FOREWORD INTRODUCTION

BARIUM BIS[2-CHLORO-5-(HYDROXY-1-NAPHTHYL)AZOTOLUENE-4–SULPHONATE
CAS N°: 5160-02-1

COVER PAGE

SIDS Initial Assessment Report
for
9th SIAM

(Paris, 29 June – 1st July 1999)

Chemical Name: Pigment Red 53:1

CAS No.: 5160-02-1

Sponsor Country: Germany

National SIDS Contact Point in Sponsor Country:

Mr. Jan Ahlers

HISTORY:

SIDS Dossier and Testing Plan were reviewed at the SIDS Review Meeting in September 1993 where the following SIDS Testing Plan was agreed.

no testing	( )
testing	(X)

Short-term toxicity test to algae was demanded. At the SIAM 5 in October 1996 it was decided that the SIAR can be prepared without conduction of an algae test.

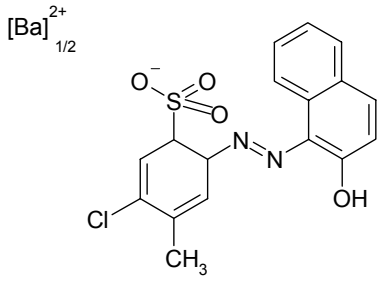
SIAR was discussed at SIAM 6 & 8. It was decided that the report will be redrafted and resubmitted to a future SIAM.

COMMENTS:

Deadline for circulation: 31 March 1999

Date of circulation: 31 March 1999
(To all National SIDS Contact Points and the OECD Secretariat)

SIDS INITIAL ASSESSMENT PROFILE

CAS Nr.	5160-02-1
Chemical Name	barium bis[2-chloro-5-[(hydroxy-1-naphthyl)azo]toluene-4-sulphonate]
Structural formula	

RECOMMENDATION OF THE SPONSOR COUNTRY

The chemical is currently of low priority for further work

SHORT SUMMARY WHICH SUPPORTS THE REASONS FOR THE RECOMMENDATIONS

Exposure

The production volume of pigment red 53:1 in 1991 was 1500 t in Germany. About 2/3 of this amount were exported. Additionally, 250 t were imported in the same year.
Pigment red 53:1 is used particularly for short-life printed matter, for colouring plastics, PVC, polyurethane foam, natural rubber stocks and in paints. Releases into the environment may mainly occur during production, formulation and paper recycling. Pigment red 53:1 is classified as "non biodegradable". In short-term tests with fish and daphnia no effect was found in concentrations up to the water solubility of 2 mg/l. As no acute effects were found no PNECaqua was derived.

Environment

The highest PEC_{local} of 41.5 µg/l was calculated resulting from paper recycling using a realistic worst case scenario. On the other hand a pigment red concentration of 3.4 µg/l was measured in one waste water sample from a German deinking plant, resulting in a PEC_{local} of 0.11 µg/l. As the water solubility of pigment red is about a factor of 50 to 10 000 higher than the estimated PECs for the scenario paper recycling it can be concluded that pigment red represents with high probability a low potential risk to the aquatic environment.

Human Health

After single oral administration pigment red 53:1 can be designated to be of low toxicity. It does not irritate the skin and eyes and is not a sensitizer. Repeated administration of high concentrations causes changes in haematological parameters as well as having an effect on spleen, liver and kidneys. A NOEL of 25 mg/kg bw was derived from a 2 year feeding study with rats. Pigment red 53:1 proved to be non-genotoxic in various in-vitro and in-vivo studies. Several long-term toxicity and carcinogenicity studies in mice and rats revealed no carcinogenicity. However, in one study high dosed male Fisher rats developed fibrosarkomas of the spleen. Since pigment red 53:1 is not genotoxic, the carcinogenic findings are considered to be a consequence of tissue damage. Studies of reproduction toxicity gave no indication of impairment of fertility. With regards to occupational exposure, no workplace measurements are available. However, based on the following theoretical worst case scenario (total dust 1mg/m^3; volume inhaled 0.8 m^3 per hour; 70% respirable; 70% sytemically available; 8 hour shift), the systemic burden of a worker would hypothetically result in 0.065 mg/kg body weight per day. In relation to the NOELs for repeated exposure of rats and mice (see 4.2b) safety margins of 385 up to 1385 are calculated. Based hereupon, no significant health risk is seen for workers.
Based on all available data pigment red 53:1 is considered to be of low potential risk for human health.

IF FURTHER WORK IS RECOMMENDED, SUMMARISE ITS NATURE

No further work is necessary

SIDS INITIAL ASSESSMENT REPORT

1. Identity

Name:	barium bis[2-chloro-5-[(hydroxy-1-naphthyl)azo]toluene-4-sulphonate]
Synonymes:	C.I. Pigment Red 53:1 D and C Red No. 9
CAS No.:	5160-02-1
Empirical Formula:	$C_{17}H_{12}ClN_2O_4S.1/2Ba$
Purity of industrial product:	ca. 99 % w/w
Major impurities	none
Water solubility:	2 mg/l
log Kow (calculated)	-0.56
pKa (calculated)	-5.49 (sulfonate group) 8.96 (phenolic group)

2. General Information on Exposure

In Germany pigment red 53:1 is only produced by one company. In 1991 the production amount was 1500 t. About 2/3 of this amount were exported. Additionally, 250 t were imported in the same year [1]. Therefore, the total amount available in Germany in 1991 was < 750 t. There is no information on other production sites.

Production of pigment red 53:1 is conducted in batch process in an open system in aqueous medium by diazotization of 2-amino-5-chloro-4-methyl benzene sulphonic acid (CLT acid) with sodium nitrite solution and hydrochloric acid. The thus produced diazonium compound is converted with ###-naphthol in alkaline solution to the azo compound, which, following the addition of aqueous barium chloride solution, is precipitated as the barium salt with low water solubility.

Pigment red 53:1 is used particularly for short-life printed matter, e.g. sheet-roller offset, intalgio and flexographic printing, for colouring plastics, PVC, polyurethane foam and natural rubber stock and in paints. In addition, smaller amounts are also used in low-requirement products, such as crayons and water colours for the office supplies industry [2].

The following use pattern is available for Germany, assuming that the imported pigment red 53:1 was put to the same use as that produced and sold in Germany [1]:

ca. 80 % in printing inks
ca. 12 % as dye in plastics

ca. 1 % in enamel paints
ca. 7 % special areas of use, e.g. office supplies

The use of pigment red 53:1 as a colouring in cosmetics has been banned in the USA since 1986 and is no longer permitted in the EU, while in Japan, when appropriately labelled as a barium salt, it is allowed in every kind of cosmetics. The banning in the USA and the EU was based on the development of fibrosarcomas after prolonged injury of the spleen in one out of several long-term feeding studies in rats. However, because this was regarded to be an indirect effect, no legal classification of pigment red 53:1 as a carcinogen has resulted in the EU.

Releases into the environment may mainly occur during production, formulation and paper recycling. The manufacturer estimated releases into the atmosphere of about 560 kg/a and into the waste water of about 12 t/a during production.

Releases into the geosphere through disposed refuses are possible, but not quantifyable. Further releases may occur through the deposition of pigment dust and through sludge application.

3. Environment

3.1 Environmental Exposure

3.1.1 General Discussion

The water solubility of pigment red was determined to 2 mg/l. The test substance was first stirred for 7 h at 80 °C and subsequently for16 h, 40 h and 64 h at room temperature. After filtration (0.05 µm) the dissolved pigment red was measured spectroscopically. It could be shown, that equilibrium was reached after 40 h. The other values for the water solubility given in the IUCLID data set could not be validated.

The log Kow cannot be estimated properly as no reliable computerized method is available to calculate the Kow for ionic organic substances. However, it is possible to calculate the Kow for the free acid and correct afterwards for the sulfonate or carboxylate anion according to Leo and Hansch (1995). With this method a log Kow of - 0.56 could be estimated. Another method for a rough estimation of the log Kow is to calculate the ratio of the chemicals solubility in water and in n-octanol. With this approach a log Kow of - 0.26 could be estimated.

Pigment red is a solid at room temperature and is melting under decomposition at 330 °C. The vapour pressure at room temperature is therefore very low and is therefore not relevant for the risk assessment.

Pigment red is described as dye of low quality and therefore its stability under environmental conditions is probably low, but no data are available.

Pigment red is not biodegradable. In a MITI-I test no biodegradation was observed within 14 days. A Zahn-Wellens test on inherent biodegradation employing a dispersion of pigment red and conducted with adapted inoculum achieved an elimination of 33 % after 21 days. About 10 % of the elimination was due to adsorption onto the sludge.

In a laboratory experiment the elimination of pigment red through precipitation and flocculation with iron sulfate was examined. 200 mg/l pigment red were stirred for 20 minutes with 1 g/l $FeSO_4$

and were then filtered through a folded filter. An elimination of 85 % was achieved leading to a pigment red concentration of 30 mg/l which is still far above the water solubility of the substance. Due to the lack of data this test cannot be validated.
In a bioaccumulation study conducted with *Oryzias latipes* in a flow-through system over a period of 6 weeks BCF of 0.9 - 1.8 and of 8.5 - 15 have been found with pigment red concentrations of 0.7 mg/l resp. 0.07 mg/l. This indicated a low potential for bioaccumulation.

Using the above cited data for the physico-chemical properties and an estimated value for the vapour pressure of 1 $\cdot 10^{-5}$ Pa it can be estimated that the hydrosphere is the target compartment for pigment red (Mackay I: 99.9%). However, it has to be kept in mind, that due to the ionic character the environmental distribution of pigment red cannot be estimated properly.

3.1.2 Predicted Environmental Concentration

3.1.2.1 Aquatic compartment

A total production volume of 1500 t/a is used for the calculation of the local PECs. In the wwtp an elimination of 10 % due to adsorption as observed in the Zahn-Wellens test is considered. Although it can be assumed that a certain degree of the substance is eliminated by flocculation this is not considered for the calculation of the PEClocal as it is not possible to transfer the result of the above cited laboratory flocculation test to the real sewage treatment plant.

In a generic (i.e. non site specific) exposure assessment the releases from production can be estimated according to appendix 1 of chapter 3 of the EU Technical Guidance Document [3], where a release fraction of 0.3 % is proposed for production (IC 14).

Using the available specific data for the German production site, in addition a more precise estimation can be performed.

Input data	generic scenario	site specific scenario
releases	0.3 % = 4.5 t/a	12 t/a
elimination	10 % by adsorption	10 % by adsorption
flow rate of receiving river	60 m^3/sec	70.5 m^3/sec
duration of emission	table B 1.2 = 180 d	300 d
PEC_{local}	4.3 µg/l	5.9 µg/l

Both local exposure concentrations are in the same order of magnitude. However, as the site specific calculation is based on more realistic data, this will be used for risk characterisation.

According to information provided by the German printing-inks association, for the formulation step no process water occurs. Therefore, releases into surface water may only result from the cleaning of the vessels and other facilities. However, about 90 % of the printing-inks are solvent based and the cleaning is normally performed with solvents that can be recycled after use. Only after formulation of aqueous based printing-inks water or alkaline solution is used for cleaning and (usually after one-site treatment) is released into waste-water treatment plants.

As pigment red is reported to be formulated at 50 different sites in Germany and only for about 10 % of the total amount of 750 t/a contaminated waste water from the cleaning of the facilities is

expected, the calculation of a local PEC for the hydrosphere seems not necessary for the lifecycle-step of formulation.

The main application area for pigment red are printing inks for short-life printed matter. Therefore from use, the main releases are expected during the paper recycling process. In a worst case exposure scenario it is assumed, that 87 % of the total volume of 750 t/a (80 % printing inks and 7 % office supplies), i.e. 650 t/a are possibly available for the paper recycling process. In chapter 7 of the EU Technical Guidance Document [3] an emission scenario document (IC 12) is proposed to estimate the releases into surface water from the de-inking process of paper recycling. In addition, site-specific information from a research report reflecting wastewater discharges of several industrial and manufactural sectors in Germany are considered [5]. The following input parameters are used for the calculation:

rate of paper recycling:	50 % (IC 12)
de-inking rate:	90 % (IC 12)
amount absorbed to de-inking sludge:	90% (IC 12)
amount absorbed to sewage sludge:	10% (Zahn-Wellens test)
number of recycling sites:	20 (specific data for Gemany)
number of working days per year:	320 d/a (research report)
flow rate of wwtp	3300 m^3/d (10%ile, research report
dilution factor in surface water:	30 (10%ile, research report)

A PEC_{local} of 41.5 µg/l is estimated.

In addition, pigment red was analysed in a sample of a waste water treatment plant effluent from a German deinking plant. The arithmetic mean of 3 runs was given as 3.4 µg/l [6]. Using a dilution factor of 30 a PEC_{local} of 0.11 µg/l is resulting. However, it has to be kept in mind that no statement is possible about the representativity of the sample.

Another application area is the use of pigment red as colouring agent for plastics. 12 % of a total volume of 750 t/a, i.e. 90 t/a are used in this sector in Germany. With the assumption that in a region 50 %, i.e. 45 t/a are used as colouring agent for plastics and using an emission factor for waste water of 0.0005 (TGD, Table A 3.11) releases of pigment red into the hydrosphere can be neglected. Therefore, no emission scenario is calculated for this application area.

Migration of pigment red out of plastics can be neglected [4].

3.1.2.2 Terrestrial compartment

Direct releases to the soil are not expected. At production, about 560 kg pigment dust are emitted into the atmosphere. It is assumed, that deposition of the dust will occur in the direct vicinity of the point source (on site) and therefore will not be of relevance for the environment.

An input through sludge application is only expected from municipal sewage sludge and therefore calculated for the paper recycling scenario. According to the models and input parameters proposed in the EU Technical Guidance Document [3] (10 years of sludge application, 30 days after the last application), the calculated sludge concentration of 0.6 g/kg results in a local soil concentration of 0.89 mg/kg.

The corresponding pore-water concentration is estimated to 4.7 mg/l However, due to the ionic character of the substance these data have only indicative character and cannot be used for a risk assessment for the terrestrial compartment.

3.1.2.3 Regional exposure

A regional exposure assessment seems not opportune for pigment red as the distribution behaviour cannot be estimated properly due to the ionic character of the substance .

3.2 Effects on the Environment

3.2.1 Aquatic effects

Short-term ecotoxicity tests have been conducted with two fish species (*Brachydanio rerio, Oryzias latipes)* and with the crustacean *Daphnia magna*. The following test results have been obtained:

Brachydanio rerio 96 h-LC_{50} > 500 mg/l
(static system, nominal concentration, test substance: Flexonyl-Red A-LCLL, dispersion)

Oryzias latipes 48 h-LC_{50} > 420 mg/l
(semistatic system, nominal concentration)

Daphnia magna 48 h-EC_0 > 2 mg/l
(test has been conducted with the saturated solution)

Although in the SIDS testing plan an acute algae test was demanded, the result from such a test may not help on the effects assessment. Moreover, the conduction of an algae test with pigment red is problematic as the substance leads to a strong colouring of the test solution and therefore to a reduction of light intensity. Therefore, the assessment is made based on the above cited short-term tests.

In none of the tests an effect has been found. As the tested concentrations in the fish-tests and in the test with *Daphnia magna* were much higher than resp. in the same order of magnitude with the water solubility, no statement about the ecotoxicity of pigment red is possible. It can only be concluded that at concentrations up to the water solubility no acute effects could be observed.

As a conclusion no PNECaqua is determined and only a qualitative risk assessment is conducted.

3.2.2 Terrestrial Effects

Tests with terrestrial organisms are not available.

3.3 Initial Assessment for the Environment

The following local PECs were calculated:

production	paper recycling (realistic worst case calculation)	paper recycling (measured value)
5.9 µg/l	41.5 µg/l	0.11 µg/l

As no PNEC was derived only a qualitative risk assessment is possible. From the reported ecotoxicological tests it can be concluded that at concentrations up to the water solubility no acute effects occur.

For the scenario production of pigment red it can be concluded that no risk for the aquatic compartment exists as the water solubility of pigment red (2 mg/l) is about a factor 500 higher than the estimated PEClocal.
For the scenario paper recycling two PECs were calculated that differ by about two orders of magnitude. The possible reasons for such large discrepancies have to be discussed for a risk estimation of pigment red due to this life-cycle step. On the one hand there is no statement possible about the representativity of the measured value as only one sample from one site was anaylzed. Therefore this measured concentration can only be used as indicative value. On the other hand a PEClocal was calculated on the basis of a generic, realistic worst-case scenario. In this scenario default values from the TGD as well as site-specific information from a research report reflecting wastewater discharges of several industrial and manufactural sectors in Germany are considered. No possible elimination due to flocculation is considered. Therefore, the statement can be made that the two derived PECs form the range of possible PECs and the „real“ PEC must be somewhere between both values. As the water solubility of pigment red is about a factor of 50 higher than the PEC based on the generic scanario and about a factor of 10 000 higher than the PEC based on the measured value it can be stated that the release of pigment red during paper recycling presents with high probability no risk for the aquatic compartment.

No risk assessment is conducted for the terrestrial compartment due to missing toxicity data with soil organisms and the questionable soil pore-water concentration values.

4. Human Health

4.1 Human Exposure

4.1.1 Occupational Exposure

At the production site occupational exposure due to inhalation and dermal exposure is largely avoided by the use of air extractors and the wearing of protective gloves. No further information concerning occupational exposure is available.

4.1.2 Consumer Exposure

The use of pigment red 53:1 as a colouring in cosmetics has been banned in the USA and is no longer permitted in the EU, while in Japan, when appropriately labelled as a barium salt, it is allowed in every kind of cosmetics. No further information is available. Other consumer exposure i.e through the use of pigment coloured materials is not expected to be of relevance.

4.2 Effects on Human Health

a) Acute Toxicity

Animal Data:

After single oral administration of pigment red 53:1 to rats and mice the compound can be considered to be of low toxicity. The LD50-values determined for both species were > 10000 mg/kg bw.

Pigment red 53:1 does not irritate the skin and eyes in respective tests with rabbits and does not show evidence of a sensitizing effect in the modified Maximization Test with guinea pigs. In earlier investigations it has been reported that D & C Red No. 9 (pigment red 53:1) is a contact sensitizer (Sugai et al. 1977). In order to determine whether the contact sensitivity is due to the color itself or the impurities contained therein, impurities were identified through the analysis of D & C Red No. 9. Analysis revealed the presence of eleven aromatic azo compounds including Sudan I (1-phenylazo-2-naphthol) which has been reported as a strong sensitizer. The modified guinea pig maximisation test showed that these impurities were contact sensitizers, while the colour itself was not. As a result of the investigation, a manufacturing process to produce the pigment free from contact sensitizers was developed (Naganuma et al. 1983).

Human Experience: no data available

b) Repeated Dose Toxicity

Animal Data:
After repeated oral administration for 90 days in rats pigment red 53:1 led in high dosages (at 3000 ppm and above) to haematological findings (depressed haemoglobin and haematocrit values) and effects on spleen (splenomegaly, haemosiderosis, fibrosis), liver and kidneys (heamosiderosis). Daily administration of pigment red 53:1 for 90 days in mice led to comparable findings. The NOEL for mice was determined as 90 mg/kg bw/day. A 20-week subacute feeding study using 5 male and 5 female weanling Osborne Mendel rats per level and levels of 2 %, 1 %, 0.5 %, 0.25 % and 0 % of D & C Red No. 9 (pigment red 53:1) in the diet produced no mortality but resulted in lowered average haemoglobin and haematocrit values. At autopsy splenomegaly was noted in rats on all substance test levels, and liver enlargement was noted at the 1 % and 0.5 % color-feeding levels. 5 groups of 50 3-week old Osborne-Mendle rats were started on a two-year feeding experiment on D & C Red No. 9 at dose levels of 1 %, 0.25 %, 0.05 %, 0.01 % and 0 % (controls). The test substance had no apparent effect on the growth rate, mortality or occurence of tumours in the test rats. Haemoglobin levels were slightly lowered and abnormal shape of red blood cells were observed in rats on the 1 % and 0.25 % feeding levels (no further information given). At autopsy, survivors on the 1 % feeding level showed moderate splenomegaly and rats on the 0.25 % level showed slight splenomegaly. Histopathologic findings attributable to the color feeding consisted of moderate splenomegaly at 1 %, slight splenomegaly at 0.25 %, and slight bone marrow hyperplasia at both levels. The 1 % feeding level rats also showed slightly increased splenic haemosiderosis and some had splenic infarcts. At 0.05 % and 0.01 % there were no gross or microscopic pathologic changes attributable to D & C Red No. 9 (pigment red 53:1). The No Observed Effect Level (NOEL) was determined as 25 mg/kg bw/day (0.05 % color in the diet).

Human Experience: no data available

c) Carcinogenicity (see also 4.2 b)

Animal data:
100 ICR (Swiss Webster derived) mice - 50 males and 50 females - received pigment red 53:1 two times per week for 18 months at the shaven back to an area of approximately 6 cm^2. Dosage levels were based on lipstick use determinations made in a group of human female volunteers. Twice each

week a 0.1 ml dose containing 1 mg of the dye was applied to the dorsal of each mouse with an automatic syringe and uniformly distributed on the exposed skin with a rubber applicator. Animals that died, those sacrificed moribund and those surviving the 18 months experimental period were necropsied. After termination of the study, tissues were selected for histopathology, sectioned, stained with hematoxylin and eosin and examined by a pathologist. The repeated application of 0.1 ml containing 1 % dye did not increase the incidence of neoplasms when compared to the vehicle controls.

Groups of 50 B6C3F1 mice received diets containing 1,000 or 2,000 ppm of test substance for 103 weeks. Groups of 50 mice of either sex served as controls. Mean body weights of male mice were comparable with those of controls. After week 50, the mean body weight of high-dose female mice was lower than that of the controls. No compound-related effect on survival or clinical signs were observed for mice of either sex. Under the conditions of this bioassay, D & C Red No. 9 (pigment red 53:1) was not carcinogenic for B6C3F1 mice of either sex.

Fisher 344 rats received 0, 50 or 150 mg pigment red 53:1/kg bw (0, 1000 or 3000 ppm) for 103 weeks with the daily feed. In males and females of the 150 mg group non-neoplastic damage to the spleen (congestion, focal and diffuse fibrosis) occured. Males of this group showed fatty changes to the spleen, which correlated with the appearance of fibrosarkomas in this organ. This treatment related effect, in the NTP bioassay report under spleen lesions, splenic capsule and splenic red pulp tabulated, is believed a consequence of prolonged splenic injury (congestion and fibrosis). There were no splenic sarcomas in low dose males or any of the female groups. It is interesting to note that in another study of FDA the significance of the formation of splenic sarcomas has been analysed. It was found that groups without splenic changes, i.e. fatty metamorphosis lesions, did not exhibit splenic tumours. There were small increases in „neoplastic nodules“ of the liver in male rats. The only malignant liver tumour occured in a control rat. It is interesting to note that the incidence of some tumours, lymphomas, leukaemias and preputial gland tumours were decreased in treated groups.

Osborne-Mendel rats received 0, 5, 25, 125 or 500 mg pigment red 53:1/kg bw (0, 100, 500, 2500 or 10000 ppm) with the daily feed for two years. Growth and mortality was unaffected. In the two highest dosage groups depressed haemoglobin levels, abnormal shape of erythrocytes (no further information given) and slight hyperplasia of bone marrow occurred. In the highest dosage group moderately - strong splenomegaly as well as haemosiderosis and partial infarction of the spleen was seen. Compared to the untreated control animals there was no increase in tumour incidence.

In a 30 months chronic toxicity and potential carcinogenicity study CD rats were subjected to in utero treatment to a high dose (10000 ppm, corresponding to ca. 500 mg/kg bw/day) of pigment red 53:1. An increased incidence of spleen tumours and phaeochromocytomas were observed. The significance of these findings is according to the opinion of the authors on account of the relatively high spontaneous tumour incidence in older CD rats questionable. In addition, treated animals showed findings in haematological parameters (reduction in red cell count, cell volume and haemoglobin content, increase of the numbers of reticulocytes), significant increase in the weight of spleen and toxic effects to the spleen (congestion, fibrosis, haemosiderosis, multifocal cell proliferation and extramedullary haematopoiesis) as well as haemosiderin deposits in a number of other organs.

Human Experience: no data available

Conclusion:

A well conducted NTP bioassay of D & C Red No. 9 (pigment red 53:1) in groups of 50 male and female F344 rats and B6C3F1 mice, at dose levels of 0, 1,000 and 3,000 ppm (rats) and 0, 1,000 and 2,000 ppm (mice) for 103 weeks showed no effect on survival and body weight effect was seen in female mice only.

There were no findings of significance in mice of either sex. In rats there was an increased incidence of splenic sarcoma (mainly fibrosarcoma) in high dose males only. These are tabulated under spleen lesions, splenic capsule and splenic red pulp. This treatment related effect is a consequence of prolonged splenic injury (congestion and fibrosis). There were no splenic sarcomas in low dose males or any of the female groups. There were small increases in neoplastic nodules of the liver in male rats. The only malignant liver tumour occurred in a control rat. It is to note that the incidence of some tumours, lymphomas, leukaemias and preputial gland tumours was decreased in treated groups.

In a longterm 2-year feeding study with Osborne-Mendel rats there was no increase in tumour incidence up to the highest feeding level of 500 mg pigment red 53:1 per kg body weight (10,000 ppm).

Charles river rats (CD strain) with in utero and lifetime exposure to D & C Red No. 9 (pigment red 53:1) in the diet reveals a small number of highly unusual mesenchymal neoplasms of the spleen. The increased incidence of these tumours was not statistically significant in the dosed animals in this study; however, due to their highly unusual nature and the possibility of tumour origin in nonneoplastic fibrosis it is highly likely that these tumours were compound induced.

ICR mice with demal exposure for 18 months showed no increased evidence of neoplasms.

In summary long-term carcinogenicity studies with Osborne Mendel rats and B6C3F1 mice gave no indication of a carcinogenic effect of pigment red 53:1. In the NTP bioassay with fisher F344 rats there was an increased incidence of splenic sarcomas only in one sex (male) and only in the highest dosage group. The development of these tumours as well as the findings with Charles River rats after in-utero and lifetime exposure can probably therefore be attributed to a toxic effect of the substance. Regarding this effect the examiners comment: „The serious non-neoplastic lesions of the spleen in the (male) rats of the highest dosage group suggest a connection between the toxicity of the administered substance and the formation of splenial neoplasms.“ A statistically significant incidence of splenic sarcoma (0/50, 0/50, 26/48, $P > 0.001$) in male rats fed with high dose levels of pigment red 53:1 is concluded to occur only above a biological threshold level at which the spleen is damaged. Provided low levels of exposure to pigment red 53:1 are maintained, potential risk resulting from use of the pigment is considered to be insignificant.

Recommendation: no need for follow-up test

Priority setting: low priority or concern

d) Reproductive Toxicity

Animal data:
The purpose of a 30-months chronic toxicity and potential carcinogenicity study in rats with in-utero and lifetime exposure to D & C Red No. 9 (pigment red 53:1) via its incorporation into the basal diets at doses of 0 and 10,000 ppm also was to evaluate the reproductive performance of the F0 generation. Rats of the charles river CD strain were 35 days of age when treatment was initiated.

After nine weeks of treatment, the animals were mated by pairing for seven days. The effect of test material for the in-utero phase was evaluated via mortality, clinical observations, body weight, food consumption, sex ratio, pup viability data and gross necropsy observations on selected animals.

Compound consumption was judged to cause orange discoloration of the animals and their feces and an enlargement of their spleens during the in-utero and chronic phases. The chronic phase revealed non-neoplastic compound related effects which included a significant decrease in the red blood cell parameters (red blood cell count, packed cell volume and haemoglobin percent) and an increase in the reticulocyte count observed after 3, 6, 12, 18 and 24 months of treatment. Compound consumption was judged to be associated with a significant increase in spleen weight, and the following non-neoplastic lesions of the spleen; extramedullary haematopoiesis, congestion, fibrosis, haemosiderosis, mesothelial hyperplasia, mesothelial cystformation, capsular fibrosis and multi-focla cellular proliferations in the capsule. The accumulation of haemsiderin in some other organs of the treated rats also suggest a compound-related effect. The combination of decreased red cell parameters, reticulocytosis and haemosiderosis supports the hypothesis that there was a compound-related decreased erythrocyte survival and a haematopoietic response to that decreased red cell survival.

The fertility and duration of gestation were as follows:

	Control	**10,000 ppm (D & C Red No. 9)**
Percent pair fertility (Litters/pair)	85 % (51/60)	92 % (55/60)
Days of gestation	22	22

Pup viability:

Indices (percent)	**Control**	**10,000 ppm (D & C Red No. 9)**
Gestation viability (Live Pups/Total Pups Day 0)	99 (666/672)	99 (706/713)
Neonate viability (Day 4/Day 0)	98 (654/666)	97 (685/706)
Late lactation viability (Day 21/Day 14)	99 (576/579)	100 (649/651)
Overall Viability (Day 21/Day 0)	86 (576/666)	92 (649/706)

Human Experience: no data available

Conclusion:
There was no evidence for an impairment of reproductive functions in animals

Recommendations: no need for follow-up tests

Priority setting: low priority or concern

e) Genetic Toxicity

Experimental data:

Pigment red 53:1 has been tested for genotoxicity in a series of Ames tests with and without metabolic activation including the Prival test, in the Cytogenetic assay with V 79 cells and with ovary cells of Chinese hamsters (CHO), in the Mouse lymphoma assay, in the Sister chromatid exchange assay with ovary cells of Chinese hamsters (CHO), the Unscheduled DNA synthesis in rat hepatocytes and the Cell transformation (Styles) test. In all these in-vitro studies pigment red 53:1 gave negative results, except in the cell transformation test with BHK21 cells. However, according to the authors the test/method is unreliable due to difficulties in obtaining reproducible results with this method as well as problems involved with conducting the test with insoluble or only slightly toxic dyes.

D & C Red No. 9 (pigment red 53:1) was assayed for genotoxicity in vivo using the rat micronucleus test, different rat ex vivo liver UDS assays and the SLRL-test in Drosophila. Uniformly negative results were obtained in all assays, even though large oral doses were used (up to 2 g/kg)

Human Experience: no data available

Conclusion: In various in-vitro and in-vivo studies pigment red 53:1 proved to be non-genotoxic. The results suggest that the tumorigenic effects of this compound in rats are mediated through a non-genotoxic rather than a genotoxic mechanism.

Recommendations: no need for follow-up test

Priority setting: low priority or concern

4.3 Initial Assessment for Human Health

After single oral administration pigment red 53:1 can be considered to be of low toxicity. It does not irritate the skin and eyes and is not a sensitizer.

Repeated administration of high concentrations causes changes in haematological parameters as well as having an effect on spleen, liver and kidneys.

Pigment red 53:1 proved to be non-genotoxic in various in-vitro and in-vivo studies.

Several long-term toxicity and carcinogenicity studies in mice and rats revealed no carcinogenicity, except in high dosed male Fisher rats. Since pigment red 53:1 is not genotoxic, these findings are considered to be a consequence of prolonged splenic injury. With regard to occupational exposure no workplace measurements are available. However, based on the following theoretical worst case scenario (total dust 1mg/m^3; volume inhaled 0.8 m^3 per hour; 70% respirable; 70% sytemically available; 8 hour shift), the systemic burden of a worker would hypothetically result in 0.065 mg/kg body weight per day. In relation to the NOEL`s for repeated exposure of rats and mice (see 4.2b) safety margins of 385 up to 1385 are calculated. Based hereupon, no significant health risk is seen for workers.

Based on all available data, the substance is considered to be of low potential risk for human health.

5. Conclusions and Recommendations

5.1 Conclusions

The assessment showed that pigment red 53:1 represents with high probability no risk to the aquatic compartment.

Based on the values for human toxicity, there is no need for further studies nor for suggestions for other measures in this field.

5.2 Recommendations

No need for further work.

6. References (not cited in the SIDS)

1: BUA report 124: Pigment Red 53:1; 1994; ISBN: 3-7776-0569-7

2: Herbst W., Hunger K. (1993): Industrial Organic Pigments. VCH, Weinheim, 316-323

3: Technical Guidance Documents in Support of the Commission Directive 93/67/EEC on the Risk Assessment for New Notified Substances and the Commission Regulation (EC) 1488/94 on Risk Assessment for Existing Substances

4.: Clariant 1997

5.: UBA 1997: „Investigation of wastewater discharges: statistical data and analysis of important industrial and manufacturing sectors with the purpose of risk assessment of substances“, UBA-FB 206 04 144/01

6.: Clariant 1998: Determination of pigment red 53:1 in waste water of a deinking plant

Full SIDS Summary
Pigment Red 53:1

CAS-NO.: 5160-02-1			PROTOCOL	RESULTS
PHYSICAL CHEMICAL				
2.1	Melting Point		NA	330°C (decomposition)
2.5	Partition Coefficient (Log Pow)		calculated	-0.56
2.6 A	Water solubility			2mg/l at 20°C
	pKa		calculated	-5.49 (sulfonate group) 8.96 (phenolic group)
ENVIRONMENTAL FATE / BIODEGRADATION				
3.1.1	Photodegradation		calc. (Atkinson)	calculation not possible
3.5	Biodegradation		OECD 301 C	not readily biodegradable
			DIN 38412	not biodegradable
ECOTOXICOLOGY				
4.1	acute/prolonged toxicity to fish	*Brachydanio rerio*	84 / 449 / EEC, C.1	LC_{50} (96 hr) > 500mg/l
4.2	acute/prolonged toxicity to aquatic invertebrates (daphnia)	*Daphnia magna*	OECD 202	EC_0 (48 hr) = >2 mg/l

CAS-NO.:5160-02-1		SPECIES	PROTOCOL	RESULTS
TOXICOLOGY				
5.1.1	acute oral toxicity	rat	Hoechst internal	LD_{50} > 10000mg/kg
		mouse	Hoechst internal	LD_{50} > 10000mg/kg
5.4	repeated dose toxicity	mouse	NTP	NOEL = 90 mg/kg
		rat	NA	NOEL = 25 mg/kg
5.5	genetic toxicity in vitro			
	bacterial test (gen mutation) ± S9-Mix including Prival-modification	Salmonella typhimurium TA 98, 100, 1535, 1537	OECD 471	Not mutagenic
	non-bacterial in vitro test (chromosomal abberations) ± metabolic activation	hamster CHO	OECD 473	Not mutagenic
		V 79 cells	OECD 473	Not mutagenic
		mouse	lymphoma assay	Negative
		hamster CHO	SCE	Not mutagenic
		rat hepatocyte	UDS	negative
5.6	genetic toxicity in vivo	rat	micronucleus	negative
		rat	UDS	negative
5.8	toxicity to reproduction	rat	NA	NOEL < 10000 ppm (general toxicity) NOEL > 10000 ppm (rep. tox. parental) NOEL > 10000 ppm (rep. tox. F1)
5.9	developmental toxicity / teratogenicity			
5.10	Carcinogenicity	Rat	Up to 30 month	Effects at organ (spleen) toxic dose
		Mice	Up to 2 years	No carcinogenic effects
5.11	experience with human exposure			

EXTRACT FROM IRPTC LEGAL FILES

```
file: 17.01 LEGAL   rn : 1470872
         !!! WARNING - not original IRPTC record - WARNING !!!
systematic name:Benzenesulfonic acid,
                5-chloro-2-[(2-hydroxy-1-naphthalenyl)azo]-4-methyl-,
                barium salt (2:1)
common name    :D and C Red No. 9
reported name  :Barium
                bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulp
                honate]
cas no         :5160-02-1           rtecs no       :DB5500000
area          : EEC                 type          : REG
 --------------------------------
|subject|specification|descriptor|
|-------+-------------+----------|
| MANUF |    INDST    |   CLASS  |
| IMPRT |    INDST    |   CLASS  |
 --------------------------------
The substance is included in a list of existing substances produced or
imported within the Community in quantities exceeding 1000 tonnes per
year. - A system of data reporting by any manufacturer who has produced
or any importer who has imported the substance, as such or in a
preparation, in quanities exceeding 10 tonnes per year is established.
entry date: AUG 1999                          effective date: 04JUN1993

title: Council Regulation (EEC) No 793/93 of 23 March 1993 on the
evaluation and control of the risks of existing substances
original : OJECFC, Official Journal of the European Communities, L84 , ,
           1 , 1993
```