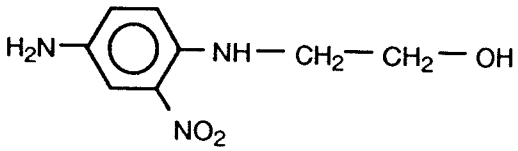
HC RED NO. 3

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Synonyms, structural and molecular data*

Chem. Abstr. Serv. Reg. No.: 2871-01-4
Chem. Abstr. Name: 2-[(4-Amino-2-nitrophenyl)amino]ethanol
Synonyms: 2-(4-Amino-2-nitroanilino)ethanol; HC Red 3; HC Red Number 3; 4-(2-hydroxyethyl)amino-3-nitroaniline; N^1-(2-hydroxyethyl)-2-nitro-*para*-phenylenediamine



$C_8H_{11}N_3O_3$ Mol. wt: 197.19

1.1.2 *Chemical and physical properties*

From US National Toxicology Program (1986)

(*a*) *Description*: Fine, dark-maroon crystals, with a greenish cast
(*b*) *Melting-point*: 124–128 °C
(*c*) *Spectroscopy data*: Infrared, ultraviolet and nuclear magnetic resonance spectral data have been reported.
(*d*) *Solubility*: Soluble in water (0.28% w/w), ethanol and acetone
(*e*) *Octanol/water partition coefficient (P)*: 1.9

1.1.3 *Trade names, technical products and impurities*

HC Red No. 3 is available commercially at a purity ≥ 95%, with aminoquinoxaline, aminonaphthyridine (US National Toxicology Program, 1986) and 2-[(4-[4-amino-2-nitrophenyl]amino-2-nitrophenyl)amino] ethanol (< 1%) as possible impurities.

1.1.4 *Analysis*

No data were available to the Working Group.

1.2 Production and use

1.2.1 *Production*

HC Red No. 3 is produced by the reaction of 4-fluoro-3-nitrobenzenamine with monoethanolamine. Production and use of this dye began in the late 1950s. Approximately 2300 kg are used annually in the USA, according to industry estimates.

1.2.2 *Use*

HC Red No. 3 is used exclusively as a dye in semi-permanent hair colour 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 Red No. 3 used in these preparations ranges from 0.1 to 5% (Frenkel & Brody, 1973; US National Toxicology Program, 1986).

1.3 Occurrence

1.3.1 *Natural occurrence*

HC Red No. 3 is not known to occur as a natural product.

1.3.2 *Occupational exposure*

No data were available to the Working Group.

On the basis of a survey conducted in the USA between 1981 and 1983, the US National Institute for Occupational Safety and Health estimated that a total of 42 485 workers, including 32 059 women, were potentially exposed as beauticians and cosmetologists to HC Red No. 3 (US National Library of Medicine, 1992).

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, eight weeks of age, were administered 0, 125 or 250 mg/kg bw HC Red No. 3 (97% pure) by gavage in corn oil on five days a week for

104 weeks and were sacrificed at 113 weeks of age. Body weight gain and survival were reduced in all groups of females because of a reproductive tract infection. Survival at the end of the study was: males—control, 30/50; low-dose, 41/50; and high-dose, 29/50; females—control, 12/50; low-dose, 8/50; and high-dose, 9/50. The incidence of hepatocellular adenomas in males was control, 11/50; low-dose, 6/50; high-dose, 16/50; the incidence of hepatocellular carcinomas was, control, 17/50; low-dose, 9/50; high-dose, 21/50. There was an increased incidence of hepatocellular adenomas and carcinomas combined in high-dose male mice (control, 25/50; low-dose, 15/50; high-dose, 35/50), which was significant ($p = 0.017$, incidental tumour test), but not after pair-wise comparison. The incidence in historical controls in the testing laboratory was 109/298 (37 ± 12%) (US National Toxicology Program, 1986). [The Working Group noted the poor survival of females and the unusually high incidence of hepatocellular carcinomas in male controls.]

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344/N rats, seven to eight weeks of age, were administered 0, 250 or 500 mg/kg bw HC Red No. 3 (97% pure) by gavage in corn oil on five days a week for 105 weeks and were sacrificed at 113–114 weeks of age. The treatment had no effect on body weight gain throughout the study and did not reduce survival in males or females. Survival at the end of the experiment was: males—control, 34/50; low-dose, 34/50; high-dose, 32/50; females—control, 39/50; low-dose, 38/50; high-dose, 34/50. The incidence of mammary gland fibroadenomas was significantly ($p = 0.019$, incidental tumour test) increased in low-dose females (control, 14/50 *versus* low-dose, 24/50) but not in high-dose females (11/50) (US National Toxicology Program, 1986). [The Working Group noted the absence of a dose–response relationship.]

4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

No data were available to the Working Group.

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

In the studies reported above, no compound-related toxic effect was reported (US National Toxicology Program, 1986).

HC Red No. 3 was present at low concentrations (0.02%) in semi-permanent hair colouring formulations evaluated in a two-year feeding study in dogs (Wernick *et al.*, 1975) and in a 20-month study of dermal toxicity in mice (Jacobs *et al.*, 1984, 0.3%), 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 formulations 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 Red No. 3 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, 0.02%; see p. 99), in a study of heritable translocation in rats (Burnett *et al.*, 1981, 0.01%; see p. 104), and in studies of teratogenesis in rats and rabbits (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 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)

HC Red No. 3 was mutagenic to *Salmonella typhimurium*.

Table 1. Genetic and related effects of HC Red No. 3

Test system	Result		Dose (LED/HID) (μg/ml)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	50.0000	Zeiger *et al.* (1988)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	1667.0000	Zeiger *et al.* (1988)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	1.7000	Zeiger *et al.* (1988)
SAS, *Salmonella typhimurium* TA97, reverse mutation	+	+	1.7000	Zeiger *et al.* (1988)

+, positive; –, negative

5. Summary of Data Reported and Evaluation

5.1 Exposure data

HC Red No. 3 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 Red No. 3 was tested for carcinogenicity by gavage in one study in mice and in one study in rats. There was a significant increase in the incidence of hepatocellular adenomas and carcinomas combined in male mice administered the high dose; poor survival precluded evaluation of the females. No increase in the incidence of treatment-related tumours was seen in rats of either sex.

5.4 Other relevant data

HC Red No. 3 was mutagenic to bacteria.

5.5 Evaluation[1]

There is *inadequate evidence* in humans for the carcinogenicity of HC Red No. 3.

There is *inadequate evidence* in experimental animals for the carcinogenicity of HC Red No. 3.

Overall evaluation

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

6. References

Burnett, C., Loehr, R. & Corbett, J. (1981) Heritable translocation study on two hair dye formulations. *Fundam. appl. Toxicol.*, **1**, 325–328

Frenkel, E.P. & Brody, F. (1973) Percutaneous absorption and elimination of an aromatic hair dye. *Arch. environ. Health*, **27**, 401–404

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

US National Library of Medicine (1992) *Registry of Toxic Effects of Chemical Substances* (RTECS No. KJ6500000), Bethesda, MD

US National Toxicology Program (1986) *Toxicology and Carcinogenesis Studies of HC Red No. 3 (CAS No. 2871-01-4) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)* (NTP Technical Report 281; NIH Publ. No. 86-2537), Research Triangle Park, NC

Wernick, T., Lanman, B.M. & Fraux, J.L. (1975) Chronic toxicity, teratologic and reproduction studies with hair dyes. *Toxicol. appl. Pharmacol.*, **32**, 450–460

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T. & Mortelmans, K. (1988) *Salmonella* mutagenicity tests: IV. Results from the testing of 300 chemicals. *Environ. mol. Mutag.*, **11** (Suppl. 12), 1–158