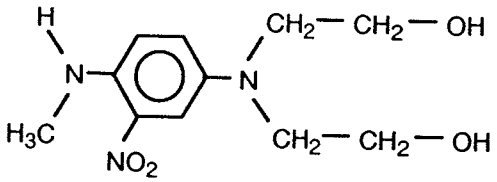
HC BLUE NO. 1

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Synonyms, structural and molecular data*

Chem. Abstr. Serv. Reg. No.: 2784-94-3
Chem. Abstr. Name: 2,2′-([4-(Methylamino)-3-nitrophenyl]imino)bis(ethanol)
Synonyms: N^4,N^4-Bis(2-hydroxyethyl)-N^1-methyl-2-nitro-*para*-phenylenediamine; HC Blue 1; HC Blue Number 1; 2,2′-([4-(methylamino)-3-nitrophenyl]imino)di(ethanol)



$C_{11}H_{17}N_3O_4$ Mol. wt: 255.27

1.1.2 *Chemical and physical properties of the substance*

From US National Toxicology Program (1985), unless otherwise specified

(*a*) *Description*: Dark-blue microcrystals or blue–black amorphous powder
(*b*) *Melting-point*: 101.5–104 °C
(*c*) *Spectroscopy data*: Infrared, ultraviolet and nuclear magnetic resonance spectral data have been reported.
(*d*) *Solubility*: Slightly soluble in water (0.38% w/w) (Frenkel & Brody, 1973); soluble in ethanol, methanol and acetone
(*e*) *Octanol/water partition coefficient (P)*: 17.2

1.1.3 *Trade names, technical products and impurities*

HC Blue No. 1 has been available commercially with a purity ≥ 95%, with 2-([4-(methylamino)-3-nitrophenyl]imino)ethanol (< 5 %) as a possible impurity.

1.1.4 *Analysis*

A thin-layer chromatographic method with spectrophotometric analysis has been reported for determination of HC Blue No. 1 in biological fluids (Frenkel & Brody, 1973).

1.2 Production and use

1.2.1 *Production*

HC Blue No. 1 was 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 was reacted with methylamine to form HC Blue No. 1.

HC Blue No. 1 is not known to be produced or used currently anywhere in the world. Production and use of HC Blue No. 1 began in the late 1960s and was discontinued in the mid-1980s. In the early 1980s, approximately 6–8 tonnes of HC Blue No. 1 were used annually in the USA, according to industry estimates.

1.2.2 *Use*

HC Blue No. 1 was used exclusively as a dye in semi-permanent hair colouring products. These products were 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. 1 used in these preparations ranged from 1 to 2% (Frenkel & Brody, 1973; US National Toxicology Program, 1985).

1.3 Occurrence

1.3.1 *Natural occurrence*

HC Blue No. 1 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

The production and use of HC Blue No. 1 were discontinued in the mid-1980s.

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, 53 days of age, were fed 0, 1500 or 3000 mg/kg (ppm) (males) or 0, 3000 or 6000 ppm (females) HC Blue No. 1 (~ 97% pure) in the

diet for 103 weeks and were killed at 112–115 weeks of age. Final mean body weights were reduced by 10–12% in treated males and 21–29% in treated females compared with controls. Survival at the end of the study was: males—control, 33/50; low-dose, 37/50; and high-dose, 30/50; females—control, 36/50; low-dose, 28/50; and high-dose, 24/50 ($p = 0.026$, life table test). Survival of female mice was reduced after 91 weeks, and many high-dose females that died early had hepatocellular carcinomas. In male and female mice, both doses of HC Blue No. 1 increased the incidence of hepatocellular carcinomas (males: control, 11/50; low-dose, 20/50; and high-dose, 30/50 ($p < 0.001$, incidental tumour trend test); females: control, 1/50; low-dose, 24/48; and high-dose, 47/49 ($p < 0.001$, incidental tumour trend test)). The incidence of hepatocellular adenomas was increased in both low-dose groups (males: control, 4/50; low-dose, 17/50 ($p = 0.003$, incidental tumour test); and high-dose, 10/50; females: control, 2/50; low-dose, 11/48 ($p = 0.004$, incidental tumour test); and high-dose, 4/49). In male mice, the incidences of thyroid follicular hyperplasia (control, 3/47; low-dose, 7/49; high-dose, 14/50) and thyroid follicular adenomas (control, 0/47; low-dose, 0/49; high-dose, 5/50 ($p = 0.027$, incidental tumour test)) were increased. The historical incidence of thyroid follicular adenomas at the study laboratory was 0–6% (US National Toxicology Program, 1985; Kari *et al.*, 1989a).

In a subsequent study, in which only the liver was examined, two groups of 48 female B6C3F$_1$ mice, six weeks of age, were fed 0.3% [3000 mg/kg of diet] (ppm) highly purified HC Blue No. 1 or recrystallized, charcoal-filtered commercial HC Blue No. 1 in the diet for 24 months. One group of 48 female mice served as controls. Highly purified HC Blue No. 1 increased the incidences of hepatocellular adenomas (10/46 *versus* 1/48 controls) and of hepatocellular carcinoms (41/46 *versus* 2/48 controls), as did commercial HC Blue No. 1 (hepatocellular adenomas: 10/46 *versus* 1/48 controls; hepatocellular carcinomas: 44/46 *versus* 2/48 controls). Three additional groups of 24 or 48 females received 0.3% recrystallized, charcoal-filtered commercial HC Blue No. 1 in the diet for nine months followed by 15 months without treatment, for 15 months in the diet followed by nine months without treatment or every other week for 24 months. All three treatment regimens resulted in an increase in the number of mice with benign and malignant hepatocellular tumours. Data from the five experiments are summarized in Table 1 (Burnett & Corbett, 1987).

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344/N rats, 53 days of age, were fed 0, 1500 or 3000 mg/kg (ppm) of diet HC Blue No. 1 (~ 97% pure) for 103 weeks and were killed at 112–115 weeks of age. From about week 50, the body weights of high-dose females were depressed by > 10% compared with controls. Survival at the end of the study was not affected by treatment (males—control, 39/50; low-dose, 34/50; and high-dose, 41/50; females—control, 40/50; low-dose, 38/50; and high-dose, 41/50). HC Blue No. 1 increased the incidences of pulmonary adenomatous hyperplasia (control, 2/50; low-dose, 5/49; high-dose, 8/50) and pulmonary adenomas/carcinomas (control, 1/50; low-dose, 3/49; high-dose, 7/50; $p = 0.034$, incidental tumour test) in females. The mean historical incidence of pulmonary adenomas and carcinomas in the laboratory was 2% (range, 0–3%). Male rats also had an increased incidence of pulmonary adenomatous hyperplasia (control, 3/50; low-dose, 5/50; high-dose, 7/50). A significant positive trend was seen in the incidence of

Table 1. Hepatocellular tumour incidences in female B6C3F$_1$ mice fed various preparations of HC Blue No. 1 in the diet

Treatment regimen	Incidence of hepatocellular tumours		
	Adenomas	Carcinomas	Combined
HC Blue No. 1 (highly purified) continuously for 24 months	10/46	41/46	45/46
HC Blue No. 1 (recrystallized, charcoal filtered) continuously for 24 months	10/46	44/46	46/46
HC Blue No. 1 (recrystallized, charcoal filtered) for 9 months followed by control diet for 15 months	10/22	13/22	20/22
HC Blue No. 1 (recrystallized, charcoal filtered) for 15 months followed by control diet for 9 months	4/23	19/23	20/23
HC Blue No. 1 (recrystallized, charcoal filtered) every other week for 24 months	21/46	25/46	40/46
Controls	1/48	2/48	3/48

From Burnett & Corbett (1987)

uterine stromal polyps (control, 5/50; low-dose, 9/50; and high-dose, 14/50; $p = 0.022$, incidental tumour trend test) and of neoplastic nodules or neoplastic nodules and carcinomas (combined) in the livers of male rats, although the incidences in the treated groups were not significantly greater than those in controls (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 1.48% HC Blue No. 1 enriched with 113.6 μCi/mg [ring-^{14}C]-labelled HC Blue No. 1 was applied on two occasions 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. On the first occasion, the hair was shaven, and radiolabel accounting for 0.09–0.15% of that applied was detected in the urine over a seven-day period; half was excreted in the urine after 18 h. On the second occasion, the hair was shaven only after 30 days: cumulative absorption was 0.15% on the first day and 0.5% on the 30th day; half of the radiolabel was excreted after 138 h (Maibach & Wolfram, 1981; Wolfram & Maibach, 1985). [The Working Group noted that in neither study were urinary metabolites identified, so that the metabolic fate of HC Blue No. 1 in humans remains unknown.]

4.1.2 *Experimental systems*

The same commercial hair dye formulation as used above was applied to the scalp hair of rhesus monkeys and allowed to remain in contact for 30 min. Radiolabel accounting for

0.12–0.13% of that applied was detected in urine over a seven-day period; half was excreted in the urine after 40 h (Maibach & Wolfram, 1981; Wolfram & Maibach, 1985).

Following intraperitoneal or subcutaneous injection of 2.5, 3.5, 4.0, 5.0 or 100 mg/kg bw HC Blue No. 1 (98% pure, containing four minor components) to adult Fischer rats or rabbits, over 90% was recovered in bile or urine within 6 h of administration. Following application of about 1 mg/cm^2 (250 μg/ml in saline) HC Blue No. 1 to the skin, about 1% of the dose was recovered in the bile and slightly more in the urine of rats and an average of 4.5% of the dose in the urine of rabbits after 48 h (Frenkel & Brody, 1973).

[The Working Group noted that urinary metabolites were not identified in these three studies].

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]HC Blue No. 1 (29 mCi/mmol [113.6 μCi/mg]; 97% pure). HC Blue No. 1 administered orally to B6C3F$_1$ mice yielded three metabolites in equal proportions, which were more water soluble than the parent compound; one was a glucuronide of the parent compound. Metabolism of [ring-^{14}C]HC Blue No. 1 (200 μM [51 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. 1 is metabolized extensively in mice to five major metabolites. Thermospray liquid chromatography–mass spectrometry of these metabolites provided tentative evidence for nitroreduction, *N*-demethylation and conjugation (glucuronidation of a demethylated product and acetylation). In rats, HC Blue No. 1 produced three metabolites similar to those found in mice (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, 1500 or 3000 ppm (mg/kg of diet) HC Blue No. 1 (~ 97% pure) were administered in the diet to Fischer 344/N rats of each sex and to male B6C3F$_1$ mice, and concentrations of 0, 3000 or 6000 ppm (mg/kg of diet) were given to female B6C3F$_1$ mice, for 103 weeks. The calculated average daily doses were 66 and 38 mg/kg bw for male rats, 74 and 153 mg/kg bw for female rats, 309 and 582 mg/kg bw for male mice, and 778 and 1634 mg/kg bw for female mice. No toxicologically significant non-neoplastic lesion was found in rats or mice of either sex (US National Toxicology Program, 1985; Kari *et al.*, 1989a).

HC Blue No. 1 was present at 1.6% in semi-permanent hair colouring formulations evaluated in a 13-week study of dermal toxicity in rabbits (Burnett *et al.*, 1976), at 0.3% in a 20-month study of dermal toxicity in mice (Jacobs *et al.*, 1984) and at 1.54% in a two-year feeding study in dogs (Wernick *et al.*, 1975), described in detail on p. 97. No treatment-related toxicity 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. 1 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 (1.54%) (Wernick *et al.*, 1975; see p. 99), in a study of heritable translocation in rats (0.5%) (Burnett *et al.*, 1981; see p. 104) and in studies of teratogenesis in rats (Wernick *et al.*, 1975, 1.54%; Burnett *et al.*, 1976, 1.6%) and rabbits (1.54%) (Wernick *et al.*, 1975) (see p. 100). 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.]

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see also Tables 2 and 3 and Appendices 1 and 2)

Both commercial preparations and purified samples of HC Blue No. 1 have been tested for genetic and related effects. The results of some tests indicate that the bacterial mutagenicity and DNA adducts seen after exposure to commercial HC Blue No. 1 are due to impurities and not to the main component (Abu-Shakra *et al.*, 1991). Commercial samples of HC Blue No. 1 induced mutations in *Salmonella typhimurium* and mutations at both the *hprt* locus of cultured Chinese hamster ovary cells and the *tk* locus of mouse lymphoma L5178Y cells. In contrast, purified samples did not induce mutation in *S. typhimurium*, *Escherichia coli*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* or *Drosophila melanogaster* or at the *hprt* locus of Chinese hamster V79 cells; a weak response was, however, obtained at the *tk* locus of mouse lymphoma L5178Y cells. The negative results in *S. typhimurium* were obtained at doses of the purified material up to 50-fold higher than the effective doses needed when commercial HC Blue No. 1 samples were tested.

Purified and commercial HC Blue No. 1 preparations induced unscheduled DNA synthesis in primary cultures of rat hepatocytes. Purified samples were found to induce unscheduled DNA synthesis in primary cultures of mouse, Syrian hamster, rabbit and rhesus monkey hepatocytes.

Sister chromatid exchange frequency was increased in cultured Chinese hamster ovary cells exposed to purified and commercial HC Blue No. 1 and in primary cultures of mouse hepatocytes exposed to a commercial preparation. Chromosomal aberrations were induced in primary mouse but not rat hepatocyte cultures by commercial HC Blue No. 1. A

commercial HC Blue No. 1 preparation increased the frequency of chromosomal aberrations in Chinese hamster ovary cells *in vitro* in the presence of a metabolic activation system, whereas one study with purified HC Blue No. 1 showed no increase in chromosomal aberrations; however, in the latter study, it was tested at a lower concentration and only in the absence of an exogenous metabolic activation system. A commercial HC Blue No. 1 was reported not to induce morphological transformation of Syrian hamster embryo cells.

Purified HC Blue No. 1 induced micronuclei in the bone marrow of female ICR mice exposed *in vivo* intraperitoneally but did not increase the frequency in the bone marrow of male CBA, male or female CD-1 or male ICR mice. A commercial sample of HC Blue No. 1 (97%) did not induce micronuclei in male or female $B6C3F_1$ mice exposed by oral administration.

Commercial HC Blue No. 1 inhibited gap-junctional intercellular communication in Chinese hamster lung V79 cells at non-cytotoxic doses.

It was reported in an abstract that presumably commercial samples (concentration unspecified) of HC Blue No. 1 induced S-phase synthesis in the livers of male but not female mice exposed *in vivo*. The samples did not induce unscheduled DNA synthesis in the livers of mice or rats exposed *in vivo* but induced unscheduled DNA synthesis in primary cultures of mouse, Syrian hamster and cynomolgus monkey hepatocytes. The samples induced hepatocyte proliferation in mice but not in rats treated *in vivo* (Mirsalis *et al.*, 1986).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

HC Blue No. 1 was used as a semi-permanent hair dye until the mid-1980s, when its production and use were discontinued.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

HC Blue No. 1 was tested for carcinogenicity by administration in the diet in two studies in mice, one of which was restricted to females, and in one study in male and female rats. In one study in male and female mice and in several experiments in the study of females, it significantly increased the incidence of hepatocellular adenomas and/or carcinomas in mice of each sex and increased the incidence of thyroid follicular-cell adenomas in males. An increase in the combined incidence of pulmonary adenomas and carcinomas was seen in female but not in male rats.

5.4 Other relevant data

Commercial samples of HC Blue No. 1 bound to DNA and induced mutation in bacteria. They induced DNA damage, gene mutation and chromosomal anomalies and inhibited intercellular communication in cultured mammalian cells.

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

Test system	Result		Dose[a] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2500.0000	Shahin & Bugaut (1983)[b]
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2500.0000	Oberly *et al.* (1990)[c]
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2500.0000	Shahin & Bugaut (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2500.0000	Oberly *et al.* (1990)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2500.0000	Shahin & Bugaut (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2500.0000	Oberly *et al.* (1990)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	2500.0000	Shahin & Bugaut (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2500.0000	Shahin & Bugaut (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2500.0000	Oberly *et al.* (1990)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	0	1000.0000	Abu-Shakra *et al.* (1991)[d]
ECW, *Escherichia coli* WP2, *uvrA*$^-$, *trp*$^-$, reverse mutation	–	–	2500.0000	Oberly *et al.* (1990)
SCG, *Saccharomyces cerevisiae*, *ade* and *trp* conversions	–	–	12700.0000	Loprieno *et al.* (1983)[e]
SZF, *Schizosaccharomyces pombe*, forward mutation, *ade* and *rad*	–	–	12700.0000	Loprieno *et al.* (1983)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	–		5100.00 injection	Darroudi *et al.* (1983)[f]
URP, Unscheduled DNA synthesis, rat primary hepatocytes	+	0	0.1000	Williams *et al.* (1989)[g]
URP, Unscheduled DNA synthesis, rat primary hepatocytes	+	0	5.0000	Hill *et al.* (1990)
UIA, Unscheduled DNA synthesis, mouse primary hepatocytes	+	0	5.0000	Hill *et al.* (1990)
UIA, Unscheduled DNA synthesis, Syrian hamster primary hepatocytes	+	0	5.0000	Hill *et al.* (1990)
UIA, Unscheduled DNA synthesis, rabbit primary hepatocytes	+	0	50.0000	Hill *et al.* (1990)
UIA, Unscheduled DNA synthesis, rhesus monkey primary hepatocytes	+	0	50.0000	Hill *et al.* (1990)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus	–	–	25500.0000	Loprieno *et al.* (1983)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus	–	(+)	25.0000	Oberly *et al.* (1990)
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	–	0	1600.0000 × 1 h	Darroudi *et al.* (1983)

Table 2 (contd)

Test system	Result		Dose[a] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	0	100.0000 × 24 h	Darroudi *et al.* (1983)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	0	400.0000	Darroudi *et al.* (1983)
UHT, Unscheduled DNA synthesis, human HeLa cells	–	–	7600.0000	Loprieno *et al.* (1983)
MVM, Micronucleus test, male CBA mouse bone marrow	–		100.0000 ip × 3	Darroudi *et al.* (1983)
MVM, Micronucleus test, female ICR mouse bone marrow	+		1000.0000 ip × 1	Parton *et al.* (1990)[h]
MVM, Micronucleus test, male ICR mouse bone marrow	–		1000.0000 ip × 1	Parton *et al.* (1990)[h]
MVM, Micronucleus test, male and female CD-1 mouse bone marrow	–		1000.0000 ip × 1	Parton *et al.* (1990)[h]
BID, Binding (covalent) to TA98 DNA *in vitro* (^{32}P-postlabelling)	–	0	12000.0000[i]	Abu-Shakra *et al.* (1991)[d]

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

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

[b]Synthesized and purified industrial sample with one recrystallization in ethanol

[c]Industrial sample with one ethanol recrystallization, purity > 98%

[d]High-performance liquid chromatography (HPLC)-purified

[e]Provided by l'Oréal (ref. MPIp37) as a pure product

[f]Pure form supplied by l'Oréal

[g]Purified preparation

[h]> 98% pure

[i]12 000 μg/ml equivalent; HPLC-purified sample derived from HPLC fractionation of 240 mg HC Blue 1 added to 20 ml incubation mixture

Table 3. Genetic and related effects of commercial HC Blue No. 1

Test system	Result		Dose[a] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	50.0000	Zeiger *et al.* (1988)[b]
SA5, *Salmonella typhimurium* TA1535, reverse mutation	-	-	500.0000	Zeiger *et al.* (1988)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	50.0000	Zeiger *et al.* (1988)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	0	1000.0000	Abu-Shakra *et al.* (1991)[c]
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	0	0.0000	Casciano *et al.* (1985)[d]; abstr.
SAS, *Salmonella typhimurium* TA98NR, reverse mutation	+	0	0.0000	Casciano *et al.* (1985)[d]; abstr.
SAS, *Salmonella typhimurium* TA97, reverse mutation	+	+	50.0000	Zeiger *et al.* (1988)
URP, Unscheduled DNA synthesis, rat primary hepatocytes	+	0	50.0000	US National Toxicology Program (1985)[b]
URP, Unscheduled DNA synthesis, rat primary hepatocytes	+	0	1.0000	Williams *et al.* (1989)[e]
UIA, Unscheduled DNA synthesis, mouse primary hepatocytes	+	0	0.0000	Mirsalis *et al.* (1986)[d]; abstr.
UIA, Unscheduled DNA synthesis, Syrian hamster primary hepatocytes	+	0	0.0000	Mirsalis *et al.* (1986)[d]; abstr.
UIA, Unscheduled DNA synthesis, cynomologus monkey primary hepatocytes	+	0	0.0000	Mirsalis *et al.* (1986)[d]; abstr.
UIH, Unscheduled DNA synthesis, human primary hepatocytes	+	0	0.0000	Mirsalis *et al.* (1986)[d]; abstr.
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus	+	0	20.0000	Myhr & Caspary (1991)[b]
GCO, Gene mutation, Chinese hamster ovary cells, *hprt* locus	+	0	0.0000	Casciano *et al.* (1985); abstr.
SIM, Sister chromatid exchange, mouse hepatocytes *in vitro*	(+)	0	10.0000	Kari *et al.* (1990a)[b]
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	(+)	119.0000	Loveday *et al.* (1990)[b]
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	-	+	960.0000	Loveday *et al.* (1990)[b]
CIM, Chromosomal aberrations, mouse hepatocytes *in vitro*	+	0	10.0000	Kari *et al.* (1990a,b)[b]
CIR, Chromosomal aberrations, rat hepatocytes *in vitro*	-	0	100.0000	Kari *et al.* (1990b)

Table 3 (contd)

Test system	Result		Dose[a] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay	–	0	50.0000	Pienta & Kawalek (1981)[d]
UPR, Unscheduled DNA synthesis, rat hepatocytes *in vivo*	–		0.0000	Mirsalis *et al.* (1986)[d]; abstr.
UVM, Unscheduled DNA synthesis, male B6C3F$_1$ mouse hepatocytes *in vivo*	–		360.0000, 7 days	Mirsalis *et al.* (1988); abstr.
UVM, Unscheduled DNA synthesis, female B6C3F$_1$ mouse hepatocytes *in vivo*	–		720.0000, 7 days	Mirsalis *et al.* (1988); abstr.
MVM, Micronucleus test, male B6C3F$_1$ mouse bone marrow			360.0000, 7 days	Mirsalis *et al.* (1988); abstr.
MVM, Micronucleus test, female B6C3F$_1$ mouse bone marrow	–		720.0000, 7 days	Mirsalis *et al.* (1988); abstr.
*, S-Phase synthesis, male B6C3F$_1$ mouse hepatocytes *in vivo*	+		360.0000, 7 days	Mirsalis *et al.* (1988)[d]; abstr.
*, S-Phase synthesis, female B6C3F$_1$ mouse hepatocytes *in vivo*	–		720.0000, 7 days	Mirsalis *et al.* (1988)[d]; abstr.
BID, Binding (covalent) to TA98 DNA *in vitro* (P^{32}-postlabelling)	+	0	12000.0000	Abu-Shakra *et al.* (1991)
ICR, Inhibition of cell-cell communication, Chinese hamster lung V79 cells	+	0	50.0000	Kari *et al.* (1990a)

+, positive; (+), weakly positive; –, negative; 0, not tested; ?, inconclusive (variable response in several experiments within an adequate study); 0.0000, dose not given

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

[b]Commercial HC Blue 1, Lot No. 3670379, approximately 97% pure

[c]Commercial HC Blue 1, Lot No. 68379913

[d]Purity unspecified

[e]Commercial sample

*Not displayed on profile

Purified samples of HC Blue No. 1 did not bind to DNA or induce mutation in bacteria. They did not induce mitotic recombination in yeasts and did not induce mutation in insects. They induced DNA damage, sister chromatid exchange and, weakly, gene mutation but not chromosomal aberrations in cultured mammalian cells. DNA damage was not induced in cultured human cells (HeLa). Micronuclei were induced in the bone marrow of female mice of one strain exposed *in vivo*.

5.5 Evaluation[1]

There is *inadequate evidence* in humans for the carcinogenicity of HC Blue No. 1.

There is *sufficient evidence* in experimental animals for the carcinogenicity of HC Blue No. 1.

Overall evaluation

HC Blue No. 1 is *possibly carcinogenic to humans (Group 2B).*

6. References

Abu-Shakra, A., Johnson, L., Earley, K., Jameson, C.W., Kari, F.W., Gupta, R. & Langenbach, R. (1991) Isolation of the mutagenic and DNA adduct-inducing components from a commercial preparation of HC Blue 1 using *Salmonella* (TA98) bioassay-directed HPLC fractionation. *Mutat. Res.*, **260**, 377–385

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

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