CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate]; C.I. Pigment Red 53:1

EC Number: **225-935-3**

CAS Number: **5160-02-1**

Index Number: **-**

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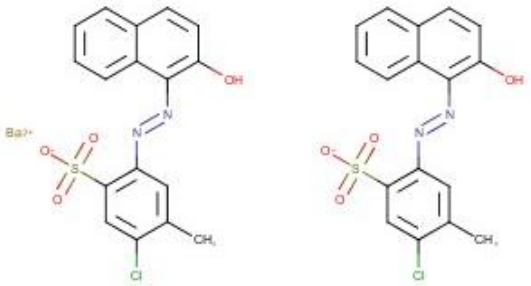
1 IDENTITY OF THE SUBSTANCE

This proposal for harmonised classification and labeling applies to the substance barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulfonate]; CI Pigment Red 53:1. This substance is available on the EU market as a nanoform. With respect to the classification and labelling, however, the assessment is not based on the properties driven by the particle form, but on the intrinsic properties.

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Barium(2+) bis(5-chloro-2-[(E)-2-(2-hydroxynaphthalen-1-yl)diazen-1-yl]-4-methylbenzene-1-sulfonate)
Other names (usual name, trade name, abbreviation)	C.I. Pigment Red 53:1 (PR 53:1), D&C Red No. 9, Benzenesulfonic acid, 5-chloro-2-[2-(2-hydroxy-1-naphthalenyl)diazenyl]-4-methyl-, barium salt (2:1)
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	225-935-3
EC name (if available and appropriate)	barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate] ; C.I. Pigment Red 53:1
CAS number (if available)	5160-02-1
Other identity code (if available)	-
Molecular formula	$C_{34}H_{24}BaCl_2N_4O_8S_2$
Structural formula	
SMILES notation (if available)	CC1=CC(=C(C=C1Cl)S(=O)(=O)[O-])N=NC2=C(C=CC3=CC=CC=C32)O.CC1=CC(=C(C=C1Cl)S(=O)(=O)[O-])N=NC2=C(C=CC3=CC=CC=C32)O.[Ba+2]
Molecular weight or molecular weight range	888.93 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	-
Degree of purity (%) (if relevant for the entry in Annex VI)	≤ 100

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate]; C.I. Pigment Red 53:1 (EC 225-935-3; CAS 5160-02-1)	≤ 100		

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

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
-				

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

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
-					

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

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
-				

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6: Proposed harmonised classification and labelling according to the CLP criteria.

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	no entry										
Dossier submitters proposal	607-RST-VW-Y	barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate]; C.I. Pigment Red 53:1	225-935-3	5160-02-1	Carc. 2	H351	GHS08, Wng	H351			
Resulting Annex VI entry if agreed by RAC and COM											

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

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

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The substance has not yet been subject to any measures regarding harmonised classification at the level of the European Union.

PR 53:1 is registered under REACH with a total tonnage band of 1 000 - 10 000 tonnes per annum.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

[A.] There is no requirement for justification that action is needed at Community level.

Substances fulfilling criteria of carcinogenicity, category 1A, 1B or 2 shall normally be subject to harmonised classification pursuant to Article 36(1) of Regulation (EC)1272/2008.

5 IDENTIFIED USES

5.1 Workers

PR53:1 belongs to the group of β-naphthol azo lake pigments which are based on monoazo dyes bearing sulfonic acid groups. The substance is an insoluble barium salt pigment. This pigment is synthesised by coupling of a diazotised 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 of workers during transfer and cleaning operations.

PR53:1 has widespread use. The most important and established use is the imparting of colour to printing inks and plastic products. In response to a survey of the evaluating Member State Competent Authority (eMSCA) during substance evaluation, some registrants indicated further uses, for example coatings (for e.g. automotive, decorative and industrial coatings) and masterbatches. Although a use in the textile and leather industry is indicated on the ECHA dissemination site, this use seems questionable according to that survey.

The use of PR53:1 in cosmetic articles is prohibited by EC Regulation No. 1223/2009, Annex 2 (EC, 2009).

5.2 Consumers

On ECHA's dissemination site as well as according to the information obtained from the survey during substance evaluation also numerous uses are indicated related to consumer exposure/use.

The main use is as heat-resistant colouring agent for inks/toners, paints, coatings and remover products. Various article categories are given on ECHA's dissemination site indicating that there are numerous articles such as toys, paper articles, and textiles which either contain PR53:1 or are treated/coated with PR53:1-containing products.

According to the information provided by some registrants, PR53:1 is mainly a part of the composition of printing inks used to print different media (e.g. labels, folding cartons, laminated packages, fast food packaging etc.). 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, include plastic and rubber products, paints, coatings, and toys.

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 behaviour of small children, resulting in oral exposure as well, 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 can be possible.

Considering the analytical data provided by the German 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 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, 2009).

Additionally, the eMSCA evaluated the information provided 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 consumers (GIFAS, 2001-2020). The use of PR53:1 in glue, as already stated above, is also supported by the information given on ECHA’s dissemination site.

There are also hints that PR53:1 is used in food contact materials. The Verband für Mineralfarbenindustrie e.V. (VdMi e.V.) and the Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers (ETAD) 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).

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 are: AC1 Other (non intended to be released): Painted articles, AC2: 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 by the registrants, no self-classification has been proposed.

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

6 DATA SOURCES

This report has been created based on the data submitted by the lead registrant in the REACH registration dossier for barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate]; C.I. Pigment Red 53:1 (CAS 5160-02-1; EC 225-935-3). In addition, further relevant data were retrieved from a literature search in PubMed, Web of Science, Embase, and Wiley (last search April 2020).

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20 °C and 101.3 kPa	Particulate material with a fine particle size distribution (nano-form)	REACH registration data*	
Melting/freezing point	Decomposed at 343 - 345 °C	IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISK OF CHEMICALS TO MAN, Some aromatic azo compounds, Volume 8, p. 107	
Boiling point	-	-	The substance is a solid which decomposes before boiling.
Relative density	1 707 kg/m³ at 20 °C	REACH registration data*	OECD Guideline 109, pycnometer method
Vapour pressure	-	-	Not applicable
Surface tension	-	-	Based on structure, surface activity is not expected.
Water solubility	Measured immediately after filtration: 2.986 mg/L at 23 °C Measured after one week: <0.01 mg/L at 23 °C	REACH registration data*	The determination was carried out by flask method based on the OECD Guideline 105 ant the ETAD method. The ETAD method was developed by intensive cooperation with the ETAD (Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers). The pigment solutions were not stable. After ca. one week an agglomeration of the pigment can be observed (especially in water). So, there are two different results given for this pigment.
Partition coefficient n-octanol/water	Measured immediately after filtration: Log P_{OW}= -0.62 at 23 °C Measured after one week: Log P_{OW}= 1.69 at 23 °C	REACH registration data*	The determination was carried out by a flask method based on OECD Guideline 105 and the ETAD method. This standard method was developed by intensive cooperation with the ETAD (Ecological and Toxicological Association of Dyes and Organic Pigments

Property	Value	Reference	Comment (e.g. measured or estimated)
			Manufacturers). The quantification was analyzed by HPLC-UV. The pigment solutions were not stable. After ca. one week an agglomeration of the pigment can be observed (especially in water). So there are two different results given for this pigment.
Flash point	Not applicable. The substance is a solid.	REACH registration data*	
Flammability	- Not highly flammable upon ignition - No pyrophoric properties - Does not liberate flammable gases on contact with water	REACH registration data*	
Explosive properties	There are chemical groups associated with explosive properties present in the molecule. The calculated oxygen balance is -129.6, which categorises the material to be a potentially explosive, as it is greater than the limit value of -200. There are no data available to decide on a non-classification.	BAM Expert judgement 2021	
Self-ignition temperature	No data available according to EU Method A.16	REACH registration data*	
Oxidising properties	No oxidising properties	REACH registration data*	
Granulometry	D10= 1.56 µm D50= 11.08 µm D90= 33.82 µm	REACH registration data*	The determination was conducted by laser diffraction method.
Stability in organic solvents and identity of relevant degradation products	Slightly soluble in ethanol, insoluble in acetone and benzene	IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISK OF CHEMICALS TO MAN, Some aromatic azo compounds, Volume 8, p. 107	

Property	Value	Reference	Comment (e.g. measured or estimated)
Dissociation constant	pKa (calculated): -5.49 (sulfonate group) 8.96 (phenolic group)	SIDS Initial Assessment Report for 9th SIAM (Paris, 29 June – 1st July 1999)	
Viscosity	-	-	The study does not need to be conducted because the substance is a solid.

*The information in this table marked with „REACH registration data“ is based on information taken from the REACH registration dossier and ECHA’s public registration information as accessed on 2021-03-11.

8 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

No reliable study on toxicokinetics of PR53:1 after oral exposure is available. Repeated dose toxicity studies (see chapter 10.10) showed haematotoxic effects in rats and mice after oral exposure so that oral absorption of PR53:1 or its metabolites can be assumed.

Regarding the inhalation route, there is only data from acute inhalation studies with PR53:1 which are insufficient with regard to transformation, clearance, accumulation in and translocation from the lung and lung-associated lymph nodes (LALN).

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

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity

Not assessed in this dossier.

10.2 Skin corrosion/irritation

Not assessed in this dossier.

10.3 Serious eye damage/eye irritation

Not assessed in this dossier.

10.4 Respiratory sensitisation

Not assessed in this dossier.

10.5 Skin sensitisation

Not assessed in this dossier.

10.6 Germ cell mutagenicity

Hazard class not assessed in this dossier. The data provided as supporting information for the assessment of carcinogenicity.

Table 9: 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	**PR53:1** 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) Bacterial strains: *S. typhimurium*: TA100, TA98, TA1537, TA1535 Test concentrations (± metabolic activation (S9 mix)): 4, 20, 100, 500, 2500, 5000 µg/plate S9: hamster liver S9, untreated and rat liver S9 Aroclor induced Vehicle: DMSO Negative/positive control: yes/yes	**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	(Hoechst AG, 1989a)
Bacterial reverse mutation test Similar to OECD TG 471 **(Prival activation and classical test protocol with S9)** Deviations: 5th strain missing GLP: yes	**PR53:1** 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) Bacterial strains: *S. typhimurium*: TA100, TA98, TA1537, TA1535 Test concentrations (± metabolic activation (S9 mix)): 4, 20, 100, 500, 2500, 5000/10 000 µg/plate S9: hamster liver (Prival activation) S9: untreated and rat liver S9 Aroclor induced (classical test protocol) Vehicle: DMSO Negative/positive control: yes/yes	**Negative** with (hamster and rat S9) and without metabolic activation Cytotoxicity: no Precipitations: yes, ≥ 100 µg/plate Neg. control: valid Pos. control: valid	(Hoechst AG, 1985a)
Bacterial reverse	**PR53:1**	**Key study**	**Negative** with and without metabolic	(Hoechst AG,

Method, guideline, deviations if any	Test substance	Relevant information about the study	Observations	Reference
mutation test Similar OECD TG 471 Deviations: none GLP: yes	CAS 5160-02-1 Purity: see confidential annex	**Reliable without restrictions** Bacterial strains: *S. typhimurium*: TA100, TA98, TA1537, TA1535, TA 1538, *Escherichia coli* WP2uvrA Test concentrations (± metabolic activation (S9 mix)): see confidential annex, guideline conform S9: see confidential annex Vehicle: see confidential annex Negative/positive control: yes/yes	activation Cytotoxicity: no Precipitations: see confidential annex Neg. control: valid Pos. control: valid	1985b)
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)** Bacterial strains: *S. typhimurium*: TA100, TA98, TA1537 Test concentrations (± metabolic activation (S9 mix)): 20, 78, 313, 1250,5000 µg/plate S9: rat liver, Aroclor-induced Vehicle: DMSO Negative/positive control: yes/yes	**Negative** with and without metabolic activation Cytotoxicity: no Precipitations: from 313 µg/plate onward Neg. control: valid Pos. control: valid	(CIBA-GEIGY Limited, 1985)
Bacterial reverse mutation test Similar to OECD TG 471 Deviations: • Documentation	**PR53:1** Purity: 33-73 %	**Disregarded study** **Not assignable (insufficient documentation and methodical deficiencies)** Bacterial strains: *S. typhimurium*: TA100, TA98, TA1537, TA1535, TA1538	**Negative** with and without metabolic activation Cytotoxicity: no data	(Brown et al., 1979)

Method, guideline, deviations if any	Test substance	Relevant information about the study	Observations	Reference
insufficient • Purity insufficient • Data on 5th strain missing • Low max. concentration • Only 3 concentrations tested • No detailed data on results (data table) GLP: no		Test concentrations (± metabolic activation (S9 mix)): 50, 100, 500 µg/plate S9: rat liver Aroclor induced Vehicle: DMSO Negative/positive control: yes/yes	Precipitations: no data Neg. control: valid Pos. control: valid	
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	PR53:1 (D&C Red No 9) CAS 5160-02-1 EC 225-935-3 Purity: unknown	**Disregarded study** **Not assignable (detailed result data missing to evaluate relevance of ambiguous result)** Bacterial strains: *S. typhimurium*: TA100, TA98, TA1537, TA1535, TA97 Test concentrations (± metabolic activation (S9 mix)): 100, 333, 1000, 3333, 10 000 µg/plate S9: hamster liver S9, untreated and rat liver S9 Aroclor-induced Vehicle: DMSO Negative/positive control: yes/yes	**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)
In vitro mammalian cell gene mutation test using the thymidine kinase gene similar to OECD TG 490	PR53:1 CAS Nr.: 5160-02-1	**Key study** **Reliable with restrictions** Cell culture: mouse lymphoma L5178Y cells Test concentrations	**Negative** (with and without metabolic activation) Cytotoxicity: no	(Myhr et al., 1991)

Method, guideline, deviations if any	Test substance	Relevant information about the study	Observations	Reference
Deviation: • No data on purity GLP: not specified	Purity: no data	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 (~ 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/positive control: yes/yes	Precipitations: yes, above 7.5 µg/mL Neg. control: valid Pos. control: valid	
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 restrictions** **(only 100 metaphases scored per concentration)** Cell culture: Chinese hamster lung fibroblasts (V79) Test concentrations: ± metabolic activation (S9 mix): 30, 150, 300 µg/mL metabolic activation: rat liver S9 induced by Aroclor Justification for top concentration: significant cytotoxicity ≥ 400 µg/mL Treatment time: ± metabolic activation: 4 and 18 h Sampling time: 4.5, 15.5, 25.5 h after beginning of treatment Vehicle: DMSO Negative/positive control: yes/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	(Hoechst AG, 1989b)
In vitro mammalian chromosomal aberration test Not similar to OECD TG	D&C Red No 9 CAS 5160-02-1 EC 225-935-3	**Disregarded study** **Not reliable** **(exposure and sampling times are not according to OECD**	**Negative** with and without metabolic activation	(Ivett et al., 1989)

Method, guideline, deviations if any	Test substance	Relevant information about the study	Observations	Reference
473 Deviation from OECD TG 473 • Continuous exposure of about 12-14 h without metabolic activation missing • Short-term treatment with and without metabolic activation not adequate (8 h and 2 h instead of 3-6 h) • Sampling time too short (2-2.5 h instead of 1.5 times the normal cell cycle length) • Only 200 (instead of 300) metaphases evaluated • No specific data on justification for top dose GLP: not specified	Purity: 89.8 %	**TG, too less cells analysed)** 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: 8 h with metabolic activation: 2 h and 8 h Sampling time: 2 - 2.5 h Vehicle: DMSO Negative/positive control: yes/yes	Cytotoxicity: no detailed data Precipitations: yes, ≥ 250 µg/mL Controls Neg. control: valid Pos. control: valid	

Table 10: 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	Observations	Reference
Unscheduled DNA synthesis (UDS) test with mammalian liver cells in vivo	PR53:1 CAS 5160-02-1 EC 225-935-3	**Supporting study*** **Reliable without restrictions*** Species: rats Piebald Virol Glaxo	**Negative** *** A negative result is not conclusive for the assessment of induction of gene mutations.**	(Westmoreland and Gatehouse, 1992)

Method, guideline, deviations if any	Test substance	Relevant information about the study	Observations	Reference
Similar to OECD TG 486 Deviation: none GLP: not specified	Purity: no data	Number of animals per group: 7 males Target organs: liver Administration route: oral (gavage), single dosage Dose level: 1000 and 2000 mg/kg bw/d Justification for top dose: limit test Sampling: **16 h** Vehicle: Corn Oil Positive control: yes (2-acetylaminofluorene for 16 h) Negative control: yes	**Results:** No marked increase in incidence of cells in repair at 16 h sampling time Toxicity: no toxicity observed Controls: Neg. control: valid Pos. control: valid	
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 (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), single dosage Dose level: 500,1000 and 2000 mg/kg bw/d Justification for top dose: limit test Sampling: **24 or 48 h** Vehicle: corn oil Positive control: yes (cyclophosphamide, 24 h) Negative control: yes	**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)

10.6.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

In vitro data

Six bacterial reverse mutation tests performed with PR53:1 are available. Four of these studies were considered reliable (CIBA-GEIGY Limited, 1985; Hoechst AG, 1985a; Hoechst AG, 1985b; Hoechst AG, 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, using 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 available bacterial reverse mutation test conducted with PR53:1.

There is one reliable *in vitro* mammalian gene mutation test available similar to OECD TG 490 (Myhr et al., 1991). This test yielded a negative result.

Two *in vitro* cytogenicity tests exist which were performed with PR53:1. The *in vitro* mammalian chromosomal aberration test (Hoechst AG, 1989b) performed similar to OECD TG 473, is considered reliable by the DS. This test yielded a negative result.

Overall, the available *in vitro* data do not indicate a concern for mutagenic action of PR53:1. However, there is no information for the 5th strain (e.g. *E.coli* WP2uvrA) in a bacterial reverse mutation test using Prival activation, which is considered most appropriate by DS as the PR53:1 is an azo dye.

In vivo data

Two *in vivo* somatic cell genotoxicity tests performed wit PR53:1 using a relevant test system are available, an unscheduled DNA Synthesis (UDS) test with mammalian liver cells (Westmoreland and Gatehouse, 1992) and a mammalian erythrocyte micronucleus test (MN) (Westmoreland and Gatehouse, 1992). Both tests yielded negative results and support the negative findings of the *in vitro* studies.

The UDS test was performed similarly to OECD TG 486 and is considered reliable without restrictions. However, it is to note that 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 a UDS assay alone is not proof that the substance does not induce gene mutations.

The available *in vivo* mammalian erythrocyte micronucleus test was performed similarly to OECD TG 474. As no evidence of exposure of the bone marrow has been provided, this *in vivo* micronucleus test is only supportive of the negative findings obtained in the *in vitro* mammalian chromosomal aberration test (Hoechst AG, 1989b).

10.6.2 Comparison with the CLP criteria

Hazard class not assessed in this dossier. The data provided as supporting information for the assessment of carcinogenicity.

10.6.3 Conclusion on classification and labelling for germ cell mutagenicity

Hazard class not assessed in this dossier. The data provided as supporting information for the assessment of carcinogenicity.

10.7 Carcinogenicity

Table 11: Summary table of animal studies on carcinogenicity

Methods	Results	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 **Reliable without restrictions** Species: rats Strain: F344 n: 50/dose group/sex Dose levels: 0, 1000, 3000 ppm Route: oral (feed) Food conversion factor: 20 (for older rats) Calculated doses*: 0, 50, 150 mg/kg bw/d Treatment time: 103 weeks, daily Post exposure period: 1 week Dose level selected based on effects observed in 91 day study *calculated by DS	**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** <u>Neoplastic lesions (0, 1000, 3000 ppm):</u> Male rats: Combined types of splenic sarcoma (0/50 (0 %), 0/50 (0 %), **26/48 (54 %)**) including fibrosarcoma (17/48) arising from red pulp or capsule of the spleen, 1 animal with leiomyosarcoma, 5 splenic osteosarcoma, 1 sarcoma and 1 fibrosarcoma of the splenic capsule, 1 fibrosarcoma of the splenic red pulp 11 of splenic tumours metastasised to peritoneal tissues, 2 sarcoma of multiple organs originated in the spleen Weeks to first observed tumor: 68 Historical control data: Fibrosarcoma (same lab) M: 0/140 and Fibrosarcoma (same lab, entire bioassay program): M: 3/2960 (0.1 %) Hepatocellular carcinoma in 1/50 (2 %) control male. Neoplastic nodules of the liver: Males: 0/50 (0 %), 6/50 (12 %), 7/49 (14 %) (hepatocytes with basophilic or eosinophilic cytoplasm) Female: 1/50 (2 %), 1/50 (2 %), 5/50 (10 %) <u>Non-neoplastic lesions (at 3000 ppm)</u>: Males: 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 <u>Survival</u>: No effects on mortality, body weight and food consumption; 6 % weight depression in high-dose females	(NTP, 1982)

Methods	Results	Reference
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 **Reliable without restrictions** Species: mice Strain: B6C3F1 n: 50/dose group/sex Dose levels: 0, 1 000, 2 000 ppm Route: oral (feed) Food conversion factor: 7 (for mice) Calculated doses*: 0, 142, 285 mg/kg bw/d Treatment time: 103 weeks, daily Post exposure period: 1 week Dose level selected based on effects observed in 91 day study *calculated by DS	**Not carcinogenic for B6C3F1 mice** <u>Neoplastic lesions (0, 1000, 2000 ppm):</u> Males: statistically significant increased incidence of hepatocellular carcinoma (4/50 (8 %), 9/50 (18 %), 11/50 (22 %)) , but not above mean historical incidence in this laboratory (65/297 – 22 %, no time range for this data reported) Females: malignant lymphomas of the hematopoietic system (2/50 (4 %), 2/50 (4 %), 7/49 (14 %) increased incidence <u>Survival</u>: No effect on mortality, body weight and food consumption, except mean body weight of treated females slightly lower in 2nd year (< 10 %)	(NTP, 1982)
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 including in utero treatment and F1 generation, no GLP **Reliable with restrictions** Species: rat Strain: Charles-River CD Sprague-Dawley n: 70/dose group/sex Dose levels: **Part I:** 0, 100, 200, 500 ppm **Part II:** 0 and 10 000 ppm in the diet (additional study with higher	**Increased incidence of splenic sarcoma in rats** <u>Neoplastic lesions:</u> 2 Haemangiosarcomas involving spleen and/or liver in control males Splenic sarcoma in 4 males and 1 female at 10 000 ppm in F1 animals, not statistically significant <u>Survival</u>: No effects on mortality, body weight, food consumption in parental animals or offspring At 10 000 ppm body weight of male and female pups decreased at weaning (day 21 postpartum); lower body weights throughout chronic phase (< 10 %) <u>Clinical findings of toxicity:</u> Signs of anaemia at 10 000 ppm in males and females Increased spleen weight in males and females (at 10 000 ppm), increased heart	(CTFA, 1982a; CTFA, 1982b) Cited in (FDA, 1986)

Methods	Results	Reference
concentration) Route: oral (feed) Corrected Dose (calculated based on food consumption): Part I: F0: 8, 17, 43 mg/kg bw/d in males; 9, 17, 42 mg/kg bw/d in females F1: 5, 10, 26 mg/kg bw/d in males; 6, 13, 32 mg/kg bw/d in females Part II: F0: 790 mg/kg bw/d for males, 894 mg/kg bw/d for females F1: no data available for males (calculated: 500 mg/kg bw/d); 521 mg/kg bw/d for females Treatment time: 30 months, daily Data of the two studies were combined	weight; increased kidney weight in females and increased testicular weight Males: splenic lesions at 10 000 ppm in males: splenic congestion, fibrosis, mesothelial hyperplasia, mesothelial cysts, haemosiderosis and splenic hematopoiesis	
2-year feeding study in rats D&C Red No. 9 (known trading name of PR53:1) (CAS 5160-02-1) Purity: 86 % Non-guideline study, No GLP **Limited reliability due to** 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 Species: rats Strain: Osborne-Mendel n: 25/dose group/sex Dose levels: 0, 100, 500, 2500, 10 000 ppm Route: oral (feed) Food conversion factor: 20 (for older rats) Calculated doses*: 0, 5, 25, 125, 500 mg/kg bw/d Treatment time: 103 weeks, daily Post exposure period: 10 days Vehicle: ethanol *calculated by DS	**No increased evidence for carcinogenicity but severe splenic effects** No effects on mortality At 10 000 ppm: moderate splenomegaly, splenic infarcts, haematomas or scars (6 rats), splenic haemosiderosis ≥ 2500 ppm: slight bone marrow hyperplasia, decreased haemoglobin, abnormal red blood cells ≥ 500 ppm: Slight to moderate splenomegaly (7/12 at 500 ppm, 4/12 at 2500 ppm, 2/12 at 10 000 ppm)	(Davis and Fitzhugh, 1962) (publication)

Methods	Results	Reference
Combined repeated dose and carcinogenicity test D&C Red No. 9 (known trading name of PR53:1) (CAS 5160-02-1) Purity: 76 % (according to FDA report) Similar to OECD TG 453, No GLP **Reliable with restrictions** Species: mice Strain: Charles-River CD1 n: 60/dose group/sex Dose levels: 0, 50, 250 and 1000 ppm Route: oral (feed) Corrected dose using food consumption: 7, 38, 147 mg/kg bw/d in males; 12, 56, 237 mg/kg bw/d in females Treatment time: 24 months/105 weeks (daily)	**Not carcinogenic in mice** <u>Survival</u>: No effects on mortality, body weight, food consumption <u>Clinical findings of toxicity</u>: Signs of anaemia at 1000 ppm in females (decreased red blood cells, increased reticulocytes, decreased haemoglobin and haematocrit), anaemia not evident at 250 ppm Decreased abs. kidney weight in male mice (1000 ppm), but rel. kidney weight similar to control	(CTFA, 1982c) Cited in (FDA, 1986)
18 month skin painting study D&C Red No. 9 (known trading name of PR53:1) (CAS 5160-02-1) Purity: 90 % Non-guideline study, No GLP **Limited reliability:** Limited reporting, no data on individual animals, limited number of organs analysed, only selected animals from solvent and positive control, study period 18 month, dermal application twice a week with very low dose, incidences only on a limited number of findings, no body weight information, no historical control data Species: mice Strain: 100 ICR n: 50/dose group/sex, 150 in control group Dose levels: dermal application to dorsal area; 0.1 mL of 1 % solution of dye (6 cm^2) twice a week for 18 months (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	**No increase in neoplasia after dermal application of the test dye compound** Single incidences of mammary gland adenosarcoma (2 females); 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 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.	(Carson, 1984) (publication)

10.7.1 Short summary and overall relevance of the provided information on carcinogenicity

Several studies are available that assess the carcinogenic potential of PR53:1 in animals (Carson, 1984; CTFA, 1982b; CTFA, 1982a; CTFA, 1982c; Davis, 1963; NTP, 1982). All studies are performed with D&C Red No. 9 which is a known trading name of PR53:1. Subsequently, always the name PR53:1 will therefore be used.

No human data are available.

Studies with rats:

2-year feeding studies in rats equivalent to OECD TG 451 (NTP guidelines) were performed by NTP (NTP, 1982). Groups of 50 male and 50 female F344 rats were administered 0, 1000 and 3000 ppm PR53:1 in feed supplied for 103 weeks (calculated doses: 0, 50, 150 mg/kg bw/d, based on a general conversion factor of 20 for older rats according to CLP guidance). 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 animals with splenic osteosarcoma. 11 of the splenic tumours metastasised to peritoneal tissues. Non-neoplastic splenic lesions were observed in high-dose animals of both sexes. 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. The incidence of fibrosarcoma in males is significantly higher than historical control records for the same lab (0/140, 0 %) and even higher than historical records for the entire bioassay program of this laboratory (3/2960 (0.1 %)).

Dose-related increased incidence of neoplastic nodules of the liver were found in both sexes, with males showing a significant increase of 6/50 animals in the mid-dose and 7/49 animals in the high-dose group, while the increase in females was less pronounced with increased incidence at the high dose group only (5/50 animals). The nodules consisted of hepatocytes with basophilic or eosinophilic cytoplasm and were of relatively small size. The incidence in males at both doses and females at the high dose were above the historical control (M/F: 5/140 (3.6 %)) in that laboratory.

Further long-term feeding studies with lifetime exposure in rats were performed according to FDA guidelines (CTFA, 1982b; CTFA, 1982a). In the first study, 70 Sprague-Dawley rats/dose/sex were treated with 0, 100, 200, 500 ppm substance in the diet (corrected doses: F0: 8/9, 17/17, 43/42 mg/kg bw/d M/F, F1: 5/6, 10/13, 26/32 mg/kg bw/d M/F; calculated doses: 0, 5, 10 and 25 mg/kg bw/d, based on a general conversion factor of 20 for older rats according to the CLP guidance) for 60 days before mating, with dietary administration of test substance continued during mating, gestation, lactation and rearing (CTFA, 1982a). 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 10 000 ppm substance in the diet; corrected dose: F0: 790/894 mg/kg bw/d M/F, F1: no data/521 mg/kg bw/d M/F; calculated dose: 0 and 500 mg/kg bw/d) using the same method, as dose levels of the first study were found too low. This was performed and is included as part II of the same report (CTFA, 1982b).

In both study parts there were no effects on survival and food consumption, but body weight of male and female pups was slightly decreased from day 21 postpartum. At 10 000 ppm, splenic sarcoma was observed in 4 males and 1 female. The pathology report states that in ‘minor cases’ some tissues could not be investigated due to autolysis, sectioning difficulties or laboratory errors, so that the number of tissues examined from each group does not necessarily represent the number of animals in that group. As details on the number of examined tissues are not given in the available report, incidence percentages calculated in Table 13 by the DS based on the group size of 70 animals/group bear uncertainties of over- as well as underestimation. However, these uncertainties are considered to be low, as the report states only missing tissues in minor cases and the authors concluded that these did not interfere with the evaluation of effects of compound administration.

Clinical findings of toxicity included signs of anaemia and increased spleen and heart weight in male and female rats of the highest dose group (10 000 ppm PR53:1). Males also showed splenic lesions such as

splenic congestion, fibrosis, mesothelial hyperplasia, mesothelial cysts, haemosiderosis and splenic haematopoiesis.

In a report by the United States Food and Drug Administration (FDA, 1986) the following limitations of the NTP data were discussed: the use of solid-bottom cages possibly leading to coprophagy and therefore higher doses of the substance and ingestion of metabolites and the presence of other carcinogens in the room, as other substances were tested at the same time. However, both points were considered not relevant as, first, the same type of tumours was detected using wire cages in the CTFA studies and, secondly, no splenic neoplasms were observed with other test substances or in control animals.

In a further study reported by Davis and Fitzhugh (1962) groups of 25 male and 25 female Osborne-Mendel rats were administered 0, 100, 500, 2500 and 10 000 ppm PR53:1 in feed supplied for 103 weeks (calculated doses: 0, 5, 25, 125, 500 mg/kg bw/d, based on a general conversion factor of 20 for older rats according to CLP guidance). 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 haemosiderosis were reported.

Studies with mice:

2-year feeding studies in mice equivalent to OECD TG 451 (NTP guidelines) were performed by NTP (NTP, 1982). Groups of 50 male and 50 female B6C3F1 mice were administered 0, 1000 and 2000 ppm PR53:1 in feed supplied for 103 weeks (calculated doses: 0, 142, 285 mg/kg bw/d, based on a general conversion factor of 7 for mice according to the CLP guidance). No effects on survival, body weight or food consumption were observed. The incidence of hepatocellular carcinoma was increased in 4/50 (8 %) and 9/50 (18 %) control and mid-dose males, respectively. Only at the high dose, this increase (11/50 or 22 %) was statistically significant compared to the control, but the incidence was not above the mean historical control incidence.

Female mice showed an increased incidence of mixed-type malignant lymphomas of the haematopoietic system in the high-dose group, which was not statistically significant in direct comparison with the controls; however, a statistically significant positive dose-response trend was found. No historical control data are available for this kind of tumours. The observed significant trend of increased incidences of malignant lymphomas of the mixed type were seen in high-dose females only. As the tumour incidences of malignant lymphomas (total) may occur spontaneously at variable incidences, no significant increase was seen for the mixed type in comparison to the internal control group, and the incidence of the number of malignant lymphomas (all types) at the high dose was not significantly different from that in the control groups, it is considered unlikely that mixed-type malignant lymphoma was a treatment-related effect.

Further long-term feeding studies with lifetime exposure in mice were performed according to FDA guidelines (CTFA, 1982c). Here, 60 mice/dose/sex were exposed to 0, 50, 250 and 1000 ppm of the substance in the diet (corrected doses: 7/12, 38/56, 147/237 mg/kg bw/d M/F; calculated doses: 0, 7, 35 and 142 mg/kg bw/d, based on a general conversion factor of 7 for mice according to the CLP guidance) for 18 months. There were no effects on survival, body weight or food consumption. No neoplastic lesions were observed.

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. 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 in the report 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 has been performed with a low dose level (based on lipstick use considerations).

Table 13 summarises the tumour incidences in studies with PR53:1 in rats and mice.

Table 12: Summary of tumour incidences in animal studies with PR53:1.

Study: 2-year feeding study in F344 rats (NTP, 1982)					
Dose in mg/kg bw/d (calculated)		**0**	**50**	**150**	**Historical control data**
Splenic sarcoma (all)	M F	0/50 0/50	0/50 0/50	**26/48 (54 %)[1,2]** 0/50	**NTP data (1984-1994)[4]:** All types of splenic sarcoma[3] M: 7/1003 (0.7 %); F: 0/1003
thereof splenic fibrosarcoma	M F	0/50 0/50	0/50 0/50	**17/48 (35 %)[1,2]** 0/50	**NTP report, 1982:** Fibrosarcoma (same lab, no time range specified): M: 0/140 Fibrosarcoma (entire bioassay program, no time range specified): M: 3/2960 (0.1 %) **NTP data (1984-1994)[4]:** Fibrosarcoma: M: 4/1003 (0.4 %), range 0-6 % F: 0/1003
Hepatic neoplastic nodules	M F	0/50 1/50 (2 %)	**6/50 (12 %)[1,2]** 1/50 (2%)	**7/49 (14 %)[1,2]** **5/50 (10 %)[2]**	**NTP report, 1982:** Neoplastic nodules in liver (same lab, no time range specified): M/F: 5/140 (3.6 %) **NTP data (1984-1994)[4]:** Neoplastic nodules in liver: no data
Hepatocellular carcinoma	M F	1/50 (2%) 0/50	0/50 0/50	0/49 0/50	**NTP data (1984-1994)[4]:** Hepatocellular carcinoma: M: 7/1002 (0.7 %), range 0-6 % F: 1/1000 (0.1 %), range 0-2 %
Study: 2-year feeding study in B6C3F1 mice (NTP, 1982)					
Dose in mg/kg bw/d (calculated)		**0**	**142**	**285**	**Historical control data**
Hepatocellular carcinoma	M F	4/50 (8 %) 4/50 (8 %)	9/50 (18 %) 2/50 (4 %)	11/50 (22 %)[1,2] 2/49 (4 %)	**NTP report, 1982:** Hepatocellular carcinoma (same lab, no time range specified): M: 65/297 (22 %) **NTP data (1984-1994)[4]:** Hepatocellular carcinoma M: 194/950(20.4 %), range 10-40 % F: 104/951(10.9 %), range 4-20 %
Haematopoietic system: malignant lymphomas, mixed type	M F	0/50 2/50 (4 %)	1/50 (2 %) 2/50 (4 %)	0/50 **7/49 (14 %)[2]**	**NTP data (1984-1994)[4]:** Haematopoietic system: malignant lymphomas, mixed type: no data
Haematopoietic system: all malignant lymphomas	M F	5/50 (10 %) 11/50 (22 %)	4/50 (8 %) 17/50 (34 %)	4/50 (8 %) 12/49 (24 %)	**NTP data (1984-1994):** M: 71/952 (7.5 %), range 2-14 % F: 167/953 (17.5 %), range 6-30 %

Study: 26-30 month dietary study (F0 and F1 dosed) including *in utero* exposure in SD rats, part 2 (CTFA, 1982b)				
Dose in mg/kg bw/d (F1) (calculated)		**0**	**500**	**Historical control data**
Splenic sarcoma (all types) [$]analysed tissue samples might be slightly lower due to minor cases of tissue lost	M F	2/70[§] (3 %) 0/70[§]	4/70[§] (F1) (6 %) 1/70[§] (F1) (1 %)	No data

[1]Statistical significant increase compared to control. [2] Statistical significant positive trend. [3] including: fibrosarcoma, splenic osteosarcoma, leiomyosarcoma, sarcoma. [4] No other time range available; source: https://ntp.niehs.nih.gov/data/controls/index.html

Furthermore, the NTP (NTP, 1994) conducted a chronic drinking water study in rats and mice using barium chloride dihydrate to assess its carcinogenic potential as is was stated by the authors (NTP, 1982) that: "*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. Thus, the DS considers the barium cation in PR35:1 will presumably not contribute to the carcinogenic effects of PR53:1.

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 (CTFA, 1982b; CTFA, 1982a; NTP, 1982). The increased incidence was statistically significant in the NTP study, but not in the CTFA study. However, results from the CTFA study are considered 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 CTFA 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 (CTFA, 1982b; CTFA, 1982a; Davis and Fitzhugh, 1962; NTP, 1982). Furthermore, the sensitivity of different rat strains concerning splenic tumours was discussed. The authors suggest that Sprague-Dawley rats appear to be less sensitive than F344 rats to aniline-related compounds, but still showed the unusual effects described in the CTFA study.

There is no evidence of carcinogenicity in mice (CTFA, 1982c; 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 lack of a carcinogenic potential for female rats and mice of both sexes could be questioned at least based on the data of the NTP study.

The high incidence of splenic sarcomas in the rat study (NTP, 1982), the diversity of sarcomas that all originated from mesenchymal tissue with different cell types as most prominent tumour cells (leio-, osteo-, and fibrosarcomas) and the frequently observed metastasis are indicating a high malignancy.

This is supported by the fact that splenic sarcoma are a rare type of tumour in animals and 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 were observed. In addition, similar splenic lesions and increased incidence of splenic sarcoma in F344 rats were described for structurally related compounds such as aniline, naphthylamine and other aromatic amines and aromatic azo compounds.

Mode of Action

The data available for PR53:1 do not suggest a genotoxic mode of action for tumour formation (see details in chapter 10.6). Goodman et al. (1984) and Weinberger et al. (1985) described possible modes of action, both discussing splenic lesions as starting point for tumour formation. Goodman et al. (1984) suggested a splenic haemosiderosis secondary to methaemoglobinemia, leading to tumour formation, whereas Weinberger et al. (1985) suggested acute vascular congestion as the initial alteration in the spleen leading to haemorrhage, fibrosis, and transformed cells.

The chronic and subchronic studies with PR53:1 (details see chapter 10.10) reveal that PR53:1 induced haemolytic anaemia in mice and rats, including a decrease in blood parameters (e.g. haemoglobin, haematocrit, and red blood cell count) accompanied by haemosiderosis of the spleen. Effects appeared less severe in mice compared to rats. In none of the available studies, methaemoglobin formation was assessed.

For the structurally related compound aniline, studies indicate that erythrocyte toxicity (indicated by methaemoglobin formation) leads to splenic lesions due to overload with cell debris, haemoglobin and redox-active iron released from damaged erythrocytes and induced oxidative stress resulting in fibrosis, fatty metamorphosis, severe splenic congestion, mesothelial hyperplasia and cyst formation, capsular fibrosis, and multifocal cellular proliferations in the spleenic capsule. This is a major cause for pre-neoplastic splenic lesions, which can result in tumour formation in rats upon chronic exposure (MAK, 2007). Species differences in methaemoglobin reductase activity, which is responsible for regeneration of functional haeme from methaemoglobin can explain lower sensitivity of mice in comparison to rats.

A similar mode of action for tumour formation as described for aniline seems plausible for PR53:1, nevertheless, the available data does not allow to draw a final conclusion with certainty.

10.7.2 Comparison with the CLP criteria

As there is no information on the carcinogenicity of PR53:1 in humans, classification in Category 1A (known human carcinogens according to CLP Regulation, Table 3.6.1) would not be appropriate.

To determine if classification into Category 1B or 2 is warranted for PR53:1, the strength of evidence in the available animal studies has to be determined as sufficient (1B) or limited (2) (CLP Regulation, Table 3.6.1).

There was an increased incidence of splenic sarcomas in male rats treated with PR53:1 in the diet. In contrast to the NTP study on F344 rats, the increased incidence of splenic sarcomas was not statistically significant in the second study with Sprague-Dawley rats. However, as splenic sarcoma is a rare type of tumour in animals and similar patterns of non-neoplastic splenic lesions such as fatty metamorphosis, focal or diffuse splenic fibrosis, unusually severe forms of splenic congestion with or without haemorrhages or infarcts, capsular fibrosis, and hyperplasia were observed in both studies, the study on Sprague-Dawley rats is considered as supportive evidence.

No tumours were seen in female rats or in mice, but non-neoplastic splenic lesions across both sexes in rats and to a lesser extent also in mice were observed. It is plausible that tumours in male rats have been a consequence of these pre-neoplastic lesions and the occurrence of splenic lesions in females and mice are thus considered as supportive evidence.

There is no information that the observed effects in rats (and mice) are not relevant to humans, thus a relevance is presumed.

In accordance with the CLP criteria and associated guidance (CLP Annex I, 3.6.2.2.6), in Table 13 the following factors are considered in proposing how to classify PR53:1 for carcinogenicity.

Table 13: Compilation of factors to be taken into consideration in the hazard assessment.

Factor	Evidence	Classification category
Tumour type and background incidence	Splenic sarcomas. High incidence (statistically significant) in F344 male rats. Increased incidence (not statistically significant) in Charles-River CD Sprague-Dawley male rats. High incidence, high malignancy and metastasis Very low spontaneous incidence.	1B or 2 1B
Multi-site responses	No	2
Progression of lesions to malignancy	Yes	1B or 2
Reduced tumour latency	Indicated by the observation that the first tumour was observed in week 68, no data on mean latency time,	No robust data
The possibility of a confounding effect of excessive toxicity at test doses	No	1B or 2
Responses in single or both sexes	Males only. Non-neoplastic splenic lesions also observed in females.	2
Responses in single or several species	Rat only. Negative in mouse, but non-neoplastic splenic lesions also observed in mice.	2
Structural similarity to substances for which there is good evidence of carcinogenicity	Similar splenic lesions and increased incidence of splenic sarcoma in F344 rats were described for structurally related compounds such as aniline and other aromatic amines and aromatic azo compounds.	Supportive information, no direct impact for classification
Route of exposure	Tumours after dietary treatment; no significant information on applicability of other routes.	No impact
Comparison of absorption, distribution, metabolism and excretion between test animals and humans	Only very limited data for animals available, no data in humans; No conclusions on differences possible.	Default assumption of relevance for humans
Mode of Action and relevance to humans	Non-genotoxic. Splenic lesions as starting point for tumour formation plausible, significant uncertainties about exact mode of action. Relevance to humans cannot be excluded.	2

Taking into account all of these factors, the observation of splenic sarcomas in male rats is considered to **present limited evidence of carcinogenicity** and thus the criteria for classification in Category 2 are met.

10.7.3 Conclusion on classification and labelling for carcinogenicity

Based on the available data, barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate]; C.I. Pigment Red 53:1 (CAS 5160-02-1; EC 225-935-3) should be classified as Carc. 2, H351 according to the criteria laid down in the CLP Regulation, Table 3.6.1. The generic concentration limit (GCL) of ≥ 1,0 % shall apply.

Barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate]; C.I. Pigment Red 53:1 is available on the EU market as a nanoform. In the registration dossier of this substance it is stated by the lead registrant, that "test materials used in this dossier are all considered to fall under the definition of nanomaterials according to the European Commission Recommendation 2011/696/EU as the synthesis and manufacturing of this pigment always yields particulate material with a fine particle size distribution". But the test material used 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 were performed in the early 1980s. However, 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 as the assessment is based on the intrinsic properties of the substance, not on properties driven by the particle form.

10.8 Reproductive toxicity

Not assessed in this dossier.

10.9 Specific target organ toxicity-single exposure

Not assessed in this dossier.

10.10 Specific target organ toxicity-repeated exposure

Hazard class not assessed in this dossier. The data provided as supporting information for the assessment of carcinogenicity.

Table 14: Summary table of oral animal studies with PR53:1 on STOT RE.

Method	Results/Observations (regarding STOT RE)	Reference (Guidance Value for STOT RE*)
2-year feeding study D & C Red No. 9 (trading name of PR53:1) Batch No: Lot No. Z-8054 Purity: 89.8 %, Impurities: sodium and barium sulfates Study according to OECD TG 451 (NTP guideline including single dose, 2-week and 13-week studies), No GLP **Reliable without restrictions** Rats, F344 (N=50/sex/dose); Mice, B6C3F1 (N=50/sex/dose) Route: oral, diet Dose rats: 0, 1000, 3000 ppm; Corr. to 0, 50, 150 mg/kg bw/d (Conversion factor 20 for older rats) Dose mice: 0, 1000, 2000 ppm; Corr. to 0, 143, 286 mg/kg bw/d (Conversion factor 7) Treatment time: 103 weeks, daily Post exposure period: 1 week Dose level selected based on effects observed in 91 day study	**Rats:** <u>Splenic lesions in males, at 150 mg/kg bw/d:</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/d:</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/d, 23 % (11/48) at 150 mg/kg bw/d**,** compared to 6 % (3/50) in controls **Mice**: No non-neoplastic findings in treated mice	(NTP, 1982) GV STOT RE 2 ≤ 12.6 mg/kg bw/d (103 weeks)
Range finding study for carcinogenicity study (13 week study) D & C Red No. 9 (trading name of PR53:1) Batch No: Lot No. Z-8054 Purity: 89.8 %, Impurities: sodium and barium sulfates Non-guideline study, No GLP **Reliable with restrictions** (no data on haematology, clinical biochemistry, urine analysis) Rat, F344 (N = 10/sex/dose); Mice, B6C3F1 (N = 10/sex/dose)	**Rats**: <u>Haemosiderosis of the liver</u>: in all dosed female rats and in 9/10 males at 625 mg/kg bw/d, 6/10 at 300 mg/kg bw/d, and 3/10 at 150 mg/kg bw/d <u>Pigment deposition in kidney tubular epithelium</u> in all dosed rats <u>Enlarged spleen (2-5 times)</u> in all dosed rats; <u>congestion and lymphoreticular hyperplasia in spleens</u> of all dosed female rats, in all male rats ≥ 300 mg/kg bw/d, and in 8/10 male rats at lowest dose (150 mg/kg bw/d) <u>Lymphoreticular hyperplasia of thymic lymph nodes</u> in 75 – 100 %	(NTP, 1982)

Method	Results/Observations (regarding STOT RE)	Reference (Guidance Value for STOT RE*)
Route: oral, diet Dose rats: 0, 3000, 6000, 12500, 25000, or 50000 ppm; Corr. to 0, 150, 300, 625, 1250, 2500 mg/kg bw/d (Conversion factor 20 for older rats) Dose mice: 0, 600, 1250, 2500, 5000 or 10000 ppm; Corr. to: 0, 86, 179, 357, 714, and 1429 mg/kg bw/d (Conversion factor 7) Treatment time: 91 d Gross necropsy of all animals, histopathology of certain tissues from controls and highest dose animals	of female rats in each dosed group (except for lowest dose, 150 mg/kg bw/d; 0/10), in 70 – 100 % of male rats in each dosed group (except for highest dose; 3/7) **Mice**: <u>Congestion of the spleen</u>: in 55/60 mice at ≥ 357 mg/kg bw/d <u>Deposits of haemosiderin</u> were present to a greater extent in all dosed animals than in controls with exception of females at 86 or 179 mg/kg bw/d and males at 86 mg/kg bw/d	GV STOT RE 2 ≤ 100 mg/kg bw/d (90 days)
30-month chronic toxicity and potential carcinogenicity study in rats with *in utero* and lifetime exposure Desert Red D & C Red No. 9 Ba. Lake (trading name of PR53:1) Batch No: #547530, C-15-I01 Purity: 76 % According to FDA guidelines, Pre-GLP **Reliable with restriction** (individual data e.g. clinical signs missing) Rat, CD [CRL:COBS CD (SD) BR] (F0/F1: N=70/sex/dose) Route: oral, diet Dose: Part I: 100, 200, and 500 ppm. Corr. to: 8, 17, 43 mg/kg bw/d in F0 males; 9, 17, 42 mg/kg bw/d in F0 females; 5, 10, 26 mg/kg bw/d in F1 males; 6, 13, 32 mg/kg bw/d F1 females Part II: 10 000 ppm. Corr. to: 790 mg/kg bw/d for F0 males, 894 mg/kg bw/d for F0 females, no data available for F1 males (calculated: 500 mg/kg bw/d); 521 mg/kg bw/d for F1 females Part I Treatment time: 8 weeks prior to mating (part I), 9 weeks prior to mating (part II), 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	<u>Haemoglobin</u>: Part I: 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/d) at month 18 Part II: 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 <u>Haematocrit:</u> Part I: Significant decrease (-6 %) in F1 high dose females (32 mg/kg bw/d) at month 3 and at month 12 (-6 %) Part II: 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 months (-8, -14, -15 %, no further data) <u>Red blood cell count</u>: Part I: Significant decrease (-10 %) in F1 high dose females (32 mg/kg bw/d) at month 12 Part II: Significant decrease in treated F1 males at month 3, 6, 12, 18, and 24 (-31, -21, -24, -10, -18 %), significant decrease in treated F1 females at 3, 12, 18, and 24 months (-32, -24 %, no further data available) <u>Reticulocyte count:</u> Part I: Significant increase (92 and 100 %) in F1 mid (12 mg/kg	(CTFA, 1982a) and (CTFA, 1982b)

Method	Results/Observations (regarding STOT RE)	Reference (Guidance Value for STOT RE*)
signs, signs of toxicity 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)	bw/d) and high (32 mg/kg bw/d) dose females at month 18 Part II: 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 and spleen weight-body weight ratio: Part I: significant increase (20.9 %, respectively 22.5 %) 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 ratio 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; Part II: Significant increase in treated F1 males (318 %, resp. 372 %) and females (210 %, resp. 246 %) at 12 months, and F1 males (183 %, resp. 175 %) and females (349 %, resp. 382 %) at terminal kill (month 30) Haemosiderosis of the spleen: Part I: F1 high dose females after 12 months Hemosiderin accumulation in liver: Part II: in dosed females (unknown incidence) Hemosiderin accumulation in kidneys: Part II: in dosed females and males (unknown incidence) Further observations (part II): Splenomegaly, splenic extramedullary haematopoiesis, splenic congestion, fibrosis, haemosiderosis, 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)	GV STOT RE 2 ≤ 11 mg/kg bw/d (120 weeks)

Method	Results/Observations (regarding STOT RE)	Reference (Guidance Value for STOT RE*)
Combined repeated dose and carcinogenicity (daily for 24 months/105 weeks) D & C Red No. 9 (trading name of PR53:1) Batch No: Lot #AA-3779 Purity: ≥ 76 % Similar to OECD TG 453, No GLP **Reliable with restrictions** (no data on clinical biochemistry of plasma or serum, no data collected for oestrus cycle or sperm parameters, no urinalysis) Mouse, CD-1 (N=60/sex/dose) Dose: 0, 50, 250 and 1 000 ppm Route: oral, diet Corr. to: 0, 7, 38, 147 mg/kg bw/d in males; 0, 12, 56, 237 mg/kg bw/d in females Haematology at 3, 6, 12, and 18 months (N=10/sex/dose)	Haemoglobin: Significant decrease in high-dose females (-11.4 % vs. control) at 18 months, in high-dose males at 6 months (-7.2 %) Haematocrit: Significant decrease in low-dose (-8.1% vs. control) and high dose females (-9.9 %) at 18 months, decrease (but not significant) at the mid-dose, (-5.1 %) Red blood cell count: Statistically increase for high dose females (14.8 % vs. control) at 3 months; significant decrease (-10.7 %) after 18 months Gross and histopathologic evaluation did not reveal any compound related findings.	(CTFA, 1982c) GV STOT RE 2 ≤ 12.6 mg/kg bw/d (103 weeks)
32 d Feeding study Test material: Confidential Annex Purity: unknown Similar to OECD TG 407, Pre-GLP **Not reliable** (insufficient characterisation of test material, no data on clinical biochemistry, main description of test conditions missing) Rat, SPF-Wistar, N=20/dose (10/sex/dose) Dose: 0, 0.2, 1, and 5 % corr. to 0, 10, 50, 250 mg/kg bw/d Route: oral, diet Treatment time: 32 d	Erythrocytes: Dose-dependent decrease in all treated groups Leucocytes: Dose-dependent increase Heinz bodies in erythrocytes: Increase in high- (100 % vs control), mid- (30 %), and low-dose groups (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	(Hoechst AG, 1973) GV STOT RE 2 ≤ 281 mg/kg bw/d (32 days)
2-year feeding experiment D&C Red No. 9 (trade name of PR53:1) Bacth No: Lot No. G4516 Purity: 86 % Non-guideline study (publication)	Slight to moderate splenomegaly: In rats of the 0.01 % (2/12 rats), 0.05 % (4/12), 0.25 % and 1.0 % (both 7/12) dose groups, significant increase in spleen weight/body weight ratio in the 0.25 % and 1.0 % dose groups; Splenic infarcts, scars, haemosiderosis, or cysts in high dose group (1 %)	(Davis and Fitzhugh, 1962) (publication)

Method	Results/Observations (regarding STOT RE)	Reference (Guidance Value for STOT RE*)
Not assignable (no full study report available) Rat, Osborne-Mendel (N= 25/sex/dose) Route: oral, diet Dose levels: 0, 0.01, 0.05, 0.25, 1 %. Corr. to 0, 5, 25, 125 and 500 mg/kg bw/d (Conversion factor 20 for older rats) 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 control groups; 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 %)	<u>Slight haematologic effects</u> (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 0.25 and 1.0 % dose groups was slightly hyperplastic compared to controls Significantly less chronic nephritis in the 0.25 and 1.0 % dose groups Light yellow, non-ferrous, granular pigment in the renal tubular epithelium in the kidneys of 0.25 % dose group animals (2/12) and of all 1 % level rats 12/12)	GV STOT RE 2 ≤ 12.6 mg/kg bw/d (103 weeks)

* Adverse effects seen at doses below the corrected guidance values may be considered for classification purposes.

10.10.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

All studies were performed with D&C Red No. 9, which is a known trading name of PR53:1. Subsequently, only the name PR53:1 is therefore used.

Rats

In a study performed according to OECD TG 451, F344 rats were exposed via the 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 (3000 ppm, corr. to 150 mg/kg bw/d) compared to controls.

Dose levels for the two-year feeding study were selected based on effects observed in a 91-day study (NTP, 1982). Here, dosed rats (0, 3000, 6000, 12 500, 25 000, or 50 000 ppm; corr. to 150, 300, 625, 1250, 2500 mg/kg bw/d) revealed enlarged spleens and pigment deposition in the renal tubular epithelium. Furthermore, haemosiderosis of the liver was observed in all dosed female rats and with a higher incidence in treated male rats, relative to controls. There were no data collected on haematology, clinical biochemistry, or urine analysis.

In another study, SD rats were exposed *in utero* and over a period of 30 months post partum to investigate the chronic toxicity and potential carcinogenicity of PR53:1 after feeding dose levels of 100, 200, and 500 ppm (corr. to 5, 10, 26 mg/kg bw/d in males and 6, 13, 32 mg/kg bw/d in females) (CTFA, 1982a). 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 the final investigations (32 months).

Exposure of rats to PR53:1 at 10 000 ppm (corr. to 500 mg/kg bw/d in males and 521 mg/kg bw/d in females) in a similar testing design markedly increased the effects seen at lower dose levels (CTFA, 1982b). Red blood cell parameters 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 ratio (> 100 %), splenomegaly, splenic extramedullary haematopoiesis, splenic congestion, fibrosis, haemosiderosis, mesothelial hyperplasia, mesothelial cyst formation, capsular fibrosis, and multifocal cellular proliferations in the splenic capsule. Haemosiderosis accumulation in liver and kidney was found in rats fed with PR53:1 (no data on incidence available).

In a 32-day feeding study in Wistar rats, oral exposure of PR53:1 (doses: 0, 0.2, 1, and 5 % corr. to 0, 10, 50, 250 mg/kg bw/d) resulted in a dose-dependent decrease of erythrocytes and an increase in leucocytes in treated animals (Hoechst AG, 1973). Furthermore, Heinz bodies (formed by irreversible precipitation of oxidative denatured haemoglobin) in erythrocytes were increased in a dose-dependent manner. There was a significant and dose-dependent increase in spleen weight accompanied by blackish-coloured and enlarged spleens and brownish-coloured kidneys in treated rats. Histological evaluation revealed a dose-dependent increase in iron storage in the liver, kidney tubular epithelium, and spleen which could be interpreted as indicative of haemosiderin (iron-positive) deposition as a consequence of (intravascular) haemolysis. The observed effects are consistent with findings in other studies, however, the study is compromised due to lacking information on the test material (unknown composition and purity) and a missing description of the detailed testing method.

Finally, Davis and Fitzhugh (1962) report a feeding study with PR53:1, which resulted in slight to moderate splenomegaly in rats at dose levels of 125 and 500 mg/kg bw/d, respectively. High dosed rats (500 mg/kg bw/d) showed splenic haemosiderosis and splenic infarcts. There is 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. Thus, these data are only supportive as similar effects were seen as in the studies by NTP and CTFA.

Mice

In a combined repeated dose and carcinogenicity study performed similarly to OECD TG 453, CD-1 mice were fed daily for 18 months with PR53:1 (50, 250 and 1 000 ppm, corr. to: 7, 38, 147 mg/kg bw/d in males; 12, 56, 237 mg/kg bw/d in females) (CTFA, 1982c). Blood parameters were investigated at months 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 months. Furthermore, the red blood cell count was statistically increased (≥ 10 %) for high-dose females in the third month. Gross and histopathologic evaluation did not reveal any compound-related findings in treated mice. Data on clinical biochemistry or urinalysis are missing.

In the 2-year feeding study conducted by NTP (1982), there was no evidence of treatment-related lesions in mice. The highest dose tested was 2000 ppm (corr. to 286 mg/kg bw/d). Dose levels for the two-year feeding study were selected based on effects observed in a 91-day study where mice were treated orally with 0, 600, 1 250, 2 500, 5 000 or 10 000 ppm (corr. to: 86, 179, 357, 714, and 1 429 mg/kg bw/d) (NTP, 1982). In the 91-day study, congestion of the spleen was observed at dose levels ≥ 357 mg/kg bw/d. Deposits of haemosiderin 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 urinalysis.

Conclusion oral repeated dose toxicity:

None of the available studies fulfils the standard requirements for a sub-chronic toxicity study (90-day) according to the current OECD test guidelines (including (detailed) clinical observations, body weight and food/water consumption, haematology, clinical biochemistry, urinalysis, and gross pathology/histo-pathology). However, the submitted studies report data on haematology and pathology/histopathology revealing adverse effects in animals treated with PR53:1. There is 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 by haemosiderosis 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 chapter 10.7) accompanied by increased incidences of diffuse/multifocal splenic and capsular fibroses and haemosiderin deposition in spleen, liver and kidneys.

No studies on repeated dose toxicity via other routes of exposure are available.

No human data are available.

10.10.2 Comparison with the CLP criteria

Hazard class not assessed in this dossier. The data provided as supporting information for the assessment of carcinogenicity.

10.10.3 Conclusion on classification and labelling for STOT RE

Hazard class not assessed in this dossier. The data provided as supporting information for the assessment of carcinogenicity.

10.11 Aspiration hazard

Not assessed in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not assessed in this dossier.

12 EVALUATION OF ADDITIONAL HAZARDS

Not assessed in this dossier.

13 ADDITIONAL LABELLING

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15 ANNEXES

- *Confidential Annex I*