd challenge Treatment group 1 animal – slight to well defined erythema		

7.9.4.1.2. Human information

Human data addressing this endpoint was not available.

7.9.4.1.3. Justification for classification or non-classification

Available data does not allow for a classification.

7.9.4.2. PR4

Skin sensitisation was not an initial concern for PR4.

7.9.4.2.1. Non-human information

Animal data on the skin sensitising potential

A reliable and conclusive *in vivo* animal study has been identified that assessed the end point skin sensitisation. Study data is summarised in Table 30. In a classical Local lymph Node Assay according to OECD TG 429 PR4 did not induce an allergenic response in mice (RCC-CCR, 2005). Due to the poor solubility of the substance, only a maximal test concentration of 17% was achieved. The deduced Stimulation Indices were well below 3 and EC3s could not be calculated. Colour of PR4 interfered with the detection of local irritation/erythema on the ear lobe, however, ear swelling indicative of an inflammatory response was not observed. Based on the available data the eMSCA can support the conclusion of the registrant that PR4 is not a skin sensitiser.

Table 30

Relevant studies related to the assessment of the skin sensitising potential of PR4			
Methods	**Results**	**Remarks**	**Reference**
Skin sensitisation LLNA	Not a skin sensitiser Up to 17 %	Reliable, valid with restriction	(RCC-CCR, 2005)

Local lymph node assay OECD TG 429 (2002; compliant) Species: Mouse CBA/ClaOlaHsd 20 females 5/group Hansa Rot R: 98,2 % Analytical certificate Vehicle: Acetone:olive oil, 4:1 (v/v) Pre-test: Test conc. 5 %, 10 %, 17 % (w/v) (17% highest conc. due to solubility issues) Stimulation index 1,17; 1,29; 1,26 Local irritation not assessable due to colour interference Positive control (α-Hexylcinnamaldehyde) GLP compliant	(judged by SI values that were below 3; EC3 could not be calculated) Test substance was soluble at max. 17%	OECD TG 429 compliant, with restriction (higher concentrations should have been tested) Local irritation not assessable due to colour interference /but no swelling of ear lobe	

7.9.4.2.2. Human information

No relevant studies were identified covering this endpoint.

7.9.4.2.3. Conclusion

Data are conclusive, no trigger for harmonized classification was identified. No concern for study request(s) under SEv for PR3 regarding skin sensitisation was identified.

7.9.4.3. PO5

Skin sensitisation was not an initial concern for P05.

7.9.4.3.1. Non-human information

Non-human information / Animal data on the skin sensitising potential

Reliable and conclusive *in vivo* animal studies have been identified that assessed the skin sensitising potential of PO5. Study data is summarised in Table 31. Valid experiments according to OECD TGs have been performed both in rats and mice with varying results. In GPMTs according to Magnusson & Kligman PO5 was shown to be a skin sensitiser. In

contrast to the GPMT data, OECD TG 429 compliant studies (LLNA) performed in mice did not show that PO5 has skin sensitising properties.

The purity of the test substance has been disclosed in some reports. The substance Dinitrochlorobenzene (1-chloro-2,4-dinitro benzene; DNCB; CAS No. 97-00-7) has been identified in relevant amounts as a synthesis dependent impurity in PO5. DNCB is a well-known and potent immunogen both in rodent and man (Loveless et al., 1996; Nakamura et al., 1994; White et al., 1986). DNCB has no harmonised classification entry in Annex VI of Regulation (EC) 1272/2008 (CLP-Regulation). Given the reported amounts of DNCB in PO5 (931ppm, (Aventis, 2002)), it is plausible to assume that the impurity DNCB is triggering the observed allergenic responses in the rat. Since the LLNA for certain substances is less sensitive than the GPMT this could explain the differences in results.

Based on the available data the eMSCA supports the conclusion of the registrant to label Pigment Orange 5 as a skin sensitiser (Skin Sens. 1 H317) dependent on the content of the impurity DNCB. The registrant has proposed that PO5 with a content of > 0.03% DNCB should be labelled. It should be noted that experimental data in the lead dossier does not allow conclusively drawing this conclusion. Negative results in the LLNA cannot be attributed to a lack of DNCB because impurities have not been disclosed. Also, only one positive GPMT assay with PO5 (Aventis, 2002) can be correlated with DNCB. In the other GPTM study by (Hoechst, 1991) that demonstrates the sensitising potential of PO5, impurities are not reported. However, taking these uncertainties into consideration the eMSCA is still of the opinion that the conclusions of the registrant can be supported regarding the labelling of PO5 with a content of DNCB >0.03% as skin sensitiser.

Table 31

Relevant studies related to the assessment of the skin sensitising potential of P05			
Methods	**Results**	**Remarks**	**Reference**
Skin sensitisation LLNA Local lymph node assay OECD TG 429 (2002; compliant) Species: Mouse CBA/ClaOlaHsd females 4/group Pigment Orange 5: 99,75% Impurities 2,4-Dinitroaniline 6ppm; ß-Naphthol 13 ppm; 2,4-Dinitrochlorobenzene << 75 ppm; CI Pigment Red 4 0,24 % Vehicle: Acetone:olive oil, 4:1 (v/v) Test conc. 2,5%, 5%, 10% (w/v) (10% (w/v) highest conc. technically achievable Positive control (α-Hexylcinnamaldehyde)	Not a skin sensitiser (up to a conc. of 10 % (w/v)) Stimulation index 1,6; 1,2; 1,3 (judged by SI values that were below 3; EC3 could not be calculated)	Valid, reliable OECD TG 429 compliant, with restriction (rather narrow testing range (max 10%). Local irritation not assessable due to colour interference	(RCC Ltd, 2003)

Skin sensitisation LLNA Local lymph node assay OECD TG 429 compliant Species: Mouse CBA/ClaOlaHsd females 4/group Pigment Orange 5: 97,8 % Impurities not reported Vehicle: Propylene glycol Test conc. 2,5%, 5%, 10% (w/v) (10% (w/v) highest conc. due to solubility issues) Local irritation not assessable due to colour interference Positive control (α-Hexylcinnamaldehyde)	<u>Not a skin sensitiser</u> (up to a conc. of 10 % (w/v)) Stimulation index 1,3; 0,7; 0,9 (judged by SI values that were below 3; EC3 could not be calculated)	Not valid /not reliable study – no original study report (copy of IUCLID data / only summary) narrow test conc. range (which is due to solubility) OECD TG 429 compliant (with restrictions?) Range finding tests indicate that PO5 won't dissolve at higher concentrations colour of test item precludes assessment of erythema (however no abnormal change in ear thickness)	(Harlan, 2012)
Skin sensitisation LLNA Local lymph node assay OECD TG 429 compliant (2002) Species: Mouse CBA/ClaOlaHsd females 5/group Pigment Orange 5: >97 % Impurities not reported Vehicle: acetone:olive oil (4:1 v/v) Test conc. 2,5%, 5%, 10% (w/v) (10% (w/v) No range finding Stimulation index 0,89; 0,87; 1,02 Local irritation not assessable due to colour interference Positive control (α-Hexylcinnamaldehyde) GLP compliant	<u>Not a skin sensitiser</u> (up to a conc. of 10 % (w/v)) Stimulation index 0,89; 0,87; 1,02 (judged by SI values that were below 3; EC3 could not be calculated)	Valid study / reliable test conc. range No range finding OECD TG 429 compliant Purity/impurities not specified rationale for test concentration – sponsor's request (higher concentrations should have been tested) colour of test item precludes assessment of erythema (however no abnormal change in ear thickness)	(RCC-CCR, 2003)
Skin sensitisation	Not a skin sensitiser in this test	Not valid/not reliable	(Hoechst, 1982)

GPMT (Buehler test w/o FCA) OECD TG 406 (1982) Species: Guinea pig Pirbright-White 15 males 10/group 5/control Hansa-Rot GG: purity not reported Vehicle: starch slime (2 %) Range finding: Identified 0,05 % (as mild irritant) Topical Induction Dermal application of 0,05 % (w/v), 9 x within 3 weeks (2,5 cm², patch, occlusive) after 6h, patch removed 16d after last application Dermal challenge: 0,5 ml (0,01% (w/v)) Assessment of skin response at 6h, 24h and 48h Re-challenge after 48h (no details)	Range finding: Treatment with 0,05 % caused slight irritant skin response (reversible) 0,01 % (non-irritating)	Not OECD TG 406 compliant No purity of compound reported No animal data reported No GLP	
Magnusson & Kligman Guinea pig maximisation test OECD TG 406 Species:Guinea pig Mol:DH (Moellegaard) females 10/group;5/control PO5: > 99 % Impurities: Pigment Red 4: 0,5 % 2,4-Dinitrochlorbenzene 931 ppm, ß-Naphthol 0,4 %, 2,4-Dinitroanilin 78 ppm Vehicle: sesame oil Intradermal induction: 2x 0,1 ml 5 % (w/v) in sesame oil/50 % FCA Dermal induction 0,5 ml 25 % (w/v) in sesame oil (2x4 cm² patch, occlusive) 1st topical challenge treatment 0,5 ml 5 % (w/v) in sesame oil (2x2 cm² patch, occlusive)	Skin sensitiser 10/10 animals showed positive response 1st dermal challenge Discrete and patchy erythema in 3 animals of control group 24h after removal of occlusive patch Treatment group 1 animal - discrete and patchy erythema 4 animals -moderate and confluent erythema 5 animals – intense erythema and swelling 48h after removal of occlusive patch	Valid study OECD TG compliant DNCB contamination?	(Aventis, 2002)

2nd topical challenge 0,5 ml 5 % (w/v) in sesame oil (2x2 cm² patch, occlusive)	Discrete and patchy erythema in 3 animals of control group Treatment group 2 animals -moderate and confluent erythema 8 animals – intense erythema and swelling Due to response in control group a second dermal challenge was performed 24h after removal of occlusive patch No response in control group Treatment group 3 animals without irritations 3 animal - discrete and patchy erythema 2 animals -moderate and confluent erythema 2 animals – intense erythema and swelling 48h after removal of occlusive patch No response in control group Treatment group 3 animal - discrete and patchy erythema 4 animals -moderate and confluent erythema 3 animals – intense erythema and swelling		
Magnusson & Kligman Guinea pig maximisation test (w. FCA) OECD TG 406 (1981) Species: Guinea pig Pirbright-White	Skin sensitiser 9/10 animals showed positive response 24h/48h after removal of occlusive patch	Valid study (minor restriction – no analytic certificate/impurities not disclosed) OECD TG 406 compliant DNCB not reported	(Hoechst, 1991)

females 10/group;5/control PO5: 96,8 % Impurities not reported Vehicle: Paraffin liq. (DAB), Vaseline (DAB) Intradermal induction: 2x 0,1 ml 5 % (w/v) Paraffin liq. (DAB)/50 % FCA Dermal induction 0,5 g of 25 % (w/w) Vaseline (DAB) (2x4 cm² patch, occlusive) 1st topical challenge treatment 0,5 g of 5 % (w/w) in Vaseline (DAB) (2x2 cm² patch, occlusive) 2nd topical challenge 0,5 ml 5 % (w/v) in sesame oil / 50% FCA (2x2 cm² patch, occlusive) GLP compliant	9/10 animals showed positive response (slight to moderate Erythema and oedema) Skin of control group animals showed no adverse effects		

7.9.4.3.2. Human information

No relevant studies were identified covering this endpoint.

7.9.4.3.3. Conclusion

The eMSCA supports the conclusion of the registrant to label Pigment Orange 5 as a skin sensitiser (Skin Sens. 1 H317) dependent on the content of the impurity DNCB. The registrant has proposed that PO5 with a content of > 0.03% DNCB should be labelled.

Data are conclusive, no trigger for harmonized classification was identified. No concern for study request(s) under SEv for PR3 regarding skin sensitisation was identified.

7.9.5. Repeated dose toxicity

Repeated dose toxicity was assessed for PR3, PR4 and PO5 to derive point of departure values for risk assessment.

7.9.5.1. PR3

7.9.5.1.1. Non-human information

7.9.5.1.1.1. Oral administration

PR3 has been investigated by the National Toxicology Program of the U.S. Department of Health and Human Services in 1992 (NTP, 1992a). Reliable 2-weeks and 13-weeks repeat dose toxicity and 2-year carcinogenicity studies in mice and rats (see Table 32) are available. The 2-year studies are evaluated on neoplastic lesions in section 7.9.7.1. (see Table 40). Other reliable studies for repeated dose toxicity have not been identified.

Table 32

Relevant studies related to the assessment of repeated dose toxicity for PR3			
Methods	**Results**	**Remarks**	**Reference**
14-day **feeding study** in rats PR3 Purity: >97 % Similar to OECD TG 407 (14 instead of 28 days) No GLP (but equivalent) Species: rats Strain: F344/N n: 5/dose group/sex Dose groups: 0 %, 0.6, 1.25, 2.5, 5, 10 % (approx.. 600, 1 250, 2 500, 5 000 and 10 000 mg/kg bw/d) Route: food	NOAEL: 5 000 mg/kg bw/d (reduction of Hb by more than 20%) No mortalities; no clinical findings indicative of chem. toxicity. Final mean body weights: females (10% group) significantly reduced (vs control); reduced feed consumption (from 2.5%) Body weight gains: females (all doses) reduced mean BWG; Liver: males (all doses) significant increased rel. liver weights. Heart: males (5 and 10%): significantly increased relative weight Blood Male rats (1.25%): significantly decreased erythrocyte counts (-5.9, -15.1, -14.8, -19.8, -31.2 % change compared to control) Males (2.5% or greater): significantly increased reticulocyte counts (0.9, 1.4, 2.0, 2.5, 3.7 fold over control) and significantly decreased haemoglobin (-7.1, -9.7, -13.6, -12.8, -11.9 % change compared to control) and haematocrit (-4.8, -7.1, -16.6, -19.4, -26.0 % change compared to control) values. Males (10%): significantly increased values for total serum bilirubin (4.0 fold increase over control), alanine aminotransferase (ALT) (4.7 fold increase over control), and cholinesterase (1.2 fold increase over control). All dosed female rat groups: significantly increased ALT values (at least 1.5 fold increase compared to control) and significantly decreased haemoglobin (-9.6, -14.1, -15.4, -14.7, -19.2 % change compared to control) values and erythrocyte counts (-12.0, -14.0, -19.8, -17.8, -27.0 % change compared to control). Female rats (2.5% or greater): significantly increased reticulocyte counts (at least 2.6 fold increase compared to control) and sorbitol dehydrogenase values and significantly decreased haematocrit (-6.4, -8.4, -13.4, -12.6, -18.8 % change compared to control) and cholinesterase levels (-22 % change compared to control). Female rats (5% or greater): significantly increased total serum	Food conversion factor: 10 (for young rats) Calculated doses: 600, 1 250, 2 500, 5 000 and 10 000 mg/kg bw/d Reliable with restrictions (14 d instead of 28 d)	(NTP, 1992a)

Relevant studies related to the assessment of repeated dose toxicity for PR3			
Methods	**Results**	**Remarks**	**Reference**
	bilirubin levels (at least 3 fold increase compared to control).		
14-day **feeding study** in mice PR3 Purity: >97 % Similar to OECD TG 407 (14 instead of 28 days) No GLP (but equivalent) Species: mice Strain: B6C3F1 n: 5/dose group/sex Dose groups: 0 %, 0.6, 1.25, 2.5, 5, 10 % (approx. 860, 1 786, 3 570, 7 143 and 14 286 mg/kg bw/d) Route: food	NOAEL: 10 000 mg/kg bw/d (no adverse effects) No mortalities; no signs of clinical findings of chemical toxicity. Female liver weight: sign. increased with 5% and 10% Rel. Brain weights decreased in females from 1.25% or greater Hb(-18.7, -8.6, -6.5, -7.2, -16.5 % change compared to control)/Ery counts (-19.4, -8.3, -7.8, -3.9, -16.9 % change compared to control) sign. decreased, both sex, 1.25%, 2.5% or 5% High dose males: increased reticulocytes, leukocytes, albumin/globulin etc. 5%/10% females: increased lymphocyte counts	Food conversion factor: 7 (for mice) Calculated doses: 860, 1 786, 3 570, 7 143 and 14 286 mg/kg bw/d Reliable with restrictions (14 d instead of 28 d)	(NTP, 1992a)
90-d **feeding study** in rats PR3 Purity: >97 % Equivalent to OECD TG 408 No GLP (but equivalent) Species: rats Strain: F344/N n: 10/dose group/sex Dose levels: 0, 0.3 %, 0.6 %, 1.25 %, 2.5 %, 5 % (approx. 150, 300, 625, 1 250, 2 500 mg/kg bw/d) Route: food	**NOAEL**: ≥300 mg/kg bw/d (liver weight rel./abs increased >20 % compared to control in males, less in females, >38 fold increased bilirubin; secondary signs of haemolytic anaemia in kidney, spleen and liver) No mortalities during study period; no clinical findings indicative of chemical toxicity. Final mean body weight significantly reduced (female, all dose groups compared to control, 5 to 10 % dose dependent reduction), mean body weight gain significantly reduced (females, ≥0.6 % mg/kg bw/d). Red stained faeces. Liver weights increased (all groups/both sex) Absolute (in % over control): 15.0, 19.9, **31.2, 32.0, 30.9** (m); 1.6, 6.8, 7.1, 14.3, 12.7 (f); Relative (in % over control): 15.8, 19.0, **27.2, 30.1, 36.8** (m); 6.6, 13.6, 14.2, 22.7, 24.5 (f)	Food conversion factor: 20 (for older rats) Calculated doses: 150, 300, 625, 1 250, 2 500 mg/kg bw/d Reliable with restrictions, e.g. lacking ED parameters (hormones, sperm); actual doses not reported, but feed consumption	(NTP, 1992a)

Relevant studies related to the assessment of repeated dose toxicity for PR3			
Methods	**Results**	**Remarks**	**Reference**
	Rel. lung weights increased (male in 2.5% or 5% dose groups up to 13 %, all female dose groups up to 23 %) Blood Sign. decreased haematocrit: males, all doses (except 2.5%): -14.1, -13.4, -14.1, 6.7 (increase), -24.1 % change compared to control); high dose females (-12.6 % versus control)) Sign. decreased erythrocyte counts (both sex, 0.6% or greater, males: -0.1, -4.3, -5.2, -2.6 , -15.9 % of control, females: -0.6, -6.4, -5.7, -3.2, -17.7 %) Ret. counts, serum albumin increased (male, all doses; females, 0.6% or greater): Hb decrease up to 10 % compared to controls (male, dose dependent) Urine Bilirubin increased (males: 13.3, 13.8, **49.2, 49.3, 55.7** fold over control; females: 18.9, 10.1, **38.0, 39.6, 52.4** fold over control) Histopathology (males, females) bone marrow, liver, spleen, kidney: signs of secondary effects to anaemia: bone marrow hyperplasia and haematopoietic cell proliferation in spleen (males: 10/10 at ≥0.3 %, females: 8/10 (BM) and 9/10 (spleen) at 0.3 % and 10/10 at ≥0.6 %) haematopoietic cell proliferation in liver (males: 0, 0, 2, 8, 10, 10 of 10; females: 0, 0, 6, 10, 10, 10) spleen congestion at all doses 10/10 (9/10 low dose females) pigments in spleen (10/10 all doses, m and f) pigments in liver and kidney at higher doses in both sex **protein casts in kidney** (males: 0, 3, 5, **10, 10, 10** out of 10, minimal severity)		
90 day **feeding study** in mice PR3 Purity: >97 % Equivalent to OECD TG 408 No GLP (but equivalent) Species: mice	NOAEL: 10 000 mg/kg bw/d (no adverse effect identified) Mortalities: One male mouse (1.25 %) and one control male mouse Food consumption, body weight and body weight gain similar to controls.	Reliable with restrictions, e.g. lacking ED parameters (hormones, sperm); actual doses not	(NTP, 1992a)

Relevant studies related to the assessment of repeated dose toxicity for PR3			
Methods	**Results**	**Remarks**	**Reference**
Strain: B6C3F1 n: 10/dose group/sex Dose levels: 0, 0.3 %, 0.6 %, 1.25 %, 2.5 %, 5 % (approx. 0, 600, 1200, 2500, 5000, 10000 mg/kg bw/d) Route: food	No clinical findings indicative of chemical toxicity. Liver weights sign. increased (males, 2.5% and 5%) There were no biologically significant changes in haematology, clinical chemistry, or urinalysis The most significant histopathologic alterations occurred in the kidney, liver, and spleen of dosed males and in the liver and spleen of dosed females. Mild cytomegaly of the renal tubule epithelium (males: 0, 0, 4, 8, 10, 10 of 10) haematopoietic cell proliferation in spleen (males: 0, 6, 6, 2, 4, 10 of 10; females: 0, 4, 4, 4, 8, 10 of 10) haematopoietic cell proliferation in liver (males: 1, 2, 3, 5, 1, 7 of 10; females: 6, 6, 9, 6, 10, 10 of 10) pigments in spleen (hemosiderin) probably associated with mild anaemia (male: 10/10 at 5%; female: 7/10 at 2.5%, 10/10 at 5%)	reported, but feed consumption Food conversion: e.g. 0.3 % group: 200 g food/kg bw/d → 600 mg/kg bw/d	
2-year feeding study in rats C.I. Pigment Red 3 (CAS 2425-85-6) Purity: >97 % Equivalent to OECD TG 451 (NTP guideline including 2-week and 13-week studies plus interim evaluation after 15 month) No GLP (but equivalent) Species: rats Strain: F344/N n: 60/dose group/sex; Additional groups of up to 10 male and 10 female rats per dose for interim evaluations (organ weights, hematology, clinical chemistry and histopathology) after 15 month Dose levels: 0, 6 000, 12 500, 25 000 ppm (approx. 0, 300, 625, 1250 mg/kg bw/d) in feed supplied weekly available ad libitum for 103 weeks Route: food	**LOAEL**: 300 mg/kg bw/d based on degenerative changes in liver (cystic degeneration, pre-neoplastic lesions e.g. foci of cellular alteration, angiectasis) and kidney (increased severity of chronic nephropathy) Non-neoplastic lesions: Male/female: eosinophilic (male: 6/50, 37/50*, 36/50*, 41/50*; female: 1/50, 7/50, 18/50*, 16/50*) or mixed type (male: 2/50, 24/50*, 21/50*, 15/50*; female: 4/50, 16/50*, 30/50*, 40/50*) foci of cellular alteration in the **liver** angiectasis (3/50, 20/50*, 21/50*, 29/50*) and cystic degeneration (9/50, 36/50*, 40/50*, 36/50*) in male and granulomas (27/50, 21/50, 42/50*, 44/50*) and cholesterol pigmentation (0/50, 3/50, 14/50*, 41/50*) in female bilary tract proliferation (female: 18/50, 12/50, 18/50, 29/50*) **Chronic nephropathy** (male: 50/50, 49/50, 50/50, 49/50; female: 49/50, 49/50, 50/50, 48/50) with increasing severity (grades: male 2.4, 3.1*, 3.6*, 3.8*; female 1.7, 2.2*, 2.4*, 2.8*)	Food conversion factor: 20 (for older rats) Calculated doses: 0, 300, 625, 1250 mg/kg bw/d Reliable without restrictions	(NTP, 1992a)

Relevant studies related to the assessment of repeated dose toxicity for PR3			
Methods	**Results**	**Remarks**	**Reference**
	Secondary to renal disease: parathyroid gland hyperplasia, fibrous osteodystrophy of the bone and mineralization of stomach, intestine, heart and blood vessels Final mean body weight >10% lower than controls: male 25 000 ppm from week 82, female ≥12 500 ppm (from week 82 low dose, week 66 mid dose, week 42 high dose) No clinical findings of toxicity; weight of liver and spleen significantly increased Haematology (15 month interim evaluation): Hct (-2.2, -6.7, -9.1 in male; -4.6, -6.8, -11.4 in female % change compared to control) and Hb (-5.6, -8.6, -11.1 in male; -3.3, -5.9, -11.2 in female % change compared to control) significantly decreased in all dose levels and erythrocyte counts (-1.0, -4.9, -6.8 in male; -5.0, -8.1, -13.2 in female % change compared to control) significantly decreased ≥12 500 ppm; platelets and bilirubin increased, MetHb in female (1.5 fold over control) Survival: male (28/50, 40/50, 28/50, 20/50) female 32/50, 41/50, 39/50, 40/50		
2-year feeding study in mice C.I. Pigment Red 3 (CAS 2425-85-6) Purity: >97 % According to OECD TG 451 (NTP guideline including 2-week and 13-week studies plus interim evaluation after 15 month) No GLP (but equivalent) Species: mice Strain: B6C3F1 n: 60/dose group/sex; additional groups of up to 10 male and 10 female rats per dose for interim evaluations (organ weights, hematology, clinical chemistry and histopathology) after 15 months Dose levels: 0, 12500, 25 000, 50 000 ppm in feed supplied weekly available ad libitum for	Non-neoplastic lesions: Male: focal renal tubule hyperplasia (0/50, 1/50, 7/50*, 7/50*) and cystic hyperplasia (0/50, 0/50, 0/50, 4/50), cytomegaly (karyomegaly) of renal tubule epithelium (0/50, 40/50*, 47/50*, 46/50*) Male/ female: chronic nephropathy (male 34/50, 39/59*, 42/50*, 45/50*; female 33/50, 45/49*, 46/49*, 45/49*), severity (grades male: 0.8, 1.0, 1.2*, 1.6*; female 0.7, 1.2*, 1.2*, 1.6*) Final mean body weight >10% lower than controls: male/ female 50 000 ppm from week 62 for male and week 38 for female No clinical findings of toxicity; liver weight significantly increased Urinalysis: total urine bilirubin increased (at least 20 fold over control) Survival: male (33/50, 28/50, 31/50, 33/50), female 39/50, 37/50, 31/50, 25/50* (survival of high-dose female significantly decreased)	Food conversion factor: 7 (for mice) Calculated doses: 0, 1785, 3571, 7142 mg/kg bw/d Reliable without restrictions	(NTP, 1992a)

Relevant studies related to the assessment of repeated dose toxicity for PR3			
Methods	**Results**	**Remarks**	**Reference**
103 weeks (approx. 0, 1785, 3571, 7142 mg/kg bw/d) Route: food Temperature 19°-27°C Relative humidity 20-85%			

Severe effects of PR3 in 2-week feeding studies in mice and rats have not been identified, except dose-related decreases in erythrocyte counts and haematocrit values and a strong increase in reticulocyte counts were observed in rats. Changes in these parameters were also observed in mice, but without clear, dose-related trends (NTP, 1992a).

In 13-week studies, toxicity of PR3 was observed in rats, i.e. bone marrow hyperplasia, congestion and hematopoietic cell proliferation of spleen; iron pigmentation of spleen, kidney and liver. Similar effects were observed in mice, additionally cytomegaly occurred in the renal tubule epithelium of the male mouse. Prominently, strong increases in relative and absolute liver weight and bilirubin excretion via urine are reported in both sexes.

In all studies, there is evidence for a PR3 induced haemolytic anaemia, including a reduction of e.g. Hb and erythrocyte counts (over 20 % reduction compared to control in male and female rats of the high dose groups in 2-week studies). The effects are more severe in rats than in mice, and are less pronounced in the 13-week studies compared to 2-week studies, indicating an adaptive response. In the corresponding 2-year studies, the organ and histological effects observed in the 90-day studies are more pronounced. In male rats, degenerative changes in liver (cystic degeneration, pre-neoplastic lesions e.g. foci of cellular alteration, angiectasis) and kidney (increased severity of chronic nephropathy) in all dose groups indicate specific toxicity to these organs, presumably secondary to a responsive haemolytic anaemia, indicated by blood parameters and severely increased bilirubin (total) excretion in urine. The findings in mice are similar, but less severe, in contrast to rats the effects are not considered adverse.

7.9.5.1.1.2. Dermal and inhalation administration

No reliable studies were identified covering this endpoint.

7.9.5.1.2. Human information

No information is available on the repeated dose toxicity of PR3 in humans.

7.9.5.1.3. Summary

PR3 induces haemolytic anaemia, which presumably leads to secondary lesions in liver, kidney and spleen in rats and mice. The effects are consistent with a (partly) compensated haemolytic anaemia, organ lesions are observed at higher doses and chronic exposure. Overall, no additional concern has been identified which would justify requesting further studies. The effects are outside the severity which would allow classification according to CLP for specific target organ toxicity after repeated exposure (STOT-RE). However, the effects reported in sub-acute, sub-chronic and chronic studies indicate adverse effects that allow identification of dose descriptors.

7.9.5.1.4. Conclusion

Overall, the eMSCA considers the available data as appropriate for substantiated evaluation of the repeated dose toxicity of PR3 and no further action is recommended.

7.9.5.2. PR4

7.9.5.2.1. Non-human information

7.9.5.2.1.1. Oral administration

For PR4, three oral repeated dose studies were identified with severely limited reliability.

- (Kupradinun et al., 2002): Published carcinogenicity study in rats, which focussed on carcinogenicity and does not report detailed results on non-neoplastic effects.
- (Gewerbetoxikologisches Laboratorium, 1959): Single-dose oral gavage 90 day study in rats with severe limitations in study design and reporting and not reliable test substance identification.
- (Gewerbe- und Arzneimitteltoxikologie, 1962): 90-day study in rats, with 1 and 5 % mixed in food with scarce reporting, limited number of animals tested, no details on methods, inspected organs and not reliable test substance identification. Due to "some changes in kidney and spleen", a LOAEL of 10,000 mg/kg diet is reported.

ECHA has requested in a recent dossier evaluation decision a combined repeated dose toxicity study with the reproduction/developmental toxicity screening study (CCH-D-2114461479-37-01/F) to fill the identified data gap.

Table 33

Relevant studies related to the assessment of repeated dose toxicity for PR4			
Methods	**Results**	**Remarks**	**Reference**
PR4 Route: oral gavage 90d, oral species: rats (albino) n=10 males No TG No GLP SID: "Permanentrot R extra" (by hand added "P.R 004") Purity: unknown ("Technisch reiner Körper" Dosing: 500 mg/kg bw on 65 of 97 days plus control group	No changes in behaviour, only faecal excretion of dyestuff, blood and (urine?) without pathological changes ("several times controlled" during study duration). No irreversible effects on organs.	Not reliable Scarce reporting, limited number of tests, no details on methods, inspected organs etc.	(Gewerbetoxikologisches Laboratorium, 1959)

Relevant studies related to the assessment of repeated dose toxicity for PR4			
Methods	**Results**	**Remarks**	**Reference**
PR4 90d, oral (food) species: rats (mixed-race albinos), m/f n = 10 at 1 % (and control), 5 at 5 % per sex No TG No GLP SID: "C Rot Nr. 41 = Permanentrot R extra Plv." Purity: unknown Dosing: 0, 1 %(10 000 mg/kg) and 5 % (50 000 mg/kg) in food Observation time 7 days and 14 days (each 50% of animals)	LOAEL: 500 mg/kg bw/d ("some changes in kidney and spleen") No altered behaviour, food intake and body weight gain "regularly and good"; no pathological changes in blood and urine status. No macroscopic findings in heart, lungs, liver, kidney, spleen. Pigmentation in kidneys (both doses) and spleen (high dose)	Not reliable Scarce reporting, limited number of tests, no details on methods, inspected organs etc. Food conversion factor: 20 (for older rats) Calculated doses: 500, 1 000 mg/kg bw/d	(Gewerbe- und Arzneimitteltoxikologie, 1962)

7.9.5.2.1.2. Dermal and inhalation administration

No reliable studies were identified covering this endpoint.

7.9.5.2.2. Human information

No information is available on the repeated dose toxicity of PR4 in humans.

7.9.5.2.3. Summary

No studies were identified which would allow reliably assessing repeated dose toxicity for PR4. From a 90-day oral gavage study in rats (Gewerbe- und Arzneimitteltoxikologie, 1962) the study authors report on "some changes in kidney and spleen" at 10 000 mg/kg diet (eq. 500 mg/kg bw/d).

Available data do not allow harmonized classification.

7.9.5.2.4. Conclusion

No conclusion possible yet as there is an ECHA decision requesting the generation of new information for repeated dose toxicity.

7.9.5.3. PO5

7.9.5.3.1. Non-human information

7.9.5.3.1.1. Oral administration

Two repeated dose studies with PO5 have been identified (see Table 34), one 90-day oral gavage study (Hoechst, 1959) and one 32-day feeding study (Hoechst, 1973a). For both studies a certificate of analysis for the substance used is not available to the eMSCA, conclusively the identities of the tested substances are uncertain. Both are reported to have used "Hansaorange RN", which is a synonym for PO5.

The (Hoechst, 1959) study is not reliable and not equivalent to OECD TG 408, there are concerns on substance purity, single and low dose testing (100 mg/kg bw/d), insufficient reporting of methods and results. The study authors only identify faecal excretion of dye as an effect of substance administration; a reliable NOAEL/LOAEL cannot be derived from this study report.

The 32-day study in rats by oral route in feed from 1973 has major deviations from OECD TG 407, e.g. insufficient number of organs investigated during necropsy, lack of clinical biochemistry, no examination of intermittently deceased animals, no detailed reporting of results, no individual animal data (averaged values per dose group and sex), no conversion of dosage in food to body weight dosage per animal weight. In all dose groups (effects were observed, e.g. changes in blood parameters (reduced Hb, reduced ERY; increased LEU), reduced body weight gain (except low dose females), excretion of dye in faeces and urine, increased splenic iron content. In addition increased high dose mortality was observed. The study has been performed with SPF Wistar rats, the age of the animals is not available (average weight at study start 92 g (m) / 82 g (f); at study termination 280 g (m) and 180 g (f)). For food conversion, the general factor of 20 for older animals (according to CLP guidance) is applied by eMSCA, as the animal age was not reported. Under this assumption, calculated doses were 100, 500 and 1250 mg/kg bw/d.

Overall, from the parameters studied and results reported, no NOAEL has been identified. The blood parameters have not been reported quantitatively in the study report available to the eMSCA. However, the described changes in blood parameters in all dose groups raise a concern for an adverse effect and a LOAEL of 100 mg/kg bw/d.

Additionally, carcinogenicity studies in rats and mice have been identified (Bio/Dynamics, 1982a; Bio/Dynamics, 1982b), assessed in (FDA, 1986; FDA, 1987; FDA, 1988), (Hart et al., 1986) and (BG RCI, 2000). These studies are evaluated on neoplastic lesions in Table 43.

The long term feeding studies in rat and mice were performed with 0, 0.02, 0.05, 0.1 % in a first and 0 and 1 % substance in a second study fed in the diet for 26 to 30 months. Calculated doses are 10, 25 and 50 mg/kg bw/d and 500 mg/kg bw/d, based on standard conversion factor for older rats according to CLP guidance.

The eMSCA does not have access to the full study reports, the published summaries lack details, but the eMSCA considers the study as reliable. There are only short quantitative descriptions on various parameters including clinical chemistry, haematology and urinalysis.

Increased liver weights in male and female rats occur at 50 and only in females at 500 mg/kg bw/d. Females at 500 mg/kg bw/d develop in addition neoplastic lesions in the liver (see Table 43). Indicative of haemolytic anaemia are changes in blood parameters such as reduced Hct, Hb and erythrocyte counts, and deposition of pigment in spleen at 500 mg/kg bw/d. Based on the increased liver weights, the eMSCA identifies a NOAEL of

25 mg/kg bw/d. At higher doses, females develop further non-neoplastic and neoplastic lesions in liver.

Table 34

Relevant studies related to the assessment of repeated dose toxicity for PO5			
Methods	**Results**	**Remarks**	**Reference**
32-day feeding study in rats Hansaorange RN 01 (trading name for PO5, but no further information on substance identity) Purity: 97.0 % Dosage: 2 000; 10 000; 25 000 ppm in food (corresponding to approx. 100, 500 and 1250 mg/kg bw/d) Exposure: 32 d SPF Wistar rats, m/f 10/group (5 per cage, m/f separated) No GLP Similar to OECD TG 407, with major deviations Average weight of animals at study termination in control group: 180g (females), 280g (males) Blood (Hb, ERY, LEU, diff. blood, Heinz-bodies) and urine (appearance, color, protein, glucose, bilirubin, spec. weight (per cage), sediment) checked prior study start	LOAEL of 100 mg/kg bw/d (based on blood parameters indicating a haemolytic anaemia). Mortality (only in high dose group): 7/10 males, 3/10 females; day 11 or later Excretion of dye in faeces and urine Food intake: reduced in high dose group, normal in other groups Body weight gain: reduced in all dose groups compared to control (exception: low dose females: no difference to control, no further details available) Total body weight reduced (control/low/mid/high dose; males: 280/259/215/94 g; females: 180/180/161/104 g) Blood: in all dose groups: reduced Hb, reduced ERY; increased LEU high and mid dose: increased neutrophile granulocytes Urine: no pathological findings Necroscopy (heart, liver, lung, kidneys, adrenal gland, spleen): no macro- or microscopic changes (versus control); increased (compared to control) splenic iron content in all dose groups.	The study is of limited value. Actual findings are reliable with restrictions, but the study as such does not fulfil the criteria for a valid OECD TG 407 study and is accordingly evaluated as not reliable. Major deviations from OECD TG 407: Limited number of organs investigated No clinical biochemistry No details on inspected organs during necroscopy No conversion from food to actual dose No clinical observation results during study period No necroscopy of intermittently deceased animals No detailed results No individual data, averaged values per dose group and sex Hormones, thymus or other ED relevant parameters not assessed Only a 17 page study summary was available to eMSCA.	(Hoechst, 1973a)
26-30 month dietary study (F0 and F1 dosed) including in utero exposure **D&C Orange No. 17*** (trading name for PO5, but no further information on substance identity) Purity: 97%	NOAEL: 25 mg/kg bw/d Increased liver weight: male rats at 50 mg/kg bw/d, female rats at 50 and 500 mg/kg bw/d Females with eosinophilic and clear cell foci in the liver (500 mg/kg bw/d) (Neoplastic lesion in liver of females (500 mg/kg bw/d))	FDA requested study Only accessible as study summaries with limited details, the evaluation of the data is largely dependent on the FDA evaluation Study according to FDA guidelines including in utero treatment and F1 generation	Unpublished report (Bio/Dynamics, 1982a) Cited in (BG RCI, 2000; FDA, 1986; FDA, 1987; FDA, 1988; Hart et al., 1986)

Relevant studies related to the assessment of repeated dose toxicity for PO5			
Methods	**Results**	**Remarks**	**Reference**
Impurities: 0.29% 2,4-dinotrobenzeneamine (dinitroaniline), 0.7% 2-naphthalenol (beta-naphthol) According to FDA guidelines Species: rats Strain: Charles River Albino n: 60/dose group/sex in F0, 70/dose group/sex in F1 Dose levels: Part I: 0, 0.02, 0.05, 0.1% of the diet (approx. 0, 10, 25 and 50 mg/kg bw/d) Part II, additional study with higher concentration: 0 and 1% of the diet (approx. 0 and 500 mg/kg bw/d) Data of the two studies were combined Experimental design: 60 days feeding period before mating; dietary administration of test substance was continued during mating, gestation, lactation and rearing Pups were weaned from their mothers 21 days after delivery 70 F1 animals selected for long-term study 12 month interim evaluation of 10 animals	Haematology: slight to statistical reduction in HCT, Hb and erythrocyte counts; increased reticulocytes (500 mg/kg bw/d) Deposition of pigment in the spleen (500 mg/kg bw/d) Body weight of male rats 18% lower at 500 mg/kg bw/d; no difference in females No effect on survival		

* The CAS number registered for „D&C Orange No. 17" corresponds to that of PO5.

7.9.5.3.1.2. Dermal and inhalation administration

No relevant studies were identified covering this endpoint.

7.9.5.3.2. Human information

No information is available on the repeated dose toxicity of PO5 in humans.

7.9.5.3.3. Summary

The available studies do not fulfil the criteria for valid repeated dose studies. In the study report for a 32-day study (Hoechst, 1973a) provided by the registrant limited parameters for haematology and urinalysis were measured and not reported quantitatively. In the short study summary for a 26-30 month dietary study (see Table 43) that was available to the eMSCA (and which has not been addressed by the registrant) parameters for haematology and urinalysis were hardly reported. Therefore the available studies fail to meet the requirements by Annex VIII of REACH. In addition, the eMSCA identifies a concern for repeated dose toxicity, i.e. haematoxicity, which is not reliably clarified by the available data.

No reliable studies were identified covering repeated dose toxicity of PO5. Study results from a 32-day feeding study (Hoechst, 1973a) with major deviations from OECD test guideline 407 in rats raise a concern for repeated dose toxicity mainly based on significant changes in blood parameters, reduced body weight gain and iron content in spleen. Excretion of the dye in urine indicates systemic availability of PO5. Based on changes in blood parameters which were not reported in detail, a precautionary LOAEL of 100 mg/kg bw/d is assumed for further risk characterisation. Uncertainties regarding the extent of changes in blood parameters and severity of presumed haemolytic anaemia exist. From carcinogenicity studies on PO5 (see Table 43), a NOAEL of 25 mg/kg bw/d for increased liver weight can be derived.

For classification as STOT-RE, Category 2 (see Guidance on the Application of the CLP criteria, section 3.9.2.2, Version 5.0), a substance needs to have an effective dose (ED) for adverse effects by oral administration between 30 and 300 mg/kg bw/d. The observed high dose mortality in the 32-day study (Hoechst, 1973a) is outside the relevant concentration range for classification. For changes in blood parameters (signs of haemolytic anaemia), the extend of e.g. reduction in Hb is not known to the eMSCA, therefore it is not possible to derive reliable effective dose and NOAEL values, therefore a classification is not possible based on the studies available to the eMSCA.

7.9.5.3.4. Conclusion

No conclusion possible yet as there is an ECHA decision requesting the generation of new information for repeated dose toxicity.

7.9.6. Mutagenicity

7.9.6.1. PR3

7.9.6.1.1. In vitro data

Table 35

Summary table of mutagenicity/genotoxicity tests *in vitro*				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
Bacterial Reverse Mutation Test (modified version for Azo-dyes) Similar to OECD TG 471 (with Prival activation) Deviation: Neither a *E.coli* WP2 strain nor the *Salmonella typhimurium* tester strain TA102 has been tested GLP: yes	C.I. Pigment Red 3 (CAS 2425-85-6) Purity: > 99 %	Key study Reliable with restriction Bacterial strains: *Salmonella typhimurium* tester strains: TA100, TA98, TA1537, TA1535 Test concentrations (with and without metabolic activation): 4, 20, 100, 500, 2500, 5000 µg/plate S9 mix: Prival S9 mix; non pre-treated Syrian hamsters (30 % S9 mix) Vehicle: DMSO Negative control: yes Positive control: yes	Positive (with metabolic activation, pre-incubation; Prival S9 mix) - with S9 mix: Positive for TA1537 (concentration related increase in the number of revertant colonies over the range tested) Cytotoxicity: no Precipitations: ≥ 500 µg/plate with and without S9 mix Controls: Negative control: valid Positive control: valid	(Hoechst, 1992)
Bacterial Reverse Mutation Test Similar to OECD TG 471 Deviations: No GLP: yes	4-Methyl-2-nitro-phenylazo-1'-naphtol-2' (CAS 2425-85-6) Purity: 99 %	Key study Reliable without restriction Bacterial strains: *Salmonella typhimurium* tester strains: TA100, TA98, TA1537, TA1535, TA1538 and E.*coli* WPuvrA Test concentrations (with and without metabolic activation (S9 mix)): 4, 20, 100, 500, 2500, 5000 µg/plate S9 mix: Rat liver S9 induced by Aroclor 1254 Vehicle: DMSO Negative control: yes Positive control: yes	Negative (with and without metabolic activation) Cytotoxicity: no Precipitations: ≥ 100 µg/plate with and without S9 mix Controls: Negative control: valid Positive control: valid	(Hoechst, 1984)
Bacterial Reverse Mutation Test (modified version for Azo-dyes) Similar to OECD TG 471 Deviations:	Pigment Red 3 (CAS 2425-85-6) Purity: > 99 %	Supporting study Reliable with restrictions Bacterial strains: Salmonella typhimurium tester strains: TA100, TA98, TA1537, TA1535 Test concentrations (with and without metabolic	Positive (with metabolic activation, Prival S9 mix) - with S9 mix: Positive for TA98 and TA100 (concentration	(Mortelmans et al., 1986) cited in (NTP, 1992b)

Summary table of mutagenicity/genotoxicity tests *in vitro*				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
Neither a E.coli WP2 strain nor the Salmonella typhimurium tester strain TA102 has been tested Hamster liver S9 also induced by Aroclor 1254 No detailed presentation of results (only mean values and standard deviation shown but no single values) GLP: no		activation (S9 mix)): 33, 100, 333, 1000, 2500, 3333 µg/plate Justification for maximum concentration: PrecipitationsS9 mix: Prival S9 mix; hamster liver S9 induced by Aroclor 1254 Vehicle: DMSO Negative control: yes Positive control: yes	related increase in the number of revertant colonies over the range tested) Precipitations: ≥ 1000 µg/plate with and without S9mix Cytotoxicity: no Controls: Negative control: valid Positive control: valid	
Bacterial Reverse Mutation Test Similar to OECD TG 471 Deviations: Neither a *E.coli* WP2 strain nor the *Salmonella typhimurium* tester strain TA102 has been tested No detailed presentation of results (only mean values and standard deviation shown but no single values) GLP: no	Pigment Red 3 (CAS 2425-85-6) Purity: > 99 %	Supporting study Reliable with restrictions Bacterial strains: *Salmonella typhimurium* tester strains: TA100, TA98, TA1537, TA1535 Test concentrations (with and without metabolic activation (S9 mix)): 33, 100, 333, 1000, 2500, 3333 µg/plate Justification for maximum concentration: Precipitations S9 mix: Rat liver S9 induced by Aroclor 1254 Vehicle: DMSO Negative control: yes Positive control: yes	Negative Cytotoxicity: TA1537 with S9 mix (at 3333 µg/plate) Precipitations: ≥ 1000 µg/plate with and without S9 mix Controls: Negative control: valid Positive control: valid	(Mortelmans et al., 1986) cited in (NTP, 1992a)
Bacterial Reverse Mutation Test No conclusion can be drawn if in accordance/similar to OECD TG 471 Deviations: only overall information on negative result without any detailed information)	Toluidine red (structure given = Pigment Red 3) Purity: No data	Disregarded study Not assignable (only overall information on negative result without any detailed information) Bacterial strains: *Salmonella typhimurium* tester strains: TA100, TA98	**Negative** (with and without metabolic activation)	(Miyagoshi et al., 1983)
Bacterial Reverse Mutation Test	Hansa Rot B,	Disregarded study	Positive	(Hoechst, 1981)

Summary table of mutagenicity/genotoxicity tests *in vitro*				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
Similar to OECD TG 471 Deviation: No data on purity GLP: yes	Substanz 196/81 (CAS 2425-85-6) Purity: No data	Not reliable (It is not possible to conclude an overall positive outcome as substance purity is not available and available key studies with the same test conditions and high substance purity gave negative results.) Bacterial strains: *Salmonella typhimurium* tester strains: TA100, TA98, TA1537, TA1535, TA1538 and E.*coli* WPuvrA Test concentrations (with and without metabolic activation (S9 mix)): 4, 20, 100, 500, 2500, 5000 µg/plate S9 mix: Rat liver S9 induced by Aroclor 1254 Vehicle: DMSO Negative control: yes Positive control: yes	(without metabolic activation) TA1537: - Increase in revertant colony numbers ≥ 2500 µg/plate in two experiments Ambiguous (with metabolic activation) TA1537: - Negative in the first experiment - Positive in the second experiment ≥ 2500 µg/plate Cytotoxicity: no Precipitations: ≥ 500 µg/plate with and without S9 mix Controls: Negative control: valid Positive control: valid	(unpublished study report)
In vitro mammalian cell gene mutation test using the thymidine kinase gene According to the recent OECD TG 490* Deviation: Expression time for experiment I was 72 h (instead of 2 d as recommended in the TG) GLP: yes *in report: referred to as OECD TG 476 (Feb. 1998)	Hansa-Rot B (C.I.Pigment Red 3) Purity: 98.2 %	Key study Reliable without restrictions Cell culture:L5178Y mouse lymphoma cells Test concentrations: Experiment I: without and with metabolic activation: 31.3, 62.5, 125, 250, 375 µg/ml Experiment II: Without metabolic activation: 62.5, 125, 250, 375, 500 µg/ml S9 mix: Rat liver S9 induced by phenobarbital/ß-naphthoflavone Justification for top concentration: Solubility and cytotoxicity Treatment time: Experiment I: 4 h Experiment II: 24 h Sampling time:	Negative (with and without metabolic activation) Cytotoxicity: at 250 µg/ml (without S9 mix) and at 500 µg/ml (with S9 mix) Precipitations: at 500 µg/ml (without S9 mix) and ≥ 62.5 µg/ml (with S9 mix) Controls: Negative control: valid Positive control: valid	(Cytotest, 2006a) (unpublished study report)

Summary table of mutagenicity/genotoxicity tests *in vitro*				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
		Experiment I: 72 h Experiment II: 24 h Vehicle: DMSO Negative control: yes Positive control: yes		
In vitro Mammalian Cell Gene Mutation tests using the Hprt gene according to OECD TG 476 Deviations: no GLP: yes	Hansa-Rot B (C.I.Pigment Red 3) Purity: 98.2 %	Key Study Reliable without restrictions Cell culture: V79 cells (Chinese hamster) Test concentrations: *Experiment I*: without metabolic activation: 15.6, 31.3, 62.5, 125, 250, 375, 500 µg/ml with metabolic activation: 31.3, 62.5, 125, 250, 375, 500 µg/ml Experiment II: without metabolic activation: 31.3, 62.5, 125, 250, 375, 500 µg/ml Justification for top concentration: Solubility S9 mix: Rat liver S9 induced by phenobarbital/ß-naphthoflavone Treatment time: Experiment I: 4 h Experiment II: 24 h Sampling time: Experiment I: 7 d Experiment II: 6 d Vehicle: DMSO Negative control: yes Positive control: yes	Negative (with and without metabolic activation) Cytotoxicity: ≥ 375 µg/ Precipitations: ≥ 250 µg/ml Controls: Negative control: valid Positive control: valid	(Cytotest, 2006b)
In vitro Mammalian Chromosomal Aberration test similar to OECD TG 473 Deviations: Short term exposure with metabolic activation is only 2h (instead of 3-6 h)	C.I. Pigment Red 3 Purity: Substance provided by NTP	Disregarded study Not reliable (due to a too short exposure time with S9 mix and the lack of short term exposure without S9 mix it is not possible to conclude an overall negative outcome) Cell culture: CHO cells (Chinese hamster)	Negative (with and without metabolic activation) Cytotoxicity: yes Precipitations: no data Controls: Negative control: valid Positive control: valid	(NTP, 1992a)

Summary table of mutagenicity/genotoxicity tests *in vitro*				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
Short term exposure is missing without metabolic activation GLP: no		Test concentrations: with and without metabolic activation (S9 mix): 50, 100, 160 µg/ml Justification for top concentration: Cytotoxicity S9 mix: Rat liver S9 induced by Aroclor 1254 Treatment times: - 2 h with S9 mix - 10 h (continuously) without S9 mix Sampling times: with S9 mix: 11 h (after end of treatment) without S9 mix: 10 h Vehicle: DMSO Negative control: yes Positive control: yes		
In vitro sister chromatid exchange assay in mammalian cells OECD TG 479 (deleted in 2014 by OECD Council decision)	C.I. Pigment Red 3 Purity: Substance provided by NTP	Disregarded study Not reliable (due to the deletion of the TG 479, this test system is not considered relevant for genotoxic assessment)	Negative (with and without metabolic activation)	(NTP, 1982)

7.9.6.1.2. In vivo data

Table 36

Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
In vivo mammalian alkaline comet assay Similar to OECD TG 489 Deviation: No positive control	Pigment Red 3 (CAS 2425-85-6) Purity: No information (purchased from Sigma)	Disregarded study Not reliable (due to the lack of positive controls the relevance of the result cannot be assessed) Species: ddY mice	Positive - positive only in colon: DNA damage observed at 24 h sampling time (negative after 3 h and 8 h sampling time) Toxicity:	(Tsuda et al., 2000)

Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
Detailed reporting of result data missing 4 animals per group (instead of 5 as recommended by the TG) No data on substance purity GLP: no		Number of animals per group: 4 males Target organs: Stomach, colon, liver, kidney, bladder, lung, brain, bone marrow Administration route: Oral (no information on treatment route) Dose: 2000 mg/kg bw Justification for top dose: Maximum limit dose (for administration period < 14 d) Treatment: Single administration Sampling: 3 h, 8 h, 24 h after treatment Vehicle: Saline Positive control: no Negative control: yes	Clinical signs: no Lethal effects: no Cytotoxicity: no Controls: Negative control: valid Positive control: no	
Unscheduled DNA Synthesis (UDS) test with mammalian liver cells in vivo According to OECD TG 486 Deviations: no GLP: yes	Pigment Red 3 (CAS 2425-85-6) Purity: 98.9 %	Key study Reliable without restrictions Species: Wistar Han rats Number of animals per group: 4 males Target organ: Liver Administration route: Oral (gavage) Dose levels: 1000 and 2000 mg/kg bw Treatment: Single gavage Sampling times: Experiment I: 16 h after dosing Experiment II 4 h after dosing Vehicle: Arachis Oil Positive control: yes Negative control: yes	Negative An negative result is not conclusive for the assessment of induction of gene mutations (see section 7.9.6.1.4., in vivo data). Toxicity: Clinical signs: no Lethal effects: no Cytotoxicity: no Controls: Negative control: valid Positive control: valid	(Harlan, 2013c)

7.9.6.1.3. Human information

No information available.

7.9.6.1.4. Summary and discussion of genotoxicity

In vitro data

Bacterial reverse mutation tests

Several bacterial reverse mutation tests are available for PR3 in the technical dossier.

Two of those (unpublished study reports by (Hoechst, 1984; Hoechst, 1992)) are judged to be key studies as performed according/similar to OECD TG 471 and GLP and are considered as reliable without restrictions by eMSCA. The test by (Hoechst, 1992) was performed using an alternative procedure referred to as "Prival-modification" (a preincubation method using S9 obtained from Syrian hamsters) which is recommended for "special cases" such as azo-dyes (see OECD TG 471, section 10). The study yielded positive results. The results indicate a potential for Pigment Red 3 to induce gene mutations in bacterial cells if the Prival modification is applied. The test by (Hoechst, 1984) was performed with the 'classical' OECD TG 471 test protocol and yielded negative results for both conditions without and with metabolic activation based on rat liver S9.

Moreover, there are two bacterial reverse mutation studies with Pigment Red 3 published (Mortelmans et al., 1986) which were performed similar to OECD TG and are considered as reliable with restrictions and supporting studies by eMSCA. In this publication, on the one hand, a test was performed also using the Prival modification which is clearly positive with metabolic activation based on Syrian hamster S9 and negative without metabolic activation. This is in line with the results obtained with the GLP test by (Hoechst, 1992). On the other hand, the same authors describe a test using the classical Ames test protocol based on rat liver S9. The result is assessed to be negative by the eMSCA which is also consistent with the result observed in the GLP study by (Hoechst, 1984) using also a rat liver S9 mix.

In addition, there are two bacterial reverse mutation studies with PR3 available (one yielding positive results (Scheerer et al., 1981) and one yielding negative results (Miyagoshi et al., 1983) which are disregarded from assessment by eMSCA as the relevance of the results can not be assessed mainly due to missing data on e.g. purity, materials and method or results. Details and restrictions of the tests are documented in Table 35.

In vitro mammalian gene mutation tests

There are two *in vitro* mammalian gene mutation tests available for PR3, one using the Hprt gene (Cytotest, 2006b), unpublished study report) and the other using the thymidine kinase gene (Cytotest, 2006a), unpublished study report). Both test were performed according to the respective OECD TG (OECD TG 476 and 490, respectively) and GLP and yielded negative results. The tests are considered to be key studies reliable without restrictions.

In vitro mammalian cytogenicity tests

There exists one *in vitro* mammalian chromosomal aberration test with PR3 which was performed similar to OECD TG 473 and yielded negative results (NTP, 1992a). However, as not all three experimental conditions were tested as recommended by OECD TG 473 (section 28) to conclude a negative outcome and as short time exposure with metabolic activation was too short (2h only instead of 3-6h as recommended in the guideline) the

negative test result is not considered to be reliable. No further *in vitro* cytogenicity test using mammalian cells is provided in the registration dossier.

Other in vitro tests

Moreover, a negative *in vitro* sister chromatid exchange assay in mammalian cells is provided in the registration dossier (NTP, 1982). Results of this test are not included in the overall assessment for the endpoint genotoxicity as this test system has been considered not relevant for testing genetic toxicity and was, consequently, deleted in 2014 by an OECD Council decision. Moreover, is not a preferred test system according to the REACH guidance IR&CSA R.7a (2017; see: table R.7.7-2).

Summarising, it can be stated that the results from reliable bacterial reverse mutation tests clearly indicate a potential for PR3 to induce gene mutations. A reliable *in vitro* cytogenicity assay is missing.

In vivo data

Two *in vivo* genotoxicity tests are provided in the registration dossier, an unscheduled DNA Synthesis (UDS) tests with mammalian liver cells *in vivo* (Harlan, 2013c), unpublished study report) and an *in vivo* mammalian alkaline comet assay (Tsuda et al., 2000)

The UDS test (Harlan, 2013b), unpublished study report) was performed similar to OECD TG 486 and is considered as reliable without restrictions. The test yielded negative results. However, according to the REACH Endpoint specific guidance (Chapter R.7a, Version 6.0) not all gene mutagens are positive in the UDS test and a negative result in an UDS assay alone is not a proof that the substance does not induce gene mutations. Thus, the UDS assay is not considered the appropriate follow-up test for the positive results observed with the bacterial reverse mutation tests for PR3.

Due to major deficiencies in experimental design and reporting, the *in vivo* mammalian comet assay, published by (Tsuda et al., 2000), is considered not to be reliable and is disregarded from the assessment. Deficiencies include lack of positive controls, no data on substance purity, missing documentation of detailed results and the testing of only 4 instead of 5 animals as recommended in the OECD TG 489. Hence, the test is not considered to be adequate.

Summarising, the available *in vivo* data are neither sufficient to clarify the concern for mutagenic effects of PR3 identified *in vitro* nor to fulfil the standard data requirement regarding a cytogenicity test.

7.9.6.1.5. Conclusion

The dossier contains positive results for *in vitro* gene mutation studies in bacteria which indicate a potential for PR3 to induce gene mutations. The available *in vivo* data are not adequate to clarify this concern. Hence, an appropriate *in vivo* follow-up mutagenicity study is necessary to address the concern identified *in vitro*. This information need is subject to the standard testing scheme of REACH and thus the generation of new information will not be requested under substance evaluation but in a compliance check.

The available *in vitro* and *in vivo* cytogenicity tests with PR3 are not considered adequate to fulfil the standard data requirement regarding a cytogenicity test. Hence, an appropriate *in vitro* cytogenicity study is necessary. This information need is subject to the standard testing scheme of REACH and thus the generation of new information will not be requested under substance evaluation but in a compliance check.

7.9.6.2. PR4

7.9.6.2.1. In vitro data

Table 37

Method, guideline, deviations if any	Test substance	Relevant information about the study	Observations	Reference
Bacterial Reverse Mutation Test According to OECD TG 471 Deviations: no GLP: yes	Hansa-Rot R/ Pigment Red 4 Purity: 98.2% (w/w)	Key study Reliable without restrictions Bacterial strains: *Salmonella typhimurium* tester strains TA 1535, TA 1537, TA 98, TA 100 and E.coli WP2 uvrA Test concentrations (with and without metabolic activation): 3, 10, 33, 100, 333, 1000, 2500, 5000 µg/plate S9 mix: plate incorporation assay with phenobarbital/ beta-naphthoflavone induced rat liver S9 mix Vehicle: DMSO Negative control: yes Positive control: yes	Positive (with metabolic activation) - TA 98 with metabolic activation (≥3 µg/plate) Cytotoxicity: no Precipitations: without S9 mix: ≥ 1000 µg/plate, with S9 mix: ≥333 µg/plate) Controls: Negative control: valid Positive control: valid	(Cytotest, 2005)
Bacterial Reverse Mutation Test (modified version for Azo-dyes) According to OECD TG 471 Deviations: no GLP: yes	Hansa-Rot R/ Pigment Red 4 Purity: 98.2% (w/w)	Key study Reliable without restrictions Bacterial strains: *Salmonella typhimurium* tester strains TA 1535, TA 1537, TA 98, TA 100 and E.coli WP2 uvrA Test concentrations (with and without metabolic activation): 33, 100, 333, 1000, 2500, 5000 µg/plate S9 mix: with non-induced hamster liver S9 mix (pre-incubation assay) Vehicle: DMSO Negative control: yes Positive control: yes	Positive (with metabolic activation; Prival activation) - TA 98 with metabolic activation (≥33 µg/plate) Cytotoxicity: no Precipitations: ≥1000 µg/plate with and without S9 mix Controls: Negative control: valid Positive control: valid	(Cytotest, 2005)
Bacterial Reverse Mutation Test Similar to OECD TG 471 Deviation:	D&C Red No. 36 Purity: 95-97%	Supporting study Reliable with restrictions Bacterial strains: TA 98, TA 100, TA 1535, TA 1537, TA 1538	Positive (with metabolic activation) - TA1538 and TA98 with S9 mix (≥100 µg/plate)	(Brown et al., 1979b)

Method, guideline, deviations if any	Test substance	Relevant information about the study	Observations	Reference
Limited reporting Neither a *E.coli* WP2 strain nor the *Salmonella typhimrium* tester strain TA102 has been tested Only 3 test concentrations tested Not evident if multiple plates were evaluated No justification for top concentration No data on cytotoxicity and precipitations Positive controls different from recommended ones in TG GLP: no		Test concentrations (with and without metabolic activation): 100, 500, 1000 µg/plate Justification for top concentration: not given S9 mix: rat liver S9 Aroclor 1254 induced(Plate incorporation assay) Vehicle: DMSO Negative control: yes Positive control: yes	Cytotoxicity: not reported Precipitations: not reported Controls: Negative control: valid Positive control: valid	
Bacterial Reverse Mutation Test Similar to OECD TG 471 Deviation: Limited reporting Only TA 98 tested Only 4 concentrations tested No justification for top concentration No data on cytotoxicity and precipitations GLP: no	D&C Red No. 36 Purity: no data	Supporting study Reliable with restrictions Bacterial strains/cell culture: TA 98 Test concentrations (with and without metabolic activation): 1 (without S9), 10, 100, 500 µg/plate Justification for top concentration: no data S9 mix: rat liver S9 Aroclor 1254 induced (Plate incorporation assay) Vehicle: DMSO Negative control: yes Positive control: yes	**Positive** (with and without metabolic activation) -TA98: Increase at 100 µg/plate with and without metabolic activation (but not at 500 µg/plate, without S9: 1.8; with S9: 1.6 fold) Cytotoxicity: no data Precipitations: no data Controls: Negative control: valid Positive control: valid	(Green and Pastewka, 1980) (Green and Pastewka, 1979) – abstract of the same data
Bacterial Reverse Mutation Test Similar to OECD TG 471 Deviation:	R-228, Parmetone red, CI 12085 Purity: no data; purified dyes	Supporting study Reliable with restrictions Bacterial strains/cell culture: TA 98, TA 100	**Positive** (with and without metabolic activation) -TA 98 with metabolic	(Miyagoshi et al., 1983)

Method, guideline, deviations if any	Test substance	Relevant information about the study	Observations	Reference
Limited reporting Only TA 98 and TA 100 tested Only four concentrations tested No justification for top concentration No data on cytotoxicity and precipitations GLP: no		Test concentrations (with and without metabolic activation (S9 mix)): 5, 50, 500, 1000 µg/plate Justification for top concentration: no data S9: Rat liver S9 (pre-incubation assay) Vehicle: DMSO Negative control: yes Positive control: yes	activation, without S9 sporadic increase of revertant numbers -TA 100 with metabolic activation Cytotoxicity: no data Precipitations: no data Controls:	
Bacterial Reverse Mutation Test Similar to OECD TG 471 Deviations: Limited reporting Result tables only for positive results and for cosmetics tested Neither a *E.coli* WP2 strain nor the *Salmonella typhimrium* tester strain TA102 has been tested No justification for top concentration No data on cytotoxicity and precipitations No data on positive control GLP: no	D&C Red No. 36 Purity: no data	Disregarded Study Not assignable (insufficient reporting, no data on positive controls) Bacterial strains: TA 98, TA 100, TA 1535, TA 1537 Justification for top concentration: not given S9 mix: Rat liver S9 Vehicle: DMSO Negative control: yes Positive control: no data	Negative (with and without metabolic activation) Cytotoxicity: no Precipitations: no Controls: Negative control: valid Positive control: no data	(Muzzall and Cook, 1979)
Bacterial Reverse Mutation Test Similar to OECD TG 471 Deviation: Limited reporting Only 3 bacterial strains tested: TA 1535, TA 1538, TA 98	Fire Red CI 12085 Purity: no data	Disregarded Study Not reliable (No detailed data on results) Bacterial strains/cell culture: TA 1535, TA 1538, TA 98 Justification for top concentration: no data S9 mix: Aroclor 1254 treated male mice 1 µg/plate Vehicle: DMSO	Negative (with and without metabolic activation) Cytotoxicity: no data Precipitations: no data	(Milvy and Kay, 1978)

Method, guideline, deviations if any	Test substance	Relevant information about the study	Observations	Reference
No detailed data on results, only given as mean number for 17 dyes together Only one low concentration No justification for top concentration No data on cytotoxicity and precipitations Positive controls different from recommended ones in TG No positive control for TA 98 Not clear if tests were performed on single plates GLP: no		Negative control: yes Positive control: yes		
In vitro Mammalian Cell Gene Mutation tests using the Hprt gene According to OECD TG 476 Deviations: no GLP: yes	Hansa-Rot R (CAS 2814-77-9) Purity: 98.2%	Key study Reliable without restrictions Cell culture: V79 cells (Chinese hamster) Test concentrations (with and without metabolic activation): *Experiment I*: 6.45, 12.9, 25.7, 51.3, 102.5, 204.9, 409.7, 819.4, 1638.7, 3277.3 µg/ml *Experiment II*: 78.1, 156.3, 312.5, 625.0, 937.5, 1250, 1875, 2500, 3277.3 µg/ml Justification for top concentration: The highest concentration produced no severe toxic effects with and without metabolic activation. Higher concentrations were not applied because of the 10 mM limitation (OECD guideline). S9 mix: phenobarbital/ beta-naphthoflavone induced rat liver S9 Treatment time(s): 4 hours	Negative (with and without metabolic activation) Cytotoxicity: no (experiment 1), moderate with the highest concentration with S9 mix (63.4% survival) (experiment 2) Precipitations: microscopic ≥ 25.7 µg/ml; macroscopic ≥ 204.9 µg/ml Controls: Negative control: valid Positive control: valid	(Aventis, 2005)

Method, guideline, deviations if any	Test substance	Relevant information about the study	Observations	Reference
		Sampling time(s): day 9 addition of 6-thioguanine; day 16 staining of colonies Vehicle: culture medium Negative control: yes Positive control: yes		
In vitro Mammalian Chromosomal Aberration Test According to OECD TG 473 Deviation: No analysis conducted to determine homogeneity, concentration or stability of test item formulation GLP: yes	Pigment Red 4 (CAS 2814-77-9) Purity: 98.5%	Key study Reliable without restrictions Cell culture: human lymphocytes Test concentrations (with and without metabolic activation): 25, 50, 100, 200, 400, 800 µg/ml Justification for top concentration: precipitation of test item at maximum dose restricting the ability to accurately score metaphases S9 mix: rat liver S9 phenobarbital/ beta-naphthoflavone induced Treatment time(s): **Experiment I**: 4 hours exposure with and without S9; 2% final concentration) and 20 hours expression period **Experiment II**: 4 hours exposure (with S9; 1% final concentration), without S9 exposure time 24 hours continuous exposure Sampling time(s): 20 hours after treatment (24 hours in total) Vehicle: DMSO Negative control: yes Positive control: yes	Negative (with and without metabolic activation) Cytotoxicity: yes, some at 800 µg/ml Precipitations: yes, at 800 µg/ml Controls: Negative control: valid Positive control: valid	(Harlan, 2013)

* The CAS number registered for Fire Red CI 12085 corresponds to that of PR4.
** The structure given for D&C Red 36 corresponds to that of PR4.
*** The CAS number registered for „R-228, Parmetone red, CI 12085" corresponds to that of PR4.

7.9.6.2.2. In vivo data

There are currently no in vivo genotoxicity studies available in the registration dossier.

7.9.6.2.3. Human information

No information available.

7.9.6.2.4. Summary and discussion of genotoxicity

In vitro data:

Bacterial reverse mutation tests

The technical dossier contains two in vitro gene mutation studies in bacteria (Ames tests) which were performed according to OECD TG 471 and are considered as key studies. Both tests yielded positive results. More specifically, the positive result is observed in the TA98 strain after metabolic activation. The positive result indicates that the substance is inducing gene mutations under the conditions of the tests.

In vitro mammalian gene mutation and cytogenicity tests

The technical dossiers further contains an in vitro mammalian cell gene mutation test using the Hprt gene and an *in vitro* mammalian chromosomal aberration test. Both tests were performed according to the respective OECD TGs, are considered by eMSCA to be reliable without restrictions and are negative. Thus, no further concern arises from those two in vitro studies.

Summarizing, available *in vitro* data (Ames tests) indicate a potential for PR4 to induce gene mutations.

In vivo data:

There are currently no *in vivo* genotoxicity tests available in the technical dossier.

Thus, an appropriate *in vivo* genotoxicity study to follow up the concern on gene mutations identified *in vitro* (Ames test) is missing.

7.9.6.2.5. Conclusion

The technical dossier contains positive results from in vitro gene mutation studies in bacteria which indicate a potential for PR4 to induce gene mutations. *In vivo* genotoxicity studies are not available in the technical dossier for the registered substance. Hence, an appropriate *in vivo* follow-up genotoxicity study is necessary to address the concern identified *in vitro*. This information need is subject to the standard testing scheme of REACH. The generation of new information regarding the genotoxicity endpoint has been already requested by ECHA in a previous compliance check.

7.9.6.3. PO5

7.9.6.3.1. In-vitro data

Table 38

Summary table of mutagenicity/genotoxicity tests *in vitro*				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
Bacterial Reverse Mutation Test According to OECD TG 471 Deviations: no GLP: yes	Pigment Orange 5 (CAS 3468-63-1) Purity: 98.78 % (w/w)	Key study Reliable without restrictions Bacterial strains: *Salmonella typhimurium* tester strains: TA100, TA98, TA1537, TA1535, *E.coli* strain: WP2 uvrA Test concentrations (with and without metabolic activation (S9 mix)): 3, 10, 33, 100, 333, 1000, 2500, 5000 µg/plate S9 mix: rat liver S9 induced by phenobarbital/b-Naphthoflavone Vehicle: DMSO Negative control: yes Positive control: yes	Positive (with and without metabolic activation) - with S9 mix: dose dependent increase in revertant colony numbers in TA98 (≥ 3 µg/plate) - without S9 mix: dose dependent increase in revertant colony numbers in TA1537 (≥ 3 µg/plate), TA98 (≥ 3 µg/plate) - Cytotoxicity: - for TA1535 with and without S9 mix at 5000 µg/plate) Precipitations: - ≥ 333 µg/plate for all tester strains with and without S9 mix Controls: Negative control: valid Positive control: valid	(Cytotest, 2007)
Bacterial Reverse Mutation Test According to OECD TG 471 Deviations: no GLP: yes	Pigment Orange 5 (3468-63-1) Purity: 99.75 %	Key study Reliable without restrictions Bacterial strains: *Salmonella typhimurium* tester strains: TA100, TA98, TA1537, TA1535, *E.coli* strain: WP2 uvrA	Positive (with and without metabolic activation) - with S9 mix: dose dependent increase in revertant colony numbers in TA1537	(Aventis, 2000)

Summary table of mutagenicity/genotoxicity tests *in vitro*				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
		Test concentrations (with and without metabolic activation (S9 mix)): 50, 160, 500, 1600, 5000 µg/plate S9 mix: rat liver S9 induced by Aroclor 1254 Vehicle: DMSO Negative control: yes Positive control: yes	(≥ 50 µg/plate) and TA98 (≥ 50 µg/plate) - without S9 mix: dose dependent increase in revertant colony numbers in TA1537 (≥ 50 µg/plate) and TA98 (≥ 50 µg/plate) Cytotoxicity: - for TA100 with and without S9 mix at 5000 µg/plate Precipitations: - ≥ 500 µg/plate for all tester strains with and without S9 mix Controls: Negative control: valid Positive control: valid	
Bacterial Reverse Mutation Test Similar to OECD TG 471 Deviations: Neither a *E.coli* WP2 strain nor the *Salmonella typhimrium* tester strain TA102 has been tested No justification why top dose tested was below 5000 µg/plate as recommended in OECD TG 471 No information on precipitations No detailed information on substance purity GLP: no	Permanent-Rot GG Purity: "chemical pure"	Supporting study Reliable with restrictions Bacterial strains: *Salmonella typhimurium* tester strains: TA100, TA98, TA1537, TA1535 Test concentrations (with and without metabolic activation (S9 mix)): 0.16, 0.8, 4, 20, 100, 500, 2500 µg/plate Justification for top concentration: no information S9 mix: rat liver S9 induced by Aroclor 1254	Positive (with and without metabolic activation) - with S9 mix: dose dependent increase in revertant colony numbers in TA98 (≥ 20 µg/plate), TA100 (≥ 500 µg/plate), TA 1537 (≥ 500 µg/plate) - without S9 mix: dose dependent increase in revertant colony numbers in TA98 (≥ 0.16 µg/plate), TA100 (≥ 500 µg/plate),	(Hoechst, 1978)

Summary table of mutagenicity/genotoxicity tests *in vitro*				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
		Vehicle: DMSO Negative control: yes Positive control: yes	TA 1537 (≥ 4 µg/plate) Cytotoxicity: no Precipitations: no information Controls: Negative control: valid Positive control: valid	
Bacterial Reverse Mutation Test Similar to OECD TG 471 Deviations: Neither a *E.coli* WP2 strain nor the *Salmonella typhimrium* tester strain TA102 has been tested No justification why top dose tested was below 5000 µg/plate for experiments with metabolic activation as recommended in OECD TG 471 No information on substance purity No information on precipitations GLP: no	Hansa Rot GG unkristall. Purity: no information	Supporting study Reliable with restrictions Bacterial strains: *Salmonella typhimurium* tester strains: TA100, TA98, TA1537, TA1535 Test concentrations (with metabolic activation (S9 mix)): 4, 20, 100, 500, 2500 µg/plate Test concentrations (without metabolic activation (S9 mix)): 0.016, 0.8, 4, 20, 500, 5000 µg/plate S9 mix: rat liver S9 induced by Aroclor 1254 Vehicle: ethanol Negative control: yes Positive control: yes	Positive (without metabolic activation) - without S9 mix: dose dependent increase in revertant colony numbers in TA98 (≥ 0.8 µg/plate) Cytotoxicity: no Precipitations: no information Controls: Negative control: valid Positive control: valid	(Hoechst, 1979a)
Bacterial Reverse Mutation Test Similar to OECD TG 471 Deviations: Tests were not performed in triplicates Only 3 test concentrations tested No information on precipitations Neither a *E.coli* WP2 strain nor the *Salmonella typhimrium*	D & C Orange No.17 (C.I.12075) Purity: 95 %	Supporting study Reliable with restrictions Bacterial strains: *Salmonella typhimurium* tester strains: TA100, TA98, TA1535, TA1537, TA1538 Test concentrations (with and without metabolic activation	Positive (with and without metabolic activation) - with S9 mix: dose dependent increase in revertant colony numbers in TA1537 (≥ 50 µg/plate), TA1538 (≥ 50 µg/plate),	(Brown et al., 1979a)

Summary table of mutagenicity/genotoxicity tests *in vitro*				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
tester strain TA102 has been tested No justification for top concentration GLP: no		(S9 mix)): 50, 100, 500 µg/plate Justification for top concentration: no information S9 mix: rat liver S9 induced by Aroclor 1254 Vehicle: DMSO Negative control: yes Positive control: yes	TA98 (≥ 50 µg/plate); TA100 (at 500 µg/plate) - without S9 mix: dose dependent increase in revertant colony numbers in TA1537 (≥ 100 µg/plate), TA1538 (≥ 50 µg/plate), TA98 (≥ 50 µg/plate); TA100 (at 500 µg/plate) Cytotoxicity: no Precipitations: no information Controls: Negative control: valid Positive control: valid	
Bacterial Reverse Mutation Test Similar to OECD TG 471 Deviation No specific data on substance identity (such as CAS/ C.I. number) No information on purity No justification why top dose tested was below 5000 µg/plate for experiments as recommended in OECD TG 471 Only one bacterial strain tested Only 4 test concentrations tested No information on precipitations GLP: no	D & C Orange No. 17 Purity: no information	Disregarded Study Not assignable (too less information available to allow firm assessment of the result) Bacterial strains: Salmonella typhimurium tester strain: TA98 Test concentrations (with and without metabolic activation (S9 mix)): 0.1, 1,10,50,100 µg/plate Justification for top concentration: no information S9 mix: rat liver S9 induced by Aroclor 1254 Vehicle: DMSO Negative control: yes	Positive (with and without metabolic activation) - with S9 mix: dose dependent increase in revertant colony numbers in TA98 (≥ 50 µg/plate) - without S9 mix: dose dependent increase in revertant colony numbers in TA98 (≥ 0.1 µg/plate) Cytotoxicity: no Precipitations: no information Controls: Negative control: valid Positive control: valid	(Green and Pastewka, 1980)

Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		Positive control: yes		
Bacterial Reverse Mutation Test Similar to OECD TG 471 Deviations: No information on substance purity Testing of only one strain: E.coli WP2 uvrA Only one experiment GLP: no	Hansa-Rot GG Purity: no information	Supporting study Reliable with restrictions Bacterial strains: *E.coli* strain: WP2 uvrA Test concentrations (with and without metabolic activation (S9 mix)): 0.8, 4, 20, 100, 500, 2500, 10000 µg/plate S9 mix: rat liver S9 induced by Aroclor 1254 Vehicle: DMSO Negative control: yes Positive control: yes	Negative (with and without metabolic activation) Cytotoxicity: no Precipitations: ≥ 100 µg/plate with and without S9 mix Controls: Negative control: valid Positive control: valid	(Hoechst, 1980b)
Bacterial Reverse Mutation Test Not in accordance with OECD TG 471 (due to the lacking data on controls) Deviations: No information on substance purity No detailed data on tested concentrations No detailed data for results shown Data on positive and negative controls are missing Neither a *E.coli* WP2 strain nor the *Salmonella typhimurium* tester strain TA102 has been tested No information on vehicle GLP: no data	Hansa-Rot GG Purity: no information	Disregarded study Not assignable (too less information available to allow firm assessment of the result) Bacterial strains: *Salmonella typhimurium* tester strains: TA100, TA98, TA1537, TA1535, TA1538 Test concentrations (with and without metabolic activation (S9 mix)): 0.0064 - 10000 µg/plate S9 mix: rat liver S9 induced by Aroclor 1254 Vehicle: no information Negative control: yes Positive control: no data	Positive (with and without metabolic activation) - no further details on results are available Cytotoxicity: no information Precipitations: no information Controls: Negative control: no detailed data Positive control: no data	(Hoechst, 1980a)

Summary table of mutagenicity/genotoxicity tests *in vitro*				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
Bacterial Reverse Mutation Test Not in accordance with OECD TG 471 (due to the lacking data on controls) Deviations: No information on substance purity Neither a *E.coli* WP2 strain nor the *Salmonella typhimrium* tester strain TA102 has been tested No detailed data on tested concentrations No detailed data for results shown Data on positive and negative controls are missing GLP: no	Hansa-Rot GG unkrist. Purity: no information	Disregarded study Not assignable (too less information available to allow firm assessment of the result) Bacterial strains: *Salmonella typhimurium* tester strains: TA98, TA100, TA1537, TA1535 Test concentrations: 0.8 – 5000 µg/plate (no data if with and without metabolic activation) S9 mix: no information Negative control: no data Positive control: no data	Positive (without metabolic activation) - no further details on results are available Cytotoxicity: no information Precipitations: no information Controls: Negative control: no data Positive control: no data	(Hoechst, 1979b)
In vitro mammalian chromosomal aberration test similar to OECD TG 473 Deviations: Only short term treatment with and without metabolic activation performed Continuous exposure without metabolic activation missing No detailed information on substance purity GLP: yes	C.I. Pigment Orange 5 (CAS: 3468-63-1) Purity: "technical pure"	Disregarded study Not reliable (due to the lack of continuous exposure without S9 mix not possible to conclude an overall negative outcome) Cell culture: V79 cells (Chinese hamster) Test concentrations: - with metabolic activation (S9 mix): 1. 200 µg/ml; 2. 20, 100, 200 µg/ml 3. 200 µg/ml -without metabolic activation (S9 mix): 1. 600 µg/ml; 2. 60, 300, 600 µg/ml; 3. 600 µg/ml Treatment time (with and without metabolic activation):	Negative (with and without metabolic activation) (only for short term exposure; long term exposure without S9 mix has not been investigated) Cytotoxicity: with and without S9 mix Precipitations: yes Controls: Negative control: valid Positive control: valid	(Hoechst, 1989a)

Summary table of mutagenicity/genotoxicity tests *in vitro*				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
		1.-3.: 4 h Sampling times after the start of treatment (with and without metabolic activation): 1. 7 h 2. 18 h 3. 28 h S9 mix: rat liver S9 induced by Aroclor 1254 Justification for top concentration: cytotoxicity Vehicle: DMSO Negative control: yes Positive control: yes Positive control: yes		
In vitro Mammalian Cell Gene Mutation tests using the Hprt genes according to OECD TG 476 Deviations: No detailed information on substance purity GLP: yes	C.I. Pigment Orange 5 (CAS: 3468-63-1) Purity: "technical pure"	Key Study Reliable without restrictions Cell culture: V79 cells (Chinese hamster) Test concentrations: - with metabolic activation (S9 mix): 50, 100, 250, 500 µg/ml - without metabolic activation: 50, 75, 100, 150 µg/ml Treatment time (with and without S9 mix): 4 h Sampling time (with and without S9 mix): after 9 days Justification for top concentration: cytotoxicity S9 mix:: rat liver S9 induced by Aroclor 1254 Vehicle: DMSO Negative control: yes Positive control: yes	Negative (with and without metabolic activation) Cytotoxicity: yes Precipitations: yes Controls: Negative control: valid Positive control: valid	(Hoechst, 1989b)

7.9.6.3.2. In vivo data

Table 39

Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo				
Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
Mammalian Bone Marrow Chromosomal Aberration Test According to OECD TG 475 Deviations: No scientific justification provided why hamsters are used Only 50 metaphases scored (instead of 200 as recommended in TG 475) No information if substance has reached bone marrow (mitotic index not depressed, no plasma or blood levels, no other ADME data) No detailed information on substance purity	Pigment Orange 5 (CAS 3468-63-1) Purity: "technical pure"	Disregarded study Not reliable (due to the lack of information if the substance has reached bone marrow cells no conclusion can be drawn on the relevance of the negative result) Species: Chinese hamster Number of animals per group: 5 Males and females Target organ: Bone marrow Administration route: Oral Dose: 5000 mg/kg bw Treatment: Dose was given in 2 equal parts within 2 h Sampling times: 12, 24, 48 h Justification for top dose: Maximum applicable dose Vehicle: Sesame oil Positive control: yes	Negative Toxicity: Clinical signs: faeces red coloured, closed palpebral fissures, impaired general condition, reduced spontaneous activity Lethal effects: no Cytotoxicity: mitotic index not depressed Controls: Positive control: valid Negative control: valid	(Hoechst, 1990)
Unscheduled DNA Synthesis (UDS) test with mammalian liver cells in vivo	Pigment Orange 5 (CAS 3468-63-1) Purity: 97.8 %	Key study Reliable without restrictions Species: Wistar Han rats	Negative A negative result is not conclusive for the assessment of induction of gene mutations (see	(Harlan, 2013b)

Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo				
Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
Similar to OECD TG 486 Deviations: no GLP: yes		Number of animals per group: 4 males Target organs: Liver Administration route: Oral (gavage) Dose levels: 1000 and 2000 mg/kg bw Treatment: Single gavage Sampling times: 4 h and16 h after dosing (begin of perfusion of liver) Justification for top dose: 2000 mg/kg bw is maximum recommended dose according to TG 486 (if no toxicity or severe clinical signs) Vehicle: Arachis Oil Positive control: yes Negative control: yes	section 7.9.6.1.4., in vivo data). Toxicity: Clinical signs: no Lethal effects: no Cytotoxicity: no Controls: Negative control: valid Positive control: valid	

7.9.6.3.3. Human information

No information is available.

7.9.6.3.4. Summary and discussion of genotoxicity

In vitro data:

Bacterial reverse mutation tests

There are two reliable bacterial reverse mutation tests (Cytotest, 2007; Aventis, 2000) available in the technical dossier which were performed according to OECD TG 471 and are considered as key studies by eMSCA. Both studies yielded clear positive results with and without metabolic activation.

In addition, there exist three bacterial reverse mutation assays (Brown et al., 1979a; Hoechst, 1979a; Hoechst, 1978) which were considered to be supporting studies by eMSCA as major deviations from OECD TG 471 were found. Nevertheless, the results are evaluated

as reliable with restrictions by eMSCA. All three tests also yielded positive results with the test substance. Furthermore, there is one bacterial reverse mutation test available which used only one strain, namely the *E.coli* WP2 uvrA strain (Hoechst, 1980b) considered as supporting study by eMSCA (reliable with restriction). The test is negative with and without metabolic activation which is in line with results for this strain in the available key studies (Cytotest, 2007; Aventis, 2000).

Three further bacterial reverse mutation tests (Hoechst, 1979b; Hoechst, 1980a; Hoechst, 1980b; Green and Pastewka, 1980) were considered as not reliable or not assignable and were disregarded from assessment by eMSCA. Reasons for the reliability assessment and details are summarised in Table 38.

In vitro mammalian gene mutation tests

There is one *in vitro* gene mutation test in mammalian cells using the Hprt genes available for PO5 (Hoechst, 1989b). The test was performed according to OECD TG 476, is considered as reliable without restrictions by eMSCA and is negative.

In vitro mammalian cytogenicity tests

There exists one *in vitro* mammalian chromosomal aberration test which was performed similar to OECD TG 473 and is negative (Hoechst, 1989a). However, due to the lack of continuous exposure without S9 mix it is not possible to conclude an overall negative outcome (see OECD TG 473, section 28) and the test is considered not to be reliable by eMSCA. Thus, the test is not considered adequate to fulfil the standard data requirement.

Summarizing, *in vitro* data in bacteria indicate a potential for PO5 to induce gene mutations. The available *in vitro* cytogenicity test with PO5 is not considered adequate to fulfil the standard data requirement.

In vivo data:

Two *in vivo* genotoxicity tests are provided for the substance in the registration dossier, an *in vivo* Mammalian Bone Marrow Chromosomal Aberration Test (Hoechst, 1990) and an Unscheduled DNA Synthesis (UDS) test with mammalian liver cells *in vivo* (Harlan, 2013b).

The *in vivo* Mammalian bone marrow chromosomal aberration test was performed according to OECD TG 475 with some deviations. The test result was negative. However, in the present test evidences of exposure to the bone marrow could not be found. The mitotic index was not depressed and data on plasma or blood levels are not available. ADME data obtained in an independent study using the same route and species are also not available. Due to the lack of this information no conclusion can be drawn if the substance has reached bone marrow cells in sufficient quantity. According to OECD TG 475 (section 44d) a test substance is considered only clearly negative if bone marrow exposure to the test substance occurred. Therefore, no conclusion can be drawn on the relevance of the negative result and the test is considered not to be adequate to fulfil the standard data requirement for a cytogenicity test.

The UDS test was performed similar to OECD TG 486 and is considered as reliable without restrictions. The test yielded a negative result. However, according to the Guidance on information requirements and chemical safety assessment (Chapter R.7a, Version 6.0, 2017, Section R.7.7.6.3) a negative result in an UDS test alone is not a proof that the substance does not induce gene mutations. Thus, this negative UDS test is not considered the appropriate follow-up test by eMSCA for the positive results observed with the bacterial reverse mutation tests for PO5.

Summarising, the available *in vivo* data are neither sufficient to clarify the concern for mutagenic effects of PO5 identified in vitro nor to fulfill the standard data requirements regarding a cytogenicity test.

7.9.6.3.5. Conclusion

The technical dossier contains positive results from *in vitro* gene mutation studies in bacteria which indicate a potential for PO5 to induce gene mutations. The available *in vivo* data are not adequate to clarify this concern. Hence, an appropriate *in vivo* follow-up mutagenicity study is necessary to address the concern identified *in vitro*. This information need is subject to the standard testing scheme of REACH and thus the generation of new information will not be requested under substance evaluation but in a compliance check.

Moreover, the available *in vitro* and *in vivo* cytogenicity tests with PO5 are not considered adequate to fulfil standard data requirement regarding a cytogenicity test. Hence, an appropriate *in vitro* cytogenicity study is necessary. This information need is subject to the standard testing scheme of REACH and thus the generation of new information will not be requested under substance evaluation but in a compliance check.

7.9.7. Carcinogenicity

7.9.7.1. PR3

7.9.7.1.1. Non- human information

7.9.7.1.1.1. Oral application

The toxicity and carcinogenicity of **Pigment Red 3** has been investigated by the US National Toxicology Program (NTP). The NTP studies were published in 1992 in the technical report 407 (NTP, 1992a). The report consists of 2-week and 13-week sub-chronic toxicity studies and 2-year carcinogenesis dietary studies (including an interim evaluation at 15 month) with rats and mice of both sexes. Additionally, genotoxicity was assayed and reported in the NTP technical report.

The authors of the NTP carcinogenesis studies in rats and mice concluded on "some evidence of carcinogenic activity" of C.I. Pigment Red 3 in male and female F344/N rats and male B6C3F_1 mice, but "no evidence of carcinogenic activity" of C.I. Pigment Red 3 in female B6C3F_1 mice. Based on these results C.I. Pigment Red 3 was evaluated as "cannot be classified as to its carcinogenicity to humans (group 3)" in the IARC Monographs on the evaluation of carcinogenic risks to humans, Volume 57 (IARC, 1993).

Table 40

Relevant studies related to the assessment of carcinogenicity for PR3			
Methods	**Results**	**Remarks**	**Reference**
2-year feeding study in rats C.I. Pigment Red 3 (CAS 2425-85-6) Purity: >97 % According to OECD TG 451 (NTP guideline including 2-week and 13-week studies plus interim evaluation after 15 month) No GLP (but equivalent)	Some evidence of carcinogenic activity in male and female rats Neoplastic lesions: Male: benign adrenal pheocromocytomas of the adrenal gland (22/50, 29/50, 35/50*, 34/50*); malignant neoplasms not increased; first incidences day 653, 605, 529, 486 Male: malignant adrenal pheocromocytomas (6/50, 7/50, 9/50, 3/50)	Food conversion factor: 20 (for older rats) Calculated doses: 0, 300, 625, 1250 mg/kg bw/d Reliable without restrictions	(NTP, 1992a)

Relevant studies related to the assessment of carcinogenicity for PR3			
Methods	**Results**	**Remarks**	**Reference**
Species: rats Strain: F344/N n: 60/dose group/sex; Additional groups of up to 10 male and 10 female rats per dose for interim evaluations (organ weights, hematology, clinical chemistry and histopathology) after 15 month Dose levels: 0, 6 000, 12500, 25 000 ppm in feed supplied weekly available ad libitum for 103 weeks Route: food	Female: hepatocellular adenomas (0/50, 0/50, 1/50, 10/50*); first incidences day -, -, 729, 553 Male: squamous cell papillomas of the skin (0/50, 4/50, 2/50, 6/50*); first incidences day -, 729, 641, 679 Male: Zymbal's gland carcinoma (0/50, 0/50, 2/50, 3/50); high-dose above historical control range Male/female: mononuclear cell leukemia, mammary gland fibroadenoma, preputial gland/clitoral gland adenoma at lower incidences compared to controls Non-neoplastic lesions: Male/female: eosinophilic (male: 6/50, 37/50*, 36/50*, 41/50*; female: 1/50, 7/50, 18/50*, 16/50*) or mixed type (male: 2/50, 24/50*, 21/50*, 15/50*; female: 4/50, 16/50*, 30/50*, 40/50*) foci of cellular alteration in the liver (angiectasis (3/50, 20/50*, 21/50*, 29/50*) and cystic degeneration (9/50, 36/50*, 40/50*, 36/50*) in male and granulomas (27/50, 21/50, 42/50*, 44/50*), and cholesterol pigmentation (0/50, 3/50, 14/50*, 41/50*) in female (bilary tract proliferation (female: 18/50, 12/50, 18/50, 29/50*) Chronic nephropathy (male: 50/50, 49/50, 50/50, 49/50; female: 49/50, 49/50, 50/50, 48/50) with increasing severity (grades: male 2.4, 3.1*, 3.6*, 3.8*; female 1.7, 2.2*, 2.4*, 2.8*) Secondary to renal disease: parathyroid gland hyperplasia, fibrous osteodystrophy of the bone and mineralization of stomach, intestine, heart and blood vessels Final mean body weight >10% lower than controls: male 25 000 ppm from week 82, female ≥12 500 ppm (from week 66 mid dose, week 42 high dose) No clinical findings of toxicity; weight of liver and spleen significantly increased		

Relevant studies related to the assessment of carcinogenicity for PR3			
Methods	**Results**	**Remarks**	**Reference**
	Haematology: Hct, Hb and erythrocytes significantly decreased; platelets and bilirubin increased, MetHb in female Survival: male (28/50, 40/50, 28/50, 20/50), female 32/50, 41/50, 39/50, 40/50		
2-year feeding study in mice C.I. Pigment Red 3 (CAS 2425-85-6) Purity: >97 % According to OECD TG 451 (NTP guideline including 2-week and 13-week studies plus interim evaluation after 15 month) No GLP (but equivalent) Species: mice Strain: B6C3F1 n: 60/dose group/sex; additional groups of up to 10 male and 10 female rats per dose for interim evaluations (organ weights, hematology, clinical chemistry and histopathology) after 15 month Dose levels: 0, 12500, 25 000, 50 000 ppm in feed supplied weekly available ad libitum for 103 weeks Route: food Temperature 19°-27°C Relative humidity 20-85%	Some evidence of carcinogenic activity in male mice; no evidence in female mice Neoplastic lesions: Male: tubule adenoma of the renal cortex (0/50, 0/50, 0/50, 6/50*); first incidences day -, -, -, 729 Male: follicular cell adenoma of the thyroid gland (0/50, 0/49, 1/50, 5/50*), also increased incidence of follicular cell hyperplasia in male and female; first incidences day -, -, 658, 557 Non-neoplastic lesions: Male: focal renal tubule hyperplasia (0/50, 1/50, 7/50*, 7/50*) and cystic hyperplasia (0/50, 0/50, 0/50, 4/50), cytomegaly (karyomegaly) of renal tubule epithelium (0/50, 40/50*, 47/50*, 46/50*) Male/ female: chronic nephropathy (male 34/50, 39/59*, 42/50*, 45/50*; female 33/50, 45/49*, 46/49*, 45/49*), severity (grades male: 0.8, 1.0, 1.2*, 1.6*; female 0.7, 1.2*, 1.2*, 1.6*) Final mean body weight >10% lower than controls: male/ female 50 000 ppm from week 62 for male and week 38 for female No clinical findings of toxicity; liver weight significantly increased Haematology: erythrocytes in male decreased; total urine bilirubin increased Survival: male (33/50, 28/50, 31/50, 33/50), female 39/50, 37/50, 31/50, 25/50* (survival of high-dose female significantly decreased)	Food conversion factor: 7 (for mice) Calculated doses: 0, 1785, 3571, 7142 mg/kg bw/d Reliable without restrictions	(NTP, 1992a)

7.9.7.1.2. Human information

No information available.

7.9.7.1.3. Summary and discussion of carcinogenicity

The 2-year carcinogenicity studies in mice and rats are equivalent to OECD TG 451 (NTP guideline).

Groups of 60 male and 60 female F344/N rats were administered 0, 6 000, 12 500, 25 000 ppm in feed supplied weekly available ad libitum for 103 weeks (Calculated dose: 0, 300, 625, 1250 mg/kg bw/d). Up to 10 rats per dose group were designated for the interims evaluations at 15 month.

Final mean body weights for male rats at the high-dose and female rats at the mid- and high-dose were more than 10% lower than controls.

Male rats showed significantly increased benign adrenal pheochromocytomas in the mid- and high-dose group with incidences exceeding the NTP historical control range for feed studies. Hepatocellular adenomas in females occurred with a dose-dependent trend which was only statistically significant in the high-dose group. Squamous cell papillomas of the skin showed a positive trend in male rats, again only statistically significant in the high-dose group. Zymbal's gland carcinoma were marginally increased in the mid- and high-dose group. Both of the latter findings are listed as uncertain findings which "may have been related to C.I. Pigment Red 3 administration". Non-neoplastic lesions include chronic nephropathy with increasing severity as well as eosinophilic and mixed cell foci in the liver of male and female rats.

Benign adrenal pheochromocytomas in male rats occurred at statistically significant incidences in the mid- and high-dose groups whereas hepatocellular adenomas in female rats occurred in high-dose group. Benign adrenal pheochromocytomas in male rats are shown at high spontaneous incidences in the internal control group. Final mean body weights for male rats in the high-dose and female rats in the mid- and high-dose were more than 10% lower than controls. Food consumption and mortalities in the treated groups were similar to the internal controls.

Groups of 60 male and 60 female B6C3F_1 mice were administered 0, 12 500, 25 000, 50 000 ppm in feed supplied weekly available ad libitum for 103 weeks (Calculated doses: 0, 1785, 3571, 7142 mg/kg bw/d). Up to 10 mice per dose group were designated for the interims evaluations at 15 months.

Final mean body weights for male and female mice at the high-dose were more than 10% lower than controls. The survival of high-dose female mice was significantly decreased.

Male mice showed significantly increased incidences in tubule adenomas of the renal cortex in the high-dose and in follicular cell adenoma of the thyroid gland in the high-dose. In female mice no increase in tumour incidences was observed. Male and female mice showed increased incidences in follicular cell hyperplasia. Statistically increased incidences in male mice were observed at very high concentrations.

Overall, the evidence of the carcinogenic potential of C.I. Pigment Red 3 in rats and mice is limited to findings which are shown to have high background incidences or appear at high dose levels. Target organs vary between sexes and species: the adrenal gland in male rats, the liver in female rats, kidney and thyroid gland in male mice and no evidence in female mice.

7.9.7.1.4. Conclusion

Overall, there is limited evidence on carcinogenicity in animals. Tumours occurred at high-dose levels where final mean body weights were more than 10% lower than controls. Benign adrenal pheochromocytomas in male rats occurred in the mid- and high-dose groups (625 and 1250 mg/kg bw/d) coinciding with high spontaneous incidences in the control group. Hepatocellular adenomas in female rats occurred in high-dose group (1250 mg/kg bw/d). Male mice showed increased incidences in tubule adenomas of the renal cortex and in follicular cell adenoma of the thyroid gland at very high concentrations (7142 mg/kg bw/d). In female mice no increase in tumour incidences was observed. Target organs vary between sexes and species.

In conclusion, the data available does not warrant classification of PR3 as carcinogen. However, additional data on a possible genotoxic mode of action would be useful. Additional genotoxicity studies are requested by ECHA in a compliance check.

7.9.7.2. PR4

Two studies investigating the carcinogenic potential of **Pigment Red 4** are a two year feeding study (Kupradinun et al., 2002) and an 18-month skin painting study performed in mice in which 14 colour materials were tested (Carson, 1984), both with limited reporting.

7.9.7.2.1. Non-human information

7.9.7.2.1.1. Oral application

Table 41

Relevant studies related to the assessment of carcinogenicity for PR4

Methods	Results	Remarks	Reference
2 year **feeding study** in rats D&C Red No. 36 (CAS 2814-77-9) Purity: >97 % Non-guideline study No GLP Species: rats Strain: Wistar n: 50/dose group/sex Dose levels: 0, 1 000, 2 000 ppm (estimated 0, 50, 100 mg/kg bw) in feed for 78 weeks and sacrificed at	Not carcinogenic Used in lipstick colouring **Table 3. Incidences of Benign Tumors in Rats Treated with D&C Red No. 36** Sex / Group / No. of effective rats / No. of rats bearing tumor (%) in: Liver[a] / Thyroid gland[b] / Adrenal gland[c] / Bladder[d] / Mammary gland[e] Male: I (Control) 50 — 10 (20.0) — 1 (2.0) — 1 (2.0) — - — - Male: II (1,000 ppm) 48 — 8 (16.7) — 1 (2.1) — - — - — - Male: III(2,000 ppm) 48 — 9 (18.8) — - — 1 (2.1) — - — - Female: I (Control) 50 — 3 (6.0) — 1 (2.0) — - — 1 (2.0) — 9 (18.0) Female: II (1,000 ppm) 47 — 6 (12.8) — 1 (2.1) — - — - — 5 (10.6) Female: III(2,000 ppm) 50 — 8 (16.0) — 2 (4.0) — 1 (2.0) — - — 5 (10.0) [a]Include hyperplastic nodule and cystic cholangioma [b]Include C-cell adenoma and follicular cell adenoma [c]Cortical adenoma [d]Transitional cell papilloma [e]Include adenoma, fibroadenoma and fibroma **Table 4. Incidences of Malignant Tumors in Rats Treated with D&C Red No. 36** Sex / Group / No. of effective rats / No. of rats bearing tumor (%) in: Lung[a] / Thyroid gland[b] / Thymus gland[c] / Soft tissue[d] / Salivary gland[e] / Uterus[f] / Others Male: I (Control) 50 — - — 1 (2.0) — - — 1 (2.0) — - — - — - Male: II (1,000 ppm) 48 — - — 2 (4.2) — - — - — - — - — - Male: III(2,000 ppm) 48 — 2 (4.2) — - — - — 1 (2.1) — - — - — 2 (4.2)[1] Female: I (Control) 50 — - — 1 (2.0) — 2 (4.0) — - — 1 (2.0) — 1 (2.0) — 1 (2.0)[2] Female: II (1,000 ppm) 47 — - — - — - — - — - — 1 (2.1) — - Female: III(2,000 ppm) 50 — 1(2.0) — 1 (2.0) — 1 (2.0) — - — 1 (2.0) — - — 1 (2.0)[3] [a]Include adenocarcinoma and mesenchymal tumor [b]Include follicular cell adenocarcinoma and C-cell carcinoma [c]Malignant thymoma [d]Include fibrosarcoma and mixed malignant fibrous histiocytoma [e]Mixed carcinoma [f]Histiocytic sarcoma [1]Include hepatocellular carcinoma, transitional cell carcinoma of urinary bladder [2]Adenocarcinoma of mammary gland [3]Wilm's tumor Benign tumours: No effect on benign tumours/ low incidences: liver, thyroid gland, adrenal gland, bladder	Limited reporting Dosing for 78 weeks plus 20 week recovery Only 2 dose groups Incidences only on a limited number of findings, historical background of findings unknown No data on individual animals	(Kupradinun et al., 2002)

week 98 (20 week recovery period) Route: food	**Female:** slight changes in **liver tumours** including hyperplastic nodules (female 3/50, 6/47, 8/50) and in mammary gland tumours (9/50, 5/47, 5/50) Malignant tumours: Lung, thyroid gland, salivary gland, uterus, soft tissue, thymus gland with low incidences No effect on body weight gain in males; lower in females No effect on survival (male 50/50, 48/50, 48/50; female 50/50, 47/50, 50/50); animals which died were excluded since they died before week 40		

7.9.7.2.1.2. Dermal application

A second study on carcinogenicity identified for Pigment Red 4 is an 18-month skin painting study performed in mice in which 14 colour materials were tested (Carson, 1984).

Table 42

Relevant studies related to the assessment of carcinogenicity for PR4			
Methods	**Results**	**Remarks**	**Reference**
18 month skin painting study D&C Red No. 36 Purity: 98 % Non-guideline study No GLP Species: mice Strain: 100 ICR n: 50/dose group/sex Dose levels: dermal application to dorsal area: 0.1 ml of 1 mg (1% solution) of dye twice a week for 18 month (mean total dose of applied material 117.1 mg) Route: dermal Vehicle: distilled water Positive control: 3,4-benzpyrene in acetone	Not carcinogenic No increase in neoplasia after dermal application of the test dye compound No effect on survival compared to control	Limited reporting No data on individual animals Limited number of organs analysed Only selected animals from solvent and positive control Study period 18 month Dermal application twice a week with very low dose Incidences only on a limited number of findings, no body weight information No historical control data Not reliable	(Carson, 1984)

7.9.7.2.2. Human information

No information available.

7.9.7.2.3. Summary and discussion of carcinogenicity

The carcinogenic potential of Pigment Red 4 was investigated in a feeding study with a 78-week treatment period plus 20 week recovery period before animals were necropsied. Rats were given D&C Red No. 36 in the diet at low concentrations (0, 50 and 100 mg/kg bw). The dye treatment had no significant effect on survival, no effects on body weight gain in males but significantly affected body weight gain in females, although there are no further details given on body weight data. Histopathological assessment included a limited number of organs. Benign as well as malignant tumours were identified with low incidences. There were small changes in the incidence of benign liver tumours, but as they include hyperplastic nodules and cystic cholangioma, the results are difficult to judge. Malignant tumours occurred at very low incidences with no clear pattern and did not reach statistical significance.

A second study on carcinogenicity identified for Pigment Red 4 is an 18-month skin painting study performed in mice. Dose levels were selected based on lipstick use assessments. An area of about 6 cm^2 was treated twice weekly with 0.1 ml suspension. The mean total dose applied was 117.1 mg D&C Red No. 36. Complete pathology was performed only on a limited number of animals, in all remaining animals any grossly abnormal organs and tissues were examined.

There was no effect on survival compared to control animals and no increase in neoplasia observed after dermal application of D&C Red No. 36. A summary table shows single incidences of any gross lesions identified, but does not include overall incidence nor is there any information on body weight or clinical observations.

Both studies are non-guideline studies, have their limitations in reporting and are performed with quite low dose levels (both based on lipstick use considerations).

7.9.7.2.4. Conclusion

In conclusion, the data reported does not warrant classification of PR4. There is no indication to request further studies under SEv.

7.9.7.3. PO5

7.9.7.3.1. Non-human information

7.9.7.3.1.1. Dermal and oral application

There are various studies evaluating the carcinogenic potential of Pigment Orange 5 including feeding studies and skin painting studies. Most of the studies are only available as short study summaries as they were reviewed by the FDA (Hart et al., 1986) and in a toxicological evaluation by (BG RCI, 2000).

Table 43

Relevant studies related to the assessment of carcinogenicity for PO5			
Methods	**Results**	**Remarks**	**Reference**
18 month skin painting study	Not carcinogenic No increase in neoplasia after dermal application of the test dye	Limited reporting No data on individual animals	(Carson, 1984)

Relevant studies related to the assessment of carcinogenicity for PO5			
Methods	**Results**	**Remarks**	**Reference**
D&C Orange No. 17 (known trading name of PO5) Purity: 97 % Non-guideline study No GLP Species: mice Strain: 100 ICR n: 50/dose group/sex Dose levels: dermal application to dorsal area; 0.1 ml of 1% solution of dye twice a week for 18 month (mean total dose of applied material 143.7 mg) Route: dermal Vehicle: distilled water Positive control: 3,4-benzpyrene in acetone	compound; single incidences of mammary gland adenosarcoma (2 female/ plus 1 female with metastasis in the lung); **hepatic cell adenoma** (2 male/ 1 male in control) No effect on survival compared to control	Limited number of organs analysed Only selected animals from solvent and positive control Study period 18 month Dermal application twice a week with very low dose Incidences only on a limited number of findings, no body weight information No historical control data Not reliable	
(Hart et al., 1986) (FDA report) and Toxicological evaluation by BG Chemie (BG RCI, 2000): short summaries of studies			
26-30 month dietary study (F0 and F1 dosed) including in utero exposure D&C Orange No. 17 (known trading name of PO5) Purity: 97% Impurities: 0.29% 2,4-dinotrobenzeneamine (dinitroaniline), 0.7% 2-naphthalenol (beta-naphthol) According to FDA guidelines Species: rats Strain: Charles River Albino n: 60/dose group/sex in F0, 70/dose group/sex in F1	**Part I:** Dosage levels too low according to FDA, additional testing with 1% (part II) High-dose females significant increase in tumours of the lymphoreticular system (lymphosarcoma, reticulum cell sarcoma and leukaemia), but none of the individual tumour types were significantly increased No effect on survival and body weight Increased liver weight at high dose males and females Part II: Positive results at 500 mg/kg bw/d Increase **in hepatocellular**	FDA requested study Only accessible as study summaries with limited details, the evaluation of the data is largely dependent on the FDA evaluation Study according to FDA guidelines including in utero treatment and F1 generation Food conversion factor: 20 (for older rats) Calculated doses: Part I: 0, 10, 25 and 50 mg/kg bw/d Part II: 0 and 500 mg/kg bw/d	Unpublished report (Bio/Dynamics, 1982a) Cited in (BG RCI, 2000; FDA, 1986; FDA, 1987; FDA, 1988; Hart et al., 1986)

Relevant studies related to the assessment of carcinogenicity for PO5			
Methods	**Results**	**Remarks**	**Reference**
Dose levels: **Part I:** 0, 0.02, 0.05, 0.1% of the diet **Part II:** 0 and 1% of the diet (additional study with higher concentration) Data of the two studies were combined Experimental design: 60 days feeding period before mating; dietary administration of test substance was continued during mating, gestation, lactation and rearing Pups were weaned from their mothers 21 days after delivery 70 F1 animals selected for long-term study 12 month interim evaluation of 10 animals	**adenomas and carcinomas in treated female rats** (21 in treated group vs. 3 in control); adenomas only (18 in treated group vs. 1 in control) Mammary fibroadenomas increased in multiplicity and total numbers (28/51, 34/59), but not significant Non-neoplastic lesions: treated females with eosinophilic and clear cell foci in the liver Haematology: slight to statistical reduction in HCT, Hb and erythrocyte counts; increased reticulocytes (no further details reported in the study summaries) Deposition of pigment in the spleen Increased liver weight of female rats No effect on survival Body weight of male rats 18% lower; no difference in females		
23-25 month dietary study D&C Orange No. 17 (known trading name of PO5) Purity: 97% Impurities: 0.29% 2,4-dinotrobenzeneamine (dinitroaniline), 0.7% 2-naphthalenol (beta-naphthol) According to FDA guidelines Species: mice	Dose related increase in hepatocellular tumours (adenomas and carcinomas) in males (8/59, 11/58, 13/57, 19/56) Slight effect on survival in treated male mice (dose-related) Haematology: slight statistical reduction in HCT, Hb and erythrocyte counts; increased reticulocytes in high-dose animals Chronic myocarditis in treated animals	FDA requested study Discussion on spontaneous incidences of liver tumours in this strain of mice – FDA concludes on equivocal results Food conversion factor: 7 (for mice) Calculated doses: 0, 36, 357 and 1428 mg/kg bw/d	Unpublished report (Bio/Dynamics, 1982b) Cited in (BG RCI, 2000; FDA, 1986; FDA, 1987; FDA, 1988; Hart et al., 1986)

Relevant studies related to the assessment of carcinogenicity for PO5			
Methods	**Results**	**Remarks**	**Reference**
Strain: CD-1 Charles River n: 60/dose group/sex Dose levels: 0, 0.025, 0.25 and 1% of the diet			
104 week dietary study D&C Orange No. 17 (known trading name of PO5) Species: rats Strain: CD Charles River n: 25/dose group/sex Dose levels: 0.025, 0.1 and 1% of the diet	Neoplastic nodules of the liver and hepatocellular carcinomas increased in high dose females (not significant) Some growth suppression at the higher level Liver weight increased in higher dose females	Food conversion factor: 20 (for older rats) Calculated doses: 0, 12.5, 50 and 500 mg/kg bw/d	(Hazelton Laboratories, 1966) Cited in (BG RCI, 2000; Hart et al., 1986)
2 year dietary study D&C Orange No. 17 (known trading name of PO5) Species: dogsStrain: beagle n: 3/dose group/sex Dose levels: 0.025, 0.125 and 1% of the diet; calculated dose 0, 0.625, 3.125 and 250 mg/kg bw	No significant pathological effects Lower body weight in treated animals	Food conversion factor: 40 (for dogs) Calculated doses: 0, 0.625, 3.125 and 250 mg/kg bw/d	(Hazelton Laboratories, 1964) Cited in (BG RCI, 2000; Hart et al., 1986)
Skin painting study (26 month) D&C Orange No. 17 (known trading name of PO5) Species: mice Strain: CF-1 Carworth n: 50/dose group/sex Dose levels: dermal application onto dorsal skin: 0.1 ml of 1% solution weekly	No carcinogenic effect		(Leberco Laboratories, 1961) Cited in (BG RCI, 2000; Hart et al., 1986)

7.9.7.3.2. Human information

No information available.

7.9.7.3.3. Summary and discussion of carcinogenicity

There was only one study on carcinogenicity of **Pigment Orange 5** identified by the registrants (Carson, 1984): an 18-month skin painting study performed in mice in which 14 colour materials were tested. Dose levels were selected based on lipstick use assessments. An area of about 6 cm^2 was treated twice weekly with 0.1 ml suspension. The mean total dose applied per animal was 143.7 mg D&C Orange No. 17. Complete pathology was performed only on a limited number of animals; in all remaining animals any grossly abnormal organs and tissues were examined. There was no effect on survival compared to control animals and no increase in neoplasia observed after dermal application of D&C Orange No. 17. A summary table shows single incidences of any gross lesions identified, but does not include overall incidence nor is there any information on body weight or clinical observations. This is a non-guideline study which has its limitations in reporting and is performed with a low dose level (based on lipstick use considerations).

Further studies assessed in several FDA reports (FDA, 1986; FDA, 1987; FDA, 1988; Hart et al., 1986) as well as in a toxicological evaluation by (BG RCI, 2000) have not been considered by the registrants. The full study reports were not available to the eMSCA, therefore the following information is taken from the study summaries.

A long term feeding study in rat and mice, requested by the FDA (FDA, 1986; FDA, 1987; FDA, 1988) to assess the carcinogenic potential of D&C Orange 17, was performed as follows: 60 rats/ dose/ sex were treated with 0, 0.02, 0.05, 0.1% substance in the diet (calculated dose: 0, 10, 25 and 50 mg/kg bw/d, based on a general conversion factor of 20 for older rats according to CLP guidance) for 60 days before mating with dietary administration of test substance continued during mating, gestation, lactation and rearing. 70 F1 pups/ dose/sex were selected for the long-term feeding study (dosing for 26-30 month). The FDA requested an additional study performed with higher concentrations (0 and 1% substance in the diet; calculated dose: 0 and 500 mg/kg bw/d) using the same method as dose levels of the first study were judged as too low. At 500 mg/kg bw/day there was a statistically significant increase in hepatocellular adenomas in treated female rats (18 in treated group vs 1 in control) as well as an increase in hepatocellular adenomas and carcinomas together (21 in treated group vs. 3 in control). Eosinophilic and clear cell foci were increased in the liver of female rats and liver weight of female rats was increased. There was no difference in body weight in female rats compared to controls.

60 mice/ dose/ sex were exposed to 0, 0.025, 0.25 and 1% of substance in the diet (calculated dose: 0, 36, 357 and 1428 mg/kg bw/d) for 23-25 month. There was a slight dose-related effect on survival in treated male mice and a dose-related increase in hepatocellular tumours (adenomas and carcinomas) (8/59, 11/58, 13/57, 19/56). The FDA concluded on an equivocal result as apparently there was some discussion on the number of spontaneous incidences of liver tumours in this strain of mice.

Further studies were discussed in these reports: two long-term dietary studies in rats and dogs and one skin painting study in mice. 25 CD Charles River rats/ dose/ sex were exposed to 0.025, 0.1 and 1% of substance in the diet (calculated doses: 0, 12.5, 50 and 500 mg/kg bw/d) for 104 weeks. Neoplastic nodules of the liver and hepatocellular carcinomas were increased in high-dose females, however, not statistically significant. A 2-year dietary study in 3 beagle dogs/ dose/ sex which were treated with 0.025, 0.125 and 1% of substance in the diet (calculated dose 0, 0.625, 3.125 and 250 mg/kg bw) showed no significant pathological effects. 50 CF-1 Carworth mice were treated with 0.1 ml of a 1% solution weekly onto the dorsal skin for 26 month. No carcinogenic effect was found.

In the report by (Hart et al., 1986) the following was concluded on the carcinogenicity of Pigment Orange 5: according to the studies requested by the FDA there is an increase in hepatocellular adenomas in treated female rats at 500 mg/kg bw/day. However, there is a lack of information on the level of impurities of the substance used in this study. In addition, there is a dose related increase in hepatocellular tumours in male mice, but the

FDA concluded that this was an equivocal result due to a discussion concerning the number of spontaneous incidences of liver tumours in this strain of mice.

7.9.7.3.4. Conclusion

Overall, the eMSCA agrees with the conclusions of Hart et al. (Hart et al., 1986), that there is limited evidence for carcinogenicity of PO5 in female rats. A feeding study showed an increase in hepatocellular adenomas in female rats at 500 mg/kg bw/d but no evidence in male rats (FDA). Feeding studies in mice, rats and dogs up to 500 mg/kg bw/d showed equivocal results, whereas no carcinogenic effects were found in skin painting studies in mice.

The full study reports, especially from the FDA studies, were not available to the eMSCA, but more detailed information would be needed to conclude on the carcinogenic potential of PO5, especially on data from individual animals regarding tumour incidences, data on body weight and survival as well as purity of the substance.

In conclusion, once additional data on genotoxicity becomes available (which have been requested by ECHA under CCH), the data on carcinogenicity should be re-evaluated to clarify if a CLH proposal should be considered. The registrants should consider all available data in their dossier.

7.9.8. Toxicity to reproduction (effects on fertility and developmental toxicity)

7.9.8.1. PR3

The only study available on reproduction toxicity with PR3 is a recent and mostly compliant with OECD TG 421 screening study (Harlan, 2013a). The study in rats by oral gavage of PR3 did not show adverse effects on the examined parameters, only discoloured faeces were noted. Other effects occurred are either not dose dependent (reduced pup weights only at low dose), or of low incidence and severity (single sperm resorptions, testes and epididymides size reduction or enlargement at high dose). Mortalities during the study were sufficiently explained and occurred during the pre-pairing period.

Table 44

Relevant studies related to the assessment of the endpoint reproduction toxicity for PR3			
Methods	**Results**	**Remarks**	**Reference**
Screening study in rats PR3 Purity 98.9 % OECD TG 421 Species: Rat Strain: RccHan: WIST(SPF) n=12 per sex and dose group Age: 11 weeks Dosing: 0, 100, 300, 1000 mg/kg bw/d Route: oral gavage (in 5ml/kg bw/d) Pre-pairing: 14d Pairing: Max. 14d Gestation. Approx. 21 d Ends: day 3 post partum (f)/day before sacrifice (m) Necropsy: day 4 pos partum (f), min. 28 d treatment (m)	Mortality: 5 (4 due to aspiration of test item, 1 unknown – no necroscopy; 0 in control, 1 at low, 1 at medium, 3 at high dose, all during pre-paring period between day 3 and 11) Clinical signs: Reddish discoloured faeces, no other test item related clinical signs Slightly reduced food consumption at mid and high dose versus control. There were only single cases (most in control) with isolated tubules that showed minor changes. Single sperm resorptions in stage VIII, IX or X tubules, some cases of single sperm retentions in a stage IX tubule, and in one case, a single tubule was completely degenerated. Testes, both sides, high dose: 1 reduced in size, 2 enlarged. Epididymides, high dose: 1 reduced in size Seminal vesicles, low dose: 1 animal with foci, 2 mm, dark red Ovaries discoloured (1 in control, 1 in mid dose) Significantly reduced pup weights (only in low dose, not dose dependent)	NOAEL: 1000 mg/kg bw/day LOAEL: NA (no adverse effect) Reliability: with restrictions shorter observation period: 4 days post-partum (vs. 13 days lactation in TG 421) No clinical biochemistry, hormone levels (but study before TG update 2016)	(Harlan, 2013a)

7.9.8.1.1. Summary

From the screening study with PR3, no adverse effects on examined parameters were identified. The study is reliable and does not raise additional concerns on reproduction toxicity.

7.9.8.1.2. Conclusion

No effects were identified that would justify classification.

The available data are considered as appropriate for an evaluation of reproduction toxicity and no further study is necessary from the point of view of the eMSCA.

7.9.8.2. PR4

7.9.8.2.1. Summary

No studies on reproduction toxicity with PR4 were identified by the eMSCA. As studies on reproduction toxicity are required standard information under REACH, a combined 28d repeated dose / screening study (OECD TG 422) has been requested by ECHA on 29 March 2019 under compliance check. The results are expected to arrive by 6 October 2020.

7.9.8.2.2. Conclusion

No conclusion possible yet as there is an ECHA decision requesting the generation of new information for reproduction toxicity.

7.9.8.3. PO5

7.9.8.3.1. Summary

No studies on reproduction toxicity with PO5 were identified by the eMSCA and there is a data gap in required standard information under REACH on reproduction toxicity.

7.9.8.3.2. Conclusion

No conclusion possible yet as there is an ECHA decision requesting the generation of new information for reproduction toxicity.

7.9.9. Hazard assessment of physico-chemical properties

Not assessed in this substance evaluation.

7.9.10. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

According to Section R.8.4 of the REACH Guidance in Information Requirements and Chemical Safety Assessment (ECHA, 2012), a DNEL for the leading health effect needs to be derived for every relevant human population and every relevant route, duration and frequency of exposure, if feasible. The registrants have calculated DNELs which are intended to protect both workers and general population from long-term systemic effects caused during inhalation to PO5.

7.9.10.1. PR3

For PR3, the eMSCA considers the available data as sufficient for the identification of dose descriptors. At sub-acute or sub-chronic study durations, signs of haemolytic anaemia are present in rats and to a lesser extend in mice, presumably secondary effects in liver, spleen and kidney match the toxicological concern. The lowest dose descriptor identified stems from a 2-year carcinogenicity study in rats (NTP, 1992a), where degenerative alterations in liver and increased chronic nephropathy were found in all tested dose groups, accordingly a LOAEL of 100 mg/kg bw/d should be used as starting point for DNEL calculation.

Table 45: PR3

Critical DNELs/DMELs				
Endpoint of concern	**Type of effect**	**Critical study(ies)**	**Corrected dose descriptor(s) (e.g. NOAEL, NOAEC)**	**Justification/ Remarks**
Repeated dose toxicity	Blood (Hb reduction by more than 20 %)	14-day, feeding study, rats, PR3 (NTP, 1992a) Calculated doses: 600, 1 250, 2 500, 5 000 and 10 000 mg/kg bw/d	NOAEL 5 000 mg/kg bw/d	
Repeated dose toxicity	liver weight rel./abs increased >20 % compared to control in males, less in females >38 fold increased bilirubin; secondary signs of haemolytic anaemia in kidney, spleen and liver	13-week, feeding study, rats, PR3 (NTP, 1992a) Calculated doses: 150, 300, 625, 1 250, 2 500 mg/kg bw/d	NOAEL 300 mg/kg bw/d	Food conversion: factor 20 for older rats

Critical DNELs/DMELs				
Endpoint of concern	**Type of effect**	**Critical study(ies)**	**Corrected dose descriptor(s) (e.g. NOAEL, NOAEC)**	**Justification/ Remarks**
Repeated dose toxicity	Degenerative alterations in liver and increased chronic nephropathy	**2-year** feeding study, rat, PR3 (NTP, 1992a) Calculated doses: 0, 300, 625, 1250 mg/kg bw/d	LOAEL 300 mg/kg bw/d	
Carcinogenicity	Benign adrenal pheocromocytomas in male rats	**2-year** feeding study, rat, PR3 (NTP, 1992a) Calculated doses: 0, 300, 625, 1250 mg/kg bw/d	NOAEL 300 mg/kg bw/d	

7.9.10.1.1. DNEL calculation for workers

Not included in the assessment of PR3.

7.9.10.1.2. DNEL calculation for Consumers / General population

For consumers, oral, dermal and inhalation routes of exposure are relevant. Repeated dose oral exposure of animals to PO5 indicates adverse effects on the blood system, i.e. reduced haemoglobin and haemolytic anaemia. This indicates systemic effects after long-term exposure for human health.

A LOAEL of 300 mg/kg bw/d based on degenerative alterations in liver and increased chronic nephropathy were observed in rats from a chronic feeding study in rats. For the DNEL calculation the eMSCA follows the specifications given in the REACH guidance chapter R.8 (ECHA, 2012a). A detailed overview of the derivation of the DNELs as conducted by the eMSCA is presented below.

Table 46

Detailed overview of the derivation of the DNEL consumer, oral, long-term, systemic effects for PR3 conducted by the eMSCA		
Description / Assessment factors (AF)	**Value**	**Remarks**
Dose descriptor	LOAEL = 300 mg/kg bw/d	This LOAEL results from a chronic (2 years) feeding toxicity study in rats (NTP, 1992a). At the lowest tested dose of 100 mg/kg bw/d, degenerative alterations in liver and increased chronic were observed.
Modified dose descriptor	LOAEL = 300 mg/kg bw/d	No modifications applied
AF for allometric scaling	4	A default AF is applied according to the REACH guidance R.8.
AF for residual interspecies differences	2.5	A default AF is applied according to the REACH guidance R.8.

AF for intraspecies differences	10	A default AF for general population is applied according to the REACH guidance R.8.because no substance-specific information is available for an adjustment.
AF for differences in exposure duration		Not applied, chronic exposure in animal study.
AF related to dose response relationship	3	A default AF for dose response relationship is applied according to the REACH guidance R.8 to extrapolate the LOAEL to an NOAEL.
AF related to quality of database		Not applied, data quality of animal study is sufficient.
Overall AFs	300	Product of all AFs
DNEL consumer, oral, long-term, systemic effects	1.000 mg/kg bw/d (~ 1 mg/kg bw/d)	

Table 47

Detailed overview of the derivation of the DNEL consumer, inhalation, long-term, systemic effects for PR3 conducted by the eMSCA		
Description / Assessment factors (AF)	**Value**	**Remarks**
Dose descriptor	LOAEL = 300 mg/kg bw/d	This LOAEL results from a chronic (2 years) feeding toxicity study in rats (NTP, 1992a). At the lowest tested dose of 100 mg/kg bw/d, degenerative alterations in liver and increased chronic were observed.
Modification of the starting point	LOAEL*100%/50% 1.15 m^3/kg bw/d → 1.7	The starting point was modified due to differences between respiratory volumes of rats and humans according to the REACH guidance R.8. The absorption rate for oral exposure in rat was set to 50% and after inhalation in humans 100%.
Modified dose descriptor	LOAEL = 521.7 mg/m^3	No modifications applied
AF for allometric scaling		Not applied, included in the modification of the starting point
AF for residual interspecies differences	2.5	A default AF is applied according to the REACH guidance R.8.
AF for intraspecies differences	10	A default AF for general population is applied according to the REACH guidance R.8.because no substance-specific information is available for an adjustment.
AF for differences in exposure duration		Not applied, chronic exposure in animal study.
AF related to dose response relationship	3	A default AF for dose response relationship is applied according to the REACH guidance R.8 to extrapolate the LOAEL to an NOAEL.
AF related to quality of database		Not applied, data quality of animal study is sufficient.
Overall AFs	75	Product of all AFs
DNEL consumer, inhalation, long-term, systemic effects	6.957 mg/m^3 (~7 mg/m^3)	

Table 48

Detailed overview of the derivation of the DNEL consumer, dermal, long-term, systemic effects for PR3 conducted by the eMSCA		
Description / Assessment factors (AF)	**Value**	**Remarks**
Dose descriptor	LOAEL = 300 mg/kg bw/d	This LOEAL results from a chronic (2 years) feeding toxicity study in rats (NTP, 1992a). At the lowest tested dose of 100 mg/kg bw/d, degenerative alterations in liver and increased chronic were observed.
Modified dose descriptor	LOAEL = 300 mg/kg bw/d	No modifications applied, the same absorption for dermal and oral exposure is assumed.
AF for allometric scaling	4	A default AF is applied according to the REACH guidance R.8.
AF for residual interspecies differences	2.5	A default AF is applied according to the REACH guidance R.8.
AF for intraspecies differences	10	A default AF for general population is applied according to the REACH guidance R.8.because no substance-specific information is available for an adjustment.
AF for differences in exposure duration		Not applied, chronic exposure in animal study.
AF related to dose response relationship	3	A default AF for dose response relationship is applied according to the REACH guidance R.8 to extrapolate the LOAEL to an NOAEL.
AF related to quality of database		Not applied, data quality of animal study is sufficient.
Overall AFs	300	Product of all AFs
DNEL consumer, oral, long-term, systemic effects	1.000 mg/kg bw/d (~ 1 mg/kg bw/d)	

7.9.10.2. PR4

ECHA has requested a combined 28-day / screening study (expected in autumn 2020). No valid studies are available covering repeated dose toxicity and reproduction toxicity. The only dose descriptor identified is based on "some changes in kidney and spleen" without further description available at lowest dose in a 90-day feeding study in rats. The LOAEL is accordingly 500 mg/kg bw/d and is used as a starting point for DNEL calculation.

Table 49: PR4

Critical DNELs/DMELs				
Endpoint of concern	**Type of effect**	**Critical study(ies)**	**Corrected dose descriptor(s) (e.g. NOAEL, NOAEC)**	**Justification / Remarks**
Repeated dose toxicity	"some changes in kidney and spleen" (no further	90 day, feeding, rats (Gewerbe- und Arzneimitteltoxikologie, 1962)	LOAEL 500 mg/kg bw/d	Study not reliable, CCH ongoing, results from 28-day study expected in autumn 2020

	description available)	Calculated doses: 500, 1 000 mg/kg bw/d		

7.9.10.2.1. DNEL calculation for workers

Not included in the assessment of PR4.

7.9.10.2.2. DNEL calculation for Consumers / General population

For consumers, oral, dermal and inhalative routes of exposure are relevant. Repeated dose oral exposure of animals to PR4 indicates adverse effects, but which are only vaguely described as "some changes in kidney and spleen". Nevertheless, this indicates systemic effects after long-term exposure also for human health.

For the DNEL calculation the eMSCA follows the specifications given in the REACH guidance chapter R.8 (ECHA, 2012a). A detailed overview of the derivation of the DNELs as conducted by the eMSCA is presented below.

Table 50:

Detailed overview of the derivation of the DNEL$_{consumer, oral, long-term, systemic effects}$ for PR4 conducted by the eMSCA		
Description / Assessment factors (AF)	**Value**	**Remarks**
Dose descriptor	LOAEL = 500 mg/kg bw/d	This LOAEL results from a sub-chronic (90 days) feeding study in rats (Gewerbe- und Arzneimitteltoxikologie, 1962). At the lowest dose of 500 mg/kg bw/d tested, "some changes in kidney and spleen" were reported.
Modified dose descriptor	LOAEL = 500 mg/kg bw/d	No modifications applied
AF for allometric scaling	4	A default AF is applied according to the REACH guidance R.8.
AF for residual interspecies differences	2.5	A default AF is applied according to the REACH guidance R.8.
AF for intraspecies differences	10	A default AF for general population is applied according to the REACH guidance R.8.because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	2	This AF was applied according to the REACH guidance R.8 to extrapolate the duration from sub-chronic to chronic.
AF related to dose response relationship	3	A default AF for dose response relationship is applied according to the REACH guidance R.8 to extrapolate the LOAEL to an NOAEL.
AF related to quality of database	2	A default AF for the database quality is applied according to the REACH guidance R.8 to be applied to compensate for the potential remaining uncertainties based on data of poor quality.
Overall AFs	1200	Product of all AFs
DNEL$_{consumer, oral, long-term, systemic effects}$	0.417 mg/kg bw/d	

Table 51

Detailed overview of the derivation of the DNEL consumer, inhalation, long-term, systemic effects for PR4 conducted by the eMSCA		
Description / Assessment factors (AF)	**Value**	**Remarks**
Dose descriptor	LOAEL = 500 mg/kg bw/d	This LOAEL results from a sub-chronic (90 days) feeding study in rats (Gewerbe- und Arzneimitteltoxikologie, 1962). At the lowest dose of 500 mg/kg bw/d tested, "some changes in kidney and spleen" were reported.
Modification of the starting point	LOAEL*100%/50% 1.15 m^3/kg bw/d → 1.7	The starting point was modified due to differences between respiratory volumes of rats and humans according to the REACH guidance R.8. The absorption rate for oral exposure in rat was set to 50% and after inhalation in humans 100%.
Modified dose descriptor	LOAEL = 869.6 mg/m^3	No modifications applied
AF for allometric scaling		Not applied, included in the modification of the starting point
AF for residual interspecies differences	2.5	A default AF is applied according to the REACH guidance R.8.
AF for intraspecies differences	10	A default AF for general population is applied according to the REACH guidance R.8.because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	2	This AF was applied according to the REACH guidance R.8 to extrapolate the duration from sub-chronic to chronic.
AF related to dose response relationship	3	A default AF for dose response relationship is applied according to the REACH guidance R.8 to extrapolate the LOAEL to an NOAEL.
AF related to quality of database	2	A default AF for the database quality is applied according to the REACH guidance R.8 to be applied to compensate for the potential remaining uncertainties based on data of poor quality.
Overall AFs	300	Product of all AFs
DNEL consumer, inhalation, long-term, systemic effects	2.899 mg/m^3	

Table 52

Detailed overview of the derivation of the DNEL consumer, dermal, long-term, systemic effects for PR4 conducted by the eMSCA		
Description / Assessment factors (AF)	**Value**	**Remarks**
Dose descriptor	LOAEL = 500 mg/kg bw/d	This LOAEL results from a sub-chronic (90 days) feeding study in rats (Gewerbe- und Arzneimitteltoxikologie, 1962). At the lowest dose of 500 mg/kg bw/d tested, "some changes in kidney and spleen" were reported.
Modified dose descriptor	LOAEL = 500 mg/kg bw/d	No modifications applied
AF for allometric scaling	4	A default AF is applied according to the REACH guidance R.8.
AF for residual interspecies differences	2.5	A default AF is applied according to the REACH guidance R.8.
AF for intraspecies	10	A default AF for general population is applied according to the REACH guidance R.8.because no substance-specific

differences		information is available for an adjustment.
AF for differences in exposure duration	2	This AF was applied according to the REACH guidance R.8 to extrapolate the duration from sub-chronic to chronic.
AF related to dose response relationship	3	A default AF for dose response relationship is applied according to the REACH guidance R.8 to extrapolate the LOAEL to an NOAEL.
AF related to quality of database	2	A default AF for the database quality is applied according to the REACH guidance R.8 to be applied to compensate for the potential remaining uncertainties based on data of poor quality.
Overall AFs	1200	Product of all AFs
$DNEL_{consumer, oral, long-term, systemic effects}$	0.417 mg/kg bw/d	

7.9.10.3. PO5

For PO5, increased liver weights from a carcinogenicity study in rats from 50 mg/kg bw/d and liver adenoma and carcinoma are reported in female rats at 500 mg/kg bw/d, and reduction of haemoglobin concentration in all dose groups (100 mg/kg bw/d or more) in a 32 d study (no details on effect levels available to eMSCA) are considered as adverse effects. It should be noted that the eMSCA considers the available repeated dose studies as not adequate to cover the information requirements for repeated dose toxicity, especially regarding the extend of primary and secondary effects of a presumed haemolytic anaemia. The NOAEL should therefore be considered as preliminary until further study data are made available. A 90-day study will be requested during SEv and might deliver potentially different dose descriptors.

For calculation of DNELs by the eMSCA the dose-descriptors are gathered from the available and relevant experimental animal studies summarised in Table 53.

Table 53

Overview of typical dose descriptors for all endpoints for PO5				
Endpoint	**Type of effect**	**Relevant Study**	**Dose descriptor**	**Remarks**
Repeated dose toxicity	Effects on blood parameters: Blood (Hb reduction, haemolytic anaemia)	32-d feeding study in rats, oral (Hoechst, 1973a)	LOAEL: 100 mg/kg bw	Study overall not reliable. Only summary available. Effects in all dose groups, adversity not assessable: preliminary LOAEL
Repeated dose toxicity	Liver weight increase, females	23-25 month feeding study in rats, incl. in utero exposure with PO5 (as summarised in (BG RCI, 2000; Hart et al., 1986) Calculated doses: 0, 10, 25 and 50 mg/kg bw/d and 500 mg/kg bw/d	NOAEL 25 mg/kg bw/d	Only summary available.

Overview of typical dose descriptors for all endpoints for PO5				
Endpoint	**Type of effect**	**Relevant Study**	**Dose descriptor**	**Remarks**
Carcinogenicity	Hepatocellular adenoma and carcinoma in female rats	23-25 month feeding study in rats, incl. in utero exposure with PO5 (BG RCI, 2000; Hart et al., 1986) Calculated doses: 0, 10, 25 and 50 mg/kg bw/d and 500 mg/kg bw/d	NOAEL 50 mg/kg bw/d	Only summary available.

7.9.10.3.1. DNEL calculation for workers

At the workplace exposure to PO5 occurs or may occur by inhalation. Therefore, a DNEL has to be derived for this route of exposure. For the DNEL calculation the eMSCA follows the specifications given in the REACH guidance chapter R.8 (ECHA, 2012a).

Data from animal experiments, where PO5 was administered orally, indicate that exposure to the substance may elicit systemic adverse effects to the human health. Especially affected is the blood, more specifically a reduction in haemoglobin and haemolytic anaemia were observed. A detailed overview of the derivation of the $DNEL_{worker,\ inhalation,\ long\text{-}term,\ systemic\ effects}$ as conducted by the eMSCA is presented in Table 54 and Table 55.

Table 54

Detailed overview of the derivation of the $DNEL_{worker,\ inhalation,\ long\text{-}term,\ systemic\ effects}$ for PO5 conducted by the eMSCA		
Description (AF=Assessment factor)	**Value**	**Remark**
Relevant dose descriptor	LOAEL = 100 mg/kg bw/d	This LOAEL results from a sub-acute (32 days) feeding toxicity study in rats (Hoechst, 1973). At the dose of 100 mg/kg bw/d a reduced haemoglobin and haemolytic anaemia were observed.
Modification of the starting point	(0.38 m^3/kg/8 h) *(6.7 m^3/10 m^3) *(7 d/5 d) *(50%/100%) ↓	Due to different exposure conditions in the animal experiment and at the workplace of humans both time scaling and a modification due to different respiratory volumes have to be applied according to the REACH guidance R.8. The absorption rate for oral exposure in rat was set to 50% and after inhalation in humans 100%.
Modified dose-descriptor	123.4 mg/m^3	
Overall AFs	450	
AF for interspecies differences	2.5	A default AF for remaining differences is applied according to the REACH guidance R.8.
AF for intraspecies differences	5	The default factor for workers is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.

AF for differences in exposure duration	6	This AF was applied according to the REACH guidance R.8 to extrapolate the duration from sub-acute to chronic.
AF related to dose response relationship	3	A default AF for dose response relationship is applied according to the REACH guidance R.8 to extrapolate the LOAEL to an NOAEL.
AF related to quality of database	2	A default AF for the database quality is applied according to the REACH guidance R.8 to be applied to compensate for the potential remaining uncertainties based on data of poor quality.
$DNEL_{worker, inhalation, long-term, systemic effects}$	0.274 mg/m^3	

Table 55

Hazard conclusion for workers - Critical DNEL for PO5					
Route	**Type of effect**	**Corrected dose descriptor**	**DNEL**	**Endpoint of concern**	**Critical study**
Inhalation	Systemic effects, long-term Effects on blood (reduction in haemoglobin and haemolytic anaemia in rats)	LOAEL = 123.4 mg/m^3	0.274 mg/m^3	Repeated dose toxicity (by inhalation)	Hoechst, 1973

7.9.10.3.2. DNEL calculation for Consumers / General population

For consumers, oral, dermal and inhalation routes of exposure are relevant. Repeated dose oral exposure of animals to PO5 indicates adverse effects on the blood system, i.e. reduced haemoglobin and haemolytic anaemia. This indicates systemic effects after long-term exposure also for human health.

A NOAEL of 25 mg/kg bw/d based on liver weight increase in female rats from a chronic feeding study in rats would lead to higher DNEL values (calculation not shown). Data from the same study show an increase in hepatocellular adenoma and carcinoma in female rats only at the highest dose. If a threshold mode of action is assumed, the DNEL would be higher than from the sub-acute study (calculation not shown).

Currently, the assumed mode of action for carcinogenicity is based on secondary effects of a haemolytic anaemia. Therefore, the non-threshold DMEL was not calculated. Results from requested studies during SEv (in vivo mutagenicity and repeated does studies) might change this assessment and require re-calculation of DNEL values. For the DNEL calculation the eMSCA follows the specifications given in the REACH guidance chapter R.8 (ECHA, 2012a). A detailed overview of the derivation of the DNELs as conducted by the eMSCA is presented below.

Table 56

Detailed overview of the derivation of the DNEL consumer, oral, long-term, systemic effects for PO5 conducted by the eMSCA		
Description / Assessment factors (AF)	**Value**	**Remarks**
Dose descriptor	LOAEL = 100 mg/kg bw/d	This LOAEL results from a sub-acute (32 days) feeding toxicity study in rats (Hoechst, 1973). At the dose of 100 mg/kg bw/d reduced haemoglobin and haemolytic anaemia were observed.
Modified dose descriptor	LOAEL = 100 mg/kg bw/d	No modifications applied
AF for allometric scaling	4	A default AF is applied according to the REACH guidance R.8.
AF for residual interspecies differences	2.5	A default AF is applied according to the REACH guidance R.8.
AF for intraspecies differences	10	A default AF for general population is applied according to the REACH guidance R.8.because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	6	This AF was applied according to the REACH guidance R.8 to extrapolate the duration from sub-acute to chronic.
AF related to dose response relationship	3	A default AF for dose response relationship is applied according to the REACH guidance R.8 to extrapolate the LOAEL to an NOAEL.
AF related to quality of database	2	A default AF for the database quality is applied according to the REACH guidance R.8 to be applied to compensate for the potential remaining uncertainties based on data of poor quality.
Overall AFs	3600	Product of all AFs
DNEL consumer, oral, long-term, systemic effects	0.028 mg/kg bw/d	

Table 57

Detailed overview of the derivation of the DNEL consumer, inhalation, long-term, systemic effects for PO5 conducted by the eMSCA		
Description / Assessment factors (AF)	**Value**	**Remarks**
Dose descriptor	LOAEL = 100 mg/kg bw/d	This LOAEL results from a sub-acute (32 days) feeding toxicity study in rats (Hoechst, 1973). At the dose of 100 mg/kg bw/d reduced haemoglobin and haemolytic anaemia were observed.
Modification of the starting point	LOAEL*100%/50% 1.15 m^3/kg bw/d → 1.7	The starting point was modified due to differences between respiratory volumes of rats and humans according to the REACH guidance R.8. The absorption rate for oral exposure in rat was set to 50% and after inhalation in humans 100%.
Modified dose descriptor	LOAEL = 173.9 mg/m^3	No modifications applied
AF for allometric scaling		Not applied, included in the modification of the starting point
AF for residual interspecies differences	2.5	A default AF is applied according to the REACH guidance R.8.
AF for intraspecies differences	10	A default AF for general population is applied according to the REACH guidance R.8.because no substance-specific information is available for an adjustment.
AF for differences	6	This AF was applied according to the REACH guidance

in exposure duration		R.8 to extrapolate the duration from sub-acute to chronic.
AF related to dose response relationship	3	A default AF for dose response relationship is applied according to the REACH guidance R.8 to extrapolate the LOAEL to an NOAEL.
AF related to quality of database	2	A default AF for the database quality is applied according to the REACH guidance R.8 to be applied to compensate for the potential remaining uncertainties based on data of poor quality.
Overall AFs	900	Product of all AFs
$DNEL_{consumer, inhalation, long-term, systemic effects}$	0.193 mg/m^3	

Table 58

Detailed overview of the derivation of the $DNEL_{consumer, dermal, long-term, systemic effects}$ for PO5 conducted by the eMSCA		
Description / Assessment factors (AF)	**Value**	**Remarks**
Dose descriptor	LOAEL = 100 mg/kg bw/d	This LOAEL results from a sub-acute (32 days) feeding toxicity study in rats (Hoechst, 1973). At the dose of 100 mg/kg bw/d reduced haemoglobin and haemolytic anaemia were observed.
Modified dose descriptor	LOAEL = 100 mg/kg bw/d	No modifications applied, the same absorption for dermal and oral exposure is assumed.
AF for allometric scaling	4	A default AF is applied according to the REACH guidance R.8.
AF for residual interspecies differences	2.5	A default AF is applied according to the REACH guidance R.8.
AF for intraspecies differences	10	A default AF for general population is applied according to the REACH guidance R.8.because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	6	This AF was applied according to the REACH guidance R.8 to extrapolate the duration from sub-acute to chronic.
AF related to dose response relationship	3	A default AF for dose response relationship is applied according to the REACH guidance R.8 to extrapolate the LOAEL to an NOAEL.
AF related to quality of database	2	A default AF for the database quality is applied according to the REACH guidance R.8 to be applied to compensate for the potential remaining uncertainties based on data of poor quality.
Overall AFs	3600	Product of all AFs
$DNEL_{consumer, dermal, long-term, systemic effects}$	0.028 mg/kg bw/d	

7.9.11. Conclusions of the human health hazard assessment and related classification and labelling

The initial human health concern were suspected C/M/R properties of PR3, PR4 and PO5. Additionally, a concern for STOT-RE (blood) has been identified during SEv.

Data gaps in standard information required under REACH do not allow conclusion on all of the addressed toxicological endpoints. Summaries of the outcomes/conclusions are listed in Table 3, Table 4 and Table 5, respectively.

To fill the existing data gaps, required studies are requested by ECHA under dossier evaluation. Whether the substance evaluation needs to be continued depends on the outcome of the requested studies.

Carcinogenicity

For PR3, PR4 and PO5, there is limited evidence on carcinogenicity in animals; but which is not sufficient for classification. Further action is not necessary at this point of time. However, once additional data on genotoxicity becomes available for PR3 and PO5, and which would potentially establish a genotoxic mode of action, the data on carcinogenicity should be re-evaluated to clarify, if a CLH proposal needs to be considered.

Mutagenicity

The dossiers for PR3, PR4 and PO5, respectively, contain positive results for *in vitro* gene mutation studies in bacteria which indicate a potential to induce gene mutations. The available *in vivo* data are not adequate to clarify this concern for all three substances. Therefore a conclusion on mutagenicity cannot be drawn before appropriate *in vivo* follow-up mutagenicity studies are available, which are requested by ECHA under dossier evaluation.

In addition, there are data gaps on *in vitro* cytogenicity for PR3 and PO5, therefore, no conclusion can be drawn on cytogenicity and appropriate studies are requested by ECHA under dossier evaluation.

Toxicity to reproduction

For PR3, available data show, that the concern for reproduction toxicity was not substantiated, no further action is necessary. For PR4 and PO5, studies have been or will be requested under compliance check.

Repeated dose toxicity

PR3 induces haemolytic anaemia, which presumably leads to secondary lesions in liver, kidney and spleen in rats and mice. But the effects are outside the severity which would allow classification according to CLP for specific target organ toxicity after repeated exposure (STOT-RE). For PR4 and PO5, studies have been or will be requested under compliance check.

Other endpoints

Available data on other human health related endpoints did not raise further concern or require further action.

7.10. Assessment of endocrine disrupting (ED) properties

Not assessed in this evaluation.

7.11. PBT and VPVB assessment

7.11.1. Persistence

7.11.1.1. Abiotic degradation

A fast hydrolysis, which could relieve a substance from P suspicion, is considered chemically improbable for the three substances. No data indicating the opposite are available.

7.11.1.2. Biotic degradation

Data exist for PR3 proving it to be not readily biodegradable (OECD 301 C). Thus, PR3 is considered to fulfil the screening P criterion as defined in the relevant guidance[17]. No biodegradation tests are available for Pigment Red 4 and Pigment Orange 5.

This accords with EPISUITE models predicting no ready biodegradation (BIOWIN 1, BIOWIN 2, BIOWIN 5 and BIOWIN 6). BIOWIN 3 predicts an ultimate biodegradation timeframe of months (value < 2.25) and BIOWIN 4 predicts a primary biodegradation timeframe of weeks. The substances are in the molecular weight range of the models and their structural fragments are represented in the training data set.

In summary, the substances fulfil the BIOWIN based screening criterion for persistence as defined in the relevant guidance. This finding is in agreement with the registrant's conclusion on ready biodegradability.

Hence, further information on degradation is necessary to prove whether or not is the substances are P and whether or not there are relevant transformation/ degradation products.

In summary, PR3, PR4 and PO5 are considered as potentially persistent based on the available screening data. The identification of relevant degradation and/or transformation products is required to check whether these might fulfil the PBT/vPvB screening criteria. In addition, in case the substances meet the screening B/vB criteria, further information on their degradation half-lives is required to conclude on P/vP. The necessary information should be generated as standard information requirement. Therefore, degradation will not be addressed under this process but under compliance check instead.

7.11.2. Bioaccumulation

A non-valid study on bioaccumulation of PR3 in fish used a test concentration above water solubility. Thus no reliable conclusion on the B properties of PR3 is possible.

All three substances are poorly soluble in both octanol and water. Log KOW values calculated based on the single solubilities in water and octanol and those based on QSAR generally differ distinctively from another. Each of the log KOW QSAR and single solubilities calculations show deficiencies restricting their validity. Thus it is presently not possible to verify if these substances meet the B/vB screening criteria and reliable information on octanol water partition is necessary.

[17] ECHA 2017. Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.11: PBT/vPvB assessment. Version 3.0, p. 49. https://echa.europa.eu/documents/10162/13632/information_requirements_r11_en.pdf/a8cce23f-a65a-46d2-ac68-92fee1f9e54f (accessed 26 September 2019)

Depending on the resulting log K_{OW} and the outcome of the P assessment a test on bioaccumulation in fish according to OECD 305 might be necessary.

However, as such testing is covered by the standard testing regime of REACH, it will not be required under substance evaluation but under compliance check.

7.11.3. Toxicity

In the short-term toxicity tests for PR3 no effects occurred up to limit of water solubility (with the test duration of the fish acute toxicity test being shorter than requested by OECD 203). A long-term toxicity test is available for algae and aquatic invertebrates (*Daphnia magna*) where no effects occurred up to the maximal water solubility concentration. No information is available on the long-term toxicity to fish.

No short-term nor long-term toxicity tests to fish, daphnia or algae are available for PR4.

Only a short-term toxicity test to aquatic invertebrates (*Daphnia magna*) is available for PO5 which did not show any effect up to the limit of water solubility of the substance. As the substance is poorly water soluble, long-term toxicity tests would be necessary to assess the toxicity of the substance to aquatic organisms. These tests are not available.

On the data basis available it is not possible to conclude on the fulfilment of the T-criterion based on aquatic toxicity. Further on-toxicity to aquatic organisms have been requested for all three substances in previous compliance checks.

However, as such testing is covered by the standard testing regime of REACH, it will not be required under substance evaluation but under compliance check.

7.12. Exposure assessment

7.12.1. Human health

An exposure assessment with regard to human health was performed for PO5 as the only one of the three substances during evaluation.

7.12.1.1. PO5

7.12.1.1.1. Worker

Overview of uses and postulated exposure scenarios

PO5 is a commercial synthetic organic monoazo pigment. This dyestuff is synthesized by coupling of diazotized 2,4-dinitroaniline to β-naphthol in a batch process (Hunger and Herbst, 2000) potentially leading to inhalation and dermal exposure during transfer and cleaning operations.

The most important and established use is the imparting of colour to a variety of materials and compositions, e.g. surface coatings for exteriors, interiors of automobiles, oil- or water-based paints for houses, wood stains, leather finishes, distempers, printing inks, inks for metal plates, textile printing inks (Kirk, 1996).

Manufacture, production and uses of the concerned pigments PR3, PR4 and PO5 are similar (Hunger and Herbst, 2000).

Based on the information provided in the chemical safety report of the lead registrant the following Table 59 gives a short description of all identified uses with their use descriptors and life cycle stages.

Table 59

Overview on Uses and PROCs provided in the updated CSR of the lead registrant (chapter 2)									
Identifiers	Short description of the identified use	Resulting life cycle stage						Sector of use (SU)	Process Category (PROC)
		Manufacture	Formulation	End use			Service life (for articles)		
				Industrial	Professional	Consumer			
M-1	Industrial manufacture of pigments or pigment additives	X		X					4, 8B
F-2	Industrial formulation of non-solid preparations containing pigment (including inks and paints)		X	X					5, 8B, 9, 14, 15
F-4	Industrial formulation of solid preparations containing pigment (including plastics)		X	X					5, 8B, 14, 15, 24
IW-8	Industrial use of pigment preparations resulting in inclusion into a matrix (including ink and paint)			X					5, 6, 7, 8A, 10, 13, 14, 21
IW-10	Industrial use of pigment preparations resulting in inclusion into a matrix (including plastic)			X					5, 8A, 14, 24
PW-13	Widespread dispersive indoor use (professional) resulting in inclusion into a matrix				X				5, 8A, 10, 11, 13, 19
PW-15	Widespread dispersive outdoor use (professional) resulting in inclusion into a matrix				X				5, 8A, 10, 11, 13, 19
PW-22	Professional removal of matrix, outdoor (e.g. abrasion)				X				24
PW-24	Professional removal of matrix, indoor (e.g. abrasion)				X				24

Scope and type of exposure

According to the lead registrant the pigments PR3, PR4 and PO5 are not classified, assuming that the impurity 1-chloro-2,4-dinitrobenzene does not exceed a concentration of 0.03 %. Therefore, the registrants did not perform an exposure assessment. However, the eMSCA had some doubts about the stated impurity concentrations in the CSR. Therefore the registrants were asked about the typical concentration of 1-chloro-2,4-dinitro benzene in their products. While three registrants answered and confirmed impurity concentrations below 0.03 %, eight registrant did not react on the request of the eMSCA.

Due to the wide dispersive use scenarios inhalation and dermal exposure is expected for a number of PROCs. Since the pigments are marketed in small particle size distribution (PR3: D50 2-28 µm; PR4: D50 2-10 µm; PO5: D50 1.1 µm) inhalation exposure may play a significant role at workplaces. Additionally, the pigments are used in surface coatings and inks which are spread or sprayed (PROC 7, 8A, 10, 11) leading to further potential inhalation and dermal exposure. However, the registrants did not provide any information about operational conditions and risk management measures for these anticipated exposure situations.

In order to gain first indications on the possible risk levels at workplaces, an exposure assessment under reasonable worst case conditions was carried out by using the ECETOC-TRA V3.0 tier 1 model.

Predicted exposure by ECETOC-TRA

The following reasonable worst case conditions were adopted for the ECETOC TRA assessment: PO5 is used as pure substance powder with high dustiness. Furthermore, the duration of activity was assumed to be more than four hours and without using suitable PSE. Depending on the type of enterprise (professional, industrial) LEV efficiencies between 75 % and 95 % were assumed. For spraying operations (PROC 7, 10, 11 and 13) involving solids suspended in liquids ECETOC TRA was not used because these scenarios are outside the scope of the model. Nevertheless, significant inhalation exposure is to be expected for spraying activities. However, higher tier exposure assessments for spraying activities (for instance with the Advanced REACH TOOL) were not feasible, since the needed model parameters were lacking.

The following table listed the predicted exposure for the provided PROCs.

Table 60

Predicted exposure for provided PROCs carried out by ECETOC-TRA V3.0								
PROCs		Exposure inhalative		Inhalative Scenario 1 Duration of activity: >4 h = 1.0	Exposure dermal (LEV is not considered)			Dermal Scenario 1.1 Duration of activity: >4 h = 1,0; Dermal protection a = 1,0
		exposure prediction [mg/m³]	LEV effectiveness [%] = 0.1	inhalative exposure [mg/m³]	initial predicted dermal exposure [µg/cm²/d]	exposed skin surface [cm²]	predicted dermal exposure [mg/kg/d]	dermal exposure [mg/kg/d]
Industrial uses	PROC 4	25	90	2,50	1000	480	6,86	6,86
	PROC 5	25	90	2,50	2000	480	13,71	13,71
	PROC 6	25	90	2,50	2000	960	27,43	27,43
	PROC 7				2000	1500	42,86	42,86
	PROC 8a	50	90	5,00	1000	960	13,71	13,71
	PROC 8b	25	95	1,25	1000	960	13,71	13,71
	PROC 9	20	90	2,00	1000	480	6,86	6,86
	PROC 10				2000	960	27,43	27,43
	PROC 13				2000	480	13,71	13,71
	PROC 14	10	90	1,00	500	480	3,34	3,34
	PROC 15	5	90	0,50	100	240	0,34	0,34
	PROC 21	10	90	1,00	100	1980	2,83	2,83
	PROC 24	10	80	2,00	100	1980	2,83	2,83
Professional uses	PROC 5	50	80	10	2000	480	13,71	13,71
	PROC 8a	50	80	10	1000	960	13,71	13,71
	PROC 10				2000	960	27,43	27,43
	PROC 11				5000	1500	107,14	107,14
	PROC 13				2000	480	13,71	13,71
	PROC 19	50	80	10	5000	1980	141,43	141,43
	PROC 24	20	75	5	100	1980	2,83	2,83

7.12.1.1.2. Consumer

Table 61

Overview on consumer uses: Uses provided in the updated (01.05.2013) CSR of the lead registrant (chapter 2)									
Identifiers	Short description of the identified use	Resulting life cycle stage		End use			Service life (for articles)	Sector of use (SU)	Process Category (PROC)
		Manufacture	Formulation	Industrial	Professional	Consumer			
C-17	Widespread dispersive indoor use (consumer) resulting in inclusion into a matrix					X			PC9a, PC18
C-18	Widespread dispersive outdoor use (consumer) resulting in inclusion into a matrix					X			PC9a
C-19	consumer indoor use of pigmented articles with low release					X			PC9a, PC18, PC32
C-20	Consumer outdoor use of pigmented articles with low release					X			PC9a, PC18, PC32
C-23	Consumer removal of matrix, outdoor (e.g. abrasion)					X			PC9a, PC18, PC32

Overview on consumer uses: Uses provided in the updated (01.05.2013) CSR of the lead registrant (chapter 2)									
Iden-tifiers	Short description of the identified use	Resulting life cycle stage		End use			Service life (for articles)	Sector of use (SU)	Process Cate-gory (PROC)
		Manu-facture	Formu-lation	Ind-ustrial	Profes-sional	Con-sumer			
C-25	Consumer removal of matrix, indoor (e.g. abrasion)					X			PC9a, PC18, PC32
SL-1	Removal of matrix (e.g. abrasion), outdoor				X	X	X		PROC 24, AC11, AC7, AC01, AC1
SL-2	Removal of matrix (e.g. abrasion), indoor				X	X	X		PROC 24, AC11, AC7, AC01, AC1
SL-3	Consumer indoor use of coloured articles					X	X		AC11, AC7, AC01, AC8, AC13
SL-4	Consumer outdoor use of coloured articles					X	X		AC11, AC7, AC01, AC8, AC13

As indicated in Table 61, PO5 is used as a colourant in several different scenarios which most likely lead to inhalation, dermal and (in some cases) oral exposure. The registrants did not perform an exposure assessment or provide any information about risk management measures due to a lacking classification of PO5. Therefore no information regarding the operational conditions is given. Because of this the relevant exposure scenarios were calculated by the German CA using Consumer-TRA 3.1 and its default values. One exception was made in this context: the concentration of PO5 in products belonging to product category 9a is set at 10%. This is an information the German CA

received from a registrant during consultation. Where necessary the exposure is calculated for children in order to follow the worst case approach and illustrate the exposure for the most sensitive affected target group.

The eMSCA is aware of the fact that this calculation is of a low tier and related to uncertainties but it also gives an indication on the possible dimension of the risk arising for the consumer using PO5 or PO5 containing products and articles. The results of the exposure calculations are summarised in Table 62.

Table 62

Results for inhalation, dermal and oral exposure of the consumer						
Descriptor	**Product subcategory**	**Dermal Exposure Estimate (mg/kg/day)**	**Oral Exposure Estimate (mg/kg/day)**	**Inhalation Exposure Estimate (mg/kg/day)**	**Inhalation Exposure Estimate (mg/m^3)**	**Target group**
PC9a: Coatings, paints, thinners, removers	Waterborne latex wall paint	7,15E+00		8,24E-10	1,64E-08	Adult
	Solvent rich, high solid, water borne paint	7,15E+00		8,24E-10	1,64E-08	Adult
	Aerosol spray can			9,44E+00	1,25E+03	Adult
PC9b: Fillers, putties, plasters, modelling clay	Plasters and floor equalizers	1,43E+02		7,49E-10	1,64E-08	Adult
PC9c: Finger paints	Finger paints	1,27E+02	6,75E+01			Child
PC12:Fertilizers	Lawn and garden preparations	7,15E+01	1,50E+01			Both
PC24: Lubricants, greases, and release products	Liquids	7,15E+01		1,50E-09	1,64E-08	Adult
	Pastes	2,86E+01				Adult
	Sprays	3,57E+01		2,02E+02	2,21E+03	Adult
PC31:Polishes and wax blends	Polishes, wax / cream (floor, furniture, shoes)	7,15E+01		1,50E-09	1,64E-08	Adult

AC8: Paper articles	Tissues, paper towels, wet tissues, toilet paper	2,86E+01		3,74E-10	1,64E-08	Adult
	Printed paper (papers, magazines, books)	7,15E-01	3,00E+00	2,99E-09	1,64E-08	Both
AC11: Wood articles	Furniture (chair)	1,46E+01		1,50E-09	1,64E-08	Adult
	Walls and flooring (also applicable to non-wood materials)	3,57E+00		2,99E-09	1,64E-08	Adult
	Small toys (car, train)	1,27E+00	1,00E+00			Child
	Toys, outdoor equipment	5,57E+00	1,00E+00			Child
AC13: Plastic articles	Toys (doll, car, animals, teething rings)	2,39E+01	4,30E-01			Child
	Plastic, small articles (ball pen, mobile phone)	3,07E+00	7,17E-01	2,99E-09	1,64E-08	Adult

7.12.2. Environment

The exposure of the environment has not been assessed by the registrants. Based on the uses of the pigments, releases might occur to surface water via sewage treatment plants, to soil via sewage sludge, or directly to soil during outdoor uses of coloured articles.

All three pigments have very low water solubilities. They will be present in the environment in a particulate form (Herbst and Hunger 2004, according to (Environment Canada, 2009a; Environment Canada, 2009b; Environment Canada, 2009c)). Vapour pressure is also very low. This indicates that these pigments will distribute into sediments via sedimentation when released to aquatic environments. If released to soil, the pigments will stay in the soil unless they are transported further with dust or runoff. So sediment and soil are expected to be the most relevant compartments for environmental exposure. If it is assumed that water solubility is very low and the pigments get incorporated in a matrix, bioavailability will be very low. In addition, these pigments are considered to be persistent in the environment. Therefore, degradation of these pigments is not expected to occur.

All three pigments have been assessed by the Canadian Agencies. Environment Canada (2009 a, b, c) concludes, that the exposure of the environment is low. Most of the substances is expected to be included in articles and will finally end up in waste. Environment Canada used a mass flow tool to estimate the potential release to the

environment. It is estimated that 93.7 % for PR3, 92.7 % for PR4 and 79 % of PO5 are transferred to waste disposal sites like landfill or incineration. Due to the low solubility of these pigments migration from landfill sites is expected to be negligible ((Environment Canada, 2009a; Environment Canada, 2009b; Environment Canada, 2009c). Only 4.7 % for PR3, 5.8 % for PR4 and 18.5 % for PO5 are expected to be released to sewer and 1.6 % for PR3, 1.5 % for PR4 and 2.5 % for PO5 are expected to be released to soil in the Canadian Assessment.

7.12.2.1. Aquatic compartment (incl. sediment)

See general exposure survey in section 7.12.2.

7.12.2.2. Terrestrial compartment

See general exposure survey in section 7.12.2.

7.12.2.3. Atmospheric compartment

See general exposure survey in section 7.12.2.

7.12.3. Combined exposure assessment

See general exposure survey in section 7.12.2.

7.13. Risk characterisation

7.13.1. PO5

7.13.1.1. Worker

Considering the physicochemical properties of PO5 and its industrial and professional uses, workplace exposure occurs mainly via inhalation and dermal contact. However, the registrants did not provide any information about operational conditions and risk management measures for these exposure situations. Thus, to gain first indications on the possible risk levels at workplaces, an exposure assessment under reasonable worst case conditions was carried out by using the ECETOC-TRA V3.0 tier 1 model. For quantitative risk characterisation of PO5, only inhalation exposure estimates were compared with the derived long-term systemic inhalation DNEL for workers.

For PO5, a long-term systemic DNEL for inhalation of 0.274 mg/m^3 was derived. The DNEL value was calculated based on a sub-acute inhalation study in rats (Hoechst, 1973). A detailed overview of how the eMSCA derived this DNEL is given in section 7.9.10.3

An overview of the RCRs calculated by the eMSCA with the derived DNEL (worker, inhalation, systemic, long-term) is given in

Table 63.

Table 63

Overview of RCRs calculated by the eMSCA in critical exposure scenarios		
Postulated worker contributing scenario	**Highest predicted inhalative exposure value [mg/m³]**	**RCR**
Industrial uses		
PROC 4	2,50	9.1
PROC 5	2,50	9.1
PROC 6	2,50	9.1
PROC 8a	5,00	18.3
PROC 8b	1,25	4.6
PROC 9	2,00	7.3
PROC 14	1,00	3.7
PROC 15	0,50	1.8
PROC 21	1,00	3.7
PROC 24	2,00	7.3
Uses by professional workers		
PROC 5	10	36.5
PROC 8a	10	36.5
PROC 19	10	36.5
PROC 24	5	18.3

Due to the remarkably low DNEL all RCRs are greater than 1. Thus a significant risk for workers is indicated. For the time being this indicates that further risk management options must be considered by the eMSCA. However, the eMSCA recommends that the registrants consider classification and the DNEL (worker, inhalation, systemic, long-term) calculated by the eMSCA and extend their safety assessment accordingly. This includes the generation of exposure scenarios. Detailed information on use and exposure might yield lower RCRs than the ones calculated with reasonable worst case assumptions.

A decision about whether these results indicate the need for regulatory action (and, if so, which) will be made once the requested information have been submitted by the registrants and are evaluated by the eMSCA.

7.13.1.2. Consumer

Consumer exposure to PO5 containing products and articles will mostly be the result of dermal contact and inhalation. Because as a worst case assumption no personal protection equipment is considered in the consumer sector it is reasonable to assume that dermal exposure is of a high relevance. No exposure assessment for consumer uses was conducted by the registrants. Therefore information regarding the operational conditions is lacking. The eMSCA conducted an assessment using ConsumerTRA 3.1 with mostly default values for the different product categories. In combination with an appropriate DNEL the derived results can be used as a basis to assess the possible risk level for the consumer arising from PO5 usage in products and articles and to derive further risk management or regulatory measures.

An overview of the calculated (combined) RCRs is given in Table 64.

In accordance with the results for the occupational sector explained above all combined RCRs are above 1. However in this case all routes are considered. Therefore and because of the even smaller inhalation DNEL for consumer and the low dermal and oral DNELs (as explained in chapter 7.9.10.3.2) the RCRs are relatively high and indicate that a risk for the consumer during the use of PO5 and products/articles containing PO5 cannot be excluded.

Table 64

Overview of RCRs calculated for the consumer					
Consumer use	**Subcategory scenario**	**Dermal RCR**	**Oral RCR**	**Inhalation RCR**	**Combined RCR**
PC9a: Coatings, paints, thinners, removers	Waterborne latex wall paint	2,55E+02		8,49E-08	2,55E+02
	Solvent rich, high solid, water borne paint	2,55E+02		8,49E-08	2,55E+02
	Aerosol spray can			6,49E+03	6,49E+03
PC9b: Fillers, putties, plasters, modelling clay	Plasters and floor equalizers	5,10E+03		8,49E-08	5,10E+03
PC9c: Finger paints	Finger paints	4,54E+03	2,41E+03		6,95E+03
PC12:Fertilizers	Lawn and garden preparations	2,55E+03	5,36E+02		3,09E+03
PC24: Lubricants, greases, and release products	Liquids	2,55E+03		8,49E-08	2,55E+03
	Pastes	1,02E+03			1,02E+03
	Sprays	1,28E+03		1,14E+04	1,27E+04
PC31:Polishes and wax blends	Polishes, wax / cream (floor, furniture, shoes)	2,55E+03		8,49E-08	2,55E+03
AC8: Paper articles	Tissues, paper towels, wet tissues, toilet paper	1,02E+03		8,49E-08	1,02E+03
	Printed paper (papers, magazines, books)	2,55E+01	1,07E+02	8,49E-08	1,33E+02
AC11: Wood articles	Furniture (chair)	5,21E+02		8,49E-08	5,21E+02
	Walls and flooring (also applicable to non-wood materials)	1,28E+02		8,49E-08	1,28E+02
	Small toys (car, train)	4,54E+01	3,57E+01		8,11E+01
	Toys, outdoor equipment	1,99E+02	3,57E+01		2,35E+02
AC13: Plastic articles	Toys (doll, car, animals, teething rings)	8,55E+02	1,54E+01		8,70E+02
	Plastic, small articles (ball pen, mobile phone)	1,10E+02	2,56E+01	8,49E-08	1,35E+02

7.14. References

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7.15. Abbreviations

B/vB	bioaccumulative / very bioaccumulative
BOD	biological oxygen demand
DOC	dissolved organic carbon
DNEL	derived no-effect level
eMSCA	evaluating Member State Competent Authority
HPLC	high performance liquid chromatography
K_{OW}	octanol water partition coefficient
NOAEL	no observed adverse effect level
Pigment Red 3 / PR3	1-(4-methyl-2-nitrophenylazo)-2-naphthol
Pigment Red 4 / PR4	1-[(2-chloro-4-nitrophenyl)azo]-2-naphthol
Pigment Orange 5 / PO5	1-[(2,4-dinitrophenyl)azo]-2-naphthol
PBT	persistent, bioaccumulative, toxic
P/vP	persistent / very persistent
QSAR	Qualitative Structure Activity Relationship
SEv	Substance Evaluation
T	toxic
vPvB	very persistent, very bioaccumulative