HC YELLOW NO. 4

1. Exposure Data

1.1 Chemical and physical data

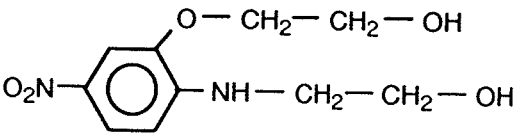
1.1.1 *Synonyms, structural and molecular data*

Confusion has existed over the structure of HC Yellow No. 4. In the second edition of the *Directory of Cosmetic and Toiletry Ingredients* (Cosmetic, Toiletry, and Fragrance Association, 1982), the structure shown for HC Yellow No. 4 had both hydroxyethyl groups on the amine and an assigned CAS No. of 52551-67-4 (2-[bis(2-hydroxyethyl)amino]-5-nitrophenol; *N,N*-bis(2-hydroxyethyl)-2-amino-5-nitrophenol). In the third edition of the *Directory of Cosmetic and Toiletry Ingredients* (Cosmetic, Toiletry, and Fragrance Association, 1991), the structure was corrected on the basis of additional structural analysis, to show one hydroxyethyl group on the amine and the other at the *ortho* position on the ring. This structure and its CAS No. (US National Toxicology Program, 1992) are given below.

Chem. Abstr. Serv. Reg. No.: 59820-43-8

Chem. Abstr. Name: 2-[(2-[2-Hydroxyethoxy]-4-nitrophenyl)amino]ethanol

Synonyms: *N,O*-Di(2-hydroxyethyl)-2-amino-5-nitrophenol; HC Yellow 4; 2-[3-nitro-6-(*beta*-hydroxyethylamino)phenoxy] ethanol



$C_{10}H_{14}N_2O_5$ Mol. wt: 242.23

1.1.2 *Chemical and physical properties of the substance*

From US National Toxicology Program (1992)

(*a*) *Description*: Fluffy, bright-yellow powder

(*b*) *Melting-point*: 145–147 °C

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

(*d*) *Solubility*: Soluble in water (0.14% w/w), ethanol and acetone

1.1.3 *Trade names, technical products and impurities*

HC Yellow No. 4 is commercially available at a purity ≥ 93%, with *N*-(2-hydroxyethyl)-2-hydroxy-4-nitroaniline (0.3–7%) (US National Toxicology Program, 1992), 2-

(2-amino-5-nitrophenoxy)ethanol (< 5%) and 2,2′-[(2-hydroxy-4-nitrophenyl)imino]bis-(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 Yellow No. 4 is produced by the reaction of 2-hydroxy-4-nitrobenzenamine with 2-chloroethanol and sodium hydroxide. Production of this dye began in the late 1950s and was estimated to be 2300 kg in the USA in 1976 (US National Toxicology Program, 1992). Currently, approximately 900 kg are used annually in the USA, according to industry estimates.

1.2.2 *Use*

HC Yellow No. 4 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 Yellow No. 4 used in these preparations ranges from 0.1 to 1.0% (Frenkel & Brody, 1973; US National Toxicology Program, 1992).

1.3 Occurrence

1.3.1 *Natural occurrence*

HC Yellow No. 4 is not known to occur as a natural product.

1.3.2 *Occupational exposure*

No data were available to the Working Group.

An estimated 4000 workers in department stores and beauty shops in the USA were exposed to HC Yellow No. 4 in 1974 (US National Toxicology Program, 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, six weeks of age, were fed diets containing 0, 5000 or 10 000 mg/kg of diet (ppm) HC Yellow No. 4 (> 93% pure; major

impurity tentatively identified as *N*-(2-hydroxyethyl)-2-hydroxy-4-nitroaniline, ~ 7%) for up to 104 weeks and were killed at 110–111 weeks of age. The mean body weights of high-dose mice were 20–30% lower than those of controls during the second year of the study. Survival at the end of the study was: males—control, 28/50; low-dose, 29/50; and high-dose, 35/50; females—control, 43/50; low-dose, 38/50; and high-dose, 43/50. No significant increase in the incidence of tumours was found in treated animals as compared with controls (US National Toxicology Program, 1992).

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344/N rats, six weeks of age, were fed diets containing 0, 2500 or 5000 mg/kg of diet (ppm) (males) and 0, 5000 or 10 000 ppm (females) HC Yellow No. 4 (> 93% pure; major impurity tentatively identified as *N*-(2-hydroxyethyl)-2-hydroxy-4-nitroaniline, ~ 7%) for up to 104 weeks and were killed at 110–111 weeks of age. The mean body weights of high-dose female rats were significantly lower than those of controls. Survival at the end of the experiment was: males—control, 21/50; low-dose, 29/50; and high-dose, 28/50; females—control, 27/50; low-dose, 31/50; and high-dose, 34/50. The incidence of adenomas of the pituitary gland was increased in male rats (control, 17/45; low-dose, 20/49; high-dose, 28/49; $p = 0.034$, logistic regression trend test), but the increase was barely significant in a pair-wise comparison ($p = 0.047$, logistic regression trend test). The incidence in historical controls in all National Toxicology Program feed studies was 29.7 ± 11.5% (range, 12–60%). Similarly, the incidence of pituitary gland hyperplasia in males was dose-dependently increased (control, 8/45; low-dose, 13/49; high-dose, 18/49; $p = 0.026$, logistic regression trend test). The incidence of pituitary gland tumours was not increased in female rats (US National Toxicology Program, 1992).

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*

During 14-day studies, groups of five Fischer 344/N rats of each sex received 0, 5000, 10 000, 20 000, 40 000 or 80 000 ppm (mg/kg) and B6C3F$_1$ mice of each sex received 0, 1250, 2500, 5000, 10 000 or 20 000 ppm HC Yellow No. 4 (purity, > 93%) in the feed. All animals survived to the end of the studies. No dose-related toxic effect was observed (US National Toxicology Program, 1992).

In 13-week studies, groups of 10 Fischer 344/N rats of each sex were fed diets containing 0, 2500, 5000, 10 000, 20 000 or 40 000 ppm (mg/kg) and 10 $B6C3F_1$ mice of each sex were fed diets containing 0, 5000, 10 000, 20 000, 40 000 or 80 000 ppm HC Yellow No. 4. All rats survived to the end of the studies. Compound-related deaths occurred in male and female mice at the two highest dose levels. Mineralization of the renal papilla occurred in all male rats fed 40 000 ppm. Thyroid pigmentation occurred in rats receiving 40 000 ppm and in 40/46 male mice; a dose-related increase in the incidence of pigmentation was observed in female mice, except for those at the highest dose, most of which died within the first two weeks of the study. The nature of the pigment was not determined. Uterine atrophy occurred in female rats fed 20 000 and 40 000 ppm and in female mice fed 40 000 and 80 000 ppm. Lymphoid depletion and atrophy of the spleen occurred in male mice that received 40 000 or 80 000 ppm and in female mice that received 80 000 ppm. Atrophy of the thymus occurred in male and female mice that received 40 000 or 80 000 ppm (US National Toxicology Program, 1992).

In the two-year studies described above, no compound-related lesion was seen in exposed rats. Male and female mice had a dose-related increase in the incidence of thyroid gland pigmentation and follicular-cell hyperplasia. The predominant impurity, tentatively identified as *N*-(2-hydroxyethyl)-2-hydroxy-4-nitroaniline, was suggested to have contributed to the increased incidence of thyroid follicular-cell hyperplasia (US National Toxicology Program, 1992).

HC Yellow No. 4 was present at a low concentration in semi-permanent hair colouring formulations evaluated in a 13-week study of dermal toxicity in rabbits (Burnett *et al.*, 1976; 0.4%) and in a two-year feeding study in dogs (Wernick *et al.*, 1975; 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 Yellow No. 4 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, 0.3%; see p. 99) and in studies of teratogenesis in rats (Wernick *et al.*, 1975, 0.3%; Burnett *et al.*, 1976, 0.4%) and rabbits (Wernick *et al.*, 1975, 0.3%) (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 Yellow No. 4 (purity > 93%) induced mutation in *Salmonella typhimurium*. In one of the two experiments, in spite of the presence of a precipitate at all doses that induced a significant response, increasing numbers of mutants were observed as increasing amounts of test material were added to the plates. HC Yellow No. 4 induced sex-linked recessive lethal mutation in *Drosophila melanogaster* after injection but not in a feeding experiment in adults. It did not induce reciprocal translocations when injected into the flies.

HC Yellow No. 4 induced sister chromatid exchange in Chinese hamster ovary cells in culture, but equivocal results were obtained for chromosomal aberration in the same cells.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

HC Yellow No. 4 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 Yellow No. 4 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 found in mice. The incidence of adenomas of the pituitary gland was increased in male rats but not in females.

5.4 Other relevant data

HC Yellow No. 4 induced gene mutation in bacteria and in insects. Chromosomal aberrations were not induced in insects, and equivocal results for this end-point were obtained in cultured mammalian cells. Sister chromatid exchange was induced in mammalian cells.

5.5 Evaluation[1]

There is *inadequate evidence* in humans for the carcinogenicity of HC Yellow No. 4.

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

Table 1. Genetic and related effects of HC Yellow No. 4

Test system	Result		Dose[a] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	5.0000[b]	Mortelmans *et al.* (1986)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	(+)	(+)	5000.0000[b]	Mortelmans *et al.* (1986)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	+	+	50.0000[b]	Mortelmans *et al.* (1986)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	50.0000[b]	Mortelmans *et al.* (1986)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	-		10000.0000 feeding	Woodruff *et al.* (1985)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	+		10000.0000 injection	Woodruff *et al.* (1985)
DMH, *Drosophila melanogaster*, reciprocal translocation	-		10000.0000 injection	Woodruff *et al.* (1985)
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	-	167.0000	US National Toxicology Program (1992)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	-	?	3000.0000	US National Toxicology Program (1992)

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

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

[b]Precipitate present in plates

There is *inadequate evidence* in experimental animals for the carcinogenicity of HC Yellow No. 4.

Overall evaluation

HC Yellow No. 4 is *not classifiable as to its carcinogenicity to humans (Group 3).*

6. References

Burnett, C., Goldenthal, E.I., Harris, S.B., Wazeter, F.X., Strausburg, J., Kapp, R. & Voelker, R. (1976) Teratology and percutaneous toxicity studies on hair dyes. *J. Toxicol. environ. Health*, **1**, 1027–1040

Cosmetic, Toiletry, and Fragrance Association (1982) *Directory of Cosmetic and Toiletry Ingredients*, 2nd ed., Washington DC

Cosmetic, Toiletry, and Fragrance Association (1991) *Directory of Cosmetic and Toiletry Ingredients*, 3rd ed., Washington DC

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

Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B. & Zeiger, E. (1986) *Salmonella* mutagenicity tests: II. Results from the testing of 270 chemicals. *Environ. Mutag.*, **8** (Suppl. 7), 1–119

US National Toxicology Program (1992) *Toxicology and Carcinogenesis Studies of HC Yellow No. 4 (CAS No. 59820-43-8) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)* (NTP Tech. Rep. No. 419; NIH Publ. No. 92-3150), 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

Woodruff, R.C., Mason, J.M., Valencia, R. & Zimmering, S. (1985) Chemical mutagenesis testing in *Drosophila*. V. Results of 53 coded compounds tested for the National Toxicology Program. *Environ. Mutag.*, **7**, 677–702