HC BLUE NO. 2

1. Exposure Data

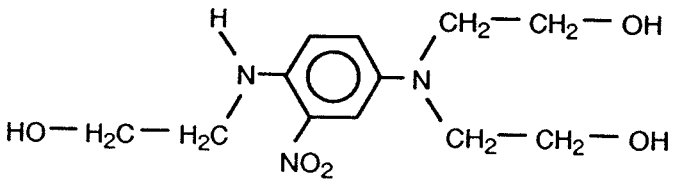
1.1 Chemical and physical data

1.1.1 *Synonyms, structural and molecular data*

Chem. Abstr. Serv. Reg. No.: 33229-34-4

Chem. Abstr. Name: 2,2′-([4-([2-Hydroxyethyl]amino)-3-nitrophenyl]imino)bis(ethanol)

Synonyms: HC Blue 2; HC Blue Number 2; 2,2′-([4-([2-hydroxyethyl]amino)-3-nitrophenyl]imino)di(ethanol); N^1,N^4,N^4-tris(2-hydroxyethyl)-2-nitro-*para*-phenylenediamine



$C_{12}H_{19}N_3O_5$ Mol. wt: 285.30

1.1.2 *Chemical and physical properties of the substance*

From US National Toxicology Program (1985)

(*a*) *Description*: Dark-blue microcrystalline (75% pure) or blackish-blue amorphous powder (98% pure), with a copper cast

(*b*) *Melting-point*: 93–98 °C (98% pure)

(*c*) *Spectroscopy data*: Infrared, ultraviolet and nuclear magnetic resonance spectral data have been reported.

(*d*) *Solubility*: Soluble in water, ethanol, methanol and acetone

(*e*) *Octanol/water partition coefficient (P)*: 1.7

1.1.3 *Trade names, technical products and impurities*

HC Blue No. 2 is available commercially with a purity ≥ 95% with possible impurities, including methylamine (≤ 1%), 1,2-dihydroxyethane (≤ 1%) and water (≤ 1%). It is also available in a technical grade containing 55–90% dye, 10–30% inorganic salts and 2,2-[(4-amino-3-nitrophenyl)imino]bis(ethanol) (≤ 7%) as a possible impurity.

1.1.4 *Analysis*

No data were available to the Working Group.

1.2 Production and use

1.2.1 *Production*

The form of HC Blue No. 2 that is ≥ 95% pure is produced commercially by the reaction of 4-fluoro-3-nitrobenzenamine with ethylene oxide (see IARC, 1985, 1987) to form 2,2-[(4-fluoro-3-nitrophenyl)imino]bis(ethanol); this intermediate is reacted with monoethanolamine to form HC Blue No. 2. The technical grade is produced by reaction of 2-nitro-*para*-phenylenediamine (see IARC, 1978) with ethylene oxide or 2-chloroethanol.

Production and use of HC Blue No. 2 began in the late 1950s. Approximately 9–11 tonnes are used annually in the USA, according to industry estimates.

1.2.2 *Use*

HC Blue No. 2 is used exclusively as a dye in semi-permanent hair colouring products. These products are generally shampooed into the hair, lathered and then allowed to remain in contact with the hair and scalp for 30–45 min. The concentration of HC Blue No. 2 used in these preparations ranges from 1.6 to 2% (Frenkel & Brody, 1973; US National Toxicology Program, 1985).

1.3 Occurrence

1.3.1 *Natural occurrence*

HC Blue No. 2 is not known to occur as a natural product.

1.3.2 *Occupational exposure*

No data were available to the Working Group.

1.4 Regulations and guidelines

No data were available to the Working Group.

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male and 50 female B6C3F$_1$ mice, seven weeks of age, were fed 0, 5000 or 10 000 mg/kg (ppm) (males) or 0, 10 000 or 20 000 ppm (females) HC Blue No. 2 (about 98%

pure) in the diet for 104 weeks and were killed at 112–113 weeks of age. Final mean body weights of treated males were similar to those of controls; however, those of treated females were 15% (low-dose) and 22% (high-dose) lower than those of controls. Survival at the end of the study was: males—control, 24/50; low-dose, 24/50; and high-dose, 34/50; females—control, 35/50; low-dose, 28/50; and high-dose, 20/50 ($p < 0.005$, life table test). The reduced survival of female mice was attributed to infection of the ovaries and uteri with *Klebsiella* sp. No significant increase in the incidence in any tumour was observed (US National Toxicology Program, 1985; Kari *et al.*, 1989a). [The Working Group noted that the reduced survival of females precluded adequate evaluation of the study.]

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344/N rats, six to seven weeks of age, were fed 0, 5000 or 10 000 mg/kg of diet (ppm) (males) or 0, 10 000 or 20 000 mg/kg ppm (females) HC Blue No. 2 (~ 98% pure) in the diet for 103 weeks and were killed at 110–112 weeks of age. Final mean body weights were depressed by less than 10% in treated males but by 13% (low-dose) and 22% (high-dose) in females. No adverse effect on survival was observed in males (control, 32/50; low-dose, 38/50; high-dose, 42/50) or females (control, 41/50; low-dose, 40/50; high-dose, 39/50). The incidence of C-cell adenomas of the thyroid in males was 7/50 control, 2/50 low-dose and 5/49 high-dose; the incidence of C-cell carcinomas was 0/50 control, 3/50 low-dose and 5/49 high-dose ($p = 0.029$, incidental tumour trend test). The trend in combined incidence (7/50, 5/50 and 10/49) was not significant. There was no excess of thyroid tumours in females. No other significant increase in the incidence of tumours was reported; however, malignant mixed mesenchymal tumours of the kidney were detected in 2/50 high-dose female rats and none of 1863 historical controls. A negative trend in the incidence of fibroadenomas of the mammary gland was seen in female rats (control, 20/50; low-dose, 10/50; high-dose, 4/50 [$p < 0.001$, Cochran Armitage test for trend]) (US National Toxicology Program, 1985; Kari *et al.*, 1989a).

4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

About 85 g of a commercial semi-permanent hair dye formulation containing 1.77% HC Blue No. 2 enriched with 378.6 μCi/mg [ring-^{14}C]-labelled HC Blue No. 2 was applied to the hair of human volunteers, worked in gently for 5–8 min and allowed to remain in contact with the hair and scalp for an additional 30 min. After 30 days, radiolabel accounting for less than 0.1% of that applied was detected in the urine; half was excreted in urine after 52 h (Wolfram & Maibach, 1985). [The Working Group noted that urinary metabolites were not identified, so that the metabolic fate of HC Blue No. 2 in humans remains unknown.]

4.1.2 *Experimental systems*

Up to 40% of radiolabel was recovered in the urine of B6C3F$_1$ mice and Fischer 344/N rats after oral administration by gavage of 100 mg/kg bw [ring-^{14}C]-labelled HC Blue No. 2

(0.1 mCi/mmol [35 μCi/mg]; 98% pure). Metabolism of HC Blue No. 2 (200 μM [57 mg]) by hepatocytes isolated from mice and rats yielded profiles similar to those seen *in vivo*. High-performance liquid chromatography separation showed that HC Blue No. 2 is metabolized extensively in mice to one major metabolite, which has not been characterized (Kari *et al.*, 1988, 1989b, 1990a,b).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Concentrations of 0, 5000 or 10 000 ppm (mg/kg) HC Blue No. 2 (98% pure) were administered in the diet to male Fischer 344/N rats for 103 weeks and to male B6C3F$_1$ mice for 104 weeks and concentrations of 0, 10 000 or 20 000 ppm (mg/kg) to female rats for 103 weeks and to female mice for 104 weeks. The calculated average daily doses were 194 and 391 mg/kg bw for male rats, 465 and 1001 mg/kg bw for female rats, 1321 and 2243 mg/kg bw for male mice, and 2362 and 5609 mg/kg for female mice. A dose-related increase in the incidence of hyperostosis of the skull was observed in male and female rats (US National Toxicology Program, 1985; Kari *et al.*, 1989a).

HC Blue No. 2 was present at low concentrations in semi-permanent hair colouring formulations evaluated in a 13-week study of dermal toxicity in rabbits (1.7%) (Burnett *et al.*, 1976) and in a two-year feeding study in dogs (1.63%) (Wernick *et al.*, 1975), described in detail on p. 97. No treatment-related adverse effect was detected. [The Working Group noted that the dose of each component of the formulation was very low and unlikely to have been toxic.]

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

No data were available to the Working Group on the reproductive and developmental effects of HC Blue No. 2 alone. The compound was present at low concentrations in semi-permanent hair colouring formulations evaluated in a study of fertility and reproductive performance in rats (Wernick *et al.*, 1975, 1.63%; see p. 99) and in studies of teratogenesis in rats (Wernick *et al.*, 1975, 1.63%; Burnett *et al.*, 1976, 1.70%) and rabbits (Wernick *et al.*, 1975, 1.63%) (see p. 100). No treatment-related adverse effect was detected. [The Working Group noted that the dose of each component of the formulations was very low and unlikely to have been toxic.]

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see also Table 1 and Appendices 1 and 2)

Although two different lots of HC Blue No. 2 have been used in most of the short-term tests (98.5% and 99.8% purity), they appear to induce similar responses when tested in the same assay system.

HC Blue No. 2 induced mutation in *Salmonella typhimurium* but did not induce reverse mutation in *Escherichia coli*. It induced mutations at the *tk* locus of mouse lymphoma L5178Y cells and induced unscheduled DNA synthesis in cultures of primary hepatocytes from mice, rats, Syrian hamsters and rabbits, but not in those from monkeys. HC Blue No. 2 induced sister chromatid exchange in cultured Chinese hamster ovary cells and, weakly, in female B6C3F$_1$ mouse primary hepatocytes. Chromosomal aberrations were not induced in Chinese hamster ovary cells or in primary cultures of rat or mouse hepatocytes.

HC Blue No. 2 did not inhibit gap-junctional communication in Chinese hamster lung V79 cells, but it elicited a slight, dose-dependent increase in communication at non-cytotoxic concentrations.

HC Blue No. 2 did not induce micronuclei in mouse bone-marrow in three assays (one of which was with the lower purity sample).

As reported in two abstracts, HC Blue No. 2 did not increase proliferation of hepatocytes from rats and mice treated *in vivo* (Mirsalis *et al.*, 1986, 1988), but it enhanced S-phase DNA synthesis in male (but not female) mouse hepatocytes. It did not induce unscheduled DNA synthesis in the livers of mice or rats in feeding studies.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

HC Blue No. 2 is used as a semi-permanent hair dye.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

HC Blue No. 2 was tested for carcinogenicity by administration in the diet in one study in mice and in one study in rats. No significant increase in tumour incidence was observed in either species, but the data on female mice could not be adequately evaluated.

5.4 Other relevant data

HC Blue No. 2 induced gene mutation in bacteria. It induced DNA damage, gene mutation and sister chromatid exchange but not chromosomal aberrations or inhibition of intercellular communication in cultured mammalian cells. Micronuclei were not induced in the bone marrow of mice exposed *in vivo*.

Table 1. Genetic and related effects of HC Blue No. 2

Test system	Result		Dose[a] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
98–98.5% Purity sample[b]				
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5000.0000	Zeiger *et al.* (1988)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5000.0000	Zeiger *et al.* (1988)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	(+)	(+)	1667.0000	Zeiger *et al.* (1988)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	500.0000	Zeiger *et al.* (1988)
SAS, *Salmonella typhimurium* TA97, reverse mutation	(+)	(+)	3333.0000	Zeiger *et al.* (1988)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus	+	0	30.0000	Myhr & Caspary (1991)
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	+	240.0000	Loveday *et al.* (1990)
SIM, Sister chromatid exchange, mouse hepatocytes *in vitro*	(+)	0	50.0000	Kari *et al.* (1990a)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	–	2500.0000	Loveday *et al.* (1990)
CIM, Chromosomal aberrations, mouse hepatocytes *in vitro*	–	0	100.0000	Kari *et al.* (1990a,b)
CIR, Chromosomal aberrations, rat hepatocytes *in vitro*	–	0	100.0000	Kari *et al.* (1990b)
ICR, Inhibition of cell–cell communication, Chinese hamster lung V79 cells	–	0	40.0000	Kari *et al.* (1990b)
99.8% Purity sample[c]				
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2500.0000	Oberly *et al.* (1990)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2500.0000	Oberly *et al.* (1990)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	(+)	2500.0000	Oberly *et al.* (1990)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	160.0000	Oberly *et al.* (1990)
ECW, *Escherichia coli* WP2, *uvrA*$^-$, *trp*$^-$, reverse mutation	–	–	2500.0000	Oberly *et al.* (1990)
URP, Unscheduled DNA synthesis, male rat primary hepatocytes	+	0	50.0000	Hill *et al.* (1990)
UIA, Unscheduled DNA synthesis, male mouse primary hepatocytes	+	0	10.0000	Hill *et al.* (1990)
UIA, Unscheduled DNA synthesis, male hamster primary hepatocytes	+	0	50.0000	Hill *et al.* (1990)
UIA, Unscheduled DNA synthesis, male rabbit primary hepatocytes	+	0	50.0000	Hill *et al.* (1990)

Table 1 (contd)

Test system	Result		Dose[a] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
UIA, Unscheduled DNA synthesis, male rhesus monkey primary hepatocytes	–	0	500.0000	Hill *et al.* (1990)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus	+	+	200.0000	Oberly *et al.* (1990)
MVM, Micronucleus test, CD-1 mouse bone marrow	–		1000.0000 ip × 1	Parton *et al.* (1990)
MVM, Micronucleus test, ICR mouse bone marrow	–		1000.0000 ip × 1	Parton *et al.* (1990)
Purity unspecified				
URP, Unscheduled DNA synthesis, rat primary hepatocytes	+	0	0.0000	Mirsalis *et al.* (1986); abstr.
UPR, Unscheduled DNA synthesis, rat hepatocytes *in vivo*	–		0.0000	Mirsalis *et al.* (1986); abstr.
UVM, Unscheduled DNA synthesis, male mouse hepatocytes *in vivo*	–		1200.0000 diet 7 days	Mirsalis *et al.* (1986); abstr.
UVM, Unscheduled DNA synthesis, female mouse hepatocytes *in vivo*	–		2400.0000 diet 7 days	Mirsalis *et al.* (1988); abstr.
MVM, Micronucleus test, male B6C3F$_1$ mouse bone marrow *in vivo*	–		1200.0000 diet 7 days	Mirsalis *et al.* (1988); abstr.
MVM, Micronucleus test, female B6C3F$_1$ mouse bone marrow *in vivo*	–		2400.0000 diet 7 days	Mirsalis *et al.* (1988); abstr.
*, S-Phase synthesis, male B6C3F$_1$ mouse hepatocytes *in vivo*	+		1200.0000 diet	Mirsalis *et al.* (1988); abstr.
*, S-Phase synthesis, female B6C3F$_1$ mouse hepatocytes *in vivo*	–		2400.0000 diet	Mirsalis *et al.* (1988); abstr.

+, positive; (+), weakly positive; –, negative; 0, not tested

[a]In-vitro tests, μg/ml; in-vivo tests, mg/kg bw

[b]Approximately 98.5% pure

[c]Lot No. 1-329, Clairol, 99.8% pure

*Not displayed on profile

5.5 Evaluation[1]

There is *inadequate evidence* in humans for the carcinogenicity of HC Blue No. 2.

There is *inadequate evidence* in experimental animals for the carcinogenicity of HC Blue No. 2.

Overall evaluation

HC Blue No. 2 is *not classifiable as to its carcinogenicity to humans (Group 3).*

6. References

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[1]For definition of the italicized terms, see Preamble, pp. 26–30.

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