**Committee for Risk Assessment**

**RAC**

Annex 1

**Background document**

to the Opinion proposing harmonised classification and labelling at EU level of

# 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**

CLH-O-0000007323-79-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

**Adopted**

**8 June 2023**

# 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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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON BARIUM BIS[2-CHLORO-5-[(2-HYDROXY-1-NAPHTHYL)AZO]TOLUENE-4-SULPHONATE]; C.I. PIGMENT RED 53:1

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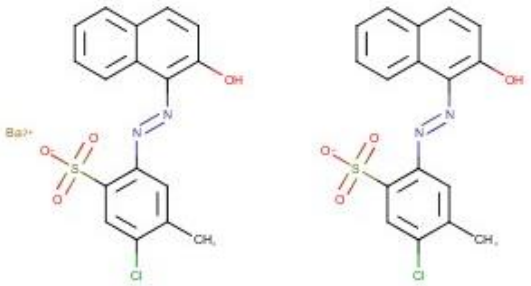
## 1 IDENTITY OF THE SUBSTANCE

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.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON BARIUM BIS[2-CHLORO-5-[(2-HYDROXY-1-NAPHTHYL)AZO]TOLUENE-4-SULPHONATE]; C.I. PIGMENT RED 53:1

## 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),<br>D&C Red No. 9,<br>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.

| **RAC general comment** |
| --- |
| Barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulfonate]; C.I. Pigment Red 53:1 (PR 53:1) belongs to the group of β-naphthol azo lake pigments with widespread uses, especially in the imparting of colour to printing inks and plastic products, but also for coating and masterbatches. The substance has no current entry in Annex VI to the CLP Regulation. The dossier submitter (DS) proposed classification as Carc. 2, H351. The DS did not assess other hazard classes in the CLH report. |

## 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:<br>2.986 mg/L at 23 °C<br><br>Measured after one week:<br><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.<br><br>The ETAD method was developed by intensive cooperation with the ETAD (Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers).<br><br>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:<br>Log $P_{OW}$= -0.62 at 23 °C<br><br>Measured after one week: | 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 |

| Property | Value | Reference | Comment (e.g. measured or estimated) |
|---|---|---|---|
| | Log $P_{OW}$= 1.69 at 23 °C | | Toxicological Association of Dyes and Organic Pigments Manufacturers). The quantification was analyzed by HPLC-UV.<br><br>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<br>- No pyrophoric properties<br>- 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<br>D50= 11.08 µm<br>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 | |

| Property | Value | Reference | Comment (e.g. measured or estimated) |
|---|---|---|---|
| | | 8, p. 107 | |
| **Dissociation constant** | pKa (calculated):<br>-5.49 (sulfonate group)<br>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**<br>Similar to OECD TG 471<br>**(Prival activation and classical test protocol with S9)**<br>Deviations: 5th strain missing<br>GLP: yes | **PR53:1**<br>CAS 5160-02-1<br>Purity: see confidential annex | **Supporting study**<br>**Reliable with restrictions** (5th strain is missing, results for TA100, TA98, TA1537, TA1535 are reliable without restrictions)<br>Bacterial strains:<br>*S. typhimurium*: TA100, TA98, TA1537, TA1535<br>Test concentrations (± metabolic activation (S9 mix)):<br>4, 20, 100, 500, 2500, 5000 µg/plate<br>S9: hamster liver S9, untreated and rat liver S9 Aroclor induced<br>Vehicle: DMSO<br>Negative/positive control: yes/yes | **Negative**<br>with (hamster and rat S9) and without metabolic activation<br>No significant increase in the number of revertants in any bacterial strains with and without Prival with and without metabolic activation.<br>Cytotoxicity: no<br>Precipitations: ≥ 500 µg/plate<br>Neg. control: valid<br>Pos. control: valid | (Hoechst AG, 1989a) |
| **Bacterial reverse mutation test**<br>Similar to OECD TG 471<br>**(Prival activation and classical test protocol with S9)**<br>Deviations: 5th strain missing<br>GLP: yes | **PR53:1**<br>CAS 5160-02-1<br>Purity: technical pure | **Supporting study**<br>**Reliable with restrictions** (5th strain is missing results for TA100, TA98, TA1537, TA1535 are reliable without restrictions)<br>Bacterial strains:<br>*S. typhimurium*: TA100, TA98, TA1537, TA1535<br>Test concentrations (± metabolic activation (S9 mix)):<br>4, 20, 100, 500, 2500, 5000/10 000 µg/plate<br>S9: hamster liver (Prival activation) S9: untreated and rat liver S9 Aroclor induced (classical test protocol)<br>Vehicle: DMSO<br>Negative/positive control: yes/yes | **Negative**<br>with (hamster and rat S9) and without metabolic activation<br>Cytotoxicity: no<br>Precipitations: yes, ≥ 100 µg/plate<br>Neg. control: valid<br>Pos. control: valid | (Hoechst AG, 1985a) |
| **Bacterial reverse** | **PR53:1** | **Key study** | **Negative** | (Hoechst AG, |

| Method, guideline, deviations if any | Test substance | Relevant information about the study | Observations | Reference |
|---|---|---|---|---|
| **mutation test**<br>Similar OECD TG 471<br>Deviations: none<br>GLP: yes | CAS 5160-02-1<br>Purity: see confidential annex | **Reliable without restrictions**<br>Bacterial strains:<br>*S. typhimurium*: TA100, TA98, TA1537, TA1535, TA 1538, *Escherichia coli* WP2uvrA<br>Test concentrations (± metabolic activation (S9 mix)): see confidential annex, guideline conform<br>S9: see confidential annex<br>Vehicle: see confidential annex<br>Negative/positive control: yes/yes | with and without metabolic activation<br>Cytotoxicity: no<br>Precipitations: see confidential annex<br>Neg. control: valid<br>Pos. control: valid | 1985b) |
| **Bacterial reverse mutation test**<br>Similar to OECD TG 471 (without Prival activation)<br>Deviations:<br>• No verification of negative result<br>• Only three strains tested (e.g. noTA 1535, E.coli WP2 missing)<br>• No data on purity<br>GLP: no | **PR53:1**<br>CAS 5160-02-1<br>Purity: no data | **Supporting study**<br>**Reliable with restrictions**<br>**(only three strains tested, no verification of negative result)**<br>Bacterial strains:<br>*S. typhimurium*: TA100, TA98, TA1537<br>Test concentrations (± metabolic activation (S9 mix)):<br>20, 78, 313, 1250,5000 µg/plate<br>S9: rat liver, Aroclor-induced<br>Vehicle: DMSO<br>Negative/positive control: yes/yes | **Negative**<br>with and without metabolic activation<br>Cytotoxicity: no<br>Precipitations: from 313 µg/plate onward<br>Neg. control: valid<br>Pos. control: valid | (CIBA-GEIGY Limited, 1985) |
| **Bacterial reverse mutation test**<br>Similar to OECD TG 471<br>Deviations: | **PR53:1**<br>Purity: 33-73 % | **Disregarded study**<br>**Not assignable (insufficient documentation and methodical deficiencies)**<br>Bacterial strains: | **Negative**<br>with and without metabolic activation | (Brown et al., 1979) |

| Method, guideline, deviations if any | Test substance | Relevant information about the study | Observations | Reference |
|---|---|---|---|---|
| • Documentation insufficient<br>• Purity insufficient<br>• Data on 5th strain missing<br>• Low max. concentration<br>• Only 3 concentrations tested<br>• No detailed data on results (data table)<br><br>GLP: no | | *S. typhimurium*: TA100, TA98, TA1537, TA1535, TA1538<br><br>Test concentrations (± metabolic activation (S9 mix)): 50, 100, 500 µg/plate<br><br>S9: rat liver Aroclor induced<br><br>Vehicle: DMSO<br>Negative/positive control: yes/yes | Cytotoxicity: no data<br><br>Precipitations: no data<br><br>Neg. control: valid<br>Pos. control: valid | |
| **Bacterial reverse mutation test**<br><br>Similar to OECD TG 471 **Prival activation and without Prival)**<br><br>Deviations:<br>• No detailed data on results (data table)<br>• 5th strain (e.g. E.coli WP2) missing<br>• No information on purity<br>• Cytotoxicity not determined<br><br>GLP: not specified | PR53:1<br>(D&C Red No 9)<br><br>CAS 5160-02-1<br><br>EC 225-935-3<br><br>Purity: unknown | **Disregarded study**<br><br>**Not assignable (detailed result data missing to evaluate relevance of ambiguous result)**<br><br>Bacterial strains:<br>*S. typhimurium*: TA100, TA98, TA1537, TA1535, TA97<br><br>Test concentrations (± metabolic activation (S9 mix)):<br>100, 333, 1000, 3333, 10 000 µg/plate<br><br>S9: hamster liver S9, untreated and rat liver S9 Aroclor-induced<br><br>Vehicle: DMSO<br>Negative/positive control: yes/yes | **Ambiguous**<br>(with and without metabolic activation)<br><br>-ambiguous for TA97 without S9 and for TA98 with and without S9<br><br>Cytotoxicity: not determined<br><br>Precipitations: ≥ 100 µg/plate<br><br>Controls:<br><br>Neg. control: valid<br>Pos. control: valid | (Zeiger et al., 1988) |
| **In vitro mammalian cell gene mutation test using the thymidine kinase gene** | PR53:1<br><br>CAS Nr.: 5160-02-1 | **Key study**<br><br>**Reliable with restrictions**<br><br>Cell culture: mouse lymphoma L5178Y cells | **Negative**<br>(with and without metabolic activation) | (Myhr et al., 1991) |

| Method, guideline, deviations if any | Test substance | Relevant information about the study | Observations | Reference |
|---|---|---|---|---|
| similar to OECD TG 490<br><br>Deviation:<br>• No data on purity<br><br>GLP: not specified | Purity: no data | Test concentrations<br>without metabolic activation: 1.25, 2.5, 5, 7.5, 15 µg/mL<br>with metabolic activation: 2, 3, 4, 5, 6 µg/mL<br><br>Justification for top concentration: solubility (~ 7.5 µg/mL)<br><br>Metabolic activation system: rat liver S9 induced by Aroclor<br><br>Treatment time(s): 4 h<br>Sampling time(s): after 2 days<br><br>Vehicle: DMSO<br>Negative/positive control: yes/yes | Cytotoxicity: no<br><br>Precipitations: yes, above 7.5 µg/mL<br><br>Neg. control: valid<br>Pos. control: valid | |
| **In vitro mammalian chromosomal aberration test**<br><br>similar to OECD TG 473<br><br>Deviations:<br>• Only 100 metaphases scored per concentration<br>• No data on purity<br><br>GLP: yes | PR53:1<br><br>CAS 5160-02-1<br><br>Purity: no data | **Key study**<br><br>**Reliable with restrictions**<br><br>**(only 100 metaphases scored per concentration)**<br><br>Cell culture: Chinese hamster lung fibroblasts (V79)<br><br>Test concentrations:<br>± metabolic activation (S9 mix): 30, 150, 300 µg/mL<br><br>metabolic activation: rat liver S9 induced by Aroclor<br><br>Justification for top concentration:<br>significant cytotoxicity ≥ 400 µg/mL<br><br>Treatment time: ± metabolic activation: 4 and 18 h<br>Sampling time: 4.5, 15.5, 25.5 h after beginning of treatment<br><br>Vehicle: DMSO<br>Negative/positive control: yes/yes | **Negative**<br>with and without metabolic activation<br><br>Cytotoxicity: significant cytotoxic ≥ 400 µg/mL<br><br>Precipitations: yes, ≥ 500 µg/mL<br><br>Controls<br><br>Neg. control: valid<br>Pos. control: valid | (Hoechst AG, 1989b) |
| **In vitro mammalian chromosomal** | D&C Red No 9 | **Disregarded study** | **Negative**<br>with and without metabolic | (Ivett et al., 1989) |

| Method, guideline, deviations if any | Test substance | Relevant information about the study | Observations | Reference |
|---|---|---|---|---|
| **aberration test**<br><br>Not similar to OECD TG 473<br><br>Deviation from OECD TG 473<br>• Continuous exposure of about 12-14 h without metabolic activation missing<br>• Short-term treatment with and without metabolic activation not adequate (8 h and 2 h instead of 3-6 h)<br>• Sampling time too short (2-2.5 h instead of 1.5 times the normal cell cycle length)<br>• Only 200 (instead of 300) metaphases evaluated<br>• No specific data on justification for top dose<br><br>GLP: not specified | CAS 5160-02-1<br>EC 225-935-3<br>Purity: 89.8 % | **Not reliable**<br>**(exposure and sampling times are not according to OECD TG, too less cells analysed)**<br>Cell culture: CHO<br><br>Test concentrations:<br>without metabolic activation (S9 mix): 37.1, 50, 123.8 µg/mL<br>with metabolic activation (S9 mix): 5, 16.7, 50 µg/mL<br><br>Metabolic activation: rat liver S9 induced by Aroclor<br><br>Justification for top concentration: no specific data<br><br>Treatment time:<br>without metabolic activation: 8 h<br>with metabolic activation: 2 h and 8 h<br><br>Sampling time: 2 - 2.5 h<br><br>Vehicle: DMSO<br>Negative/positive control: yes/yes | activation<br><br>Cytotoxicity: no detailed data<br><br>Precipitations: yes, ≥ 250 µg/mL<br><br>Controls<br><br>Neg. control: valid<br>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 |
|---|---|---|---|---|

| 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**<br>Similar to OECD TG 486<br>Deviation: none<br>GLP: not specified | PR53:1<br>CAS 5160-02-1<br>EC 225-935-3<br>Purity: no data | **Supporting study***<br>**Reliable without restrictions***<br>Species: rats Piebald Virol Glaxo<br>Number of animals per group: 7 males<br>Target organs: liver<br>Administration route: oral (gavage), single dosage<br>Dose level: 1000 and 2000 mg/kg bw/d<br>Justification for top dose: limit test<br>Sampling: **16 h**<br>Vehicle: Corn Oil<br>Positive control: yes (2-acetylaminofluorene for 16 h)<br>Negative control: yes | **Negative**<br>*** A negative result is not conclusive for the assessment of induction of gene mutations.**<br>**Results:**<br>No marked increase in incidence of cells in repair at 16 h sampling time<br>Toxicity: no toxicity observed<br>Controls:<br>Neg. control: valid<br>Pos. control: valid | (Westmoreland and Gatehouse, 1992) |
| **Mammalian erythrocyte micronucleus test**<br>Similar to OECD TG 474<br>Deviation:<br>No evidence of exposure of bone marrow<br>GLP: not specified | PR53:1<br>CAS 5160-02-1<br>EC 225-935-3<br>Purity: no data | **Supporting study**<br>**Not reliable (no evidence of exposure of bone marrow shown)**<br>Species: rats Piebald Virol Glaxo<br>Number of animals per group: 7 males<br>Target organs: bone marrow<br>Administration route: oral (gavage), single dosage<br>Dose level: 500,1000 and 2000 mg/kg bw/d<br>Justification for top dose: limit test<br>Sampling: **24 or 48 h**<br>Vehicle: corn oil<br>Positive control: yes (cyclophosphamide, 24 h)<br>Negative control: yes | **Negative**<br>**Results:**<br>No increase in the frequency of micronuclei<br>Toxicity: no toxicity observed<br>**Evidence of exposure of bone marrow: no, as ratio PCE/NCE not decreased, no other evidences**<br>Controls:<br>Neg. control: valid<br>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 5$^{th}$ strain (*E.coli* WP2 uvrA), negative results were obtained with and without metabolic activation using the classical test protocol. Test results for the 5$^{th}$ 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 5$^{th}$ 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**<br><br>D&C Red No. 9 (known trading name of PR53:1) (CAS 5160-02-1)<br>Purity: 89.8 %, impurities sodium and barium sulfates<br><br>According to OECD TG 451 (NTP guideline), no GLP<br>**Reliable without restrictions**<br><br>Species: rats<br>Strain: F344<br>n: 50/dose group/sex<br><br>Dose levels: 0, 1000, 3000 ppm<br>Route: oral (feed)<br>Food conversion factor: 20 (for older rats)<br>Calculated doses*: 0, 50, 150 mg/kg bw/d<br><br>Treatment time: 103 weeks, daily<br>Post exposure period: 1 week<br><br>Dose level selected based on effects observed in 91 day study<br><br>*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**<br><br><u>Neoplastic lesions (0, 1000, 3000 ppm):</u><br>Male rats:<br>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<br><br>11 of splenic tumours metastasised to peritoneal tissues, 2 sarcoma of multiple organs originated in the spleen<br><br>Weeks to first observed tumor: 68<br><br>Historical control data: Fibrosarcoma (same lab) M: 0/140 and Fibrosarcoma (same lab, entire bioassay program): M: 3/2960 (0.1 %)<br><br>Hepatocellular carcinoma in 1/50 (2 %) control male.<br><br>Neoplastic nodules of the liver:<br>Males: 0/50 (0 %), 6/50 (12 %), 7/49 (14 %) (hepatocytes with basophilic or eosinophilic cytoplasm)<br>Female: 1/50 (2 %), 1/50 (2 %), 5/50 (10 %)<br><br><u>Non-neoplastic lesions (at 3000 ppm)</u>:<br>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<br>Areas of fibrosis in 2 control males<br><br>Females: 25/50 multifocal, diffuse or focal fibrosis of the spleen<br><br><u>Survival</u>:<br>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**<br><br>D&C Red No. 9 (known trading name of PR53:1) (CAS 5160-02-1)<br>Purity: 89.8 %, impurities sodium and barium sulfates<br><br>According to OECD TG 451 (NTP guideline including single dose, 2-week and 13-week studies), no GLP<br>**Reliable without restrictions**<br><br>Species: mice<br>Strain: B6C3F1<br>n: 50/dose group/sex<br>Dose levels: 0, 1 000, 2 000 ppm<br>Route: oral (feed)<br>Food conversion factor: 7 (for mice)<br>Calculated doses*: 0, 142, 285 mg/kg bw/d<br>Treatment time: 103 weeks, daily<br>Post exposure period: 1 week<br><br>Dose level selected based on effects observed in 91 day study<br>*calculated by DS | **Not carcinogenic for B6C3F1 mice**<br><br><u>Neoplastic lesions (0, 1000, 2000 ppm):</u><br>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)<br>Females: malignant lymphomas of the hematopoietic system (2/50 (4 %), 2/50 (4 %), 7/49 (14 %) increased incidence<br><br><u>Survival</u>:<br>No effect on mortality, body weight and food consumption, except mean body weight of treated females slightly lower in 2$^{nd}$ year (< 10 %) | (NTP, 1982) |
| **26-30 month dietary study (F0 and F1 dosed) including *in utero* exposure**<br><br>D&C Red No. 9 (known trading name of PR53:1) (CAS 5160-02-1)<br>Purity: 76 %<br><br>According to FDA guidelines including in utero treatment and F1 generation, no GLP<br>**Reliable with restrictions**<br><br>Species: rat<br>Strain: Charles-River CD Sprague-Dawley<br>n: 70/dose group/sex<br>Dose levels:<br>**Part I:** 0, 100, 200, 500 ppm | **Increased incidence of splenic sarcoma in rats**<br><br><u>Neoplastic lesions:</u><br>2 Haemangiosarcomas involving spleen and/or liver in control males<br>Splenic sarcoma in 4 males and 1 female at 10 000 ppm in F1 animals, not statistically significant<br><br><u>Survival</u>:<br>No effects on mortality, body weight, food consumption in parental animals or offspring<br>At 10 000 ppm body weight of male and female pups decreased at weaning (day 21 postpartum); lower body weights throughout chronic phase (< 10 %)<br><br><u>Clinical findings of toxicity:</u><br>Signs of anaemia at 10 000 ppm in males and females | (CTFA, 1982a; CTFA, 1982b)<br><br>Cited in (FDA, 1986) |

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

| Methods | Results | Reference |
|---|---|---|
| *calculated by DS | | |
| **Combined repeated dose and carcinogenicity test**<br><br>D&C Red No. 9 (known trading name of PR53:1) (CAS 5160-02-1)<br>Purity: 76 % (according to FDA report)<br><br>Similar to OECD TG 453, No GLP<br>**Reliable with restrictions**<br><br>Species: mice<br>Strain: Charles-River CD1<br>n: 60/dose group/sex<br>Dose levels: 0, 50, 250 and 1000 ppm<br>Route: oral (feed)<br>Corrected dose using food consumption:<br>7, 38, 147 mg/kg bw/d in males; 12, 56, 237 mg/kg bw/d in females<br>Treatment time: 24 months/105 weeks (daily) | **Not carcinogenic in mice**<br><br><u>Survival</u>:<br>No effects on mortality, body weight, food consumption<br><br><u>Clinical findings of toxicity</u>:<br>Signs of anaemia at 1000 ppm in females (decreased red blood cells, increased reticulocytes, decreased haemoglobin and haematocrit), anaemia not evident at 250 ppm<br>Decreased abs. kidney weight in male mice (1000 ppm), but rel. kidney weight similar to control | (CTFA, 1982c)<br><br>Cited in (FDA, 1986) |
| **18 month skin painting study**<br><br>D&C Red No. 9 (known trading name of PR53:1) (CAS 5160-02-1)<br>Purity: 90 %<br><br>Non-guideline study, No GLP<br>**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<br><br>Species: mice<br>Strain: 100 ICR<br>n: 50/dose group/sex, 150 in control group<br>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)<br>Route: dermal | **No increase in neoplasia after dermal application of the test dye compound**<br><br>Single incidences of mammary gland adenosarcoma (2 females); hepatic cell carcinoma (1 male/1 male in control), reticulum cell sarcoma (1 male)<br><br>No effect on survival compared to control<br><br>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)<br><br>(publication) |

| Methods | Results | Reference |
|---|---|---|
| Treatment time: 483 days<br>Vehicle: distilled water<br>Positive control: 3,4-benzpyrene in acetone | | |

### 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<br>F | 0/50<br>0/50 | 0/50<br>0/50 | **26/48 (54 %)[1,2]**<br>0/50 | **NTP data (1984-1994)[4]:**<br>All types of splenic sarcoma[3]<br>M: 7/1003 (0.7 %); F: 0/1003 |
| thereof splenic fibrosarcoma | M<br>F | 0/50<br>0/50 | 0/50<br>0/50 | **17/48 (35 %)[1,2]**<br>0/50 | **NTP report, 1982:**<br>Fibrosarcoma (same lab, no time range specified):<br>M: 0/140<br>Fibrosarcoma (entire bioassay program, no time range specified):<br>M: 3/2960 (0.1 %)<br><br>**NTP data (1984-1994)[4]:**<br>Fibrosarcoma:<br>M: 4/1003 (0.4 %), range 0-6 %<br>F: 0/1003 |
| Hepatic neoplastic nodules | M<br>F | 0/50<br>1/50 (2 %) | **6/50 (12 %)[1,2]**<br>1/50 (2%) | **7/49 (14 %)[1,2]**<br>**5/50 (10 %)[2]** | **NTP report, 1982:**<br>Neoplastic nodules in liver (same lab, no time range specified):<br>M/F: 5/140 (3.6 %)<br><br>**NTP data (1984-1994)[4]:**<br>Neoplastic nodules in liver: no data |
| Hepatocellular carcinoma | M<br>F | 1/50 (2%)<br>0/50 | 0/50<br>0/50 | 0/49<br>0/50 | **NTP data (1984-1994)[4]:**<br>Hepatocellular carcinoma:<br>M: 7/1002 (0.7 %), range 0-6 %<br>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<br>F | 4/50 (8 %)<br>4/50 (8 %) | 9/50 (18 %)<br>2/50 (4 %) | 11/50 (22 %)[1,2]<br>2/49 (4 %) | **NTP report, 1982:**<br>Hepatocellular carcinoma (same lab, no time range specified):<br>M: 65/297 (22 %)<br><br>**NTP data (1984-1994)[4]:**<br>Hepatocellular carcinoma<br>M: 194/950(20.4 %), range 10-40 %<br>F: 104/951(10.9 %), range 4-20 % |
| Haematopoietic system: malignant lymphomas, mixed type | M<br>F | 0/50<br>2/50 (4 %) | 1/50 (2 %)<br>2/50 (4 %) | 0/50<br>**7/49 (14 %)[2]** | **NTP data (1984-1994)[4]:**<br>Haematopoietic system: malignant lymphomas, mixed type: no data |
| Haematopoietic system: all malignant lymphomas | M<br>F | 5/50 (10 %)<br>11/50 (22 %) | 4/50 (8 %)<br>17/50 (34 %) | 4/50 (8 %)<br>12/49 (24 %) | **NTP data (1984-1994):**<br>M: 71/952 (7.5 %), range 2-14 %<br>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)<br><br>$analysed tissue samples might be slightly lower due to minor cases of tissue lost | M<br>F | 2/70§ (3 %)<br>0/70§ | 4/70§ (F1) (6 %)<br>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.<br>High incidence, high malignancy and metastasis<br>Very low spontaneous incidence. | 1B or 2<br>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.<br>Non-neoplastic splenic lesions also observed in females. | 2 |
| **Responses in single or several species** | Rat only.<br>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.

## RAC evaluation of carcinogenicity

### Summary of the Dossier Submitter's proposal

The DS reported the following *in vivo* carcinogenicity studies (cf. Table 12 and 13 of the CLH report):

| **Reference, Method, guideline, deviations if any** | **Test substance** | **Relevant information about the study including rationale for dose selection (as applicable)** | **Results** | **Historical control data (HCD)** |
|---|---|---|---|---|
| **Studies on rats** | | | | |
| NTP, 1982a<br>2-year feeding study in rats<br>Non-GLP<br>According to OECD TG 451<br>Reliability without restriction according to the DS | PR 53:1<br>Purity 89.8 %<br>Impurities sodium and barium sulfates | Species: rats<br>Strain: F344<br>Route: oral (feed)<br>Number: 50/dose group/sex<br>Treatment time: 103 weeks, daily<br>Post exposure period: 1 week<br>Dose levels: 0, 1 000, 3 000 ppm[1]<br>Food conversion factor: 20 (for older rats)<br>Calculated doses[2]: 0, 50, 150 mg/kg bw/d | Carcinogenicity:<br>- Increased incidence of sarcoma of the spleen and dose-related increase in neoplastic nodules of the liver in male rats; no effects in female rats<br>Spleen neoplastic lesions in male rats:<br>- Combined types of splenic sarcoma (0, 1 000, 3 000 ppm): 0/50 (0 %); 0/50 (0 %), 26/48 (54 %)[*,#]<br>- Fibrosarcoma (17/48) (35 %)[*,#] arising from red pulp or capsule of the spleen, leiomyosarcoma (1/48), splenic osteosarcoma (5/48), | Splenic neoplastic lesions<br>NTP data (1984-1994)[4]:<br>- All types of splenic sarcoma[5]: 7/1 003 (0.7 %) in males and 0/1 003 in females<br>- Fibrosarcoma: 4/1 003 (0.4 %), range 0-6 % in males; 0/1 003 in females<br>NTP report (1982):<br>- Fibrosarcoma in males: 0/140 |

| | | | | |
|---|---|---|---|---|
| | | | sarcoma (1/48), fibrosarcoma of the splenic capsule (1/48), fibrosarcoma of the splenic red pulp (1/48). 11 of splenic tumors metastasized to peritoneal tissues, 2 sarcoma of multiple organs originated in the spleen.<br><br>Liver neoplastic lesions (0, 1 000, 3 000 ppm):<br><br>- Hepatocellular carcinoma 1/50 (2 %), 0/50, 0/49 in males and 0/50, 0/50, 0/50 in females<br><br>- Neoplastic nodules of the liver 0/50 (0 %), 6/50 (12 %), 7/49 (14%)*,# (hepatocytes with basophilic or eosinophilic cytoplasm) in males and 1/50 (2 %), 1/50 (2 %), 5/50 (10 %)* in females<br><br>Non-neoplastic lesions (3 000 ppm):<br><br>- 14/48: congestion of splenic parenchyma; 23/48 focal or multifocal area of fibrosis; 3/48 diffuse fibrosis; 13/48 areas of fatty metamorphosis in the spleen in males<br><br>- Areas of fibrosis in 2 control males<br><br>- 25/50 multifocal, diffuse or focal fibrosis of the spleen in females<br><br>Survival:<br><br>- No effects on mortality, body weight (BW) and food consumption; 6 % weight depression in high-dose female rats | (same lab), 3/2 960 (0.1 %) (same lab, entire bioassay program)<br><br>Liver neoplastic lesions<br><br>NTP data (1984-1994)[4]:<br><br>- Hepatic neoplastic nodules: no data<br><br>- Hepatocellular carcinoma: 7/1 002 (0.7 %), range 0-6 % in males;<br><br>1/1 000 (0.1 %), range 0-2 % in females<br><br>NTP report (1982):<br><br>- Neoplastic nodules in liver (same lab, no time range specified): M/F: 5/140 (3.6 %) |
| CTFA, 1982a; CTFA, 1982b<br><br>Both studies cited in FDA, 1986<br><br>26-30 months dietary study (F0 and F1 dosed) including in-utero | PR 53:1<br><br>Purity: 76 % | Species: rats<br><br>Strain: Charles-River CD Sprague-Dawley<br><br>Route: oral (feed)<br><br>Number: 70/dose group/sex<br><br>Treatment time: | Carcinogenicity:<br><br>- Increased incidence of splenic sarcoma in rats<br><br>Neoplastic lesions:<br><br>- Haemangiosarcomas in 2/70[&] (3 %) male control animals involving spleen | No data |

| | | | | |
|---|---|---|---|---|
| exposure<br>Non-GLP<br>According to FDA guidelines including in utero treatment and F1 generation<br>Reliable with restriction<br>[&]uncertainty regarding the number of tissues examined from each group, incidence percentages calculated by the DS based on the group size of 70 animals/group bear uncertainties | | 30 months, daily<br>Dose levels:<br>Part I: 0, 100, 200, 500 ppm<br>Part II: 0, 10 000 ppm<br>Corrected doses[3]:<br>Part I<br>F0: 8, 17, 43 mg/kg bw/d in males; 9, 17, 42 mg/kg bw/d in females<br>F1: 5, 10, 26 mg/kg bw/d in males; 6, 13, 32 mg/kg bw/d in females<br>Part II<br>F0: 790 mg/kg bw/d for males, 894 mg/kg bw/d for females<br>F1: no data available for males (calculated: 500 mg/kg bw/d); 521 mg/kg bw/d for females | and/or liver<br>- Splenic sarcoma in 4/70[&] (6 %) males and 1/70[&] (1 %) female at 10 000 ppm in F1 animals (not statistically significant)<br>Survival:<br>- no effects on mortality and food consumption in parental animals or offspring<br>- slight BW decrease from day 21 postpartum in male and female pups at 10 000 ppm; lower BW through chronic phase (< 10 %)<br>Clinical findings at 10 000 ppm:<br>- Sings of anemia in males and females<br>- Increase in spleen and heart weight in males and females; increased kidney weight in females and increased testicular weight in males<br>Splenic lesions at 10 000 ppm in males:<br>- Splenic congestion, fibrosis, mesothelial hyperplasia, mesothelial cysts, haemosiderosis and splenic hematopoiesis | |
| Davis and Fitzhugh, 1962, publication<br>2-year feeding study<br>Non-guideline study<br>Non-GLP<br>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 | PR 53:1<br>Purity: 86 %<br>Vehicle: ethanol | Species: rats<br>Strain: Osborne-Mendel<br>Route: oral (feed)<br>Number: 25/dose group/sex<br>Treatment time: 103 weeks, daily<br>Dose levels: 0, 100, 500, 2 500, 10 000 ppm<br>Food conversion factor: 20 (for older rats)<br>Calculated doses2: 0, 5, 25, 125, 500 mg/kg bw/d<br>Post exposure period: | Carcinogenicity:<br>- No increased evidence for carcinogenicity but severe splenic effects<br>Survival:<br>- No effects on mortality<br>10 000 ppm:<br>- Moderate splenomegaly, splenic infarcts, haematomas or scars (6 rats), splenic haemosiderosis<br>≥ 2 500 ppm:<br>- Slight bone marrow hyperplasia, decreased hemoglobin, abnormal red blood cells | No data |

| | | | | |
|---|---|---|---|---|
| information, according to the DS | | 10 days | ≥ 500 ppm:<br>- Slight to moderate splenomegaly (7/12 at 500 ppm, 4/12 at 2 500 ppm, 2/12 at 10 000 ppm) | |
| **Studies on mice** | | | | |
| NTP, 1982b<br>2-year feeding study<br>OECD TG 451 (NTP guideline including single dose, 2-week and 13-week studies)<br>Non-GLP<br>Reliable without restriction according to the DS | PR 53:1<br>Purity: 89.8 %<br>Impurities: sodium and barium sulfates | Species: mice<br>Strain: B6C3F1<br>Number: 50/dose group/sex<br>Route: oral (feed)<br>Treatment time: 103 weeks, daily<br>Dose levels: 0, 1 000, 2 000 ppm<br>Food conversion factor: 7 (for mice)<br>Calculated doses[2]: 0, 142, 285 mg/kg bw/d<br>Post exposure period: 1 week<br>Dose level selected based on effects observed in 91 day study | Carcinogenicity:<br>- No carcinogenicity<br>Hepatic neoplastic lesions (0, 1 000, 2 000 ppm):<br>- Hepatocellular carcinoma 4/50 (8 %), 9/50 (18 %), 11/50 (22 %)[*,#] (not above HCD in the same laboratory) in males; 4/50 (8 %), 2/50 (4 %), 2/49 (4 %) in females<br>Haematopoietic system neoplasias (0, 1 000, 2 000 ppm):<br>- Malignant lymphomas, mixed type: 0/50, 1/50 (2 %), 0/50 in males and 2/50 (4 %), 2/50 (4 %), 7/49 (14 %)[*] in females<br>- All malignant lymphomas 5/50 (10 %), 4/50 (8 %), 4/50 (8 %) in males; 11/50 (22 %), 17/50 (34 %), 12/49 (24 %) in females<br>Survival:<br>- No effect on mortality, body weight and food consumption, except mean body weight of treated females slightly lower in 2nd year (< 10 %) | Liver neoplastic lesions<br>NTP report (1982):<br>- Hepatocellular carcinoma (same lab, no time range specified): 65/297 (22 %) in males<br>NTP data (1984-1994)[4]:<br>- Hepatocellular carcinoma:<br>194/950 (20.4 %), range 10-40 % in males;<br>104/951 (10.9 %), range 4-20 % in females<br>Haematopoietic system neoplasias<br>NTP data (1984-1994):<br>- No data for malignant lymphomas, mixed type<br>- All malignant lymphomas:<br>71/952 (7.5 %), range 2-14 % in males;<br>167/953 (17.5 %), range 6-30 % in females |
| CTFA, 1982c<br>Cited in FDA, 1986<br>Combined repeated dose and carcinogenicity test<br>Similar to OECD TG 453 | PR 53:1<br>Purity: 76 % (according to FDA report) | Species: mice<br>Strain: Charles-River CD1<br>Number: 60/dose group/sex<br>Route: oral (feed)<br>Treatment time: 24 months/105 weeks, | Carcinogenicity:<br>- No carcinogenicity<br>Survival:<br>- No effects on mortality, body weight, food consumption<br>Clinical findings of toxicity:<br>- Signs of anemia at | No data |

| | | | | |
|---|---|---|---|---|
| Non-GLP<br>Reliable without restriction according to the DS | | daily<br>Dose levels: 0. 50, 250, 1 000 ppm<br>Corrected dose using food consumption: 7, 38, 147 mg/kg bw/d in males; 12, 56, 237 mg/kg bw/d in females | 1 000 ppm in females (decreased red blood cells, increased reticulocytes, decreased hemoglobin and hematocrit), anemia not evident at 250 ppm<br>- Decreased absolute kidney weight in males (1 000 ppm), but relative kidney weight similar to control | |
| Carson, 1984, publication<br>18-month skin painting study<br>Non-guideline study<br>Non-GLP<br>Limited reliability according to the DS: 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 HCD | PR 53:1;<br>Purity: 90 %;<br>Vehicle: distilled water<br>Positive control: 3,4-benzpyrene in acetone | Species: mice<br>Strain: 100 ICR<br>Number: 50/dose group/sex, 150 in control group<br>Route: dermal, application to dorsal area<br>Treatment time: 483 days<br>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) | Carcinogenicity:<br>- No carcinogenicity<br>- Single incidences of mammary gland adenosarcoma (2 females); hepatic cell carcinoma (1 male/1 male in control), reticulum cell sarcoma (1 male)<br>Survival:<br>- No effect on survival compared to control<br>Full histopathology of a low number of randomly chosen animals did not give an indication of systemic toxicity or carcinogenicity | No data |

*statistically significant positive trend; #statistically significant increase compared to control; [1]Dose level selected based on effects observed in 91 day study; [2]Doses calculated by the DS; [3]Doses calculated based on food consumption; [4]No other time range available; source: https://ntp.niehs.nih.gov/data/controls/index.html; [5]including fibrosarcoma, splenic osteosarcoma, leiomyosarcoma, sarcoma

There were seven studies available that investigate carcinogenic potential of PR 53;1 (4 in rats and 3 in mice). No human data were available.

***Studies in rats***

A 2-year feeding study in rats, conducted according to NTP guidelines and equivalent to OECD TG 451 was performed by NTP using 50 male and 50 female F344 rats (NTP, 1982a). Doses of 0, 1 000 and 3 000 ppm PR 53:1 were administered in feed daily for 103 weeks (calculated doses based on a general conversion factor of 20 for older rats are as follows: 0, 50, 150 mg/kg bw/d). No effects on survival, body weight, or food consumption were reported except for high-dose females that showed a 6 % lower body weight compared with

the control. In the male high-dose group, 26/48 (54 %) combined types of splenic sarcomas were reported, including fibrosarcoma (17/48 (35 %)) arising from red pulp or capsule of the spleen, leiomyosarcoma (1/48) and splenic osteosarcoma (5/48). Eleven of the splenic tumours metastasised to peritoneal tissues. Non-neoplastic spleen lesions were observed in the high-dose group in both sexes. In males, 14/48 animals showed congestion of splenic parenchyma, 23/48 focal or multifocal area of fibrosis, 3/48 diffuse fibrosis and 13/48 areas of fatty metamorphosis in the spleen. In females, in the high-dose group 25/50 animals reported multifocal, diffuse or focal fibrosis of the spleen. The increased incidence of fibrosarcoma in males was significantly above the HCD from the laboratory (0/140) and above the historical records for the entire bioassay program of the laboratory (3/2 960, 0.1 %). Males and females reported a dose-related increase of neoplastic nodules in liver above the HCD (M/F: 5/140 (3.6 %)). The increase was more significant in males (6/50 and 7/49 males in the mid-dose and high-dose groups) than in females (only 5/50 animals affected in the high-dose group). The nodules consisted of hepatocytes with basophilic or eosinophilic cytoplasm and were of relatively small size.

A report from United States Food and Drug Administration (FDA, 1986) discussed some limitations of this study regarding the use of solid-bottom cages that can lead to coprophagy. This can determine the ingestion of higher doses of chemical and its metabolites and the presence of other carcinogens in the room, as other substances were also tested at the same time. These limitations were considered to be not relevant as also in the CTFA studies where wire cages were used, the same type of tumours were observed and due to the fact that no splenic neoplasms were observed with other test substances or in control animals in other studies (according to the HCD).

Long-term feeding studies with lifetime exposure in rats were performed according to the FDA guidelines (CTFA, 1982b; CTFA, 1982a). In the first study, 70 Sprague-Dawley rats/dose/sex were treated with 0, 100, 200, 500 ppm of PR 53:1 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, during mating, gestation, lactation and rearing (CTFA, 1982a). Seventy F1 pups/dose/sex were selected for the long-term feeding study dosing for 30 months (CTFA, 1982b). The dose levels of the first study were found too low and a new experiment was required by the FDA with higher concentrations (0 and 10 000 ppm PR 53:1 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 for M: 0 and 500 mg/kg bw/d) using the same method (CTFA, 1982b). No effects on survival and food consumption were observed in both studies, while a slight decrease in body weight was observed in male and female pups from day 21 postpartum. At 10 000 ppm, splenic sarcoma was observed in 4 males and 1 female. Details on the number of examined tissues from each group is not reported in the study report and it is stated that in 'minor cases' some tissues could not be investigated due to autolysis, sectioning difficulties or laboratory errors. These uncertainties could lead to underestimation of the effects. The evaluation of clinical signs of toxicity reveals anaemia and increase in spleen and heart weight in both sexes at 10 000 ppm. In males, splenic lesions such as splenic congestion, fibrosis, mesothelial hyperplasia, mesothelial cysts, haemosiderosis and splenic haematopoiesis were also reported.

In the study by David and Fitzhugh (1962), 25 Osborne-Mendel rats/sex/dose were administered 0, 100, 500, 2 500 and 10 000 ppm PR 53:1 by feed 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 study report has several limitations as only 6 animals

from each group were examined histopathologically and a limited number of findings were reported. Carcinogenicity was not observed, but the evaluated animals showed slight to moderate splenomegaly (7/12 at 500 ppm, 4/12 at 2 500 ppm, 2/12 at 10 000 ppm), slight bone marrow hyperplasia, decreased haemoglobin, abnormal red blood cells at 2 500 ppm and above, and moderate splenomegaly, splenic infarcts, haematomas or scars (6 rats), splenic haemosiderosis at 10 000 ppm.

***Studies in mice***

In the NTP 2-year feeding study (1982b) equivalent to OECD TG 451 (NTP guidelines) 50 B6C3F1 mice/sex/dose group were administered 0, 1 000 and 2 000 ppm, PR 53:1 in feed 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). The exposure determined no effects on survival, body weight or food consumption. A positive trend of hepatocellular carcinoma incidence was reported, 4/50 (8 %), 9/50 (18 %) and 11/50 (22 %) in 0, 1 000 and 2 000 ppm males. Statistically significant increase compared with the control was reported only in the high-dose group males and in all dose groups the incidence did not exceed the HCD. In female mice at 2 000 ppm, an increased incidence in mixed-type malignant lymphomas of the haematopoietic system was reported without statistical difference compared with the control, although a positive dose-response trend was established. No HCD were available.

In another study conducted according to FDA guidelines (CTFA, 1982c) 60 Charles-River CD1 mice/sex/dose were administered 0, 50, 250 and 1 000 ppm PR 53:1 (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. No effects on survival, body weight or food consumption and no neoplastic lesions were reported.

An 18-month skin painting study investigated the toxicity of PR 53:1 in dose levels based on lipstick use assessments via dermal administration (Carson, 1984). A skin area of 6 $cm^2$ from the animal dorsal region was treated twice weekly with 0.1 mL suspension; the mean total dose applied was 134.7 mg. The study has several limitations as complete pathology was performed only on a limited number of animals. No effects on survival or increase of neoplasia compared with the control group were reported. Only single incidences of any gross lesions were reported.

A chronic drinking water study in both rats and mice investigating barium chloride dihydrate carcinogenic potential is available (NTP, 1994). The study author stated that "PR53:1 is a barium-containing pigment" and that "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." The study reported no evidence of carcinogenic activity of barium chloride dehydrate and the author's conclusion was that barium cation in PR 53:1 presumably does not contribute to the carcinogenic effects of PR 53:1.

The outcome of the overall evaluation of animal studies regarding the carcinogenic potential of PR 53:1 is that there is evidence of carcinogenic potential based on an increased incidence of splenic sarcomas in male rats, a rare type of tumours in this organ (CTFA, 1982b; CTFA, 1982a; NTP, 1982a). This incidence was statistically significant and above the HCD in the NTP study (1982a), but not in the CTFA study. The results of CTFA study are considered only supportive evidence as a similar pattern of non-neoplastic splenic lesions were observed in

both studies (CTFA, 1982b; CTFA, 1982a; NTP, 1982a). FDA report (1986) also stated a common pattern of splenic lesions in NTP and CTFA studies that include 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). It is considered that Sprague-Dawley rats are less sensitive than F344 rats to aniline-related compounds, but still show the pre-neoplastic trend observed in the F344 rats.

The studies on mice reported no carcinogenic effects (CTFA, 1982c; NTP, 1982b). The NTP report for rat and mouse studies however states that "with the possible exception of female mice, all other dosed groups of rats or mice might have tolerated higher doses" and that "thus a clear maximum tolerated dose may not have been utilized in this study."

***Supporting information from specific target organ toxicity – repeated exposure studies***

Specific target organ toxicity-repeated exposure was not evaluated in the CLH report for classification but data were provided to support the assessment of carcinogenicity.

| **Reference, Method, guideline, deviations if any** | **Test substance** | **Relevant information about the study including rationale for dose selection (as applicable)** | **Results** | **Guidance Value for STOT RE** |
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| **Studies on rats** | | | | |
| NTP, 1982a<br>2-year feeding study in rats<br>No GLP<br>According to OECD TG 451<br>Reliability without restriction according to the DS | PR53:1,<br>Purity 89.8 %<br>Impurities sodium and barium sulfates | Species: rats<br>Strain: F344<br>Route: oral (feed)<br>Number: 50/dose group/sex<br>Treatment time: 103 weeks, daily<br>Post exposure period: 1 week<br>Dose levels: 0, 1 000, 3 000 ppm[1]<br>Food conversion factor: 20 (for older rats)<br>Calculated doses[2]: 0. 50, 150 mg/kg bw/d | Spleen lesions in male (150 mg/kg bw/d):<br>- Congestion of the splenic parenchyma (14/48), focal or multifocal areas of fibrosis (23/48), diffuse fibrosis (3/48), areas of fatty metamorphosis in the spleen (13/48)<br>Spleen lesions in female (150 mg/kg bw/d):<br>- Multifocal, diffuse, or focal fibrosis (25/50)<br>Areas of fibrosis present in 2/50 control male rats<br>Increased incidence of testis/tubule degeneration:<br>- 10 % (5/50), 23 % (11/48) at 50 and 150 mg/kg bw/d compared to 6 % (3/50) in controls | GV STOT RE 2 ≤ 12.6 mg/kg bw/d for 103-week exposure |
| NTP, 1982c<br>Range finding study for carcinogenicity - feeding study in rats (13-week study)<br>No GLP | PR53:1<br>Purity 89.8 %<br>Impurities sodium and barium | Species: rats<br>Strain: F344<br>Route: oral (feed)<br>Number: 10/dose group/sex<br>Treatment time: 91 | Haemosiderosis of the liver in all dosed female rats, and in 3/10, 6/10, 9/10 males at 150, 300, 625 mg/kg bw/d<br>Pigment deposition in kidney tubular epithelium in all dosed rats | GV STOT RE 2 ≤ 100 mg/kg bw/d for 13-week exposure |

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| Reliability with restriction according to the DS (no data on haematology, clinical biochemistry, urine analysis) | sulfates | days, daily<br>Dose levels: 0, 3 000, 6 000, 12 500, 25 000, 50 000 ppm<br>Food conversion factor: 20 (for older rats)<br>Calculated doses: 0, 150, 300, 625, 1 250, 2 500 mg/kg bw/d | Enlarged spleen (2-5 fold) in all dosed rats; congestion and lymphoreticular hyperplasia in spleens of all dosed female rats, and in 8/10 male rats at 150 mg/kg bw/d and in all male rats ≥ 300 mg/kg bw/d<br>Lymphoreticular hyperplasia of thymic lymph nodes:<br>- 75-100 % of female rats in each dosed group except 0/10 at 150 mg/kg bw/d<br>- 70-100 % of male rats in each dosed group except 3/7 at 2 500 mg/kg bw/d | |
| CTFA, 1982a and CTFA, 1982b<br>30-months chronic toxicity and potential carcinogenicity study with in utero and lifetime exposure<br>According to FAD guidelines<br>Pre-GLP<br>Reliable with restrictions (individual data e.g. clinical signs missing) | PR 53:1,<br>Purity: 86 %<br>Vehicle: ethanol | Species: rats<br>Strain: CD [CRL:COBS CD (SD) BR]<br>Route: oral (feed)<br>Number: F0/F1:70/dose group/sex<br>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<br>Dose levels:<br>Part I: 0, 100, 500, ppm<br>Part II: 0, 10 000 ppm<br>Doses calculated based on food consumption:<br>Part I<br>8, 17, 43 mg/kg bw/d in F0 males;<br>9, 17, 42 mg/kg bw/d in F0 females;<br>5, 10, 26 mg/kg bw/d in F1 males;<br>6, 13, 32 mg/kg bw/d F1 females<br>Part II<br>10 000 ppm corresponds to 790 and 894 mg/kg bw/d for F0 males and | Haemoglobin decrease<br>Part I:<br>- Statistically significant (-8 %) in F1 high dose females (12 months)<br>- Statistically significant (-6 %) in F1 mid dose males (18 months)<br>Part II:<br>- Decrease in all treated groups<br>- Statistically significant in treated F1 males at month 3, 12, 18, and 24 (-9, -18, -10, -9 %)<br>- Statistically significant in treated F1 females at 3, 12, 18, and 24 month (-14, 18 %, no further data available)<br>Haematocrit decrease<br>Part I:<br>- Statistically significant in F1 high dose females at 3 and 12 months (-6 %)<br>Part II:<br>- Statistically significant in F1 males at 3, 12, 18, and 24 month (-8, -10, -8, -9 %)<br>- Statistically significant in treated F1 females at 3, 6, 12, 18 and 24 months (-8, -14, -15 %, no further data)<br>Red blood cell count decrease<br>Part I:<br>- Statistically significant (-10 %) in F1 high dose females | GV STOT RE 2 ≤ 11 mg/kg bw/d for 30-month exposure |

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| | | females<br><br>No data available for F1 males (calculated: 500 mg/kg bw/d); 521 mg/kg bw/d for F1 females | (12 months)<br><br>Part II:<br><br>- Statistically significant in treated F1 males at month 3, 6, 12, 18, and 24 (-31, -21, -24, -10, -18 %)<br><br>- Statistically significant in treated F1 females at 3, 12, 18, and 24 months (-32, -24 %, no further data available)<br><br>Reticulocyte count increase<br><br>Part I:<br><br>- Statistically significant (92 and 100 %) in F1 mid and high dose females at month 18<br><br>Part II:<br><br>- Statistically significant 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<br><br>Spleen weight and spleen weight-body weight ratio increase<br><br>Part I:<br><br>- Statistically significant (20.9 %, respectively 22.5 %) in F1 high dose females at month 12<br><br>- Spleen weight of high dose males increased (not statistically significant)<br><br>Spleen weight and spleen weight-body weight ratio values for F1 high dose of both sexes were increased compared to combined control (control 1 plus control 2), but not statistically significant at 30 month; high mean 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;<br><br>Part II:<br><br>- Statistically significant in treated F1 males (318 %, respectively 372 %) and females (210 %, respectively | |

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| | | | 246 %) at 12 months, and F1 males (183 %, respectively 175 %) and females (349 %, respectively 382 %) at terminal kill (month 30)<br><br>Haemosiderosis of the spleen<br><br>Part I:<br><br>- F1 high dose females after 12 months<br><br>Hemosiderin accumulation in liver<br><br>Part II:<br><br>- dosed females (unknown incidence)<br><br>Hemosiderin accumulation in kidneys<br><br>Part II:<br><br>- dosed females and males (unknown incidence)<br><br>Further observations (part II)<br><br>- 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<br><br>- Testis weight and testis weight/bw: Statistically significant decrease in F1 mean weight (-24 %) and weight/bw percentage (-26.1 %), compared to controls at terminal kill (month 30) | |
| Hoechst AG, 1973<br><br>32-days feeding study<br><br>Similar to OECD TG 407<br><br>Pre-GLP<br><br>Not reliable (insufficient characterisation of test material, no data on clinical biochemistry, main description of test conditions missing) | Mixture of two azo dyes<br><br>Purity: unknown | Species: rats<br><br>Strain: SPF-Wistar<br><br>Route: oral (feed)<br><br>Number: 10/dose group/sex<br><br>Treatment time: 32 days<br><br>Dose levels: 5 %, 1 % and 0.2 %<br><br>Calculated doses: 0, 10, 50, 250 mg/kg bw/d | Erythrocytes:<br><br>- Dose-dependent decrease in all treated groups<br><br>Leucocytes:<br><br>- Dose-dependent increase<br><br>Heinz bodies in erythrocytes:<br><br>- increase in high, mid and low dose groups (100 %, 30 % and 10 %)<br><br>Spleen weight:<br><br>- Statistically significant and dose-dependent increase; enlarged and blackish coloured spleen and brownish coloured | GV STOT RE 2 ≤ 281 mg/kg bw/d for<br><br>32-day exposure |

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| | | | kidneys in mid and high dose animals<br>Iron storage:<br>- Dose-dependent increase in liver, kidney tubular epithelium (except for low dose group), moderate to strong increase in iron levels in spleen in all treatment groups | |
| Davis and Fitzhugh, 1962, publication<br>2-year feeding study<br>Non-guideline study<br>Reliability: Not assignable (no full study report available) | PR 53:1,<br>Purity: 86 % | Species: rats<br>Strain: Osborne-Mendel<br>Route: oral (feed)<br>Number: 25/dose group/sex<br>Treatment time: 103 weeks<br>Dose levels: 0, 0.01, 0.05, 0.25, 1 %. Calculated doses: 0, 5, 25, 125 and 500 mg/kg bw/d | Slight to moderate splenomegaly:<br>- In rats of the 0.01 % (2/12 rats), 0.05 % (4/12), 0.25 % and 1.0 % (both 7/12) dose groups<br>- significant increase in spleen weight/body weight ratio in the 0.25 % and 1.0 % dose groups<br>Splenic infarcts, scars, haemosiderosis, or cysts in high dose group (1 %)<br>Slight hematologic effects (slight decrease of haemoglobin, presence of abnormal circulating red blood cells) noted early in the test, did not increase in severity (no raw data)<br>Bone marrow of 0.25 and 1.0 % dose groups was slightly hyperplastic compared to controls<br>Significantly less chronic nephritis in the 0.25 and 1.0 % dose groups<br>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 for 2-year exposure |
| Studies on mice | | | | |
| NTP, 1982b<br>2-year feeding study in mice<br>Non-GLP<br>According to OECD TG 451<br>Reliability without restriction according to the DS | PR53:1,<br>Purity 89.8 %<br>Impurities sodium and barium sulfates | Species: mice<br>Strain: B6C3F1<br>Route: oral (feed)<br>Number: 50/dose group/sex<br>Treatment time: 103 weeks, daily<br>Post exposure period: 1 week<br>Dose levels: 0, 1 000, | No non-neoplastic findings in treated mice | GV STOT RE 2 ≤ 12.6 mg/kg bw/d for<br>2-year exposure |

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| | | 2 000 ppm<br>Food conversion factor: 7<br>Calculated doses: 0, 142, 285 mg/kg bw/d | | |
| NTP, 1982<br>Range finding study for carcinogenicity - feeding study in rats (13-week study)<br>Non-GLP<br>Reliability with restriction according to the DS (no data on haematology, clinical biochemistry, urine analysis) | PR53:1<br>Purity 89.8 %<br>Impurities sodium and barium sulfates | Species: mice<br>Strain: B6C3F1<br>Route: oral (feed)<br>Number: 10/dose group/sex<br>Treatment time: 91 day, daily<br>Dose levels: 0, 600, 1 250, 2 500, 5 000 or 10 000 Food conversion factor: 7<br>Calculated doses: 0, 86, 179, 357, 714, and 1 429 mg/kg bw/d | Congestion of the spleen in 55/60 mice at ≥ 357 mg/kg bw/d<br>Deposits of haemosiderin 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 for 13-week exposure |
| CTFA, 1982c<br>Combined repeated dose and carcinogenicity (daily for 24 months/105 weeks)<br>Similar to OECD TG 453<br>Non-GLP<br>Reliable with restrictions according to the DS: no data on clinical biochemistry of plasma or serum, no data collected for oestrus cycle or sperm parameters, no urinalysis | PR 53:1;<br>Purity: ≥ 76 % | Species: mice<br>Strain: CD-1<br>Number: 60/dose group/sex,<br>Route: oral, diet<br>Treatment time: 105 weeks<br>Dose levels: 0, 50, 250, 1 000 ppm<br>Calculated doses: 0, 7, 38, 147 mg/kg bw/d in males; 0, 12, 56, 237 mg/kg bw/d in females | Haemoglobin:<br>- Statistically significant decrease in high-dose females (-11.4 % vs. control) at 18 months, in high-dose males at 6 months (-7.2 %)<br>Haematocrit:<br>- Statistically significant decrease in low-dose (-8.1 % vs. control) and high dose females (-9.9 %) at 18 months<br>- decrease (but not significant) at the mid-dose, (-5.1 %)<br>Red blood cell count:<br>- Statistically significant increase for high dose females (14.8 % vs control) at 3 months<br>- Statistically significant decrease (-10.7 %) after 18 months<br>Gross and histopathologic evaluation did not reveal any compound related findings. | GV STOT RE 2 ≤ 12.6 mg/kg bw/d for 103 week exposure) |

[1]Dose level selected based on effects observed in 91 day study; [2]Doses calculated by the DS

Six studies in rats were assessed as supporting information to carcinogenicity for specific target organ toxicity and non-neoplastic effects produced by PR 53:1. The first study performed according to OECD TG 451 on F344 rats exposed over a period of 2 years (NTP, 1982) showed that non-neoplastic lesions in the spleen including focal, multifocal and diffuse fibrosis were significantly increase in both sexes at high dose group compared to control. The

second study was the 91-day study used for dose selection for the two-years feeding study (NTP, 1982c). At all the tested doses (3 000, 6 000, 12 500 and 50 000 ppm corresponding to 150, 300, 625, 1 250 and 2 500 mg/kg bw/d) showed enlarged spleen and pigment deposition in the renal tubular epithelium. In a 30-month chronic toxicity study with in utero and lifetime exposure levels of 5, 10, 26 mg/kg bw/d in males and 6, 13, 32 mg/kg bw/d in females (CTFA, 1982a), 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) were reported. A similar study design using higher doses (500 mg/kg bw/d in males and 521 mg/kg bw/d in females) reported that the effects observed at lower doses were increased (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 were found in rats fed with PR 53:1 (no data on incidence available) (CTFA, 1982a; CTFA, 1982b). A dose-dependent decrease in erythrocytes and an increase in leucocytes in treated animals were reported in a 32-days feeding study (Hoechst AG, 1973) but the study is not reliable as it does not provide information on the composition and purity of test material. Furthermore, Heinz bodies (formed by irreversible precipitation of oxidative denatured haemoglobin) in erythrocytes were increased in a dose-dependent manner in the study, and 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 study by Davis and Fitzhugh (1962) showed a slight to moderate splenomegaly at 125 and 500 mg/kg bw/d. High dosed rats (500 mg/kg bw/d) showed splenic haemosiderosis and splenic infarcts.

Three studies in mice were assessed as supporting information to carcinogenicity for specific target organ toxicity and non-neoplastic effects produced by PR 53:1. A 2-year feeding study reported no non-neoplastic findings at doses up to 285 mg/kg bw/d (NTP, 1982b). This study was conducted based on a 91-day study results reporting congestion of the spleen at doses above 375 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 or 179 mg/kg bw/d and 86 mg/kg bw/d males. There were no data collected on haematology, clinical biochemistry, or urinalysis. A combined repeated dose and carcinogenicity study similar to OECD TG 453 on CD-1 mice treated for 18 months with PR 53:1 (doses 7, 38, 147 mg/kg bw/d in males and 12, 56, 237 mg/kg bw/d in females) (CTFA, 1982c) reported a significant decrease in haemoglobin (≥ -10 %), haematocrit, and red blood cell count in high dose females at 18 months. Furthermore, the red blood cell count was statistically increased (≥ 10 %) for high-dose females at 3 months.

The reported data on haematology and pathology/histopathology reveal adverse effects on animals treated with PR 53:1. There is consistency between the different studies in rats and mice that PR 53:1 induced haematolytic anaemia, 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 PR 53:1 in rats resulted in neoplastic lesions of the spleen accompanied by increased incidences of diffuse/multifocal splenic and capsular fibroses, and haemosiderin deposition in spleen, liver and kidneys.

***Germ cell mutagenicity studies***

Germ cell mutagenicity was not evaluated in the CLH report but data from mutagenicity studies were provided to support the assessment of carcinogenicity.

The DS reported the following *in vitro* and *in vivo* mutagenicity/genotoxicity studies (cf. Table 10 and 11 of the CLH report and information regarding study design in Annex I):

| **Reference, method, guideline, deviations if any** | **Test substance** | **Relevant information about the study including rationale for dose selection (as applicable)** | **Results** | **Reliability** |
|---|---|---|---|---|
| ***In vitro* studies** | | | | |
| Hoechst AG, 1989, unpublished study report<br>Bacterial Reverse Mutation Test<br>GLP study<br>Similar to OECD TG 471 (Ames test)<br>Deviations: 5th strain missing | PR 53:1<br>Purity: technically pure<br>Vehicle: DMSO<br>Positive controls: yes<br>Negative controls: yes | Supporting study according to the DS<br>Strains: *S. typhimurium* TA100, TA98, TA1537, TA1535<br>Metabolic activation system: S9 mix-hamster liver S9, untreated and rat liver S9 Aroclor induced<br>Test concentrations (± metabolic activation (S9 mix)): 4, 20, 100, 500, 2 500, 5 000 µg/plate | Genotoxicity: negative with (hamster and rat S9) and without metabolic activation.<br>No significant increase in the number of revertants in any bacterial strains with and without Prival with and without metabolic activation.<br>Cytotoxicity: no<br>Precipitations: ≥ 500 µg/plate<br>Neg. control: valid<br>Pos. control: valid | Reliable with restriction according to the DS<br>(5th strain missing, results for TA100, TA98, TA1537, TA1535 are reliable without restrictions) |
| Hoechst AG, 1985a<br>Bacterial Reverse Mutation Test<br>GLP study<br>Similar to OECD TG 471 (Ames test)<br>Deviations: 5th strain missing | PR 53:1<br>Purity: technically pure<br>Vehicle: DMSO<br>Positive controls: yes<br>Negative controls: yes | Supporting study according to the DS<br>Strains: *S. typhimurium* TA100, TA98, TA1537, TA1535<br>Metabolic activation system: S9: hamster liver (Prival activation) S9: untreated and rat liver S9 Aroclor-induced (classical test protocol)<br>Test concentrations (± metabolic activation (S9 mix)): 4, 20, 100, 500, 2 500, 5 000/10 000 µg/plate | Genotoxicity: negative with (hamster and rat S9) and without metabolic activation.<br>Cytotoxicity: no<br>Precipitations: ≥ 100 µg/plate<br>Neg. control: valid<br>Pos. control: valid | Reliable with restrictions according to the DS (5th strain missing results for TA100, TA98, TA1537, TA1535 are reliable without restrictions) |

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| Hoechst AG, 1985b, unpublished study report<br>Bacterial Reverse Mutation Test<br>GLP study<br>Similar to OECD TG 471 (Ames test)<br>Deviations: none | PR 53:1<br>Purity: technically pure<br>Vehicle: DMSO<br>Positive controls: yes<br>Negative controls: yes | Key study according to the DS<br>Strains: *S. typhimurium* TA100, TA98, TA1537, TA1535, TA 1538, Escherichia coli WP2uvrA<br>Metabolic activation system: S9: Aroclor 1254 induced rat liver<br>Test concentrations (± metabolic activation (S9 mix)): 4, 20, 100, 500, 2 500, 10 000 µg/plate | Genotoxicity: negative with and without metabolic activation.<br>Cytotoxicity: no<br>Precipitations: ≥ 100 µg/plate<br>Neg. control: valid<br>Pos. control: valid | Reliable without restrictions according to the DS |
| CIBA-GEIGY Limited, 1985<br>Bacterial Reverse Mutation Test<br>Non-GLP<br>Similar to OECD TG 471 (Ames test) (without Prival activation)<br>Deviations:<br>No verification of negative result<br>Only three strains tested (e.g. no TA 1535, *E.coli* WP2 missing)<br>No data on purity | PR 53:1<br>Purity: no data<br>Vehicle: DMSO<br>Positive controls: yes<br>Negative controls: yes | Supporting study according to the DS<br>Strains: *S. typhimurium* TA100, TA98, TA1537<br>Metabolic activation system: S9: rat liver, Aroclor-induced<br>Test concentrations (± metabolic activation (S9 mix)): 20, 78, 313, 1 250, 5 000 µg/plate | Genotoxicity: negative with and without metabolic activation.<br>Cytotoxicity: no<br>Precipitations: ≥ 100 µg/plate<br>Neg. control: valid<br>Pos. control: valid | Reliable with restrictions according to the DS (only three strains tested, no verification of negative results) |
| Brown *et al*., 1979<br>Bacterial Reverse Mutation Test<br>Non-GLP<br>Similar to OECD TG 471 (Ames test)<br>Deviations:<br>•Documentation insufficient<br>•Purity insufficient | PR 53:1<br>Purity: 33-73 %<br>Vehicle: DMSO<br>Positive controls: yes<br>Negative controls: yes | Disregarded study according to the DS<br>Strains: *S. typhimurium* TA100, TA98, TA1537, TA1535, TA1538<br>Metabolic activation system: S9: rat liver, Aroclor-induced<br>Test concentrations (± metabolic activation (S9 mix)): 50, 100, 500 µg/plate | Genotoxicity: negative with and without metabolic activation.<br>Cytotoxicity: no data<br>Precipitations: no data<br>Neg. control: valid<br>Pos. control: valid | Not assignable (insufficient documentation and methodical deficiencies) |

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| •Data on 5th strain missing<br>•Low max. concentration<br>•Only 3 concentrations tested<br>•No detailed data on results (data table) | | | | |
| Zeiger *et al.*, 1988<br>Bacterial Reverse Mutation Test<br>GLP not specified<br>Similar to OECD TG 471 (Ames test) (Prival activation and without Prival)<br>Deviations:<br>No detailed data on results<br>No information on purity<br>Data on 5th strain missing<br>Cytotoxicity not determined | PR 53:1<br>Purity: unknown<br>Vehicle: DMSO<br>Positive controls: yes<br>Negative controls: yes | Disregarded study according to the DS<br>Strains : *S. typhimurium* TA100, TA98, TA1537, TA1535, TA97<br>Metabolic activation system: S9: hamster liver S9, untreated and rat liver S9 Aroclor-induced<br>Test concentrations (± metabolic activation (S9 mix)): 100, 333, 1 000, 3 333, 10 000 µg/plate | Genotoxicity: ambiguous with and without metabolic activation: ambiguous for TA97 without S9 and for TA98 with and without S9<br>Cytotoxicity: not determined<br>Precipitations: ≥ 100 µg/plate<br>Neg. control: valid<br>Pos. control: valid | Not assignable (detailed result data missing to evaluate relevance of ambiguous results) |
| Myhr *et al.*, 1991<br>*In vitro* mammalian cell gene mutation test using the thymidine kinase gene<br>GLP not specified<br>Similar to OECD TG 490<br>Deviation:<br>No data on purity | PR 53:1<br>Purity: no data<br>Vehicle: DMSO<br>Positive controls: yes<br>Negative controls: yes | Key study according to the DS<br>Cell culture: mouse lymphoma L5178Y cells<br>Metabolic activation system: S9: rat liver S9 Aroclor-induced<br>Test concentrations: without metabolic activation (S9 mix)): 1.25, 2.5, 5, 7.5, 15 µg/mL<br>With metabolic activation: 2, 3, 4, 5, 6 µg/mL<br>Treatment time(s): 4 h<br>Sampling time(s): after 2 days | Genotoxicity: negative with and without metabolic activation:<br>Cytotoxicity: no<br>Precipitations: ≥ 7.5 µg/mL<br>Neg. control: valid<br>Pos. control: valid | Reliable with restrictions |
| Hoechst AG, | PR 53:1 | Key study according to | Genotoxicity: negative | Reliable with |

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| 1989b<br>*In vitro* mammalian chromosomal aberration test<br>GLP study<br>Similar to OECD TG 473<br>Deviation:<br>Only 100 metaphases scored per concentration<br>No data on purity | Purity: no data<br>Vehicle: DMSO<br>Positive controls: yes<br>Negative controls: yes | the DS<br>Cell culture: Chinese hamster lung fibroblasts (V79)<br>Metabolic activation system: S9: rat liver S9 Aroclor-induced<br>Test concentrations: ± metabolic activation (S9 mix)): 30, 150, 300 µg/mL<br>Treatment time(s): 4 and 18 h<br>Sampling time(s): 4.5, 15.5, 25.5 h after beginning of treatment<br>Justification for top concentration: significant cytotoxicity ≥ 400 µg/mL | with and without metabolic activation<br>Cytotoxicity: significant cytotoxic effect ≥ 400 µg/mL<br>Precipitations: ≥ 500 µg/mL<br>Neg. control: valid<br>Pos. control: valid | restrictions (only 100 metaphases scored per concentration) |
| Ivett *et al.*, 1989<br>*In vitro* mammalian chromosomal aberration test<br>GLP: not specified<br>Not similar to OECD TG 473<br>Deviation:<br>Continuous exposure of about 12-14 h without metabolic activation missing<br>Short-term treatment with and without metabolic activation not adequate (8 h and 2 h instead of 3-6 h)<br>Sampling time too short (2-2.5 h instead of 1.5 times the normal cell cycle length)<br>Only 200 | PR 53:1<br>Purity: 89.8 %<br>Vehicle: DMSO<br>Positive controls: yes<br>Negative controls: yes | Disregarded study according to the DS<br>Cell culture: CHO<br>Metabolic activation system: S9: rat liver S9 Aroclor-induced<br>Test concentrations: without metabolic activation (S9 mix)): 37.1, 50, 123.8 µg/mL<br>with metabolic activation (S9 mix)): 5, 16.7, 50 µg/mL<br>Treatment time(s):<br>Without metabolic activation: 8 h<br>With metabolic activation: 2 h and 8 h<br>Sampling time(s): 2-2.5 h<br>Justification for top concentration: no specific data | Genotoxicity: negative with and without metabolic activation:<br>Cytotoxicity: no detailed data<br>Precipitations: ≥ 250 µg/mL<br>Neg. control: valid<br>Pos. control: valid | Not reliable (exposure and sampling times are not according to OECD TG, too few cells analysed) |

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| (instead of 300) metaphases evaluated<br>No specific data on justification for top dose | | | | |
| ***In vivo* studies** | | | | |
| Westmoreland and Gatehouse, 1992<br>Unscheduled DNA synthesis (UDS) test with mammalian liver cells *in vivo*<br>GLP: not specified<br>Similar to OECD TG 486 | PR 53:1<br>Purity: no data<br>Vehicle: Corn oil<br>Positive controls: yes (2-acetylaminofluorene for 16 h)<br>Negative controls: yes | Supporting study according to the DS<br>Species: rats Piebald Virol Glaxo<br>Number of animals per group: 7 males<br>Target organs: liver<br>Administration route: oral (gavage), single dosage<br>Dose level: 1 000 and 2 000 mg/kg bw/d<br>Justification for top dose: limit test<br>Sampling: 16 h | Genotoxicity: No marked increase in incidence of cells in repair at 16 h sampling time<br>Toxicity: no toxicity observed.<br>Controls were valid. | Reliable without restrictions |
| Westmoreland and Gatehouse, 1992<br>Mammalian erythrocyte micronucleus test<br>GLP: not specified<br>Similar to OECD TG 474<br>Deviation:<br>No evidence of exposure of bone marrow | PR 53:1<br>Purity: no data<br>Vehicle: Corn oil<br>Positive controls: yes (cyclophosphamide, 24 h)<br>Negative controls: yes | Supporting study according to the DS<br>Species: rats Piebald Virol Glaxo<br>Number of animals per group: 7 males<br>Target organs: bone marrow<br>Administration route: oral (gavage), single dosage<br>Dose level: 500, 1 000 and 2 000 mg/kg bw/d<br>Justification for top dose: limit test<br>Sampling: 24 or 48 h | Genotoxicity: negative, no increase in the frequency of micronuclei<br>Toxicity: no toxicity observed<br>Evidence of exposure of bone marrow: no, as ratio PCE/NCE not decreased, no other evidence<br>Controls were valid. | Not reliable (no evidence of exposure of bone marrow shown) |

There are six *in vitro* bacterial reverse mutation tests performed using PR 53:1, four of them being considered reliable (CIBA-GEIGY Limited, 1985; Hoechst AG, 1985a; Hoechst AG, 1985b; Hoechst AG, 1989a). All the reliable studies reported negative results for the strains *S. typhimurium* TA100, TA98, TA1537 and TA1535 with and without metabolic activation (all six studies used S9 obtained from Aroclor-induced rat liver, and two studies additionally with Prival activation). PR 53:1 is an azo dye and according to the OECD TG 471 the use of reductive metabolic activation system (Prival activation) is considered more appropriate than the classical test protocol using S9 obtained from Aroclor-induced rat liver. 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 PR 53:1. These results are supported by one reliable *in vitro* mammalian gene mutation test similar to OECD TG 490 (Myhr *et al*., 1991). There are also two *in vitro* cytogenicity tests. One of the cytogenicity studies is an *in vitro* mammalian chromosomal aberration test (Hoechst AG, 1989b) similar to OECD TG 473, and considered reliable by the DS supporting the negative results. In conclusions, *in vitro* data indicates no mutagenic effects for PR 53:1 with some limitations (no information for the 5th strain in a bacterial reverse mutation test using Prival activation).

There are two *in vivo* somatic cells genotoxicity tests performed with PR 53:1. The unscheduled DNA Synthesis (UDS) test with mammalian liver cells (Westmoreland and Gatehouse, 1992) and the mammalian erythrocyte micronucleus test (MN) (Westmoreland and Gatehouse, 1992) both reported negative results. The UDS test is similar to OECD TG 486 and is considered reliable without restrictions by the DS. As specified in 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 mammalian erythrocyte micronucleus test is used only as supportive study as the exposure of bone marrow was not demonstrated.

According to the classification criteria, even if germ cell mutagenicity is not assessed in this dossier, the information supports the conclusion that the carcinogenic effects of PR 53:1 occurs through a non-genotoxic mode of action.

The possible non-genotoxic mode of action of PR 35:1 was described by Goodman *et al*. (1984) and Weinberger *et al*. (1985). Both authors consider that splenic lesions are the starting point for tumour formation. Goodman *et* at. (1984) suggested splenic haemosiderosis secondary to methaemoglobinemia led 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 data from chronic and sub-chronic studies revealed the induction of 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. The effects were less severe in mice compared to rats. Methaemoglobin formation was not evaluated in any of the studies.

For the structurally related compound aniline, data suggests 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 splenic capsule. This is a major cause for pre-neoplastic splenic lesions related to aniline, which can result in tumour formation in rats upon chronic exposure (MAK-Collection for Occupational Health and Safety, 2007). The lower sensitivity of mice in comparison to rats can be explained by species differences in methaemoglobin reductase activity, which is responsible for regeneration of functional haeme from methaemoglobin.

The non-genotoxic mode of action of aniline that explain the tumour formation can be plausible also for PR 53:1, but a final conclusion on the mode of action cannot be drawn due to lack of available data.

## Comments received during consultation

Six comments were received during public consultation, five from industry and 1 from a Member State Competent Authority (MSCA). The comment received from the MSCA was in agreement with the DS conclusion that PR 53:1 should be classified as Carc. 2, H351. The other five comments received from the industry are all against PR 53:1 classification as Carc.2, H351 pointing out that the chemical has no genotoxic potential and does not act as a primary carcinogen, and that the adverse effects seen in liver and spleen are more likely to appear due to metabolites leading to hemosiderosis in both target organs and to fibrosis and promotion of tumour formation in spleen. The comments by industry noted that the incidence of tumours was seen only in one study, one species (rat) and one sex (males). The industry comments emphasised that PR 53:1 is handled safely by workers and professionals. Personal precautions, protective equipment as well as protective clothing, have been established to minimize the risk of exposure in manufacturing and processing by inhalation or dermal contact, and by accidental oral exposure. Additionally, PR 53:1 is not handled by the general population. In consumer articles the material is included at very low concentrations, embedded in a matrix, e.g. a polymer matrix or binders matrix. Uptake of the substance at dose level relevant for adverse toxic effects is not expected.

The DS responded that the classification of a chemical as a carcinogen is based on its potential to induce tumours, to increase tumour incidence and/or malignancy, or shorten the time to tumour occurrence, and that the mode of action (as genotoxic carcinogen or not) is not relevant for classification. The studies included in the CLH report provide evidence of carcinogenic potential of PR 53:1 based on the increased incidence of splenic sarcomas in male rats, a rare type of tumour in this organ (CTFA, 1982b; CTFA, 1982a; NTP, 1982a). In the NTP study there is a statistically significant increase in splenic sarcoma incidence in male rats of the high dose group that is above the HCD and is reported with frequently observed metastases that indicate a high malignancy. The results of the CTFA studies are considered supportive, as similar patterns of non-neoplastic splenic lesions were observed in both studies. The study by Davis and Fitzhugh (1962) showed no tumour formation, but severe splenic effects. According to the DS, the study has limited reliability due to limited reporting, no data were provided for individual animals, only six animals from each group were examined histopathologically and no body weight information or HCD were provided. Similar limitations were also identified for the dermal study published by Carson (1984). The DS proposed classification of Pigment Red 53:1 as carcinogen, Category 2. According to Regulation (EC)1272/2008 category 2 is fulfilled, when there is limited evidence of carcinogenicity.

## Assessment and comparison with the classification criteria

For PR 53:1, no human evidence for carcinogenicity is available to support the classification in category 1A for carcinogenicity.

A high incidence of splenic sarcoma (statistically significant) in F344 male rats and increased incidence (not statistically significant) in Charles-River CD Sprague-Dawley male rats was reported for PR 53:1. The study in F344 rats showed high incidence, high malignancy and metastasis, with low spontaneous incidence according to HCD and also proofs of progression of lesions to malignancy. These data can be supportive for classification in category 1B, but are not definitive based on the total weight of evidence, effects being observed only in one

experiment that according to Annex I: 3.6.2.2.3 represents a limited evidence of carcinogenicity. The criteria for classification as Carc.1B are therefore not fulfilled.

The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

One animal study on F344 rats reported a statistically significant increase in splenic sarcomas in males above the HCD, with high malignancy and metastasis potential and low spontaneous incidence. The effects are supported by another study on Charles-River CD Sprague-Dawley rats reporting a similar trend without statistical significance. There is no possibility of confounding effects of excessive toxicity at the tested doses. The effects were observed only in males. In females, an increasing trend of non-neoplastic spleen lesions was reported, the lesions being also reported in studies with mice. Splenic lesions are considered as a starting point for tumour formation. The possibility of a genotoxic mode of action is dismissed based on the available negative *in vitro* and *in vivo* genotoxicity studies. The non-genotoxic mode of action starting from splenic lesions is plausible and its relevance to humans cannot be excluded. Similar findings and mode of action is described also for aniline and other aromatic amines and aromatic azo compounds that are structurally related with PR 53:1. Taking all the above into consideration, the increasing incidence of splenic sarcoma in male rats is considered as limited evidence of carcinogenicity, and the criteria for classification in Category 2 are met. The generic concentration limit (GCL) of ≥ 1.0 % shall apply. RAC agrees with the DS classification proposal and with the non-genotoxic mode of action.

RAC agrees with the DS proposal that **classification of PR 53:1 in Category 2 (H351) for carcinogenicity is warranted.**

## 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**<br><br>D & C Red No. 9 (trading name of PR53:1)<br>Batch No: Lot No. Z-8054<br>Purity: 89.8 %, Impurities: sodium and barium sulfates<br><br>Study according to OECD TG 451 (NTP guideline including single dose, 2-week and 13-week studies), No GLP<br><br>**Reliable without restrictions**<br><br>Rats, F344 (N=50/sex/dose);<br>Mice, B6C3F1 (N=50/sex/dose)<br>Route: oral, diet<br>Dose rats: 0, 1000, 3000 ppm; Corr. to 0, 50, 150 mg/kg bw/d (Conversion factor 20 for older rats)<br>Dose mice: 0, 1000, 2000 ppm; Corr. to 0, 143, 286 mg/kg bw/d (Conversion factor 7)<br>Treatment time: 103 weeks, daily<br>Post exposure period: 1 week<br>Dose level selected based on effects observed in 91 day study | **Rats:**<br><br><u>Splenic lesions in males, at 150 mg/kg bw/d:</u><br>14/48, congestion of the splenic parenchyma,<br>23/48, focal or multifocal areas of fibrosis,<br>3/48, diffuse fibrosis,<br>13/48, areas of fatty metamorphosis in the spleen<br><br><u>Splenic lesions in females, at 150 mg/kg bw/d:</u><br>25/50, multifocal, diffuse, or focal fibrosis<br><br>Areas of fibrosis present in 2/50 control male rats<br><br>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<br><br>**Mice**:<br>No non-neoplastic findings in treated mice | (NTP, 1982)<br><br>GV STOT RE 2<br>≤ 12.6 mg/kg bw/d<br>(103 weeks) |
| **Range finding study for carcinogenicity study (13 week study)**<br><br>D & C Red No. 9 (trading name of PR53:1)<br>Batch No: Lot No. Z-8054<br>Purity: 89.8 %, Impurities: sodium and barium sulfates<br>Non-guideline study, No GLP<br><br>**Reliable with restrictions** (no data on haematology, clinical biochemistry, urine analysis)<br><br>Rat, F344 (N = 10/sex/dose);<br>Mice, B6C3F1 (N = 10/sex/dose) | **Rats**:<br><br><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<br><br><u>Pigment deposition in kidney tubular epithelium</u> in all dosed rats<br><br><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) | (NTP, 1982) |

| Method | Results/Observations (regarding STOT RE) | Reference (Guidance Value for STOT RE*) |
|---|---|---|
| Route: oral, diet<br><br>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)<br>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)<br><br>Treatment time: 91 d<br><br>Gross necropsy of all animals, histopathology of certain tissues from controls and highest dose animals | Lymphoreticular hyperplasia of thymic lymph nodes in 75 – 100 % 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)<br><br>**Mice**:<br><br>Congestion of the spleen: in 55/60 mice at ≥ 357 mg/kg bw/d<br><br>Deposits of haemosiderin 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<br>≤ 100 mg/kg bw/d (90 days) |
| **30-month chronic toxicity and potential carcinogenicity study in rats with *in utero* and lifetime exposure**<br><br>Desert Red D & C Red No. 9 Ba. Lake (trading name of PR53:1)<br>Batch No: #547530, C-15-I01<br>Purity: 76 %<br><br>According to FDA guidelines, Pre-GLP<br><br>**Reliable with restriction** (individual data e.g. clinical signs missing)<br><br>Rat, CD [CRL:COBS CD (SD) BR] (F0/F1: N=70/sex/dose)<br><br>Route: oral, diet<br><br>Dose:<br>Part I: 100, 200, and 500 ppm.<br>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<br><br>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<br><br>Part I<br><br>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 | Haemoglobin:<br>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<br>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<br><br>Haematocrit:<br>Part I: Significant decrease (-6 %) in F1 high dose females (32 mg/kg bw/d) at month 3 and at month 12 (-6 %)<br><br>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)<br><br>Red blood cell count:<br>Part I: Significant decrease (-10 %) in F1 high dose females (32 mg/kg bw/d) at month 12<br><br>Part II: Significant decrease in treated F1 males at month 3, 6, 12, 18, and 24 (-31, -21, -24, -10, -18 %),<br>significant decrease in treated F1 females at 3, 12, 18, and 24 months (-32, -24 %, no further data available) | (CTFA, 1982a) and (CTFA, 1982b) |

| Method | Results/Observations (regarding STOT RE) | Reference (Guidance Value for STOT RE*) |
|---|---|---|
| weaning<br><br>Examination F0: mortality, body weights, food consumption, general physical signs, signs of toxicity<br><br>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<br><br>Clinical pathology at months 3, 6, 12, 18, and 24 (N=10/sex/dose) | Reticulocyte count:<br>Part I: Significant increase (92 and 100 %) in F1 mid (12 mg/kg bw/d) and high (32 mg/kg bw/d) dose females at month 18<br><br>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<br><br>Spleen weight and spleen weight-body weight ratio:<br>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<br><br>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;<br><br>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)<br><br>Haemosiderosis of the spleen:<br>Part I: F1 high dose females after 12 months<br><br>Hemosiderin accumulation in liver:<br>Part II: in dosed females (unknown incidence)<br><br>Hemosiderin accumulation in kidneys:<br>Part II: in dosed females and males (unknown incidence)<br><br>Further observations (part II):<br>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 | GV STOT RE 2 |

| Method | Results/Observations (regarding STOT RE) | Reference (Guidance Value for STOT RE*) |
|---|---|---|
| | 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) | ≤ 11 mg/kg bw/d (120 weeks) |
| **Combined repeated dose and carcinogenicity (daily for 24 months/105 weeks)**<br><br>D & C Red No. 9 (trading name of PR53:1)<br>Batch No: Lot #AA-3779<br>Purity: ≥ 76 %<br><br>Similar to OECD TG 453, No GLP<br><br>**Reliable with restrictions** (no data on clinical biochemistry of plasma or serum, no data collected for oestrus cycle or sperm parameters, no urinalysis)<br><br>Mouse, CD-1 (N=60/sex/dose)<br>Dose: 0, 50, 250 and 1 000 ppm<br>Route: oral, diet<br>Corr. to: 0, 7, 38, 147 mg/kg bw/d in males; 0, 12, 56, 237 mg/kg bw/d in females<br><br>Haematology at 3, 6, 12, and 18 months (N=10/sex/dose) | <u>Haemoglobin:</u><br>Significant decrease in high-dose females (-11.4 % vs. control) at 18 months, in high-dose males at 6 months (-7.2 %)<br><br><u>Haematocrit:</u><br>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 %)<br><br><u>Red blood cell count:</u><br>Statistically increase for high dose females (14.8 % vs. control) at 3 months; significant decrease (-10.7 %) after 18 months<br><br>Gross and histopathologic evaluation did not reveal any compound related findings. | (CTFA, 1982c)<br><br>GV STOT RE 2<br>≤ 12.6 mg/kg bw/d (103 weeks) |
| **32 d Feeding study**<br><br>Test material: Confidential Annex<br>Purity: unknown<br><br>Similar to OECD TG 407, Pre-GLP<br><br>**Not reliable** (insufficient characterisation of test material, no data on clinical biochemistry, main description of test conditions missing)<br><br>Rat, SPF-Wistar, N=20/dose (10/sex/dose)<br><br>Dose: 0, 0.2, 1, and 5 % corr. to 0, 10, 50, 250 mg/kg bw/d<br><br>Route: oral, diet<br><br>Treatment time: 32 d | <u>Erythrocytes:</u> Dose-dependent decrease in all treated groups<br><br><u>Leucocytes:</u> Dose-dependent increase<br><br><u>Heinz bodies in erythrocytes</u>: Increase in high- (100 % vs control), mid- (30 %), and low-dose groups (10 %)<br><br><u>Spleen weight</u>: Significant and dose dependent increase; enlarged and blackish-coloured spleen and brownish-coloured kidneys in mid- and high-dose animals<br><br><u>Iron storage</u>: 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)<br><br>GV STOT RE 2<br>≤ 281 mg/kg bw/d (32 days) |
| **2-year feeding experiment**<br><br>D&C Red No. 9 (trade name of PR53:1)<br>Bacth No: Lot No. G4516 | <u>Slight to moderate splenomegaly:</u><br>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 | (Davis and Fitzhugh, 1962) |

| Method | Results/Observations (regarding STOT RE) | Reference (Guidance Value for STOT RE*) |
|---|---|---|
| Purity: 86 %<br><br>Non-guideline study (publication)<br><br>**Not assignable** (no full study report available)<br><br>Rat, Osborne-Mendel (N= 25/sex/dose)<br><br>Route: oral, diet<br><br>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)<br><br>From week 103 on, survivors were sacrificed and autopsied;<br><br>Organ weights: Heart, liver, spleen, kidneys, and testes;<br><br>Viscera, pituitary, gross lesions, and one hind leg from each rat were fixed for pathologic study;<br><br>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 %) | weight ratio in the 0.25 % and 1.0 % dose groups;<br><br>Splenic infarcts, scars, haemosiderosis, or cysts in high dose group (1 %)<br><br><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)<br><br>Bone marrow of 0.25 and 1.0 % dose groups was slightly hyperplastic compared to controls<br><br>Significantly less chronic nephritis in the 0.25 and 1.0 % dose groups<br><br>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) | (publication)<br><br>GV STOT RE 2<br>≤ 12.6 mg/kg bw/d<br>(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*