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

Barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate]

EC No 225-935-3

CAS RN 5160-02-1

Evaluating Member State(s): Germany

Dated: 06 December 2021

Evaluating Member State Competent Authority

BAuA

Federal Institute for Occupational Safety and Health
Division 5 - Federal Office for Chemicals
Friedrich-Henkel-Weg 1-25
D-44149 Dortmund, Germany

Year of evaluation in CoRAP: 2020

Member State concluded the evaluation without any further need to ask more information from the registrants under Article 46(1) decision.

Further information on registered substances here:

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

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

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 Substance, Barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate] (Pigment Red 53:1; PR53:1) (EC number 225-935-3) was originally selected for substance evaluation in order to clarify concerns about:

- suspected carcinogenic properties,
- wide dispersive use and
- exposure of workers.

During the evaluation, the following were identified as additional concerns:

- repeated dose toxicity,
- substance identity regarding nanoforms and
- exposure of consumers

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

A dossier evaluation process has been conducted by ECHA resulting in additional requests to generate information to fulfil standard information requirements.

For PR53:1, ECHA issued a decision on 9 April 2018. The standard data submitted for human health endpoints (OECD TG 414) was considered in this substance evaluation.

A further compliance check procedure should be opened by ECHA as the evaluating Member State Competent Authority (eMSCA) considers that standard information is still necessary to clarify the identified concerns.

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the Substance has led the evaluating Member State to conclude that:

- A further compliance check procedure should be opened by ECHA as the evaluating Member State Competent Authority (eMSCA) considers that standard information is still necessary to clarify the identified concerns.
- A need for regulatory follow-up action has been identified (see Table 1).

Table 1

CONCLUSION OF SUBSTANCE EVALUATION	
Conclusions	
Need for follow-up regulatory action at EU level	x
Harmonised Classification and Labelling	x
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	
No need for regulatory follow-up action at EU level	

The need to re-open a Substance Evaluation will be determined based on the outcome of the new information generated via a Compliance Check.

4. FOLLOW-UP AT EU LEVEL

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

A Harmonised Classification and Labelling for the endpoint carcinogenicity will be initiated by the eMSCA.

Also, the need for further measures will be re-evaluated once standard information requested via Compliance Check is available.

4.1.1. Harmonised Classification and Labelling

Based on the existing information on PR53:1 the eMSCA considers that a harmonised classification of the substance as carcinogenic (Carc. 2) is necessary, a hazard class for which PR53:1 currently neither possesses a harmonised classification nor for which it is self-classified in the registrations.

The eMSCA considers a new entry of the harmonised classification with regards to carcinogenicity as the most important measure to drive further risk management of PR53:1. The labelling of a mixture containing PR53:1 according to the CLP Regulation (EC, 2008) as Carc. 2, H351, may result in an increased substitution pressure for the manufacturer of the product. It is envisaged that the harmonised classification of PR53:1 as Carc. 2, H351, is expected to result in a reduction of consumer exposure (see Section 7.12.1.2) e.g. regarding the use in toys/finger paint. The eMSCA will submit a proposal for an Annex VI entry in CLP for PR53:1.

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

N/A

4.1.3. Restriction

N/A

4.1.4. Other EU-wide regulatory risk management measures

N/A

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

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

N/A

5.2. Other actions

N/A

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**
CLH Dossier	2022	DE CA
Compliance Check	N/A	ECHA

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

The Substance, Barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate] (Pigment Red 53:1; PR53:1) (EC number 225-935-3) was originally selected for substance evaluation in order to clarify concerns about:

- suspected carcinogenic properties,
- wide dispersive use and
- exposure of workers.

During the evaluation, the following were identified as additional concerns:

- repeated dose toxicity,
- substance identity regarding nanoforms and
- exposure of consumers

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome / conclusion**
Substance Identity	Concern unresolved. Incomplete standard information regarding the characterisation of the nanoform. eMSCA recommends that ECHA requests further standard information via compliance check.
Carcinogenicity	Concern confirmed. Based on available information, classification of PR53:1 as Carc. 2 is considered appropriate.
Repeated dose toxicity	Concern unresolved. Incomplete standard information for inhalation route based on nanoform of PR53:1. eMSCA recommends that ECHA requests further standard information via compliance check.
Wide dispersive use	The substance is used by consumers and workers. See below for individual conclusions for both endpoints.
Exposure of consumer	The eMSCA considers that the exposure of consumers via the three routes (inhalation, dermal, oral) is possible (see subsection 7.12.1.2). Whether or not this exposure pose a risk for consumers could not be concluded in the substance evaluation. The envisaged harmonised classification of the substance as Carc. 2, H351, is expected to result in a reduction of consumer exposure (see subsection 7.13.2).
Exposure of workers	Identified uses for workers (industrial and professional) were evaluated. RCRs > 1 were calculated. However, the calculation of the dermal RCRs are based on the worst case assumption of 100 % dermal absorption, which is considered to be not realistic. For details see section 7.13.1. The eMSCA currently considers no further action as necessary.
Additional endpoints	

Toxicokinetics	Incomplete standard information according to REACH Annexes to address nanoforms. Hand over to compliance check to assess the need to require this information for nanoforms.
Mutagenicity	Incomplete standard information set. eMSCA recommends that ECHA requests further standard information via compliance check. An indication of mutagenic activity of the Substance could influence the current CLH on Carcinogenicity, i.e. Carc 1B or Carc 2.
Reproductive Toxicity	Based on available information, a trigger for a EOGRTS has been identified. eMSCA recommends that ECHA requests further standard information via compliance check.
Sensitisation	Incomplete standard information. eMSCA recommends that ECHA requests further standard information via compliance check.

The conclusions are based on the assumption that the test materials used in the available toxicity studies are representative of the Substance. PR53:1 is registered as nanomaterial by the lead registrant. In the dossier of the lead registrant it is stated, “test materials used in this dossier are all considered to fall under the definition of nano-materials according to the European Commission Recommendation 2011/696/EU”. However, the test material in the available toxicity studies is not adequately characterised according to the adapted REACH Annex to address nanoforms of substances entered into force in 2020, mainly due to the fact that the studies are very old. According to the lead registrant two different nanoforms should be covered by the registration: the first one is spherical and the second one elongated. Based on the boundary composition, no bulk form is covered by the registration.

During the substance evaluation the eMSCA noted that a characterisation of the individual nanoforms covered by the different registrations is lacking. It is unclear whether the boundary composition provided by the lead registrant covers all nanoforms of the co-registrants. Next to this it is unclear if some of the registrants manufacture or import the bulk form of the substance and how this is addressed in the lead dossier.

Thus, a final conclusion can only be drawn when the substance identity is clarified regarding the forms of the substance covered by this registration.

The available data provided by the lead registrant and supporting information are sufficient to propose a classification as Carc. 2 for PR53:1. The synthesis and manufacturing of PR53:1 generally yields particulate material with a fine and ultrafine particle size distribution. The available data do not suggest that a distinction has to be made between the nanoform and the bulk form of the substance regarding the proposed classification for carcinogenicity.

7.2. Procedure

The Substance, PR53:1, was included in the Community Rolling Action Plan for substance evaluation (CoRAP) 2016 as suspected carcinogen. The evaluation was started on 18 March 2020 following the adoption of the respective CoRAP update.

7.3. Identity of the substance

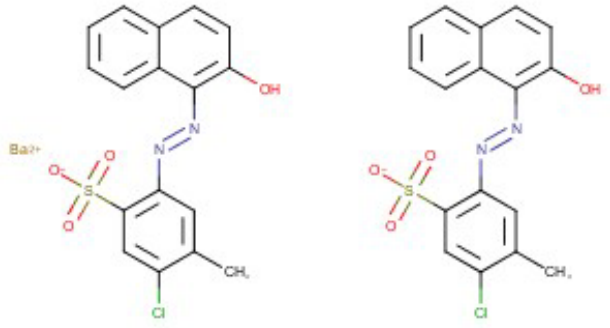
Table 4

SUBSTANCE IDENTITY

Public name:	Barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate]
EC number:	225-935-3
CAS number:	5160-02-1
Index number in Annex VI of the CLP Regulation:	N/A
Molecular formula:	$C_{34}H_{24}BaCl_2N4O_8S_2$
Molecular weight range:	888.9646 g/mol
Synonyms:	C.I. Pigment Red 53:1; PR53:1; Benzenesulfonic acid, 5-chloro-2-[2-(2-hydroxy-1-naphthalenyl)diazenyl]-4-methyl-, barium salt (2:1); Lake Red CBA; Irgalite Red D; D&C Red No. 9

Type of substance ☒ Mono-constituent ☐ Multi-constituent ☐ UVCB

Structural formula:



The lead registrant provided information regarding the characterisation of the nanoform and a boundary composition.
However, a characterisation of the individual nanoforms covered by the different registrations is lacking. Only one registrant of the joint submission updated their registration dossier with regards to the characterisation of the nanoform at the end of the substance evaluation year. Update of registration dossiers to inform on the nanoforms was expected due to the new obligations according to the adapted REACH Annex entered into force in January 2020. It is not clear, if the boundary composition provided by the lead registrant covers all nanoforms of the co-registrants.

The eMSCA proposes to clarify the substance identity of the registered substance with regard to the characterisation of nanoforms as part of a compliance check. Next to this it needs to be clarified whether bulk forms of the substance are also covered by the registration and how this is addressed in the lead dossier.

7.4. Physico-chemical properties

Table 5

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20 °C and 101.3 kPa	Red powder, it is conceivable that the substance subject to registration could be considered as falling within the boundaries of the nanomaterial definition.
Vapour pressure	Not applicable; The substance decomposes before melting

Water solubility	- Measured immediately after filtration: 3 mg/L at 23 °C - Measured after one week: <0.01 mg/L at 23 °C
Partition coefficient n-octanol/water (Log Kow)	- Measured immediately after filtration: Log Pow = -0.62 at 23 °C - Measured after one week: Log Pow = 1.69 at 23 °C
Granulometry	MMD = 11 µm D10 = 1.5 µm D90 = 33 µm Remark: conducted by laser diffraction method, it is conceivable that the substance subject to registration could be considered as falling within the boundaries of the nanomaterial definition.
Stability in organic solvents and identity of relevant degradation products	In dimethylsulfoxide (DMSO) stable for one week In dimethylformamide (DMF) stable for one week
Dissociation constant	pKa = -5.49 at 20 °C, sulfonate group pKa = 8.96 at 20 °C, phenolic group

7.5. Manufacture and uses

7.5.1. Quantities

Table 6

AGGREGATED TONNAGE (PER YEAR)				
☐ 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 7

USES	Use(s)
Manufacture	Synthesis of pigment PROC 1, 3, 5, 8a, 8b, 9
Formulation	- Ind. formulation of non-solid preparations containing pigment (incl. inks, paints); PROC: 3, 5, 8b, 9, 14, 15 - Use in textile/leather/fishing; PROC: 1, 2, 3, 5, 8a, 9, 14 - Ind. formulation of solid preparations containing pigment (incl. plastics); PROC: 5, 8b, 9,14, 15, 24, 28 - Formulation of pigment product; PROC: 1, 3, 5, 8a, 8b, 9 - Use in laboratory; PROC: 15 - Ind. formation of inks (water and solvent based) & toner; PROC: 3, 5, 8b, 9, 15, 28 - Ind. manufacture of coatings and inks; PROC: 1, 2, 3, 5, 8a, 8b, 9, 14 - Coating, ink, plastic applications: manufacture of powder products; PROC: 1, 2, 3, 5, 8a, 8b, 9 - Used in paints; PROC: 1, 2, 3, 5, 8a, 8b, 9, 14 - Used in plastic masterbatches; PROC: 1, 2, 3, 5, 8a, 8b, 9, 14

Uses at industrial sites	- Ind. use of pigment preparations resulting in inclusion into a matrix (incl. ink and paint); PROC: 5, 6, 7, 8a, 10, 13, 14, 21 - Ind. use of pigment preparations resulting in inclusion into a matrix (incl. plastics); PROC: 5, 8a, 14, 24 - Use in laboratory; PROC: 15 - Industrial application of printing inks (water-based, solvent-based); PROC: 5, 8b, 15, 28 - Ind. Use of pigment preparations resulting in inclusion into a matrix (incl. inks and paints); PROC: 5,6,7,8a,10,13,14,21 - Ind. removal of matrix (e.g. abrasion); PROC: 24 - Ind. application of automotive, decorative and industrial coatings; PROC: 5, 7, 8b, 10, 13, 15, 21, 28 - Use in paints; PROC: 1, 2, 3, 5, 6, 7, 8a, 8b, 9, 13, 14 - Coating, ink, plastic applications: industrial; PROC: 1, 2, 3, 4, 5, 6, 7, 8a, 8b, 9, 10, 13, 14, 21, 24 - Ind. application of masterbatches and compounds – production of plastic articles; PROC: 5, 6, 8b, 15, 24, 28
Uses by professional workers	- Widespread dispersive outdoor use (prof.) resulting in inclusion into or onto a matrix; PROC: 5, 8a, 10, 11, 13, 19 - Widespread dispersive indoor use (prof.) resulting in inclusion into a matrix; PROC: 5, 8a, 10, 11, 13, 19 - Prof. application of paints, coatings – widespread dispersive indoor use; PROC: 5, 8a, 10, 11, 13, 28 - Prof. application of coatings and inks; PROC: 5, 6, 7, 8a, 8b, 9, 10, 13, 14 - Prof. application of inks (water- and solvent-borne); PROC: 5, 8a, 8b, 10, 28 - Coating, ink, plastic applications: prof.; PROC: 2, 3, 4, 5, 8a, 10, 11, 13, 19, 21 - Used in paints; PROC: 5, 6, 7, 8a, 8b, 9, 10, 13, 14 - Auxiliary activities in prof. applications od coatings; PROC: 5, 6, 7, 8a, 8b, 9, 10, 13, 14 - Prof. removal of matrix, outdoor (e.g. abrasion); PROC: 24 - Prof. removal of matrix, indoor (e.g. abrasion); PROC: 24
Consumer Uses	Widespread dispersive indoor and outdoor use (consumer) resulting in inclusion into or onto a matrix, removal of matrix indoor and outdoor (e.g. abrasion), consumer indoor and outdoor use of pigmented articles with low release, use of printing mixtures and toners, inks PC 1: Adhesives, sealants PC 8: Biocidal products (e.g. disinfectants, pest control) 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; consumer indoor and outdoor use of coloured articles; use in paints AC 01: Other (not intended to be released): Painted 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 6: Leather articles

	AC7a: Metal articles: Large surface area articles AC7c: Metal articles: Packaging (excluding food packaging) AC 8: Paper articles AC10a: Rubber articles: Large surface area articles AC10b: Rubber articles: Toys intended for children's use (and child dedicated articles) AC10c: Rubber articles: Packaging (excluding food packaging) AC11a: Wood articles: Large surface area articles AC11b: Wood articles: Toys intended for children's use (and child dedicated articles) AC11c: Wood articles: Packaging (excluding food packaging) AC11e: Wood articles: Furniture & furnishings AC 13: Plastic article

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

No harmonised classification is available.

7.6.2. Self-classification

In the registration(s): not classified.

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

- Acute Tox. 3 (H301)
- Acute Tox. 4 (H332)
- Skin Sens. 1 (H317)

7.7. Environmental fate properties

Not assessed in the course of this evaluation.

7.8. Environmental hazard assessment

Not assessed in the course of this evaluation.

7.9. Human Health hazard assessment

Notes for consideration regarding substance identity for human health hazard assessment:

The Substance, PR53:1, is registered as a nanomaterial by some registrants. In the dossier of the lead registrant (since March 2020), it is stated, “test materials used in this dossier are all considered to fall under the definition of nano-materials according to the European Commission Recommendation 2011/696/EU”.

However, it is not clear if the test materials used in the available toxicity studies is representative for the registered Substance. In the available toxicity studies the test substance is not adequately characterised according to the adapted REACH Annex to address nanoforms of substances entered into force in 2020, mainly due to the fact that the studies are very old. The synthesis and manufacturing of this pigment generally yields particulate material with a fine and ultrafine particle size distribution. However, variations in generating the small particles by the individual registrant may result in different particle size distributions.

Based on the available data it cannot be evaluated whether these differences in particle size (if existing) have a significant impact on the toxicity of the pigment. **The eMSCA**

proposes to clarify the substance identity of the registered substance as part of a compliance check.

7.9.1. Toxicokinetics

No reliable study on toxicokinetics of PR53:1 after oral exposure is available. Repeated dose toxicity studies (see chapter 7.9.4.) showed haematotoxic effects in rats and mice after oral exposure, so that an oral absorption of PR53:1 or its metabolites can be assumed.

Regarding the inhalation route, the available data are insufficient with regard to transformation, clearance, accumulation in and translocation from the lung and lung-associated lymph nodes (LALN) of PR53:1.

No adequate study on dermal absorption of PR53:1 is available.

7.9.1.1. Conclusion

In compliance with adaptation of the REACH Annexes to address nanoforms of substances, enforced as from 01 January 2020, in the 10-100 tonnage band a toxicokinetic study shall be proposed or may be required by the Agency in accordance with Article 40 or 41 in case such an assessment cannot be performed on the basis of the relevant available information (REACH Annex 8.8.1).

The eMSCA notes that available information for assessment is limited. In particular, no repeated dose toxicity study via inhalation is available that could have provided at least some relevant toxicokinetic information. **Therefore, the eMSCA proposes that a toxicokinetic study in a compliance check would be required when the substance identity is clarified regarding the nanoform.**

7.9.2. Acute toxicity and Corrosion / Irritation

In this section the data for acute toxicity after inhalation exposure were reviewed.

7.9.2.1. Acute toxicity after inhalation exposure

Table 8

Relevant studies related to the assessment of the endpoint acute toxicity (inhalation)			
Methods	**Results**	**Remarks**	**Reference**
Acute inhalation toxicity study - Equivalent to OECD TG 403 (Acute Inhalation Toxicity) GLP: yes Test material: 5313C (known trading name of PR53:1) Purity: not specified Rat, SD 5/sex Inhalation: dust aerosol (MMAD 2.6 µm, σg 0.28 µm) Exposure concentration (actual): mean 5.24 mg/L (range 4.25 – 6.33 mg/L) Exposure: 4h, nose-only (observation for 14 days)	No mortality LC_{50} > 5.24 mg/L In addition to common clinical signs, incidents of gasping and noisy respiration; reversible within 2-3 days (except staining of the fur).	Reliable without restrictions	TL 1993a

Relevant studies related to the assessment of the endpoint acute toxicity (inhalation)			
Methods	**Results**	**Remarks**	**Reference**
Acute inhalation toxicity study - Equivalent to OECD TG 403 (Acute Inhalation Toxicity) GLP: yes Test material: Permanent-Lackrot C (known trading name of PR53:1) Purity: 98.1% Rat, SPF Wistar 5/sex Inhalation: dust (MMAD 1.9 µm, σg 2.3 µm) Exposure concentration (actual): mean 4.13 mg/L (range 3.81 – 4.40 mg/L) Exposure: 4h, nose-only (observation for 15 days)	One male died accidentally (black discolouration of the lungs and dark discolouration of the liver) LC_{50} > 4.13 mg/L In addition to non-specific clinical signs, impairments of respiration and motility, narrowed palpebral fissures and trembling; reversible on day 7 (except staining of the fur)	Reliable without restrictions	TL 1993b

Two OECD TG 403 inhalation studies have been submitted. In both studies no treatment-related mortality was observed (TL 1993a, TL 1993b). The eMSCA notes that as the updated REACH Annexes have become effective for nanoforms of substances in 2020, inhalation is the default route for standard acute toxicity testing.

7.9.2.2. Conclusion

No acute toxic effects after inhalation exposure were identified which would justify classification. Assuming that the test substance is representative for the registered substance, the available data is considered as appropriate for an evaluation of acute toxicity after inhalation exposure.

7.9.3. Sensitisation

Table 9

Animal data on skin sensitisation				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
GPMT – Similar to OECD TG 406) GLP not specified Protocol according to Sato et al. (1981) Guinea pig, Hartley, N=10/ dose Day 1, 2, and 3: Intradermal injection of FCA water-emulsion, cross-cross lattice of abrasions was made at site of injection; at the same time 0.1 ml (0.1 g) test sample was applied occlusively; Day 8: 10 % sodium lauryl sulfate were applied Day 9: Topical induction for 48h	D&C Red No 9 Purity: no information, stated as "purified" Analysis of D&C Red No. 9 revealed the presence of eleven aromatic azo compounds	**Not reliable** Publication, No study report available Concentration for induction and challenge not reported, individual readings in animals not reported, control (positive/ negative) data not reported	Negative "*Results of the modified guinea pig maximization test indicated that each of the subsidiary colors was a contact sensitizer, while D&C Red No. 9 itself was not.*"	Naganuma et al., 1983

Animal data on skin sensitisation				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
Day 21: Topical application of 50 mg test substance in petrolatum for 24 h Reading: 24 and 48 h after application, excess of coloring substance was removed by washing with suitable solvent at 24 h GLP: not specified				

7.9.3.1. Non-human information

The lead registrant submitted negative animal testing data for the sensitising potential of PR53:1. A read-across for an LLNA performed according to OECD TG 429 conducted with an analogue (source) substance, Pigment Red 49:2, was submitted as key study (TL, 2012a). The read-across was justified by the structural similarity between PR53:1 and Pigment Red 49:2. However the justification is considered as insufficient by the eMSCA.

Furthermore, the registrant referenced a study of a guinea pig maximisation test (Naganuma et al., 1983) performed according to a protocol of (Sato et al., 1981). Eight samples of D&C Red No. 9 (trade name for PR53:1) were investigated for impurities revealing the presence of eleven aromatic azo compounds. The authors reported that "*every subsidiary color* [of D&C Red No. 9] *is a contact sensitizer, though their allergenic capabilities are different from each other. This is why the contact sensitivity of D&C Red No. 9 is considered to be mainly caused by the subsidiary colors*". Furthermore, the authors stated that "*purified D&C Red No. 9 did not contain the subsidiary colors, and the contact sensitivity was not recognized*". However, relevant information on the testing procedure (e.g. concentration for induction or challenge, vehicle for induction, individual readings of guinea pigs treated with the test substance or controls) are missing so that this study is considered as not reliable.

7.9.3.2. Human information

Table 10

Human data on skin sensitisation				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
Patch test data from dermatological department Selected dermatitis patients	Red Lake C barium salt (trade name of PR53:1) 5 % in 88 % PEG 400 and 12 % PEG 6000	During 2 years, 53 patients with pigmented cosmetic dermatitis were patch- and photo-patch-tested with the patient's own cosmetics and representative coal tar dyes, according to standard method recommended by ICDRG (Fregert and Bandmann, 1975). 28 subjects were patch-tested with PR53:1.	**17.9 %** (5/28) positive reactions (4 weak reactions, 1 moderate reactions) **Positive** **High frequency** Previous exposure to PR53:1 not documented, no sub-categorisation possible	Sugai et al., 1977

Human data on skin sensitisation				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
Patch test data Consecutive dermatitis patients Short communication	D&C Red No 9 Barium Lake 30 % in olive oil	12/1979-01/1980, 50 patients with eczema were tested with PR53:1 as part of the routine series, by the North American Contact Dermatitis Research Group	0/50 positive reactions **Negative**	Mitchell et al., 1982

Human data on the skin sensitising potential of PR53:1 comprise two diagnostic patch tests from dermatological clinics. Patch testing with PR53:1 (5%) in patients with pigmented cosmetic dermatitis resulted in a high frequency (17.9%) of positive reactions (Sugai et al., 1977). Consecutive dermatitis patients patch-tested to a 30% solution did not show positive patch test reactions (Mitchell et al., 1982).

7.9.3.3. Conclusion

The lead registrant submitted a read-across for an LLNA performed according to OECD TG 429 with an analogue (source) substance Pigment Red 49:2 (CAS RN 1103-39-5). However, the read-across justification is considered as insufficient by the eMSCA. Furthermore, a negative skin sensitisation potential of PR53:1 in a guinea pig maximisation test was cited in the literature, but this data is of low reliability due to the lack of main study information. Furthermore, human data reveal that PR53:1 elicited skin sensitisation at a relatively high frequency in patients with pigmented cosmetic dermatitis. Consecutive dermatitis patients did not show skin sensitisation after patch-testing to PR53:1. Positive patch test in selected dermatitis patents support that PR53:1 acts as a skin sensitiser. However, positive patch test data were collected in just one dermatological clinic. Altogether, the available data do not allow to evaluate, if PR53:1 has a skin sensitisation potential. Thus, an information need is identified for the endpoint skin sensitisation.

As this information need is subject to the standard testing scheme of REACH the generation of new information will not be requested under substance evaluation and are to be addressed in a compliance check.

7.9.4. Repeated dose toxicity

7.9.4.1. Repeated dose toxicity: oral

Animal (rodent) studies

Table 11

Repeated dose toxicity data (oral route) for PR53:1				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
Combined repeated dose and carcinogenicity - **Similar to OECD TG 453** No GLP	D & C Red No. 9 Lot #AA-3779	**Reliable with restriction** **Key study** No data on clinical biochemistry of plasma or serum	<u>Haemoglobin:</u> Significant decrease in high dose females (-11.4% vs. control) at 18 months, in high dose males at 6 months (-7.2%) <u>Haematocrit:</u>	TL, 1981

Repeated dose toxicity data (oral route) for PR53:1				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
Exposure for 18 months / 105 weeks (daily) Mouse, CD-1 (N=60/sex/dose) Dose: 50, 250 and 1 000 ppm corr. to: 7, 38, 147 mg/kg bw in males; 12, 56, 237 mg/kg bw in females Oral, diet Haematology at 3, 6, 12, and 18 months (N=10/sex/dose)	Purity: Not less than 76 %	No data collected for oestrus cycle or sperm parameters No urine analysis	Significant decrease in low dose (-8.1 % vs. control) and high dose females (-9.9 %) at 18 month, decrease (but not significant) at mid dose, (-5.1 %) <u>Red blood cell count:</u> Statistically increase for high dose females (14.8 % vs. control) at 3 month; significant decrease (-10.7 %) after 18 month Gross and histopathologic evaluation did not reveal any compound related findings.	
2-year feeding study - **According to OECD TG 451** (NTP guideline including single dose, 2-week and 13-week studies) No GLP Rats, F344 (N=50/sex/dose) Mice, B6C3F1 (N=50/sex/dose) Dose rats: 0, 1000, 3000 ppm Dose mice: 0, 1000, 2000 ppm Oral, diet Treatment time: 103 weeks, daily Post exposure period: 1 week	D & C Red No. 9 Lot No. Z-8054 Purity: 89.8%, impurities sodium and barium sulfates	**Reliable without restriction** Dose level selected based on effects observed in 91 day study	**Rat:** Conversion factor 20 (older rat): 50, 150 mg/kg bw <u>Splenic lesions in males, at 150 mg/kg bw:</u> 14/48, congestion of the splenic parenchyma, 23/48, focal or multifocal areas of fibrosis, 3/48, diffuse fibrosis, 13/48, areas of fatty metamorphosis in the spleen; <u>Splenic lesions in females, at 150 mg/kg bw:</u> 25/50, multifocal, diffuse, or focal fibrosis Areas of fibrosis present in 2/50 control male rats Increased incidence of testis/tubule degeneration: 10 % (5/50) at 50 mg/kg bw, (23 %) 11/48) at 150 mg/kg bw**,** compared to 6 % (3/50) in controls **Mouse**: Conversion factor 7 : 143, 286 mg/kg bw No non-neoplastic findings in treated mice	NTP, 1982
Range finding study for carcinogenicity study (13 week study) - **Non-guideline study** No GLP Rat, F344, N=20 (10/sex/dose);	D & C Red No. 9 Lot No. Z-8054 Purity: 89.8%, impurities sodium and	**Supporting study** **Reliable with restriction** No data on haematology	**Rat**: Conversion factor 20 (older rat): 150, 300, 625, 1 250, 2 500 mg/kg bw <u>Hemosiderosis of the liver</u>: in all dosed female rats and in 9/10 males at 625 mg/kg bw,	NTP, 1982

Repeated dose toxicity data (oral route) for PR53:1				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
B6C3F1 mice, N=20 (10/sex/dose) Dose rats: 0, 3000, 6000, 12500, 25000, or 50000 ppm; Mice: 0, 600, 1250, 2500, 5000 or 10000 ppm Oral, diet Treatment time: 91 d Gross necropsy of all animals, histopathology of certain tissues from controls and highest dose animals	barium sulfates	No data on clinical biochemistry No data on urinalysis	6/10 at 300 mg/kg bw, and 3/10 at 150 mg/kg bw Pigment deposition in kidney tubular epithelium in all dosed rats Enlarged spleen (2-5 times) in all dosed rats; congestion and lymphoreticular hyperplasia in spleens of all dosed female rats, in all male rats ≥300 mg/kg bw, and in 8/10 male rats at lowest dose (150 mg/kg bw) Lymphoreticular hyperplasia of thymic lymph nodes in 75 %-100 % of female rats in each dosed group (except for lowest dose, 150 mg/kg bw; 0/10), in 70 %-100 % of male rats in each dosed group (except for highest dose; 3/7) **Mouse**: Conversion factor 7 : 86, 179, 357, 714, and 1 429 mg/kg bw Congestion of the spleen: in 55/60 mice at ≥357 mg/kg bw; Deposits of hemosiderin were present to a greater extent in all dosed animals than in controls with exception of females at 86 or 179 mg/kg bw and males at 86 mg/kg bw	
30-month chronic toxicity and potential carcinogenicity study in rats with *in utero* and lifetime exposure - **According to FDA guidelines** Pre-GLP Rat, CD [CRL:COBS CD (SD) BR], F1: N=70/sex/dose Dose: 100, 200, and 500 ppm corr. to: 8, 17, 43 mg/kg bw in F0 males; 9, 17, 42 mg/kg bw in F0 females; 5, 10, 26 mg/kg bw in F1 males; 6, 13, 32 mg/kg bw F1 females	Desert Red D & C Red No. 9 Ba. Lake Batch #547530, C-15-I01 Purity: 76%	**Supporting study** **Reliable with restriction** Individual data e.g. clinical signs missing Not included in registration dossier	Haemoglobin: Significant decrease (-8 %) in F1 high dose females (32 mg/kg) vs. control at month 12; significant decrease (-6%) in F1 mid dose males (10 mg/kg bw) at month 18 Haematocrit: Significant decrease (-6 %) in F1 high dose females (32 mg/kg bw) at month 3 and at month 12 (-6 %) Red blood cell count: Significant decrease (-10 %) in F1 high dose females (32 mg/kg bw) at month 12 Reticulocyte count: Significant increase (92 % and 100 %) in F1 mid (12 mg/kg bw) and	TL, 1982a

Repeated dose toxicity data (oral route) for PR53:1				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
Oral, diet Treatment time: 8 weeks prior to mating, mating, gestation, and lactation; females were allowed to litter and raise their pups until weaning; F1 generation rats exposed for 30 months after weaning Examination F0: mortality, body weights, food consumption, clinical signs Examination F1: mortality, body weight, food consumption, general physical appearance, signs of toxicity, haematology, clinical chemistry, urinalysis, organ weight, organ weight-body weight percentage, gross necropsy, and histopathological data Clinical pathology at months 3, 6, 12, 18, and 24 (N=10/sex/dose)			high (32 mg/kg bw) dose females at month 18 Spleen weight (20.9 %) and spleen weight-body weight percentages (22.5 %) significantly increased in F1 high dose females at month 12; spleen weight of high dose males increased, but not significantly Spleen weight and spleen weight-body weight percentages values for F1 high dose of both sexes were elevated compared to combined control (control 1 plus control 2), but not statistically significant at 30 month; high mean value of spleen weight and spleen weight-body weight percentages for control 1 males at 30 months terminal kill were due to an extremely enlarged spleen in one individual; Hemosiderosis of the spleen: F1 high dose females after 12 month	
30-month chronic toxicity and potential carcinogenicity study in rats with *in utero* and lifetime exposure - **According to FDA guidelines** Pre-GLP Rat, CD [CRL:COBS CD (SD) BR], F1: N=70/sex/dose Dose: 10 000 ppm corr. to: 790 mg/kg bw for F0 males, 894 mg/kg bw for F0 females No data available for F1 males (calculated: 500 mg/kg bw); 521 mg/kg bw for F1 females Oral, diet	D & C Red No. 9 Batch #547530 Purity: 76%	**Supporting study** **Reliable with restriction** Individual data e.g. clinical signs missing Not included in registration dossier	Haemoglobin: Decrease in all treated rats; significant decrease in treated F1 males at month 3, 12, 18, and 24 (-9, -18, -10, -9 %), significant decrease in treated F1 females at 3, 12, 18, and 24 month (-14, -18 %, no further data available), compared to controls Haematocrit: Significant decrease in treated F1 males at 3, 12, 18, and 24 month; -8, -10, -8, -9 %), significant decrease in treated F1 females at 3, 6, 12, 18 and 24 month (-8, -14, -15 %, no further data) compared to controls Red blood cell count: Significant decrease in treated F1 males at month 3, 6, 12,	TL, 1982b

Repeated dose toxicity data (oral route) for PR53:1				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
Treatment time: 9 weeks prior to mating; continued during mating, gestation and lactation; females were allowed to litter and raise their pups until weaning; F1 generation rats exposed for 30 months after weaning Examination F0: mortality, body weights, food consumption, general physical signs, signs of toxicity Examination F1: mortality, body weight, food consumption, general physical signs, signs of toxicity, haematology, clinical chemistry, urinalysis, organ weight, organ weight-body weight percentage, and histopathological data Clinical pathology at months 3, 6, 12, 18, and 24 (N=10/sex/dose)			18, and 24 (-31, -21, -24, -10, -18 %), significant decrease in treated F1 females at 3, 12, 18, and 24 month (-32, -24 %, no further data available) Reticulocyte count: Significant increase in treated F1 males (468, 223, 142, 60, 139 %) and females 526, 127, 99 %, no further data) after 3, 6, 12, 18, and 24 months Spleen weight: Significant increase in treated F1 males (318 %) and females (210 %) at 12 month, and F1 males (183 %) and females (349 %) at terminal kill (month 30); Spleen weight-body weight percentages: Significant increase in treated F1 males (372 %) and females (246 %), compared to control at 12 month, and F1 males (175 %) and females (382 %) at terminal kill Hemosiderin accumulation in liver: in dosed females (unknown incidence) Hemosiderin accumulation in kidneys: in dosed females and males (unknown incidence) Splenomegaly, splenic extramedullary haematopoiesis, splenic congestion, fibrosis, hemosiderosis, mesothelial hyperplasia, mesothelial cyst formation, capsular fibrosis, and multifocal cellular proliferations in the spleenic capsule of dosed rats Statistically significant reduction in F1 mean testis weight (-24 %) and testis weight/body weight percentage (-26.1 %), compared to controls at terminal kill (month 30)	

Repeated dose toxicity data (oral route) for PR53:1				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
32 d feeding study - **Similar to OECD TG 407** Pre-GLP Rat, SPF-Wistar, N=20/dose (10/sex/dose) Dose: 5 %, 1 %, 0.2 %, corr. to 0, 10, 50, 250 mg/kg bw Oral, diet	Test material: Confidential Annex Purity: unknown	**Disregarded study** **Not reliable** Insufficient characterisation of test material No data on clinical biochemistry Main description of test conditions missing	Erythrocytes: Dose-dependent decrease in all treated groups Leucocytes: Dose-dependent increase Heinz bodies in erythrocytes: Increase in high dose group (100 % vs control), mid dose (30 %), and (low dose (10 %) Spleen weight: Significant and dose dependent increase; enlarged and blackish coloured spleen and brownish coloured kidneys in mid and high dose animals Iron storage: Dose dependent increase in liver, kidney tubular epithelium (except for low dose group), moderate to strong increase of iron levels in spleen in all treatment groups	TL 1973
2-year feeding experiment Rat, Osborne-Mendel N= 25/sex/dose Dose level: 1 %, 0.25 %, 0.05 %, 0.01 %, and 0 % Oral, diet From week 103 on, survivors were sacrificed and autopsied; Organ weights: Heart, liver, spleen, kidneys, and testes; Viscera, pituitary, gross lesions, and one hind leg from each rat were fixed for pathologic study; Histologic examination: Heart, lung, liver, spleen, kidney, stomach, intestine, pancreas, pituitary, thyroid, adrenal, bone, and either testis and prostate or ovary and uterus (first six males and first six females) of high dose (1 %) and	D&C Red No. 9 Lot No. G4516 Purity: 86 %	**Disregarded study** **Not assignable** **No study report available** **Paper publication**	Conversion factor 20 (older rats): 500, 125, 25, and 5 mg/kg bw Slight to moderate splenomegaly: In rats of the 1.0 % and 0.25 % (7/12 rats), 0,05 % (4/12), and 0.01 % (2/12) dose group, significant increase in spleen weight/body weight ratio in the 1,0 % and 0.25 % dose group; Splenic infarcts, scars, hemosiderosis, or cysts in high dose group (1 %) Slight haematologic effects (slight lowering of haemoglobin, presence of abnormal circulating red blood cells) noted early in the test, did not increase in severity (no raw data) Bone marrow of 1 % and 0.25 % dose group was slightly hyperplastic, compared to controls Significantly less chronic nephritis in the 1.0 % and 0.25 % dose group	Davis and Fitzhugh, 1962 (publication)

Repeated dose toxicity data (oral route) for PR53:1				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
control group; Bone, spleen, adrenal, pituitary, liver, and kidney sections (first six males and first six females) from 0.25 % and 0.05 % dose groups; Spleen sections (first six males and six females) from the lowest dose group (0.01%)			Light yellow nonferrous, granular pigment in the renal tubular epithelium of all 1 % level rats (N=12), and in the kidneys of the 0.25 % dose group (2/12)	

- In a combined repeated dose and carcinogenicity study performed similar to **OECD TG 453**, mice were fed daily for 18 months with PR53:1 (TL, 1981). Blood parameters were investigated at month 3, 6, 12, and 18. There was a significant decrease in haemoglobin (≥ 10%), haematocrit, and red blood cell count in high dose females (237 mg/kg bw/d) at 18 month. Furthermore, the red blood cell count was statistically increased (≥ 10%) for high dose females at 3. month. Gross and histopathologic evaluation did not reveal any compound related findings in treated mice. Data on clinical biochemistry or urine analysis are missing.
- Furthermore, in a study performed according to **OECD TG 451**, rats and mice were exposed via diet with PR53:1 over a period of two years (NTP, 1982). Non-neoplastic lesions in the spleen, including focal, multifocal, and diffuse fibrosis were significantly increased in high dose rats of both sexes (150 mg/kg bw/d) compared to controls. There was no evidence of treatment-related lesions in mice (highest dose tested: 286 mg/kg bw/d).
- Dose level for the **two-year** feeding study were selected based on effects observed in a 91-day study (NTP, 1982). Dosed rats (150, 300, 625, 1 250, and 2 500 mg/kg bw/d) revealed enlarged spleens and pigment deposition in the renal tubular epithelium. Furthermore, hemosiderosis of the liver was observed in all dosed female rats and with a higher incidence in treated male rats, relative to controls. In mice treated with PR53:1 (86, 179, 357, 714, and 1 429 mg/kg bw/d), congestion of the spleen was observed at dose levels ≥357 mg/kg bw/d. Deposits of hemosiderin were present to a greater extent in the spleen of all dosed mice, compared to controls, with exception of females at 86 (lowest dose) or 179 mg/kg bw/d and lowest-dosed males. There were no data collected on haematology, clinical biochemistry, or urine analysis.
- In the study (TL, 1982a), rats were exposed *in utero* and during lifetime over a period of **30 months** to investigate chronic toxicity and potential carcinogenicity of PR53:1 after feeding dose levels of 5, 10, 26 mg/kg bw in males and 6, 13, 32 mg/kg bw in females. There was a significant change in red blood cell parameters in high dose females, including a decrease in haemoglobin (<10 %), haematocrit (<10 %), and red blood cell count (-10 %), at interim withdrawal and an increase in reticulocyte count (100 %) at final investigations (32 months).
- Exposure of rats with PR53:1 at 500 mg/kg bw in males and 521 mg/kg bw in females in a similar testing design (TL, 1982b) markedly increased the effects seen at lower dose levels. Red blood cell parameter were significantly decreased in dosed males and females (haemoglobin (≥10 %), haematocrit (≤10%), and red blood cell count (≥10 %) at several time points investigated during the study. Treated rats of both sexes showed an increased spleen weight and spleen weight-body weight percentages (>100 %), splenomegaly, splenic extramedullary hematopoiesis, splenic congestion, fibrosis, hemosiderosis, mesothelial hyperplasia, mesothelial cyst formation, capsular fibrosis, and multifocal cellular proliferations in the splenic capsule. Hemosiderosis

accumulation in liver and kidney were found in rats fed with PR53:1 (no data on incidence available).

- A **32-day** feeding study in rats was submitted for PR53:1 (TL 1973). Oral exposure of the test material resulted in a dose-dependent decrease of erythrocytes and increase in leucocytes in treated animals. Furthermore, Heinz bodies in erythrocytes were increased in a dose-dependent manner. There was a significant and dose-dependent increase in the spleen weight accompanied with blackish coloured and enlarged spleens and brownish coloured kidneys in treated rats. Histological evaluation revealed a general disturbance of the iron metabolism with a dose-dependent increase in iron storage in the liver, kidney tubular epithelium, and spleen. However, this study is considered as not reliable, due to the lacking information on the test material (unknown composition and purity) and missing description of the testing method.
- Finally, in a **2-year** study from the literature (Davis and Fitzhugh, 1962), feeding of PR53:1 resulted in slight to moderate splenomegaly in rats at dose levels of 125 and 500 mg/kg bw, respectively. High dosed rats (500 mg/kg bw) showed splenic hemosiderosis and splenic infarcts. Data support effects seen in the studies submitted by the registrant. However there was no study report available to get detailed information on housing and feeding conditions, preparation of the animals and doses, body weight and food consumption, and individual data and analysis are missing.

Human information

No data available.

Conclusion

The lead registrant submitted repeated dose toxicity studies and chronic toxicity/ carcinogenicity studies in mice and rats for exposure of PR53:1 via diet. None of the submitted studies fulfils the standard information requirement of a sub-chronic toxicity study (90-day) according to Annex IX 8.6.2 (including (detailed) clinical observations, body weight and food/water consumption, haematology, clinical biochemistry, urinalysis, and gross pathology/histopathology). However, the submitted studies report data on haematology and pathology/histopathology revealing adverse effects in animals treated with PR53:1.

There is also consistent evidence from several studies that PR53:1 induced haematolytic anaemia in mice and rats, including a decrease in blood parameters (e.g. haemoglobin, haematocrit, and red blood cell count) accompanied with hemosiderosis of the spleen. Effects appeared less severe in mice compared to rats. Chronic exposure of PR53:1 in rats resulted in neoplastic lesions of the spleen (see 7.9.6) accompanied by increased incidences of diffuse/multifocal splenic and capsular fibroses and hemosiderin deposition in spleen, liver and kidneys. However, the dose at which the non-neoplastic effects occurred are not sufficiently low in comparison to the CLP guidance values to allow classification for specific organ toxicity from repeated exposure (STOT-RE). Conducting a sub-chronic study to fill the data gap for clinical biochemistry and urinalysis may strengthen the presence of the current effects (e.g. on haemolytic anaemia). However, it is questionable whether significant additional information (expected e.g. on bilirubin content and excretion) will result in a different outcome with regard to the major toxicity or target organs.

Although none of the available studies were performed according to OECD TG 408/407 and key parameters are missing in the single studies, in a weight of evidence considering the available chronic toxicity studies, the available data on repeated dose toxicity are considered adequate for evaluation of this endpoint assuming that the used test materials are representative for the registered substance. No further action is recommended.

7.9.4.2. Repeated dose toxicity: inhalation

No repeated dose toxicity study via inhalation is available. The lead registrant justifies waiving of a corresponding study by investigations of the static and dynamic dissolution in phagolysosomal simulant fluid as well as surface reactivity (two studies in abiotic condition

and one *in vitro* study) of PR53:1. The lead registrant concluded that PR53:1 is of low toxicity after respiratory exposure. However, these non-guideline, *in vitro* investigations are not sufficient for waiving the repeated dose toxicity study via inhalation.

It is noted that the TEM images provided by the lead registrant show elongated, needle-like particles. Considering this morphology and in combination with the crystallinity and possibly low solubility of PR53:1, cellular toxicity and/or injury in the lung and pleura cannot be excluded.

Conclusion

The eMSCA concludes that the provided information is insufficient to allow a decision on repeated dose toxicity via inhalation. According to REACH Annex IX (column 2) testing by inhalation route is appropriate if exposure of humans via inhalation is likely taking into account the vapour pressure of the substance and/or the possibility of exposure to aerosols, particles or droplets of an inhalable size. As reported by the lead registrant, human exposure to dust of PR53:1 is considered as the most critical scenario. Therefore, based on ECHA's feedback, **the eMSCA proposes to address this data gap as part of a compliance check when the substance identity is clarified regarding the nanoform**. The eMSCA notes that the high concentration (approximately 5 mg/L) in the acute toxicity study after inhalation exposure should be considered when determining the maximum tolerated dose in a repeated dose toxicity study via inhalation.

7.9.5. Mutagenicity

7.9.5.1. In vitro data

Only studies performed with the Substance (registered) and using a relevant *in vitro* genotoxicity test system (see Table R.7.7-2 in Chapter R.7a of the REACH endpoint specific guidance) are included in Table 12.

Table 12

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 - Similar to OECD TG 471 **(Prival activation and classical test protocol with S9)** Deviations: • 5th strain missing GLP: yes	**PR 53:1** CAS 5160-02-1 Purity: see confidential annex	**Supporting study** **Reliable with restrictions** (5th strain is missing, results for TA100, TA98, TA1537, TA1535 are reliable without restrictions) - (Registrant ev.: reliable with restriction) Bacterial strains: *Salmonella typhimurium* tester strains: TA100, TA98, TA1537, TA1535 Test concentrations (with and without metabolic activation (S9	**Negative** with (hamster and rat S9) and without metabolic activation No significant increase in the number of revertants in any bacterial strains with and without Prival with and without metabolic activation. Cytotoxicity: no Precipitations: ≥ 500 µg/plate Neg. control: valid Pos. control: valid	TL 1989a

Summary table of mutagenicity/genotoxicity tests in vitro				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
		mix)): 4, 20, 100, 500, 2500, 5000 µg/plate S9: hamster liver S9, untreated and rat liver S9 Aroclor induced Vehicle: DMSO Negative control: yes Positive control: yes		
Bacterial Reverse Mutation Test - Similar to OECD TG 471 (**Prival activation and classical test protocol with S9)** Deviations: • 5th strain missing GLP: yes	**PR 53:1** CAS 5160-02-1 Purity: technical pure	**Supporting study** **Reliable with restrictions** (5th strain is missing results for TA100, TA98, TA1537, TA1535 are reliable without restrictions) - (Registrant ev.:reliable with restriction) Bacterial strains: *Salmonella typhimurium* tester strains: TA100, TA98, TA1537, TA1535 Test concentrations (with and without metabolic activation (S9 mix)): 4, 20, 100, 500, 2500, 5000/10000 µg/plate S9: hamster liver (Prival activation) S9: untreated and rat liver S9 Aroclor induced (classical test protocol) Vehicle: DMSO Negative control: yes Positive control: yes	**Negative** with (hamster and rat S9) and without metabolic activation Cytotoxicity: no Precipitations: yes, ≥ 100 µg/plate Neg. control: valid Pos. control: valid	TL 1985a
Bacterial Reverse Mutation Test - Similar OECD	**PR 53:1** CAS 5160-02-1	**Key study** **Reliable without restriction**	**Negative** with and without metabolic activation	TL 1985b

Summary table of mutagenicity/genotoxicity tests in vitro				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
TG 471 Deviations: none GLP: yes	Purity: see confidential annex	(Registrant ev.:disregarded study, not reliable) Bacterial strains: *Salmonella typhimurium* tester strains: TA100, TA98, TA1537, TA1535, TA 1538, *Escherichia coli* WP2uvrA Test concentrations (with and without metabolic activation (S9 mix)): see confidential annex, guideline conform S9: see confidential annex Vehicle: see confidential annex Negative control: yes Positive control: yes	Cytotoxicity: no Precipitations: see confidential annex Neg. control: valid Pos. control: valid	
Bacterial Reverse Mutation Test - Similar to OECD TG 471 (without Prival activation) Deviations: • No verification of negative result • Only three strains tested (e.g. noTA 1535, E.coli WP2 missing) • No data on purity GLP: no	**PR53:1** CAS 5160-02-1 Purity: no data	**Supporting study** **Reliable with restrictions** (only three strains tested, no verification of negative result) (Registrant ev.: supporting study, not assignable) Bacterial strains: *Salmonella typhimurium* tester strains: TA100, TA98, TA1537 Test concentrations (with and without metabolic activation (S9 mix)): 20, 78, 313, 1250,5000 µg/plate S9: rat liver, Aroclor induced	**Negative** with and without metabolic activation Cytotoxicity: no Precipitations: from 313 µg/plate onward Neg. control: valid Pos. control: valid	TL 1985c

Summary table of mutagenicity/genotoxicity tests in vitro				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
		Vehicle: DMSO Negative control: yes Positive control: yes		
Bacterial Reverse Mutation Test - Similar to OECD TG 471 Deviations: • Documetation insufficient • Purity insufficient • Data on 5th strain missing • Low max. concentration • Only 3 concentrations tested • No detailed data on results (data table) GLP: no	**PR53:1** Purity: 33-73%	**Disregarded** **Not assignable (insufficient documentation and methodical deficiencies)** (Registrant ev.: supporting study, not assignable) Bacterial strains: *Salmonella typhimurium* tester strains: TA100, TA98, TA1537, TA1535, TA1538 Test concentrations (with and without metabolic activation (S9 mix)):50, 100, 500 plate S9: rat liver Aroclor induced Vehicle: DMSO Negative control: yes Positive control: yes	**Negative** with and without metabolic activation Cytotoxicit: no data Precipitations: no data Controls: Neg. control: valid Pos. control: valid	Brown et al., 1979
Bacterial Reverse Mutation Test - Similar to OECD TG 471 (**Prival activation and without Prival)** Deviations: • No detailed data on results (data table) • 5th strain (e.g. E.coli WP2) missing • No information on purity • Cytotoxicity not determined GLP: not specified	D&C Red No 9 (PR53:1) CAS 5160-02-1 EC 225-935-3 Purity: unknown	**Disregarded study** **Not assignable** (detailed result data missing to evaluate relevance of ambiguous result) (Registrant ev.: supporting study, reliable with restriction) Bacterial strains: *Salmonella typhimurium* tester strains: TA100, TA98, TA1537, TA1535, TA97	**Ambiguous** (with and without metabolic activation) -ambiguous for TA97 without S9 and for TA98 with and without S9 Cytotoxicity: not determined Precipitations: ≥ 100 µg/plate Controls: Neg. control: valid Pos. control: valid	Zeiger et al., 1988

Summary table of mutagenicity/genotoxicity tests in vitro				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
		Test concentrations (with and without metabolic activation (S9 mix)): 100, 333, 1000, 3333, 10000 µg/plate S9: hamster liver S9, untreated and rat liver S9 Aroclor induced Vehicle: DMSO Negative control: yes Positive control: yes		
In vitro Mammalian Cell Gene Mutation tests using the Thymidine Kinase Gene - similar to OECD TG 490 Deviation: • No data on purity GLP: not specified	PR 53:1 CAS Nr.: 5160-02-1 Purity: no data	**Key Study** **Reliable with restriction -** (Registrant ev.: key study, reliable without restriction) Cell culture: mouse lymphoma L5178Y cells Test concentrations without metabolic activation: 1.25, 2.5, 5, 7.5, 15 µg/ml with metabolic activation: 2,3,4,5,6 µg/ml Justification for top concentration: solubility (about 7.5 µg/ml) Metabolic activation system: rat liver S9 induced by Aroclor Treatment time(s): 4 h Sampling time(s): after 2 days Vehicle: DMSO Negative control: yes	**Negative** (with and without metabolic activation) Cytotoxicity: no Precipitations: yes, above 7.5 µg/ml Neg. control: valid Pos. control: valid	Myhr et al., 1991

Summary table of mutagenicity/genotoxicity tests in vitro				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
		Positive control: yes		
In vitro mammalian chromosomal aberration test - similar to OECD TG 473 Deviations: • Only 100 metaphases scored per concentration • No data on purity GLP: yes	PR53:1 CAS 5160-02-1 Purity: no data	**Key study** **Reliable with restriction** (only 100 metaphases scored per concentration) (Registrant ev.: key study, reliable with restrictions) Cell culture: Chinese hamster lung fibroblasts (V79) Test concentrations: With and without metabolic activation (S9 mix): 30,150,300 µg/ml metabolic activation: rat liver S9 induced by Aroclor Justification for top concentration: significant cytotoxic ≥ 400 µg/ml Treatment time: With and without metabolic activation: 4 and 18 h Sampling time: 4.5, 15.5, 25.5 h after beginning of treatment Vehicle: DMSO Negative control: yes Positive control: yes	**Negative** with and without metabolic activation Cytotoxicity: significant cytotoxic ≥ 400 µg/ml Precipitations: yes, ≥ 500 µg/ml Controls Neg. control: valid Pos. control: valid	TL 1989b
In vitro mammalian chromosomal aberration test - Not similar to OECD	D&C Red No 9 CAS 5160-02-1	**Disregarded Study** **Not reliable** **(exposure and**	**Negative** with and without metabolic activation Cytotoxicity: no detailed	Ivett et al., 1989

Summary table of mutagenicity/genotoxicity tests in vitro				
Method, guideline, deviations if any	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
TG 473 Deviation from OECD TG 473 • Continuous exposure of about 12-14h without metabolic activation missing • short term treatment with and without metabolic activation not adequate (8h and 2h instead of 3-6 h) • Sampling time too short (2-2.5h instead of 1.5 normal cell cycle length) • Only 200 (instead of 300) metaphases evaluated • No specific data on justification for top dose GLP: not specified	EC 225-935-3 Purity: 89.8%	**sampling times are not according to OECD TG, too less cells analysed)** (Registrant ev.: key study, reliable without restriction) Cell culture: CHO Test concentrations: without metabolic activation (S9 mix): 37.1, 50, 123.8 µg/ml with metabolic activation (S9 mix): 5, 16.7, 50 µg/ml metabolic activation: rat liver S9 induced by aroclor Justification for top concentration: no specific data Treatment time: without metabolic activation: 8h with metabolic activation: 2h and 8h Sampling time: 2-2.5h Vehicle: DMSO Negative control: yes Positive control: yes	data Precipitations: yes, ≥ 250 µg/ml Controls Neg. control: valid Pos. control: valid	

7.9.5.2. In vivo data

Only studies performed with the registered substance and using a relevant *in vivo* genotoxicity test system (see Table R.7.7-3 and R.7.7-4 in Chapter R.7a of the REACH endpoint specific guidance) are included in Table 13.

Table 13

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**
Unscheduled DNA Synthesis (UDS) test with mammalian liver cells in vivo - Similar to OECD TG 486 Deviation: none GLP: not specified	PR53:1 CAS 5160-02-1 EC 225-935-3 Purity: no data	**Supporting study*** **reliable without restrictions*** Species: rats Piebald Virol Glaxo Number of animals per group: 7 males Target organs: liver Administration route: oral (gavage) Dose level: 1000 and 2000 mg/kg bw Justification for top dose: limit test Treatment: single dosage Sampling: **16 h** Vehicle: Corn Oil Positive control: yes (2-Acetylamino-fluorene for 16-h,) 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).** **Results:** • No marked increase in incidence of cells in repair 16 h sampling time Toxicity: no toxicity observed Controls: Neg. control: valid Pos. control: valid	(Westmoreland and Gatehouse, 1992
Mammalian erythrocyte micronucleus test Similar to OECD TG 474 Deviation: • No evidence of exposure of bone marrow GLP: not specified	PR53:1 CAS 5160-02-1 EC 225-935-3 Purity: no data	**Supporting study** **Not reliable (Result not reliable as no evidence of exposure of bone marrow shown)** Species: rats Piebald Virol Glaxo Number of animals per group: 7 males Target organs: bone marrow Administration route: oral (gavage) Dose level: 500,1000 and 2000 mg/kg bw Justification for top dose: limit test	**Negative** **Results:** • No increase in the frequency of micronuclei Toxicity: no toxicity observed **Evidence of exposure of bone marrow: no, as ratio PCE/NCE not decreased, no other evidences** Controls: Neg. control: valid Pos. control: valid	Westmoreland and Gatehouse, 1992

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**
		Treatment: single dosage Sampling: **24 or 48h** Vehicle: Corn Oil Positive control: yes (cyclophosphamide, 24h) Negative control: yes		

7.9.5.3. Human information

No information available.

7.9.5.4. Summary and discussion of genotoxicity

***In vitro* data**

Bacterial reverse mutation tests

Six bacterial reverse mutation tests performed with PR53:1 are available in the registration dossier (c.f. Table 12).

- Four of these studies were considered as reliable by eMSCA (TL, 1985c; TL, 1985a; TL, 1985b; TL, 1989a). In those studies negative results were obtained for the strains *S. typhimurium* TA100, TA98, TA1537 and TA1535 with and without metabolic activation and both with the classical test protocol with S9 obtained from Aroclor induced rat liver and with Prival activation. As PR53:1 is an azo-dye the using of a reductive metabolic activation system (Prival activation) is considered more appropriate than the classical test protocol (see section 16 of OECD TG 471). For the 5th strain (*E.coli* WP2 uvrA) negative results were obtained with and without metabolic activation using the classical test protocol. **Test results for the 5th strain using Prival activation are not present in any bacterial reverse mutation test conducted with the registered substance available in the registration dossier.**
- In addition, there is one reliable bacterial reverse mutation test available in the registration dossier (TL, 2012b) which was not performed with the registered substance but with an analogue substance, **calcium bis[2-[(2-hydroxynaphthyl)azo]naphthalenesul-phonate]** (EC number 214-161-1, CAS RN 1103-39-5) and using Prival activation. The registrants provided a read-across justification. However, the eMSCA considers that too less genotoxicity data are available for the source substance calcium bis[2-[(2-hydroxynaphthyl)azo]-naphthalenesulphonate] to fill the standard data requirement for the 5th strain of a bacterial reverse mutation test (using Prival activation) for PR53:1.

In vitro mammalian gene mutation tests

One *in vitro* mammalian gene mutation test is available in the registration dossier performed with the Substance and similar to OECD TG 490 in (Myhr et al., 1991), and the test yielded a negative result. This test is considered reliable by eMSCA.

In vitro mammalian cytogenicity tests

Only one of the two available *in vitro* cytogenicity tests performed with the Substance and similar to OECD TG 473 (TL, 1989b), is considered reliable by eMSCA, and yielded a negative result.

Overall, the available *in vitro* data do not indicate a concern for mutagenic action of the registered substance. However, an information need has been identified by the eMSCA for the 5th strain (e.g. *E.coli* WP2uvrA) in a bacterial reverse mutation test using Prival activation as the registered substance is an azo-dye. The 5th strain may detect e.g. oxidising mutagens, cross-linking agents and hydrazines. In addition the 5th strain detects mutations on AT base pairs (while the four standard *S. typhimurium* strains detect mutations at GC base pairs). Thus, only testing of all strains provides full information on the genotoxic mode of action in this test system. Testing of the 5th strain and application of Prival activation for azo-dyes is recommended in OECD TG 471. Therefore, missing data on the 5th strain using Prival activation is considered as a gap in standard data requirement.

In vivo **data**

Two *in vivo* somatic genotoxicity tests performed using a relevant *in vivo* genotoxicity test system are provided in the registration dossier, an unscheduled DNA Synthesis (UDS) test with mammalian liver cells *in vivo* (Westmoreland and Gatehouse, 1992) and a mammalian erythrocyte micronucleus test (MN) (Westmoreland and Gatehouse, 1992).

UDS Test

The UDS test 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. While the test supports the negative results obtained from *in vitro* gene mutation tests available (Ames tests and MLA test), it cannot fulfill the identified data gap for the 5th strain in a Ames test using Prival activation.

MN *in vivo*

The available *in vivo* mammalian erythrocyte micronucleus test was performed similar to OECD TG 474. The test yielded negative results and supports the negative findings obtained in an *in vitro* mammalian chromosomal aberration test (TL, 1989b). However, as no evidence of exposure of the bone marrow has been provided, this *in vivo* micronucleus test alone would not be a proof that the substance does not induce clastogenic effects. Overall, there is no standard data gap for cytogenic effects.

Summarising, the available *in vivo* data support the negative findings of the *in vitro* studies but they are not sufficient to fulfill the data gap for a ‘complete’ Ames test using Prival activation.

Conclusion

The eMSCA supports the Registrant’s view that based on the results of all available relevant and reliable *in vitro* and *in vivo* genotoxicity studies in the registration dossier a concern for mutagenic effects of the registered substance is not indicated.

However, reliable data for the 5th strain in a bacterial reverse mutation assay using Prival activation for the registered substance are missing in the registration dossier. The eMSCA considers that this data gap cannot be filled by the provided ‘complete’ bacterial reverse mutation test with the analogue substance, other *in vitro* tests available or the given *in vivo* studies.

Thus, the eMSCA has identified an information need regarding the 5th strain in a bacterial reverse mutation test using Prival activation. In addition, it has to be noted that according to ECHA Guidance (2017), the Ames test (OECD TG 471) is not recommended for the investigation of the genotoxicity of nanomaterials.

As this information need is subject to the standard testing scheme of REACH the generation of new information will not be requested under substance evaluation but should be addressed in a compliance check.

7.9.6. Carcinogenicity

7.9.6.1. Non-human information

The carcinogenic potential of PR53:1 has been investigated in several feeding and skin painting studies in rats and mice.

One of these studies was performed by the US National Toxicology Program (NTP, 1982). The NTP studies were published in 1982 in the technical report 225. The report consists of single-day dosing, 2-week and 13-week sub-chronic toxicity studies and 2-year carcinogenesis studies with rats and mice of both sexes. Single-day dosing, 2-week and 13-week sub-chronic toxicity studies were performed with 5 animals/sex/group and were used as range-finding studies.

The authors of the NTP carcinogenesis studies in rats and mice concluded that PR53:1 "*was carcinogenic for male F344 rats causing an increased incidence of sarcomas of the spleen and a dose related increase in neoplastic nodules of the liver. PR53:1 was not considered to be carcinogenic to female F344 rats, although the increased incidence of neoplastic nodules of the liver may have been associated with administration of the test chemical.*" *"No evidence of carcinogenic activity of PR53:1 in B6C3F1 mice of either sex."*

There are further studies evaluating the carcinogenic potential of PR53:1 including feeding studies and a skin painting study (Carson, 1984; TL, 1981; TL, 1982b; TL, 1982a; Davis and Fitzhugh, 1962). These studies were also reviewed by the (Food and Drug Administration (FDA), 1986) and (Hart et al., 1986). The TL 1981, 1982a and 1982b studies were not considered by the registrants for the endpoint carcinogenicity.

Table 14

Relevant studies related to the assessment of carcinogenicity for PR53:1			
Methods	**Results**	**Remarks**	**Reference**
2-year feeding study in rats D&C Red No. 9 (known trading name of PR53:1) (CAS 5160-02-1) Purity: 89.8%, impurities sodium and barium sulfates According to OECD TG 451 (NTP guideline) No GLP Species: rats Strain: F344 n: 50/dose group/sex Dose levels: 0, 0.1%, 0.3% (0, 1000, 3000 ppm)	**Carcinogenic for male F344 rats causing increased incidence of sarcoma of the spleen and dose-related increase in neoplastic nodules of the liver, not carcinogenic for female F344 rats** Neoplastic lesions: Male rats: Combined types of splenic sarcoma (0/50, 0/50, 26/48) including fibrosarcoma (17/48) arising from red pulp or capsule of the spleen, 1 animal with leiomyosarcoma, 5 splenic osteosarcoma, 11 of splenic tumours metastasised to peritoneal tissues, 2 sarcoma of multiple organs originated in the spleen, 1 sarcoma and 1 fibrosarcoma of the splenic capsule, 1 fibrosarcoma of the splenic red pulp Neoplastic nodules of the liver: Males: 0/50, 6/50, 7/49 (hepatocytes with basophilic or	Food conversion factor: 20 (for older rats) Calculated doses: 0, 50, 150 mg/kg bw/d Reliable without restrictions	NTP, 1982

Relevant studies related to the assessment of carcinogenicity for PR53:1			
Methods	**Results**	**Remarks**	**Reference**
Route: oral (feed) Treatment time: 103 weeks, daily Post exposure period: 1 week Dose level selected based on effects observed in 91 day study	eosinophilc cytoplasm) Hepatocellular carcinoma in 1/50 control male Female rats: Neoplastic nodules of the liver 1/50, 1/50, 5/50 Non-neoplastic lesions: Males at 3000 ppm 14/48: congestion of splenic parenchyma; 23/48 focal or multifocal areas of fibrosis; 3/48 diffuse fibrosis; 13/48 areas of fatty metamorphosis in the spleen Areas of fibrosis in 2 control males Females: 25/50 multifocal, diffuse or focal fibrosis of the spleen Survival: no effects on mortality, body weight and food consumption; 6% weight depression in high dose females		
2-year feeding study in mice D&C Red No. 9 (known trading name of PR53:1) (CAS 5160-02-1) Purity: 89.8%, impurities sodium and barium sulfates According to OECD TG 451 (NTP guideline including single dose, 2-week and 13-week studies) No GLP Species: mice Strain: B6C3F1 n: 50/dose group/sex Dose levels: 0, 0.1%, 0.2% (0, 1000, 2000 ppm) Route: oral (feed) Treatment time: 103 weeks, daily Post exposure period: 1 week	**Not carcinogenic for B6C3F1 mice** Neoplastic lesions: Males: statistically significant increased incidence of hepatocellular carcinoma (4/50, 9/50 (18%), 11/50 (22%)) ↑, but not above mean historical incidence in this laboratory (65/297 – 22%) Females: malignant lymphomas of the hematopoietic system (2/50, 2/50, 7/49) increased incidence Survival: No effect on mortality, body weight and food consumption, except mean body weight of treated females slightly lower in 2nd year (< 10%)	Food conversion factor: 7 (for mice) Calculated doses: 0, 142, 285 mg/kg bw/d Reliable without restrictions	NTP, 1982

Relevant studies related to the assessment of carcinogenicity for PR53:1			
Methods	**Results**	**Remarks**	**Reference**
Dose level selected based on effects observed in 91 day study			
26-30 month dietary study (F0 and F1 dosed) including *in utero* exposure D&C Red No. 9 (known trading name of PR53:1)(CAS 5160-02-1) Purity: 76% According to FDA guidelines No GLP Species: rat Strain: Charles-River CD Sprague-Dawley n: 70/dose group/sex Dose levels: **Part I:** 0, 0.01%, 0.02%, 0.05% **Part II:** 0 and 1% of the diet (additional study with higher concentration) Route: oral (feed) Treatment time: 30 months, daily Data of the two studies were combined	**Increased incidence of splenic sarcoma in rats** Neoplastic lesions: 2 haemangiosarcoma involving spleen and/or liver in control males Splenic sarcoma in 4 males and 1 female at 1% Highly unusual mesenchymal neoplasms of the spleen at 1% in F1 animals, not statistically significant Survival: No effects on mortality, body weight, food consumption in parental animals or offspring At 1% body weight of male and female pups decreased at weaning (day 21 postpartum) and lower body weights throughout chronic phase (<10%) Clinical findings of toxicity Signs of anaemia at 1% in males and females Increased spleen weight in males and females (1%), increased heart weight; increased kidney weight in females and increased testicular weight Males: splenic lesions at 1.0% in males: splenic congestion, fibrosis, mesothelial hyperplasia, mesothelial cysts, hemosiderosis and splenic hematopoiesis	Study according to FDA guidelines including in utero treatment and F1 generation Food conversion factor: 20 (for older rats) Calculated doses: Part I: 0, 5, 10, 25 mg/kg bw/d Part II: 0 and 500 mg/kg bw/d Reliable with restrictions	TL, 1982b; TL, 1982a Cited in (Food and Drug Administration (FDA), 1986) Study not in dossier
Combined repeated dose and carcinogenicity - Similar to OECD TG 453 D&C Red No. 9 (known trading name of PR53:1) (CAS 5160-02-1) Purity: 76% (according to FDA report) No GLP Species: mice	**Not carcinogenic in mice** Survival: No effects on mortality, body weight, food consumption in parental animals or offspring Clinical findings of toxicity: Signs of anaemia at 0.1% in females (decreased red blood cells, increased reticulocytes, decreased haemoglobin and haematocrit), anaemia not evident at 0.2% Decreased kidney weight in male mice (0.2%)	Food conversion factor: 7 (for mice) Calculated doses: Part I: 0, 7, 35, 142 mg/kg bw/d Reliable with restrictions	TL, 1981 Cited in (Food and Drug Administration (FDA), 1986) Study not in dossier

Relevant studies related to the assessment of carcinogenicity for PR53:1			
Methods	**Results**	**Remarks**	**Reference**
Strain: Charles-River CD1 n: 60/dose group/sex Dose levels: 0, 50, 250 and 1 000 ppm Route: oral (feed) Treatment time: 18 month / 105 weeks (daily)			
2-year feeding study in rats - Non-guideline study D&C Red No. 9 (known trading name of PR53:1) (CAS 5160-02-1) Purity: 86% No GLP Species: rats Strain: Osborne-Mendel n: 25/dose group/sex Dose levels: 0, 0.01%, 0.05%, 0.25%, 1% (0, 100, 500, 2500, 10000 ppm) Route: oral (feed) Treatment time: 103 weeks, daily Post exposure period: 10 days Vehicle: ethanol	**No increased evidence for carcinogenicity but severe splenic effects** No effects on mortality At 1%: moderate splenomegaly, splenic infarcts, haematomas or scars (6 rats), splenic hemosiderosis ≥0.25%: slight bone marrow hyperplasia, decreased haemoglobin, abnormal red blood cells ≥0.01%: Slight to moderate splenomegaly (2/12 at 0.01%, 4/12 at 0.05%, 7/12 at 0.25%)	Food conversion factor: 20 (for older rats) Calculated doses: 0, 5, 25, 125, 500 mg/kg bw/d Limited reporting No data on individual animals Only 6 animals from each group examined histopathologically Incidences only on a limited number of findings, no body weight information No historical control data Not reliable	Davis and Fitzhugh, 1962 (publication)
18-month skin painting study - Non-guideline study D&C Red No. 9 (known trading name of PR53:1) (CAS 5160-02-1) Purity: 90% No GLP Species: mice Strain: 100 ICR	**No increase in neoplasia after dermal application of the test dye compound** Single incidences of mammary gland adenosarcoma (2 female); hepatic cell carcinoma (1 male/ 1 male in control), reticulum cell sarcoma (1 male) No effect on survival compared to control Dermal application of 1 mg per mouse twice per week for 18 months	Limited reporting No data on individual animals Limited number of organs analysed Only selected animals from solvent and positive control Study period 18 month	Carson, 1984 (publication)

Relevant studies related to the assessment of carcinogenicity for PR53:1			
Methods	**Results**	**Remarks**	**Reference**
n: 50/dose group/sex, 150 in control group Dose levels: dermal application to dorsal area; 0.1 ml of 1% solution of dye (6cm^2) twice a week for 18 month (mean total dose of applied material 134.7 mg) Route: dermal Treatment time: 483 days Vehicle: distilled water Positive control: 3,4-benzpyrene in acetone	did not cause skin cancer. Full histopathology of a low number of randomly chosen animals did not give an indication of systemic toxicity or carcinogenicity.	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	

7.9.6.2. Human information

No data available.

7.9.6.3. Summary and discussion of carcinogenicity

Several studies allow to assess the carcinogenic potential of PR53:1 (Carson, 1984; TL, 1981; TL, 1982b; TL, 1982a; Davis, 1963; NTP, 1982).

- Two 2-year feeding studies in rats and mice were performed by NTP (1982) equivalent to OECD TG 451 (NTP guidelines).
  Groups of 50 male and 50 female F344 rats were administered 0, 0.1% and 0.3% PR53:1 in feed supplied for 103 weeks (calculated dose: 0, 50, 150 mg/kg bw/d). No effects on survival, body weight or food consumption were observed, only high-dose females showed a 6% lower body weight than controls. 26/48 male rats of the high-dose group showed combined types of splenic sarcoma, including fibrosarcoma (17/48) arising from red pulp or capsule of the spleen, 1 animal with leiomyosarcoma, 5 splenic osteosarcoma and 11 of the splenic tumours metastasized to peritoneal tissues. Non-neoplastic splenic lesions were observed in high-dose animals. 14/48 male rats had congestion of splenic parenchyma, 23/48 had focal or multifocal areas of fibrosis, 3/48 diffuse fibrosis and 13/48 had areas of fatty metamorphosis in the spleen. 25/50 high-dose female rats showed multifocal, diffuse or focal fibrosis of the spleen.
  Groups of 50 male and 50 female B6C3F1 mice were administered 0, 0.1% and 0.2% PR53:1 in feed supplied for 103 weeks (calculated dose: 0, 142, 285 mg/kg bw/d). No effects on survival, body weight or food consumption were observed. The incidence of hepatocellular carcinoma in high-dose males was increased but not above the mean historical control incidence. Female mice showed an increased incidence of malignant lymphomas of the hematopoietic system in the high-dose group, which was not statistically significant.
- In the Food and Drug Administration report (FDA, 1986), the following limitations of the data were discussed: the use of solid bottom cages possibly leading to coprophagy and therefore higher doses of the Substance and ingestion of metabolites as well as the presence of other carcinogens in the room as other substances were tested at the same time. However, both points were considered by the eMSCA as not relevant as, firstly, the same type of tumours was detected using wire cages in the TL 1981, 1982a,1982b,

1983 studies and, secondly, no splenic neoplasms were observed with other test substances or in control animals.

- Further long-term feeding studies with lifetime exposure in rats and mice were performed according to FDA guidelines (TL, 1981; TL, 1982b; TL, 1982a).
  70 Sprague-Dawley rats/dose/sex were treated with 0, 0.01%, 0.02% or 0.05% substance in the diet (calculated dose: 0, 5, 10 and 25 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 30 months). 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. There were no effects on survival and food consumption, body weight of male and female pups was slightly decreased from day 21 postpartum. At 500 mg/kg bw/d, splenic sarcoma were observed in 4 males and 1 female.
  Clinical findings of toxicity included signs of anaemia and increased spleen and heart weight in male and female rats of the highest dose group (1 % PR53:1). Males also showed splenic lesions such as splenic congestion, fibrosis, mesothelial hyperplasia, mesothelial cysts, hemosiderosis and splenic haematopoiesis.
  60 mice/dose/sex were exposed to 0, 50, 250 and 1000 ppm of the substance in the diet (calculated dose: 0, 7, 35 and 142 mg/kg bw/d) for 18 months. There were no effects on survival, body weight or food consumption. No neoplastic lesions were observed in mice.
- A study reported by Davis and Fitzhugh (1962) groups of 25 male and 25 female Osborne-Mendel rats were administered 0, 0.01%, 0.05%, 0.25% and 1% PR53:1 in feed supplied for 103 weeks (Calculated doses: 0, 5, 25, 125, 500 mg/kg bw/d). The reporting of the study is very limited. Only 6 animals from each group were examined histopathologically and only a limited number of findings are reported.
  No increased evidence for carcinogenicity was observed but splenic effects such as moderate splenomegaly, splenic infarcts, haematomas or scars and splenic hemosiderosis were reported.
- An 18-month skin painting study was performed in mice in which 14 colour materials were tested (Carson, 1984). 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 134.7 mg PR53:1 (described as D&C Red No. 9 in the publication). 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 PR53:1.
  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).

Furthermore, the NTP (NTP, 1994) conducted a chronic drinking water study in rats and mice using barium chloride dihydrate assessing its carcinogenic potential as is was stated by the authors (NTP, 1982): "*PR53:1 is a barium-containing pigment. Barium and its salts are known to be toxic to muscle and nervous tissue. Although the toxicity of this metal is limited due to the insolubility of barium salts, a potential for barium toxicity must be recognized.*" However, there was no evidence of carcinogenic activity of barium chloride dehydrate under the conditions of this drinking water study in rats and mice.

7.9.6.4. Conclusion

Overall, there is evidence of a carcinogenic potential of PR53:1 based on an increased incidence of splenic sarcomas in male rats, a rare type of tumour in this organ (TL, 1982b; TL, 1982a; NTP, 1982). Although the increased incidence was not statistically significant in the TL 1981, 1982a, 1982b, 1983 studies in contrast to the NTP study, the eMSCA

considers it as supportive evidence as similar patterns of non-neoplastic splenic lesions were observed in both studies.

This is in line with the conclusions by the FDA (1986). The FDA report discussed a common pattern of splenic lesions in the NTP and TL 1981, 1982a, 1982b, 1983 studies including fatty metamorphosis, focal or diffuse splenic fibrosis, unusually severe forms of splenic congestion with or without haemorrhages or infarcts, capsular fibrosis and hyperplasia and the association of these splenic lesions with the occurrence of fibrosarcoma in male rats (TL, 1982b; TL, 1982a; Davis and Fitzhugh, 1962; NTP, 1982). Furthermore, the sensitivity of different rat strains concerning splenic tumours was discussed. The authors suggest that the Sprague-Dawley rats appears to be less sensitive than the F344 rats to aniline related compounds, but still showed the unusual effects described in the study.

There is no evidence of carcinogenicity in mice (TL, 1981; NTP, 1982). However, the following is stated in the NTP report: "*With the possible exception of female mice, all other dosed groups of rats or mice might have tolerated higher doses, thus a clear maximum tolerated dose may not have been utilized in this study.*" Therefore, the carcinogenic potential for female rats and mice of both sexes could be questioned at least in the NTP study.

Taken together, splenic sarcoma are a rare type of tumour in animals and there is supportive evidence showing a similar pattern of non-neoplastic splenic lesions across both sexes in rats and to a lesser extent in mice as possible pre-neoplastic lesions. Similar splenic lesions were described for aniline and other aromatic amines and aromatic azo compounds, structurally related compounds. All of these compounds also showed an increased incidence of splenic sarcoma in F344 rats.

The data available for PR53:1 do not suggest a genotoxic mode of action in tumour formation. Goodman et al. (1984) and Weinberger et al. (1985) described possible modes of action, both discussing splenic lesions as staring point to tumour formation. Goodman et al. (1984) suggested a splenic hemosiderosis secondary to methaemoglobinemia leading to tumours formation whereas Weinberger et al. (1985) suggested acute vascular congestion as the initial alternation in the spleen leading to haemorrhage, fibrosis and transformed cells.

In contrast to the statement of the IARC on the evaluation of carcinogenic risks to humans, Volume 57 (IARC, 1993) where PR53:1 "cannot be classified as to its carcinogenicity to humans (group 3)", the eMSCA is of the opinion that a classification of PR53:1 as carcinogen Cat. 2 is warranted. From the eMSCA point of view the requested clarification of the substance identity regarding the nanoform will have no impact on this conclusion.

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

7.9.7.1. Effects on fertility

Table 15

Relevant studies related to the assessment of the endpoint fertility			
Methods	**Results**	**Remarks**	**Reference**
30-month chronic toxicity and potential carcinogenicity study in rats with in utero and lifetime exposure (Part I) According to FDA Guideline, meets main principles of the OECD TG 415 (One-	There were no test substance-related adverse effects on reproductive performance at any	Supporting information Reliable with restrictions - Individual raw data missing	TL, 1982a (Part I)

Relevant studies related to the assessment of the endpoint fertility			
Methods	**Results**	**Remarks**	**Reference**
Generation Reproduction Toxicity Study, deleted in 2019) Pre-GLP Test material: D&C Red No. 9 (CAS 5160-02-1) - Purity: 76% (Batch #547530) Rat, CD [CRL:COBS CD (SD) BR], F0: 60/sex/dose F1: 70/sex/dose (including 10/sex/dose for 12 month interim kill) Oral: diet F0: 0, 100, 200, and 500 ppm corr. to: 0, 8, 17, 43 mg/kg bw in F0 males; 0, 9, 17, 42 mg/kg bw in F0 females; F1: 0, 100, 200, and 500 ppm corr. to: 0, 5, 10, 26 mg/kg bw in F1 males; 0, 6, 13, 32 mg/kg bw F1 females Exposure: 8 weeks prior to mating; continued during mating, gestation and lactation; females were allowed to litter and raise their pups until weaning; F1 generation exposed for 30 months after weaning Examination F0: mortality, clinical signs, body weight, food consumption, reproductive performance Examination F1 pups: survival, body weight, sex ratio Examination F1: mortality, clinical signs, body weight, food consumption, haematology, clinical chemistry, urinalysis, organ weight, gross necropsy, ophthalmology, histopathology	dose in F0 generation.	(appendices of study report not available) - exposure duration for males prior to mating too short (requested are 10 weeks) - no gross necropsy was performed for F0 parents and F1 pups (which died during lactation) - No data on haematology, clinical biochemistry, urine analysis for F0 parents - no histopathology was performed for F0 parents - No data on oestrus cycling, no sperm parameters	
30-month chronic toxicity and potential carcinogenicity study in rats with in utero and lifetime exposure (Part II) According to FDA Guideline, meets main principles of the OECD Test Guideline 415 (One-Generation Reproduction Toxicity Study, deleted 2019) Pre-GLP Test material: D&C Red No. 9 (CAS 5160-02-1) Purity: 76% (Batch #547530) Rat, CD [CRL:COBS CD (SD) BR] F0: 60/sex/dose F1: 70/sex/dose (including 10/sex/dose for 12 month interim kill) Oral: diet	There were no test substance-related adverse effects on reproductive performance in F0 generation. Statistically significant reduction in F1 mean testis weight (-24 %) and testis weight/body weight percentage (-26.1 %) at 10,000 ppm (converted to 500 mg/kg bw), compared to controls at terminal kill (30 month)	Supporting information Reliable with restrictions - Individual raw data missing (appendices of study report not available) - exposure duration for males prior to mating too short (requested are 10 weeks) - No data on haematology, clinical biochemistry, urine analysis, organ weights for F0 parents	TL 1982b (Part II) Study not in dossier

Relevant studies related to the assessment of the endpoint fertility			
Methods	**Results**	**Remarks**	**Reference**
F0: 0, 10,000 ppm corr. to: 790 ± 200 mg/kg bw for F0 males, 894 ± 208 mg/kg bw for F0 females F1: 0, 10,000 ppm corr. to: No data available for F1 males (Food conversion factor: 20 (for older rats) results in calculated dose of 500 mg/kg bw/d); 521 mg/kg bw for F1 females Exposure: 9 weeks prior to mating; continued during mating, gestation and lactation; females were allowed to litter and raise their pups until weaning; F1 generation rats exposed for 30 months after weaning Examination F0: mortality, general physical appearance and signs of toxicity, body weight, food consumption, reproductive performance, gross necropsy Examination F1 pups: survival, body weight, sex ratio, gross necropsy Examination F1: mortality, general physical appearance and signs of toxicity, body weight, food consumption, haematology, clinical chemistry, urinalysis, organ weight, gross necropsy, ophthalmology, histopathology		- no histopathology was performed for F0 parents - No data on oestrus cycling, no sperm parameters	
2-year feeding study - according to OECD TG 451 (NTP guideline including single dose, 2-week and 13-week studies) No GLP Test material: D&C Red No. 9 (CAS 5160-02-1), Lot No. Z-8054 Purity: 89.8%, impurities sodium and barium sulfates Rats, F344 (N=50/sex/dose) Mice, B6C3F1 (N=50/sex/dose) Dose rats: 0, 1000, 3000 ppm Dose mice: 0, 1000, 2000 ppm Oral, diet Treatment time: 103 weeks, daily Post exposure period: 1 week	**Rat:** Conversion factor 20 (older rat): 50, 150 mg/kg bw Increased incidence of testis/ tubule degeneration: 10 % (5/50) at 50 mg/kg bw, 23 % (11/48) at 150 mg/kg bw, compared to 6 % (3/50) in controls Slightly increased incidence of males with testis atrophy at 150 mg/kg bw: 22 % (11/50) at 50 mg/kg bw, 29 % (14/48) at 150 mg/kg bw, compared to 20% (10/50) in controls **Mouse**: Conversion factor 7: 143, 286 mg/kg bw No non-neoplastic findings in treated mice	Supporting information Reliable without restrictions Dose level selected based on effects observed in 91 day study	NTP, 1982

As summarised in the table above, there is a study performed according to FDA guidelines in CD rats with D&C Red No. 9 (known trading name of PR53:1) available, which is comparable with OECD TG 415 (One-Generation Reproduction Toxicity Study, TL, 1982b; TL, 1982a). The study was performed in two parts. Part I of the study was done at concentrations of 100, 200 and 500 of PR53:1 in the diet. Part II of the study was performed with 10,000 ppm in the diet. In 2017 ECHA concluded in a compliance check that this study is inadequate because the doses are too low. However, part II of the study with a dosing of 10,000 ppm was not available to ECHA. No adverse effects on reproductive performance were observed for F0 generation up to 10,000 ppm in the diet.

For F1 males receiving 10,000 ppm (converted to 500 mg/kg bw) of D&C Red No. 9, there was a statistically significant reduction in mean testes weight (24%) and testes/body weight percentage (26%) compared to the control group at terminal kill (after 30 months) which was not apparent for interim kill (after 12 months). No histopathological changes in testes of F1 males were described in the study report (raw data are missing).

In the NTP study (NTP, 1982), where F344 rats received 1000 ppm (converted to 50 mg/kg bw) and 3000 ppm (converted to 150 mg/kg bw) of D&C Red No. 9 in the diet for 103 weeks, histopathological changes in the testes were observed, such as a dose-dependent increase in the incidence of males with degeneration of testis/tubule (6%, 10%, 23%) as well as a slight higher incidence of males with testis atrophy at the highest dose of 3000 ppm can be found in the raw data (20%, 22%, 29%). No information on statistical significance is given. Organ weights were not determined in this study.

In addition, a three generation study in rats with D&C Red No. 9 (known trading name of PR53:1), not performed to any guideline, is available (TL, 1972c). No adverse effects were observed in this study. However, the doses are too low (0.05, 0.5, 1.5, and 5 mg/kg bw). Therefore, the study is inadequate for risk assessment.

7.9.7.2. Conclusion

No adequate study on effects on fertility is available. The repeated dose toxicity studies (TL, 1982b; TL, 1982a; NTP, 1982) after oral exposure show some adverse effects on the male reproductive system (reduced testes weight at 10,000 ppm (converted to 500 mg/kg bw), degeneration of testis/ tubule at 3,000 ppm (converted to 150 mg/kg bw). Therefore, the eMSCA considers that an EOGRTS is triggered for the substance to investigate possible effects of PR53:1 exposure on the male reproductive organs. The eMSCA concludes that these effects should be followed up in a compliance check by ECHA.

7.9.7.3. Developmental toxicity

Table 16

Relevant studies related to the assessment of the endpoint developmental toxicity			
Methods	**Results**	**Remarks**	**Reference**
Developmental toxicity study according to OECD Test Guideline 414 (Prenatal Developmental Toxicity Study) GLP: yes Test material: PR53:1 Purity: ≥ 90% Rat, Wistar [rat] - Crl:WI(Han) 25 time-mated females/dose oral: gavage	<u>Maternal animals:</u> NOAEL (maternal toxicity: 3 mg/kg bw/d (nominal) At doses of 10 mg/kg bw/d first signs of hemolytic anemia were observed in dams. Fetuses: NOAEL (developmental toxicity): 30 mg/kg bw/d (nominal)	Key study Reliable without restrictions	TL 2019

Relevant studies related to the assessment of the endpoint developmental toxicity			
Methods	**Results**	**Remarks**	**Reference**
0, 3, 10 and 30 mg/kg bw/d (nominal) Vehicle: CMC (carboxymethyl cellulose) - 0.5 % suspension in deionized water Exposure: GD 6 to GD 19, once daily	There were no test substance-related adverse effects on fetuses at any dose.		

As result of a compliance check in 2017, ECHA requested a developmental toxicity study (OECD TG 414). The results are now available and summarized in the table above. No test substance-related adverse effects on fetuses were observed in Wistar rats after oral exposure to PR53:1 (highest dose level: 30 mg/kg bw/d) (TL 2019).

Furthermore, there are two older PNDT studies similar to OECD TG 414 in different species (rat and rabbit) with D&C Red No. 9 (known trading name of PR53:1) (TL, 1972a; TL, 1972b). No treatment-related maternal or developmental toxicity was observed in either study. However, the doses in both PNDT studies are too low (high-dose level: 15 mg/kg bw/d). As a consequence, ECHA has requested a new PNDT study during a compliance check in 2017.

7.9.7.4. Conclusion

No developmental toxic effects were identified after oral administration of PR53:1 that would justify classification. Assuming that the test substance is representative for the registered substance, the available data is considered as appropriate for an evaluation of developmental toxicity.

7.9.8. Hazard assessment of physico-chemical properties

Not assessed in the course of this evaluation.

7.9.9. 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 dose descriptors are gathered from the available and relevant experimental animal studies for PR53:1.

Table 17

OVERVIEW OF DOSE DESCRIPTORS AS RESULT OF HAZARD ASSESSMENT				
Endpoint of concern	**Type of effect**	**Critical study(ies)**	**Corrected dose descriptor(s) (e.g. NOAEL, NOAEC)**	**Justification/ Remarks**
Repeated dose toxicity	Splenic lesions (fibrosis)	2-year feeding study in **rats** (NTP, 1982)	**NOAEL** **50 mg/kg bw/d**	Study reliable without restrictions

		Calculated doses: 50, 150 mg/kg bw		
	Blood (Hb reduction, haemolytic anaemia)	Combined repeated dose and carcinogenicity (18 month/ 105 weeks (daily)) feeding study in **mice** (TL, 1981) Doses: 7, 38, 147 for males; 12, 56, 237 mg/kg bw for females	**NOAEL** **56 mg/kg bw/d**	Study reliable with restrictions
Carcinogenicity	Fibrosarcoma in male rats	2-year feeding study in **rats** (NTP, 1982) Calculated doses: 0, 50, 150 mg/kg bw/d	**NOAEL** **50 mg/kg bw/d**	Study reliable without restrictions

7.9.9.1. DNEL calculation for workers

At the workplace exposure to PR53:1 occurs or may occur mainly by inhalation. Dermal exposure can also be assumed. For this reason, DNELs were calculated for both exposure routes. For the DNEL calculation, the eMSCA follows the specifications given in the REACH guidance chapter R.8 (ECHA, 2012a).

In animal studies where PR53:1 was administered orally the substance causes remarkable effects in rats and mice from app. 100 mg/kg bw/d onward. These effects are systemic and include splenic lesions (fibrosis), haematotoxicity, and nodules in the liver in both species (NTP, 1982; TL, 1981). At doses of 50 mg/kg bw/d no effects in rats were observed, therefore this value was used as NOAEL and as starting point for DNEL derivation.

Table 18

DNEL DERIVATION FOR WORKER, INHALATION, LONG-TERM, SYSTEMIC EFFECTS		
Description (AF=Assessment factor)	**Value**	**Specification**
Relevant dose descriptor	NOAEL = 50 mg/kg bw/d	This NOAEL results from a 2-year feeding study in rats (NTP, 1982). In the next higher dose group of 150 mg/kg bw/d lesions like fibrosis in the spleen of rats were observed.
Modification of the starting point	50 mg/kg bw/d 0.38 m^3/kg bw = 131.6 mg/m^3	Application of different physiological default parameters under the allometric scaling principle to adapt the different exposure conditions in the animal experiment to the workplace of humans (according to REACH guidance R.8). The absorption rate for oral exposure in rat was set to 50% and after inhalation in humans 100%.
	131.6 mg/m^3 *(6.7 m^3/10 m^3) *(7 d/5 d) *(50%/100%) ↓	
Modified dose-descriptor	**61.7 mg/m^3**	
Overall AFs	12.5	

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	-	The application of this AF is not necessary because the animal experiment was a chronic study.
AF related to dose response relationship	-	The application of this AF is not necessary because starting point for the derivation was already a NOAEL.
AF related to quality of database	-	The application of this AF is not necessary because the study used is of good quality and reliability.
DNEL worker, inhalation, long-term, systemic effects	**4.9 mg/m³**	

Table 19

DNEL DERIVATION FOR WORKER, DERMAL, LONG-TERM, SYSTEMIC EFFECTS		
Description (AF=Assessment factor)	**Value**	**Specification**
Relevant dose descriptor	NOAEL = 50 mg/kg bw/d	This NOEAL results from a 2-year feeding study in rats (NTP, 1982). In the next higher dose group of 150 mg/kg bw/d lesions like fibrosis in the spleen of rats were observed.
Modification of the starting point	*(7 d/5 d) ↓	The dose descriptor was only modified regarding the different exposure times in animal experiments and at the workplace.
Modified dose-descriptor	**35.7 mg/kg bw/d**	
Overall AFs	50	
AF for interspecies differences - allometric scaling - remaining differences	 4 2.5	For interspecies differences, default factors are applied for allometric scaling to take into account the difference between the experimental animal and humans and for remaining differences 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	-	The application of this AF is not necessary because the animal experiment was a chronic study.
AF related to dose response relationship	-	The application of this AF is not necessary because starting point for the derivation was already a NOAEL.
AF related to quality of database	-	The application of this AF is not necessary because the study used is of good quality and reliability.
DNEL worker, dermal, long-term, systemic effects	**0.7 mg/kg bw/d**	

7.9.9.2. DNEL calculation for Consumers/General population

No quantitative risk assessment is included in this SEv for consumers due to standard data gaps identified resulting in further data being generated.

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

According to the lead registrant PR53:1 fulfils the definition of a nanomaterial as included in Annex VI of the REACH Regulation as of 01 January 2020. During the SEv it has been noticed that a characterisation of the individual nanoforms covered by the different registrations is lacking. It is not clear, if the boundary composition provided by the lead registrant covers all nanoforms of the co-registrants. The eMSCA proposes to clarify the substance identity of the registered substance as part of a compliance check. The following conclusions are based on the assumption that the test materials used in the available toxicity studies are representative for the registered substance.

The initial human health concern for this substance evaluation was the suspected carcinogenic property of PR53:1. Additionally, a concern for STOT-RE (blood, spleen) has been identified during SEv; available data were not sufficient for CLH (STOT-RE).

A two-year carcinogenicity study (NTP, 1982) provides clear evidence of a carcinogenic potential of PR53:1 in male rats based on increased incidence of sarcomas of the spleen and a dose-related increase in neoplastic nodules of the liver. In the view of the eMSCA the evidence from existing data on carcinogenicity of PR53:1 indicate that classification as Carc. 2, H351, is required.

For specific target organ toxicity arising from repeated exposure toxic effects such as haemolytic anaemia presumably leading to lesions in the spleen have been identified. However, the effects at doses found in the available studies would not allow classification according to the criteria laid down in the CLP Regulation for specific target organ toxicity after repeated exposure (STOT-RE).

During its evaluation, the eMSCA assessed other toxicological endpoints and identified data gaps which are considered standard information requirements under REACH. Therefore the eMSCA recommends that the studies are requested by ECHA under dossier evaluation:

- toxicity to reproduction (a trigger for an EOGRTS has been identified)
- germ cell mutagenicity (5th strain in Ames test with Prival activation)
- skin sensitisation
- furthermore, based on the nanoform of PR53:1 there are also data gaps related to sub-chronic toxicity via the inhalation route, and toxicokinetics.

Whether the substance evaluation needs to be re-opened subsequently depends on the outcome of the studies that will be requested.

The eMSCA considers the following classification and labelling for PR53:1 as necessary based on the available information.

Table 20

Classification and labelling for PR53:1 according to the assessment of the evaluating member state	
Classification	
Hazard Class and Category Codes	**Hazard statement codes**
Carc. 2	H351

7.10. Assessment of endocrine disrupting (ED) properties

There is no information about ED properties of the substance available. Not assessed in the course of this evaluation.

7.11. PBT and VPVB assessment

Not assessed in the course of this evaluation.

7.12. Exposure assessment

7.12.1. Human health

7.12.1.1. Worker

Overview of uses and postulated exposure scenarios (ES)

PR53:1 belongs to the group of β-naphthol azo pigment lakes which are based on monoazo dyes bearing sulfonic acid groups. The substance is an insoluble barium salt pigment. This pigment is synthesized by coupling of a diazotized aniline sulfonic acid with β-naphthol which yields a monoazo dye that is converted to the pigment by lake formation. This batch process is potentially leading to inhalation and dermal exposure during transfer and cleaning operations.

An overview of uses is given in Table 7. The most important and established use is the imparting of colour to printing inks and plastic products. As a response to a survey of the eMSCA, some registrants indicated further uses, for example coatings (for e.g. automotive, decorative and industrial coatings) and masterbatches. However, PR53:1 is not used in the textile and leather industry, this use on the dissemination site seems to be obsolete. Furthermore, the use of PR53:1 in cosmetic articles is prohibited by EC Regulation No. 1223/2009, Annex 2 (EC, 2009b).

Scope and type of exposure

According to the lead registrant PR53:1 is not classified. Nevertheless, the lead registrant performed an exposure and a risk assessment for 13 worker ES (see Table 21). The lead registrant used EasyTRA 4.3.0 and ART (Advanced REACH Tool) for estimating the inhalation and dermal exposure. Due to the nature of the described uses inhalation and dermal exposure is expected. Since the pigment is marketed as a powder with a small particle size distribution (D50: ca. 46.9 nm) inhalation exposure to dust may play a significant role at workplaces. Additionally, the pigments are used in surface coatings and inks which are spread or sprayed (PROC 7, 10, 11) leading to further potential inhalation and dermal exposure.

Table 21: Overview of uses for which the lead registrant provided ES

Overview on uses and PROCs provided in the updated CSR of the lead registrant							
Short description of the identified use	Resulting life cycle stage						Process Category (PROC)
	Manuf.	Formulation	End use			Service life (for articles)	
			Ind.	Prof.	Cons.		
Industrial formulation of non-solid preparations containing pigment including paints		X	X				3, 5, 8B, 9, 15, 28
Industrial formulation of inks (water- and solvent based) & toner		X	X				3, 5, 8B, 9, 15, 28
Industrial formulation of mixtures for master-batching and compounding industry		X	X				5, 8B, 9, 14, 15, 24A, 28
Industrial application of automotive, decorative and industrial coatings			X				5, 7, 8B, 10, 13, 15, 21, 28
Industrial application of masterbatches and compounds – production of plastic articles			X				5, 6, 8B, 14, 15, 24A, 28
Industrial application of printing inks (water-based, solvent-based)			X				5, 8B, 15, 28
Professional application of inks (water- and solvent-based)				X			5, 8A, 8B, 10, 28
Professional application of paints, coatings – widespread dispersive indoor use				X			5, 8A, 10, 11, 13, 28
Professional application of paints, coatings – widespread dispersive outdoor use				X			5, 8A, 10, 11, 28
Professional removal of matrix, indoor (e.g. abrasion)				X			24A
Professional removal of matrix, outdoor (e.g. abrasion)				X			24A
Handling/use of pigment-containing coated/painted articles for workers				X		X	21, 24A
Handling/processing of plastic articles/objects and/or coated/painted articles for workers				X		X	21, 24A

The eMSCA has recalculated the exposure and risk assessment provided by the registrant by using the tier 1 model ECETOC TRA v3.0. The model EasyTRA used by the registrant is not in the frame of the ECHA Guidance R14 (ECHA, 2016). Furthermore, ART, a higher tier model, was used for estimating the ES with potential for aerosol formation, as this would be outside of the scope of ECETOC TRA.

Predicted exposure by ECETOC-TRA

The following conditions were adopted for the ECETOC TRA assessment:
PR53:1 is used as powder with moderate dustiness. For dermal exposure of high or moderate dust it is stated in the ECETOC TRA Technical Report No. 114 (ECETOC, 2012) that modifying factors are not allowed for the parameter “duration of activity”. Thus, the factor 1 was used. Furthermore, the use of an enhanced ventilation of 70 % effectiveness was stated by the lead registrant leading to a modifying factor of 0.3, which was used by the eMSCA as well. This factor was only used for calculating the inhalation exposure.

EasyTRA estimates the dermal exposure by using a linear relationship between the concentration of product/duration of activity and the exposure level. This is not in the scope of the original ECETOC TRA model. Thus, given exact concentrations and working durations in the CSR of the lead registrant were allocated to the band model of ECETOC TRA.

Some of the registrants used additional modifying factors for inhalation and dermal personal protection. For example, for the ES 9.6, 9.10 an additional dermal protection factor of 50% was used with the justification “It is expected that all professional ink makers/printers should be well educated and use adequate protective equipment (clothing, gloves, mask)”. These factors are not within the scope of ECETOC TRA, as well. Thus, the eMSCA did not used these additional factors.

Table 22 lists the modifying factors used for the exposure estimation with ECETOC TRA.

Table 22

Modifying factors used for the exposure estimation with ECETOC TRA				
PROC	Duration of activity		Concentration	Protective Gloves
	Inhalation	Dermal		
ES 9.1: Professional removal of matrix indoor (e.g. abrasion)				
24	>4h: 1	>4h: 1	5-25 %: 0.6	90 %: 0.1
ES 9.2: Handling/processing of plastic articles/objects and/or coated/painted articles for workers				
21	>4h: 1	>4h: 1	5-25 %: 0.6	90 %: 0.1
24	>4h: 1	>4h: 1	5-25 %: 0.6	90 %: 0.1
ES 9.4: Industrial formulation of mixtures for master-batching and compounding industry				
5	1-4h: 0.6	>4h: 1	5-25 %: 0.6	95 %: 0.05
8B	1-4h: 0.6	>4h: 1	5-25 %: 0.6	95 %: 0.05
9	>4h: 1	>4h: 1	5-25 %: 0.6	95 %: 0.05
14	>4h: 1	>4h: 1	5-25 %: 0.6	95 %: 0.05
15	>4h: 1	>4h: 1	5-25 %: 0.6	95 %: 0.05
24	>4h: 1	>4h: 1	5-25 %: 0.6	95 %: 0.05
ES 9.6: Professional application of Inks (water- and solvent-based)				
5	1-4h: 0.6	>4h: 1	5-25 %: 0.6	90 %: 0.1
8A	1-4h: 0.6	>4h: 1	5-25 %: 0.6	90 %: 0.1
8B	1-4h: 0.6	>4h: 1	5-25 %: 0.6	90 %: 0.1
10	ART	>4h: 1	5-25 %: 0.6	90 %: 0.1
ES 9.8: Industrial application of masterbatches and compounds - production of plastic articles				
5	1-4h: 0.6	>4h: 1	>25 %: 1	95 %: 0.05
6	1-4h: 0.6	>4h: 1	>25 %: 1	95 %: 0.05
8B	1-4h: 0.6	>4h: 1	>25 %: 1	95 %: 0.05
14	1-4h: 0.6	>4h: 1	>25 %: 1	95 %: 0.05

15	>4h: 1	>4h: 1	>25 %: 1	95 %: 0.05
24	>4h: 1	>4h: 1	>25 %: 1	95 %: 0.05
ES 9.9:Professional removal of matrix outdoor (e.g. abrasion)				
24	>4h: 1	>4h: 1	5-25 %: 0.6	90 %: 0.1
ES 9.10: Professional application of paints, coatings - widespread dispersive outdoor use				
5	1-4h: 0.6	>4h: 1	5-25 %: 0.6	90 %: 0.1
8A	1-4h: 0.6	>4h: 1	5-25 %: 0.6	90 %: 0.1
10	ART	>4h: 1	<5 %: 0.2	90 %: 0.1
11	ART	>4h: 1	<5 %: 0.2	90 %: 0.1
ES 9.12: Industrial application of printing inks (water-based, solvent-based)				
5	1-4h: 0.6	>4h: 1	5-25 %: 0.6	95 %: 0.05
8B	1-4h: 0.6	>4h: 1	5-25 %: 0.6	95 %: 0.05
15	1-4h: 0.6	>4h: 1	5-25 %: 0.6	95 %: 0.05
ES 9.15: Industrial formulation of inks (water- and solvent-based) & toner				
3	>4h: 1	>4h: 1	5-25 %: 0.6	95 %: 0.05
5	1-4h: 0.6	>4h: 1	5-25 %: 0.6	95 %: 0.05
8B	1-4h: 0.6	>4h: 1	5-25 %: 0.6	95 %: 0.05
9	>4h: 1	>4h: 1	5-25 %: 0.6	95 %: 0.05
15	>4h: 1	>4h: 1	5-25 %: 0.60.6	95 %: 0.05
ES 9.16: Industrial formulation of non-solid preparations containing pigment including paints				
3	>4h: 1	>4h: 1	5-25 %: 0.6	95 %: 0.05
5	1-4h: 0.6	>4h: 1	5-25 %: 0.6	95 %: 0.05
8B	1-4h: 0.6	>4h: 1	5-25 %: 0.6	95 %: 0.05
9	1-4h: 0.6	>4h: 1	5-25 %: 0.6	95 %: 0.05
15	>4h: 1	>4h: 1	5-25 %: 0.6	95 %: 0.05
ES 9.17: Handling/use of pigment-containing coated/painted articles for workers				
21	>4h: 1	>4h: 1	5-25 %: 0.6	90 %: 0.1
24	>4h: 1	>4h: 1	5-25 %: 0.6	90 %: 0.1
ES 9.18: Industrial application of automotive, decorative and industrial coatings				
5	1-4h: 0.6	>4h: 1	5-25 %: 0.6	95 %: 0.05
7	ART	>4h: 1	5-25 %: 0.6	95 %: 0.05
8B	1-4h: 0.6	>4h: 1	5-25 %: 0.6	95 %: 0.05
10	ART	>4h: 1	5-25 %: 0.6	95 %: 0.05
13	15min to 1h: 0.2	>4h: 1	5-25 %: 0.6	95 %: 0.05
15	>4h: 1	>4h: 1	5-25 %: 0.6	95 %: 0.05
21	>4h: 1	>4h: 1	5-25 %: 0.6	95 %: 0.05
ES 9.19: Professional application of paints, coatings - widespread dispersive indoor use				
5	1-4h: 0.6	>4h: 1	5-25 %: 0.6	90 %: 0.1
8A	1-4h: 0.6	>4h: 1	5-25 %: 0.6	90 %: 0.1
10	ART	>4h: 1	<5 %: 0.2	90 %: 0.1
11	ART	>4h: 1	<5 %: 0.2	90 %: 0.1
13	1-4h: 0.6	>4h: 1	5-25 %: 0.60	90 %: 0.1

The Table 23 lists the inhalation and dermal exposure (both long term) assessed by the eMSCA with ECETOC TRA for the provided ES and PROCs.

Table 23:

Inhalation and dermal exposure estimated with ECETOC TRA			
Exposure scenario	**PROC**	**inhalation exposure [mg/m^3]**	**dermal exposure [mg/kg/d]**
ES 9.1	24	0.9	0.170
ES 9.2	21	0.9	0.170
	24	0.9	0.170
ES 9.4	5	0.54	0.4113
	8B	0.108	0.4113
	9	0.9	0.2058
	14	0.18	0.1029

	15	0.09	0.0102
	24	0.54	0.0849
ES 9.6	5	0.54	0.8226
	8A	1.08	0.8226
	8B	0.54	0.8226
	10	ART	1.6458
ES 9.8	5	0.9	0.6855
	6	0.9	1.3715
	8B	0.18	0.6855
	14	0.18	0.1715
	15	0.15	0.017
	24	0.9	0.1415
ES 9.9	24	0.9	0.1698
ES 9.10	5	0.54	0.8226
	8A	1.08	0.8226
	10	ART	0.5486
	11	ART	2.1428
ES 9.12	5	0.54	0.4113
	8B	0.108	0.4113
	15	0.054	0.0102
ES 9.15	3	0.18	0.0207
	5	0.54	0.4113
	8B	0.108	0.4113
	9	0.9	0.2058
	15	0.09	0.0102
ES 9.16	3	0.18	0.0207
	5	0.54	0.4113
	8B	0.108	0.4113
	9	0.54	0.2058
	15	0.09	0.0102
ES 9.17	21	0.9	0.1698
	24	0.9	0.1698
ES 9.18	5	0.54	0.4113
	7	ART	1.2849
	8B	0.108	0.4113
	10	ART	0.8229
	13	0.036	0.4113
	15	0.68	0.0102
	21	0.54	0.0849
ES 9.19	5	0.54	0.8226
	8A	1.08	0.8226
	10	ART	0.5486
	11	ART	2.1428
	13	0.54	0.8226

Predicted exposure by ART (Advanced REACH Tool)

For the estimation of the inhalation exposure during spraying operations (PROC 7, 11) and roller application (PROC 10) involving solids suspended in liquids ECETOC TRA was not used because these scenarios are outside the scope of the model. Thus, higher tier exposure assessment for spraying and spreading operations was performed with ART. The ART parameters used for the exposure assessment of PROC 10 (Roller application or brushing) are listed in Table 24.

Table 24

ART parameters set for the exposure assessment of PROC 10					
		ES 9.6	ES 9.10	ES 9.18	ES 9.19
PROC		10			

Scenario type		Powders dissolved in a liquid or incorporated in a liquid matrix (e.g. copper in anti-fouling paint)			
		spreading of liquid products			
Duration		5 min	240 min		
Containment		low	low	medium	low
Substance emission potential	**Substance product type**	liquids			
	Process T	room T			
	Vapour Pressure	1E-05 Pa			
	Liquid weight fraction	minor (5-10%)	small (1-5%)	substantial (10-50%)	substantial (10-50%)
	Viscosity	medium			
Activity emission potential	**Activity class**	Handling of contaminated objects	Spreading of liquid products		
	Situation	Activities with treated/contaminated objects (surface 0.1 - 0.3 m²)	Spreading of liquids at surfaces or work pieces > 3 m² / hour	Spreading of liquids at surfaces or work pieces 0.3 - 1.0 m² / hour	Spreading of liquids at surfaces or work pieces 1.0 - 3.0 m² / hour
	Contamination of the surface of the objects	Contamination > 90 % of surface			
Surface contamination	**Process fully enclosed?**	no			
	Effective housekeeping practices in place?	yes			
Dispersion	**Working area**	indoors	outdoors	indoors	indoors
	Source located close to buildings?		no		
	Room size	any size workroom		large workrooms only	any size workroom
	Ventilation rate	0.3 air changes per hour (ACH)		mechanical ventilation giving at least 1 ACH	0.3 air changes per hour (ACH)
Localised controls	**Primary**			Medium level containment (99.00 % reduction)	Low level containment (90.00 % reduction)
	Secondary	no			

The ART parameters used for the exposure assessment of PROC 7 and 11 (Industrial and non-industrial spraying) are listed in Table 25.

Table 25

ART parameters set for the exposure assessment of PROC 7 and 11			
	ES 9.10	**ES 9.18**	**ES 9.19**
PROC	**11**	**7**	**11**
Scenario type	Powders dissolved in a liquid or incorporated in a liquid matrix (e.g. copper in anti-fouling paint)		
	Spray application of liquids		
Duration	240 min	5 min	120 min
Containment	low		

Substance emission potential	**Substance product type**	liquids		
	Process T	room T		
	Vapour Pressure	1E-05 Pa		
	Liquid weight fraction	small (1-5 %)	substantial (10-50 %)	small (1-5 %)
	Viscosity	low		
Spray application of liquids	**Activity class**	Surface spraying of liquids		
	Situation	Moderate application rate (0.3 - 3 l/minute)		
	Spray direction	Only horizontal or downward		
	Spray technique	Spraying with no or low compressed air use		
Surface contamination	**Process fully enclosed?**	no	yes	no
	Effective housekeeping practices in place?	yes		
Dispersion	**Working area**	outdoors	indoors	indoors
	Source located close to buildings?	no		
	Room size		Any size workroom	
	Ventilation rate		0.3 air changes per hour (ACH)	Only good natural ventilation
Localised controls	**Primary**	Low level containment (90.00 % reduction)	Fixed capturing hood (90.00 % reduction)	Low level containment (90.00 % reduction)
	Secondary	no		

Table 26 lists the predicted 90th percentile full-shift inhalation exposure for the spraying and spreading operations (PROC 7, 10, 11) in the ES 9.6, ES 9.10, ES 9.18 and ES 9.19 estimated with ART.

Table 26

Inhalation exposure for spraying and spreading operations estimated with ART		
Exposure Scenario	**PROC**	**Inhalation exposure [mg/m³]**
ES 9.6	10	0.000021
ES 9.10	10	0.0094
	11	0.032
ES 9.18	7	0.028
	10	0.0014
ES 9.19	10	0.041
	11	0.046

7.12.1.2. Consumer

According to the information given on ECHAs dissemination site PR53:1 is mainly used as a heat-resistant colouring agent for inks/toners, paints, coatings and remover products. Matching these information, various article categories (see Table 7 for all categories) are named by the same source indicating that there are numerous articles (e.g. toys, paper articles, textiles) which either contain PR53:1 or are treated/coated with PR53:1 containing products. Furthermore, the product categories for fingerpaints, “fillers, putties, plasters and modelling clay” and “adhesives and sealants” are mentioned under the heading of the use in paints and coatings (ECHA, 2020). Especially the use of fingerpaints is of high importance when it comes to the assessment of a possible risk because it not only includes a high dermal exposure but additionally, as it is used by children frequently, oral exposure

is expected. As PR53:1 is also used in coatings and paints which are *inter alia* used for toys, the mouthing behavior of small children which also results in oral exposure, has to be considered in a comprehensive approach. Furthermore, as PR53:1 is used in products belonging to e.g. the remover or adhesive category, inhalation exposure is assumed to be possible.

Considering the analytical data provided by the Federal Office of Consumer Protection and Food Safety (BVL) PR53:1 was detected in footwear (2015), “tattoo colours for permanent make up” (2018) and vehicle maintenance and cleaning products (2019). The latter one indicates the potential for both inhalation and dermal exposure. In previous years (2006-2014) PR53:1 was detected in various cosmetic products (BVL, 2006-2019). By now, the use of PR53:1 in cosmetics is prohibited by the regulation (EC) No 1223/2009 on cosmetic products (inclusion in Annex II). (EC, 2009b)

Additionally the BfR evaluated the information given by the German GIFAS product database (Giftinformations- und Archivierungssystem). Ten non-industrial/non-professional products containing PR53:1 were identified. Two of them, glue and ink, are further classified and considered to be relevant for the consumer (GIFAS, 2001-2020). The use of PR53:1 in glue, as already stated above, is also supported by the information given on ECHAs dissemination site.

The main use of PR53:1 according to the information provided by some registrants is as a part of the composition of printing inks used to print different medias (e.g. labels, folding cartons, laminated packages, fast food packaging etc.). According to the registrants, the printing inks are not directly supplied to consumers. However, articles printed with PR53:1 containing inks are most likely to come into contact with consumers during their life cycle leading to dermal exposure. Additional PR53:1 containing products named, matching the already given information, are plastic and rubber products, paints, coatings and toys.

Further hints regarding the use of PR53:1 in food contact materials are given by the Verband für Mineralfarbenindustrie e.V. (VdMi e.V.) and the Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers (ETAD). Both organisations support the inclusion of PR53:1 into Annex 14 Table 2 of the printing ink ordinance (Hofherr, 2014; Liewald, 2014). This means that PR53:1 is intended to be used in products/articles which have direct contact to food (e.g. in napkins, paper packaging or to print on plastics, silicones etc) (BMEL, 2017).

In the chemical safety report (CSR) the lead registrant states that PR53:1 is used in inks, printing mixtures and toners, plastic and/or painted articles (AC1, AC8, AC13), paint remover products (indoor/outdoor) and paints and coatings (indoor/outdoor). Further Article categories potentially contributing to the consumer exposure to PR53:1 as named on the dissemination site and the registrants dossier are: AC 01 Other (non intended to be released): Painted articles, AC 2: Machinery, mechanical appliances, electrical/electronic articles, AC7a: Metal articles: Large surface area articles, AC7c: Metal articles: Packaging (excluding food packaging), AC10a: Rubber articles: Large surface area articles, AC10b: Rubber articles: Toys intended for children's use (and child dedicated articles), AC10c: Rubber articles: Packaging (excluding food packaging), AC11a: Wood articles: Large surface area articles, AC11b: Wood articles: Toys intended for children's use (and child dedicated articles), AC11c: Wood articles: Packaging (excluding food packaging), AC11e: Wood articles: Furniture & furnishings. As so far no hazard was identified for PR53:1, the registrants did not provide an exposure assessment for any of the mentioned uses referring to Article 14(3) of the REACh regulation.

The described information regarding the possible uses of PR53:1 leads to the conclusion that an exposure of the consumer over the three routes (inhalation, dermal, oral) is possible.

7.12.2. Environment

Not assessed in the course of this evaluation.

7.12.3. Combined exposure assessment

Not assessed in the course of this evaluation.

7.13. Risk characterisation

7.13.1. Worker

Considering the physicochemical properties of PR53:1 and its industrial and professional uses, workplace exposure occurs via inhalation and dermal contact. The lead registrant provided information about operational conditions and risk management measures for 13 worker ES and performed an exposure and risk assessment for these scenarios. However, the lead registrant performed the exposure estimation by EasyTRA which is not in the frame of the ECHA Guidance R14 (ECHA, 2016). Thus, the eMSCA estimated the exposure using ECETOC-TRA v3. The inhalation exposure for spraying and spreading operations (PROC 7, 10, 11) is not in the scope of ECETOC-TRA and EasyTRA. Thus, the estimation of the inhalation exposure for these operations was performed with ART.

For the quantitative risk characterisation of PR53:1, estimates of inhalation and dermal exposure were compared with the corresponding derived long-term systemic DNELs for workers.

For PR53:1, a long-term systemic DNEL for inhalation of 4.9 mg/m^3 and for dermal exposure of 0.7 mg/kg bw/d were derived. The derivation of both DNELs is based on the same chronic feeding study in rats (NTP, 1982). A detailed overview of how the eMSCA derived this DNEL is given in section 7.9.9.1.

An overview of the RCRs calculated by the eMSCA with the derived DNEL (long-term, systemic, worker, inhalation or dermal exposure) is given in Table 27.

Table 27:

Overview of RCRs for inhalation and dermal exposure and combined						
Exposure scenario	PROC	Inhalation		Dermal		RCR combined
		Exposure mg/m³	RCR	Exposure mg/kg/d	RCR	
ES 9.1	24	0.90	0.18	0.17	0.24	0.42
ES 9.2	21	0.90	0.18	0.17	0.24	0.42
	24	0.90	0.18	0.17	0.24	0.42
ES 9.4	5	0.54	0.11	0.41	0.59	0.70
	8B	0.11	0.02	0.41	0.59	0.61
	9	0.90	0.18	0.21	0.30	0.48
	14	0.18	0.04	0.10	0.14	0.18
	15	0.09	0.02	0.01	0.01	0.03
	24	0.54	0.11	0.08	0.11	0.22
ES 9.6	5	0.54	0.11	0.82	**1.17**	**1.28**
	8A	1.08	0.22	0.82	**1.17**	**1.39**
	8B	0.54	0.11	0.82	**1.17**	**1.28**
	10	0.000021	0.00	1.65	**2.36**	**2.36**
ES 9.8	5	0.90	0.18	0.69	0.99	**1.17**
	6	0.90	0.18	1.37	**1.96**	**2.14**
	8B	0.18	0.04	0.69	0.99	**1.03**
	14	0.18	0.04	0.17	0.24	0.28
	15	0.15	0.03	0.02	0.03	0.06
	24	0.90	0.18	0.14	0.20	0.38
ES 9.9	24	0.90	0.18	0.17	0.24	0.42
ES 9.10	5	0.54	0.11	0.82	**1.17**	**1.28**
	8A	1.08	0.22	0.82	**1.17**	**1.39**
	10	0.0094	0.00	0.55	0.79	0.79
	11	0.032	0.01	2.14	**3.06**	**3.07**

ES 9.12	5	0.54	0.11	0.41	0.59	0.70
	8B	0.11	0.02	0.41	0.59	0.61
	15	0.05	0.01	0.01	0.01	0.03
ES 9.15	3	0.18	0.04	0.02	0.03	0.07
	5	0.54	0.11	0.41	0.59	0.70
	8B	0.11	0.02	0.41	0.59	0.61
	9	0.90	0.18	0.21	0.30	0.48
	15	0.09	0.02	0.01	0.01	0.03
ES 9.16	3	0.18	0.04	0.02	0.03	0.07
	5	0.54	0.11	0.41	0.59	0.70
	8B	0.11	0.02	0.41	0.59	0.61
	9	0.54	0.11	0.21	0.03	0.14
	15	0.09	0.02	0.01	0.01	0.03
ES 9.17	21	0.90	0.18	0.17	0.24	0.42
	24	0.90	0.18	0.17	0.24	0.42
ES 9.18	5	0.54	0.11	0.41	0.59	0.70
	7	0.028	0.01	1.28	**1.80**	**1.81**
	8B	0.11	0.02	0.41	0.59	0.61
	10	0.0014	0.00	0.82	**1.17**	**1.17**
	13	0.04	0.01	0.41	0.59	0.60
	15	0.68	0.14	0.01	0.01	0.15
	21	0.54	0.11	0.08	0.11	0.22
ES 9.19	5	0.54	0.11	0.82	**1.17**	**1.28**
	8A	1.08	0.22	0.82	**1.17**	**1.39**
	10	0.041	0.01	0.55	0.79	0.80
	11	0.046	0.01	2.14	**3.06**	**3.07**
	13	0.54	0.11	0.82	**1.17**	**1.28**

The calculated RCRs for inhalation exposure with values from 0.02 to 0.22 are far below 1 and are therefore acceptable from a risk assessment point of view.

In contrast, the RCRs for the dermal exposure reach 3.06 for some uses, and therefore are above the acceptable value of 1. However, despite these significantly increased RCRs, the eMSCA does not see any evidence of risk to workers. The worst case assumption of 100 % dermal absorption made during DNEL derivation is considered to be not realistic. This is supported by an *in vitro* skin penetration study where the percutaneous absorption of PR53:1 through human skin was found to be very low (TL, 1983). Very little colorant was found in the epidermis or dermis, less than 0.3 % in all cases. The vast majority of the unabsorbed material remained on the surface of the skin and was found in the skin wash. This amount varied from 85-103 %.
From the perspective of the eMSCA, a risk for workers due to dermal exposure can be neglected. The TL 1983 study was not used to derive the dermal DNEL because of its limitations. However, it can be used to justify why the increased RCRs for dermal exposure are not a reason for concern and why further regulatory measures are not envisaged.

7.13.2. Consumer

Regulatory effects of a harmonised Carc. 2 classification of PR53:1 from the consumer point of view

Mixtures/articles under REACH and CLP

The labelling of a mixture containing PR53:1 according to the CLP Regulation (EC, 2008) as Carc. 2, H351, may result in an increased substitution pressure for the manufacturer of the product. In addition, according to Article 14(4) of REACH (Regulation No.1907/2006), a classification of the substance triggers the obligation to perform a Chemical Safety Assessment according to article 14 of the regulation, including an exposure and a risk assessment in the chemical safety report (EC, 2006). This is expected to provide significantly more information on the consumer uses of the substance (e.g. concentrations of PR53:1, information on the use in articles) and require the registrants of the substance to demonstrate that the identified uses are safe.

Toys and finger paints

According to Directive 2009/48/EG of the European parliament and of the council on the safety of toys CMR-substances must not be used in toys, except:

- The concentration of the substance is below a generic or a substance specific limit
- They are not accessible by children at all, or
- There is an exemption for which the use is authorized

In the case of a harmonised classification as Carc. 2, H351, the generic limit is at a concentration of 1% (EC, 2009a). A specific threshold for PR53:1 is not defined so far.

As described above in addition to the limit of 1% the registrants have to prove the safe use of PR53:1 in their products by providing an exposure and an risk assessment (Art. 14(4) REACH Regulation (EC, 2006). The combination of both is expected to restrict and limit the use of PR53:1 in toys, in case the classification as Carc. 2, H351, is adopted.

Finger paints are further regulated by the European norm EN 71-7. According to this norm, colorants shall only be used in finger paints if they are not carcinogenic. Therefore the usage of PR53:1 would be prohibited in finger paints after a successful harmonised classification as Carc. 2, H351 (Technical committee - Safety of Toys, 2018).

Food contact materials

Printing inks for food packaging material are currently not specifically regulated in on a European level. However they are governed by the framework for food contact materials (Reg (EC) No. 1935/2004) which states that “food contact materials shall be manufactured [...] so that, under normal or foreseeable conditions of use, they do not transfer their constituents to food in quantities which could [...] endanger human health.” (EC, 2004). This has to be proven by the manufacturer. A harmonised classification as Carc. 2, H351, would increase the pressure for the manufacturer to prove that the uses of PR53:1 are safe or to substitute the substance.

In Germany a printing ink ordinance is currently foreseen (notified, but not in force yet). PR53:1 is not yet listed in this regulation which means it can only be used on the side of the food packaging which is not in contact with the food and only if it is not a CMR substance. According to the regulation after a successful classification as Carc. 2, H351, PR53:1 could not be used anymore in food contact materials at all. Nevertheless it is possible for the registrants to prove the safe use (by providing a risk assessment) of the substance which then could be included in a positive list of this regulation. Thereafter, it could be used in food contact materials according to the conditions listed in the regulation (BMEL, 2017).

Currently it cannot be fully clarified under substance evaluation whether risks for consumers from the current uses of PR53:1 are controlled due to lack of toxicological and exposure data. However the envisaged harmonised classification of PR53:1 as Carc. 2, H351, is expected to result in a reduction of consumer exposure (see Section 7.12.1.2) e.g. regarding the use in toys/ finger paint.

7.14. References

BMEL (2017): Twenty-First Ordinance amending the Consumer Goods Ordinance (Bedarfsgegenständeverordnung) of ... 2016. https://www.bmel.de/SharedDocs/Downloads/EN/_Consumer-Protection/DruckfarbenVO-EN.pdf?__blob=publicationFile&v=3 (last accessed on 26.11.2020)

Brown J.P., Dietrich P.S., and Bakner C.M. (1979): Mutagenicity testing of some drug and cosmetic dye lakes with the Salmonella/mammalian microsome assay. Mutation Research 66 (2), 181-185. DOI: 10.1016/0165-1218(79)90064-8

BVL (2006-2019): BVL data set regarding the use of PR53 in consumer products

Carson S. (1984): Skin Painting Studies in Mice with 14 Fd-and-C and D-and-C Colors - Fd-and-C Blue No-1, Red No-3, and Yellow No-5, D-and-C Red No-7, Red No-9, Red No-10, Red No-19, Red No-21, Red No-27, Red No-31, Red No-36, Orange No-5, Orange No-10, and Orange No-17. Journal of Toxicology - Cutaneous and Ocular Toxicology 3 (4), 357-370. DOI: 10.3109/15569528409036288

Davis K.J. (1963): 3. Chronic toxicity of D & C Red No. 9. Food and Cosmetics Toxicology 1, 99-100. DOI: 10.1016/S0015-6264(63)80466-6

Davis K.J. and Fitzhugh O.G. (1962): Pathologic changes noted in rats fed D&C Red No. 9 for two years. Toxicology and Applied Pharmacology 4 (2), 200-205. DOI: 10.1016/0041-008X(62)90058-3

EC (2004): REGULATION (EC) No 1935/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. European Commission

EC (2006): REGULATION (EC) No 1907/2006 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. European Commission

EC (2008): REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. European Commission

EC (2009a): DIRECTIVE 2009/48/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 18 June 2009 on the safety of toys. European Commission

EC (2009b): REGULATION (EC) No 1223/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 30 November 2009 on cosmetic products. European Commission

ECETOC (2012): Technical Report No. 114 - ECETOC TRA version 3: Background and Rationale for the Improvements, date: 7/2012. Chemicals E.C.f.E.a.T.o., Brussels. http://members.ecetoc.org/Documents/Document/20120810142457-ECETOC_TR_114.pdf

ECHA (2016): Guidance on Information Requirements and Chemical Safety Assessment Chapter R.14: Occupational exposure assessment. https://echa.europa.eu/documents/10162/13632/information_requirements_r14_en.pdf/bb14b581-f7ef-4587-a171-17bf4b332378

ECHA (2020): Barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate]. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14894 (last accessed on 26.11.2020)

Food and Drug Administration (FDA) (1986): Listing of D&C Red No. 8 and D&C Red No. 9 for use in ingested drag and cosmetic up products and externally applied drugs and cosmetics. Federal Register (FD) 51 (234), 43877-43900

Fregert S. and Bandmann H.J. (1975): Patch Testing. Berlin: Springer-Verlag

GIFAS (2001-2020): Data set regarding PR53 in products which were reported to the GIFAS

Goodman D.G., Ward J.M., and Reichardt W.D. (1984): Splenic fibrosis and sarcomas in F344 rats fed diets containing aniline hydrochloride, p-chloroaniline, azobenzene, o-toluidine hydrochloride, 4, 4′-sulfonyldianiline, or D & C red no. 9. Journal of the National Cancer Institute 73 (1), 265-273. DOI: 10.1093/jnci/73.1.265

Hart R.W., Freni S.C., Gaylor D.W., Gillette J.R., Lowry L.K., Ward J.M., Weisburger E.K., Lepore P., and Turturro A. (1986): Final Report of the Color Additive Scientific Review Panel. Risk Analysis 6 (2), 117-154. DOI: 10.1111/j.1539-6924.1986.tb00202.x

Hofherr W. (2014): Stellungnahme zum Verordnungsvorhaben "21. Verordnung zur Veranderung der Bedarfsgegenständeverordnung" (nationale Druckfarbenverordnung). The Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers ETAD

IARC (1993): D&C RED NO. 9 (CI Pigment Red 53:1). IARC MONOGRAHS (International Agency for Research on Cancer) 57, 203 - 212

Ivett J.L., Brown B.M., Rodgers C., Anderson B.E., Resnick M.A., and Zeiger E. (1989): Chromosomal aberrations and sister chromatid exchange tests in Chinese hamster ovary cells in vitro. IV. Results with 15 chemicals. Environmental and molecular mutagenesis 14 (3), 165-187. DOI: 10.1002/em.2850140306

Liewald H. (2014): Stellungnahme zum Verordnungsvorhaben „21. Verordnung zur Änderung der Bedarfsgegenständeverordnung“ (nationalen Druckfarbenverordnung) (Entwurf, Stand: 14.07.2014). Verband der Mineralfarbenindustrie (VdMi e.V.)

Mitchell J.C., Adams R.M., Glendenning W.E., Fisher A., Kanof N., Larsen W., Maibach H.I., Rudner E.J., Schnorr W., Storks F., and Taylor J.S. (1982): Results of standard patch tests with substances abandoned. Contact Dermatitis 8 (5), 336-337. DOI: 10.1111/j.1600-0536.1982.tb04242.x

Myhr B.C., Caspary W.J., and Holden H.E. (1991): Chemical mutagenesis at the thymidine kinase locus in L5178Y mouse lymphoma cells: Results for 31 coded compounds in the national toxicology program. Environmental and molecular mutagenesis 18 (1), 51-83. DOI: 10.1002/em.2850180109

Naganuma M., Ohtsu Y., Katsumura Y., Matsuoka M., Morikawa Y., Tanaka M., and Mitsui T. (1983): Analysis of subsidiary colors in D and C Red No-9 and Its purification - Development of non-allergenic D and C Red No-9. Journal of the Society of Cosmetic Chemists 34 (6), 273-284

NTP (1982): Carcinogenesis Bioassay of D & C Red No. 9 (CAS No. 5160-02-1) in F344 Rats and B6C3F1 Mice (Feed Study). National Toxicology Program technical report series 225, 1-168

NTP (1994): Toxicology and Carcinogenesis Studies of Barium Chloride Dihydrate in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). National Toxicology Program technical report series 432, 1-291

Sato Y., Katsumura Y., Ichikawa H., Kobayashi T., Kozuka T., Morikawa F., and Ohta S. (1981): A modified technique of Guinea-Pig testing to identify delayed-hypersensitivity allergens. Contact Dermatitis 7 (5), 225-237. DOI: 10.1111/j.1600-0536.1981.tb04058.x

Sugai T., Takahasmi Y., and Takagi T. (1977): Pigmented cosmetic dermatitis and coal tar dyes. Contact Dermatitis 3 (5), 249-256. DOI: 10.1111/j.1600-0536.1977.tb03670.x

Technical committee - Safety of Toys (2018): Safety of toys - Part 7: Finger paints - Requirements and test methods; German version EN 71-7:2014+A2:2018

Testing Laboratory (1972a): D&C Red 9 - Teratology study in rabbits. Unpublished study report

Testing Laboratory (1972b): D&C Red 9 - Teratology study in rats. Unpublished study report

Testing Laboratory (1972c): Multi-generation reproduction study in rats. Unpublished study report

Testing Laboratory (1973): Lackrottoner LCLL - CM 71685 - 32 Tage-Fütterungsversuch

Testing Laboratory (1981): Chronic Toxicity and Potential Carcinogenicity Study in the Mouse.

Testing Laboratory (1982a): 30-month chronic toxicity and potential carcinogenicity study in rats with in utero and lifetime exposure to D&C Red No. 9 in the diet. Final Report

Testing Laboratory (1982b): 30-month chronic toxicity and potential carcinogenicity study in rats with in utero and lifetime exposure to D&C Red No. 9 in the diet. Final Report Volume I

Testing Laboratory (1983): Percutaneous absorption of D & C Red No. 9 through human skin in vitro. Final report

Testing Laboratory (1985a): Permanent-Lackrot LC - Ames Test (Salmonella/mammalian-microsome mutagenicity test - Standard plate test) and Prival modification (Salmonella/mammalian-microsome mutagenicity test - preincubation test).

Testing Laboratory (1985b): Permanent-Lackrot LC - Study of the mutagenic potential in strains of salmonella typhimurium (Ames Test) and Escherichia coli.

Testing Laboratory (1985c): Salmonella mutagenicity test with three strains with TK 11 450 (IRGALITE Red CBNO) (Test for mutagenic properties in bacteria).

Testing Laboratory (1989a): Permanent-Lackrot LC - Ames Test (Salmonella/mammalian-microsome mutagenicity test - Standard plate test) and Prival modification (Salmonella/mammalian-microsome mutagenicity test - preincubation test).

Testing Laboratory (1989b): Permanent-Lackrot LC - Chromosome Aberrations in vitro in V79 Chinese Hamster Cells.

Testing Laboratory (1993a): Acute inhalation toxicity study - Four-hour exposure (nose only) in the rat.

Testing Laboratory (1993b): Permanent-Lackrot LC - Testing for acute dust inhalation toxicity in the male and female SPF Wistar rat 4-hour LC 50.

Testing Laboratory (2012a): Local lymph node assay (LLNA) in mice with pigment red 49:2.

Testing Laboratory (2012b): Salmonella Typhimurium and Escherichia Coli reverse mutation assay modified version for azo-dyes with Pigment Red 49:2.

Testing Laboratory (2019): Pigment Red 53:1 - Prenatal Developmental Toxicity Study in Wistar Rats Oral Administration (Gavage).

Weinberger M.A., Albert R.H., and Montgomery S.B. (1985): Splenotoxicity associated with splenic sarcomas in rats fed high doses of d ' c red no. 9 or aniline hydrochloride. Journal of the National Cancer Institute 75 (4), 681-690. DOI: 10.1093/jnci/75.4.681

Westmoreland C. and Gatehouse D. (1992): D and C Red No. 9: Genotoxic or non-genotoxic carcinogen? Mutation Research Letters 281 (3), 163-167. DOI: 10.1016/0165-7992(92)90003-Z

Zeiger E., Anderson B., Haworth S., Lawlor T., and Mortelmans K. (1988): Salmonella mutagenicity tests: IV. Results from the testing of 300 chemicals. Environmental and molecular mutagenesis 11 Suppl 12, 1-157. https://www.ncbi.nlm.nih.gov/pubmed/3277844 (last accessed on 26.11.2020)

7.15. Abbreviations

AF	Assessment Factor
bw	body weight
CLH	Harmonized Classification and Labelling
CLP	Classification, labelling, and packaging of substances
CoRAP	Continuous rolling action plan
CSR	chemical safety report
DMEL	Derived minimum effect level
DNEL	Derived no effect level
ECHA	European Chemical Agency
eMSCA	evaluating Member State Competent Authority
ES	exposure scenarios
FDA	U.S. Food and Drug Administration
GIFAS	poison information and archiving system (Giftinformations- und Archivierungssystem)
GLP	Good laboratory practice
GPMT	guinea pig maximisation test
LC50	Lethal concentration to 50% of test animals
LALN	Lung-associated lymph nodes
LLNA	Local lymph node assay
NOAEC	No observed adverse effect concentration
NOAEL	No observed adverse effect level
NTP	National Toxicology Program
PC	Product category
PROC	Process category
PR	Pigment Red
RCR	risk characterisation ratio
RDT	Repeated Dose Toxicity
Reg	Regulation
SEv	Substance Evaluation
STOT-RE	Specific Target Organ Toxicity – Repeated exposure
TG	Testing guideline
TL	Testing laboratory