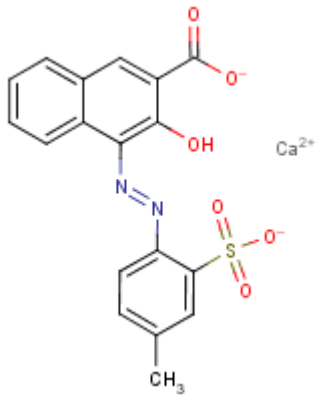
LITHOL RUBINE BK

SYNONYMS

2-Naphthalenecarboxylic acid, 3-hydroxy-4-((4-methyl-2-sulfophenyl)azo)-, calcium salt;
3-Hydroxy-4-((4-methyl-2-sulfophenyl)azo)-2-naphthalenecarboxylic acid, calcium salt;
CI 15850:1 (Ca salt);
D & C Red no. 7;
Lithol rubin B ca;
2-Naphthalenecarboxylic acid, 3-hydroxy-4-((4-methyl-2-sulfophenyl)azo)-, calcium salt (1:1);
3-Hydroxy-4-((2-sulfo-p-tolyl)azo)-2-naphthalenecarboxylic acid, calcium salt (1:1);
3-Hydroxy-4-((4-methyl-2-sulfophenyl)azo)-2-naphthalenecarboxylic acid, calcium salt;
C.I. Pigment Red 57, calcium salt (1:1);
Brilliant Carmine 6B

CHEMICAL STRUCTURE



CHEMICAL FORMULA

$C_{18}H_{14}N\text{-}O_6S.Ca$

IDENTIFIER DETAILS

CAS Number	:	5281-04-9
CoE Number	:	-
FEMA	:	-
EINECS Number	:	226-109-5
E Number	:	E180

CLP CLASSIFICATION

Ingredient CLP Classification: No

Endpoint	Classification	Category
Acute Oral Toxicity	-	-
Acute Dermal Toxicity	-	-
Acute Inhalation Toxicity	-	-
Skin Corrosive/irritant	-	-
Eye Damage/Irritation	-	-
Respiratory Sensitisation	-	-
Skin Sensitisation	-	-
Mutagenicity/Genotoxicity	-	-
Carcinogenicity	-	-
Reproductive Toxicity	-	-
Specific Target Organ Toxicity	-	-
Aspiration Toxicity	-	-

REACH Statement

This ingredient has been registered under REACH. Under REACH, registrants have an obligation to provide information on substances they manufacture or import. This information includes data on hazardous properties (covering various toxicological endpoints), guidance on safe use and classification and labelling. The European Chemicals Agency (ECHA) makes this information publicly available on its website: http://echa.europa.eu/.

SPECIFICATIONS

Melting Point: 375°C

Boiling point: 791°C

STATUS IN FOOD, TOBACCO AND DRUG LAWS

CoE limits:

Beverages (mg/kg)	Food (mg/kg)	Exceptions (mg/kg)
-	-	-

Acceptable Daily Intake:

ADI (mg/kg)	ADI Set by	Date Set	Comments
None allocated 5 mg/day 0 – 1.5 mg/kg	JECFA FDA SCF	1986 1982 1983	Group ADI = 0.15 mg/kg

FDA Status [CFR 21]:

Section Number	Comments
74.1307	Listing of colour additives subject to certification

HUMAN EXPOSURE

Natural Occurrence: Does not occur in nature.

Reported Uses: Dye is approved as Ca-salt (E180) for food use (treatment of cheese rind) in the EU.

Sources other than foods: Dye (sodium 5858-81-1 and calcium salt 5281-04-9) is approved in the EU for general uses in cosmetics. In the US, both salts are approved for usage in drugs and cosmetics (with the exception of eye area use).

TOXICITY DATA

In Vivo Toxicity Status

Species	Test	Route	Dose
Rat	LD_{50}	Oral	9.8 g/kg bw
Rat	LD_{50}	Oral	5 g/kg bw
Dog	LD_{50}	Oral	9.8 g/kg bw [SCF, 2004].

Male and female Sprague-Dawley rats were orally administered (gavage) with 100, 300 and 1000 mg/kg/day (period of time not specified). Male rats that received 300 mg/kg, or greater, showed significantly decreased levels for serum calcium and phosphorous. Significant decreases in serum potassium and total cholesterol levels and significant increases in chloride were also shown in males that received 1000 mg/kg. No other significant differences in clinical parameters were observed in the dosed male groups. Male rats that received 1000 mg/kg showed a significant increase in relative kidney weights, and females that received 100 or 1000 mg/kg showed decreases in thymus weights in comparison with controls. Histopathological examination revealed dose-dependent lesions (regenerated tubular epithelium) in the kidney of the male rats receiving ≥ 300 mg/kg. There were no histopathological changes in the sexual organs of the female. The NOEL of this chemical for repeated dose toxicity was 300 and 100 mg/kg in the female and male rats, respectively [OECD SIDS, 1994].

Administration of 1 g/kg bw/day of the Lithol Rubine BK calcium salt by stomach tube, 5 days/week over 30 days (22 doses in total), to groups of 20 male and 20 female rats slightly reduced growth (food consumption was unaffected), increased kidney weight and kidney damage was observed microscopically. The effects were reversible over a two week recovery period. There were no effects on the blood or urine, or on the weight or microscopic

appearance of the liver, adrenals and spleen. There was obvious red colouration of the faeces which the author concluded may be indicative that Lithol Rubine BK is not absorbed readily by the gastrointestinal tract [SCF, 2004].

Groups of five male and five female rats were fed diets containing 0.25, 0.5, 1 or 2 % Lithol Rubine BK (calcium salt) (approximately 125, 250, 500 or 1000 mg/kg bw/day, respectively) for 18 weeks. There were no effects on food intake, body weight, blood composition, organ weights or the microscopic appearance of a number tissues [SCF, 2004].

Dogs were administered increasing doses of Lithol Rubine BK (calcium salt) in the diet, over a period of 13 weeks (0.5 % in week 1 – 2; 1.0 % week 2 – 3; 1.5 % week 5 – 10 and 2.0 % week 11 – 13), to one animal per sex. The only effects noted were diarrhoea and vomiting, as the dose increased [SCF, 2004].

Groups of 3 dogs per sex were fed 0.015, 0.1 and 1.0 % (approximately 250 mg/kg bw/day) of Lithol Rubine LK (calcium salt) for 2 years. A NOEL was determined at a dose of 1.0 %, due to no substance related effects on body weight, organ weight and pathology, food consumption, survival rate, blood and clinical chemistry and urinalysis. There was a slight increase in thyroid weights but this was not considered as a pathological effect by the authors [SCF, 2004].

Rats (70 males and 70 females) were fed 0.05, 0.3 or 2 % of pigment red 57 (sodium salt) (approximately 25, 150 or 1000 mg/kg bw/day) for 2 years. These rats were the offspring of rats which had been treated at the same levels for 60 days prior to mating and throughout pregnancy and lactation. Effects included a dose-related growth retardation, particularly in the male rats, and increased mortality was observed in the male rats at the top dose. Blood composition was unaffected and apart from the colour of the urine, no treatment-related effects were noted in urinalysis. The weights and gross appearance of the major organs were unaffected, except at the high dose where male rats showed variations in the organ weights. Microscopic examination of the controls and high dose animals only revealed kidney changes and males and females. The NOAEL was reported to be 150 mg/kg bw/day [SCF, 2004].

Groups of 60 males and 60 female mice were fed 0.05, 1.0 or 5.0 % (approximately 75, 1500 or 7500 mg/kg bw/day) Pigment Red 57 (sodium salt) in the diet for 2 years. There were no changes in food consumption, body weight gain or blood composition. Treated males showed increased mortality, which was statistically significant in the high dose group, and microscopic examination of the control and high dose group only, revealed degenerative kidney changes in the treated males [SCF, 2004].

Carcinogenicity and Mutagenicity

Groups of 60 males and 60 female mice were fed 0.05, 1.0 or 5.0 % (approximately 75, 1500 or 7500 mg/kg bw/day) Pigment Red 57 (sodium salt) in the diet for 2 years. There was an increased incidence of alveolar adenomas in the high dose males [SCF, 2004].

Rats (70 males and 70 females) were fed 0.05, 0.3 or 2 % of pigment red 57 (sodium salt) (approximately 25, 150 or 1000 mg/kg bw/day) for 2 years. These rats were the offspring of rats which had been treated at the same levels for 60 days prior to mating and throughout pregnancy and lactation. There was a slight increase in the incidence of Leydig cell adenomas in the high dose males (6 % as compared to 2 % in controls) [SCF, 2004].

Tumour incidence was not increased in 50 male or 50 female mice given uncovered applications of 1 % aqueous suspension of Pigment Red 57 (calcium salt) (approximately 50 mg/kg bw/application) for 18 months [SCF, 2004].

Pigment red 57 (calcium salt) was not tumourigenic when applied to the skin of male and female mice at 50 mg/kg per application (in a 1 % aqueous solution) twice a week for 18 months [Carson et al., 1984].

Dermal Toxicity

A continuous 21 day covered patch test in ten healthy women with a 50 % mixture of lithol rubine BK in talc, produced a minimal irritant effect [BIBRA, 1993].

Human subjects (25) were given five 48 h covered patch tests with 50 % lithol rubine BK mixed with talc. No skin reactions were seen when the volunteers were challenged, generally after a 10 – 14 day rest period [BIBRA, 1993].

A 1 % aqueous suspension of Lithol Rubine BK (calcium salt) (approximately 50 mg/kg bw/application) was applied to 50 male and 50 female mice twice weekly for 18 months. There was no difference in the survival rate when compared to controls, and there were no clear effects on the gross or microscopic appearance of a range of tissues [SCF, 2004].

A 10 % dilution of Pigment Red 57 (salt not indicated) in propylene glycol was applied to the skin of three New Zealand albino rabbits once a day, 5 days/week for two weeks. The noted grade of irritation was 0 (no irritation) and there was no significant increase in follicular keratosis [SCF, 2004].

A 10 % aqueous solution of pigment red 57 (salt not indicated) was applied to the conjunctival sac of one eye of 6 or more albino rabbits, twice daily, 5 days/week for 4 weeks. The Pigment Red 57 solution did not cause staining of any orbital tissues. One hour after application, an irritation score of 2 was noted, and 24 h after application no irritation was noted [SCF, 2004].

Solutions of Pigment Red 57 (25 µl) [in DMSO or water:acetone (1:1) with olive oil (4:1)] (salt not indicated) was applied to the ears at 0.5, 1.0, 2.0 and 4

% for three consecutive days. On day 5 the mice received an intravenous injection of 250 µl of phosphate buffered saline containing 21.4 µCi of [H^3] methyl thymidine. Five hours later, the mice were sacrificed and the draining auricular lymph node was removed and weighed. And radioactivity determined. There were no changes in clinical signs or mortalities and the weight of the lymph nodes did not increase due to treatment with Pigment Red 57. Pigment Red 57 did not reveal any potential to be a skin sensitiser in the two vehicles [SCF, 2004].

A case is described in which a 22 year old woman developed allergic pigmented lip dermatitis following the use of a lipstick containing Lithol Rubine BCA (D&C Red No. 7). Patch testing was carried out and of the tested substances, only Lithol Rubine BCA showed an allergic reaction [Hayakawa, 1994].

Reproductive and Developmental Toxicity

Lithol Rubine BK (calcium salt) was administered at doses of 5, 16 or 50 mg/kg bw/day by stomach tube on days 6 – 15 pregnancy to 20 female rats. There were no adverse maternal effects or effects on embryo resorptions, foetal weight and viability, litter size or the incidence of foetal malformations or skeletal aberrations [SCF, 2004].

Lithol Rubine B (sodium salt) had no effect on fertility, pregnancy or lactation when administered to groups of 60 male and 60 female rats in the diet at 0.05, 0.3 or 2.0 % (approximately 25, 150 or 1000 mg/kg bw/day) prior to mating and throughout pregnancy and lactation. The offspring (70 males and 70 females) were maintained on the same diet for the following two years. The high dose males showed an acceleration of testicular changes (degeneration of the testicular tubules) from 12 months onwards, which can be a common aging effect in ageing rats. The increased incidence was not of statistical significance [SCF, 2004].

Groups of 10 male and 20 female rats were fed 0.5, 5, 15 or 50 mg/kg bw/day Pigment Red 57 (salt not indicated) in a three generation study. There were no effects on maternal or foetal body weights, number of resorptions or survival of the offspring. There was a reduction in fertility in the second generation at 50 mg/kg bw/day but this was not seen in the third generation at any dose level [SCF, 2004].

Groups of 10 female rabbits were administered 5, 16 or 50 mg/kg bw/day Pigment Red 57 (salt not indicated) by stomach tube on days 6 – 18 of pregnancy. There were no adverse effects on maternal weight gain, number of resorptions, litter size, foetal weight and viability, or the incidence of foetal malformations [SCF, 2004].

The oral toxicity of D&C Red No.7 was studied in rats according to the OECD combined repeated dose and reproductive/developmental toxicity test [OECD TG 422] at doses of 0, 100, 300 and 1000 mg/kg. Mating performance, duration of gestation, pup viability, body weight and sex distribution, and gross

anomalies were determined. No treatment-related adverse effects were detected. The NOEL for reproductive/developmental toxicity of the rats in this study was 1000 mg/kg/day [OECD SIDS, 1994].

Behavioural Data

No data identified

***In Vitro* Toxicity Status**

Carcinogenicity and Mutagenicity

Pigment red 57 (sodium salt) was not mutagenic with or without metabolic activation in the Ames assay (strains TA98, TA100, TA102, TA1535, TA1537) at 1 – 5000 µg/plate. There was a small increase in the number of revertants in the TA102 strain, but the authors concluded it was without biological significance [SCF, 2004].

Pigment Red 57 (salt not indicated) was not active for the induction of gene mutations in the mouse lymphoma L5178Y assay at 10 – 320 µg/ml with and without metabolic activation [SCF, 2004].

The mutagenicity of the smoke condensate was assayed in the *Salmonella* plate incorporation [Ames] assay with the tester strain TA98 in the presence of an S9 metabolic activation system. It was concluded that the *in vitro* mutagenicity of the cigarette smoke condensate was not increased by the addition of a mixture of ingredients at 600 ppm, which included Pigment red 57 (5281-04-9) at levels up to 100 ppm.

A battery of tests was used to compare the toxicology of mainstream smoke from experimental cigarettes manufactured with different monogram inks including lithol rubine BK. Experimental cigarettes were monogrammed using high-level print on the paper toward the filter end of the cigarette. The mass of the printed text was not quantified. There were no observed differences in cytotoxicity and Ames test between experimental cigarettes printed with lithol rubine BK. The incorporation of lithol rubine BK slightly increased the styrene levels but did not affect the other smoke constituents (Smith *et al*., 2013).

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