GENERAL REMARKS

This fifty-seventh volume of *IARC Monographs* contains 18 monographs in which the carcinogenicity of 17 chemicals is evaluated. The Working Group prepared a monograph on occupational exposure of hairdressers and barbers and the exposure of users of hair colourants. They also considered both nitro aromatic amines used in hair colouring formulations and benzidine congener-derived azo dyes used as industrial colourants. Four aromatic amines, three of which are used in dyestuff manufacture and have been found as pollutants in the general environment, are also included; the fourth is 4,4′-methylene bis(2-chloroaniline) (MOCA), used principally as a curing agent in certain castable polyurethane products. Previous monographs on 1,4-diamino-2-nitrobenzene (IARC, 1978a), D&C Red No. 9 (IARC, 1975), magenta (IARC, 1974a, 1987a) and MOCA (IARC, 1974b, 1987b) were updated, because new data had become available. In 1982, a working group at IARC surveyed the epidemiological evidence relevant to hair dyes and cancer (IARC, 1982a) and included studies of hairdressers. Since that time, new epidemiological studies have become available, and a monograph was prepared.

The Group noted the lack of quantitative and detailed qualitative information on the potential exposures of hairdressers and barbers and of users of particular hair colouring products. Those groups are potentially exposed to many chemical products (estimated to be over 5000), both during hair treatments, such as shampooing, conditioning, styling and waving, and in the use of skin and nail products. The activities probably include frequent exposures to volatile solvents, propellants, formaldehyde (see IARC, 1982b), methacrylates (see IARC, 1979) and traces of nitrosamines (see IARC, 1978b). The composition of many of the products used by hairdressers has changed gradually with time. For example, some ingredients have been dropped from hair dyes for a variety of reasons, including regulatory activity, technical deficiencies and availability. New materials have been introduced to maintain the range of colours. Examples of this evolution are the declining use of 2-amino-4-nitrophenol and 2-amino-5-nitrophenol and the introduction of their *O*- and *N*-hydroxyalkyl derivatives and certain isomers of the parent compounds.

As has been noted in previous *Monographs*, the present Working Group recognized the importance of data on the purity of the chemicals that were tested in carcinogenicity experiments in animals and in mutagenicity tests but were faced with the fact that many of the compounds considered were of technical grades, varying in purity from relatively high (> 95%) to indeterminate: Magenta, for example, is a mixture of various proportions of three or more components. The chemical analyses that were reported were often limited to identification of the major component of a dye mixture; minor contaminants were usually not addressed. Commercially available chemicals were often used as such, and no attempt was made to purify the compounds further. That the use of low-purity chemicals may lead to

erroneous conclusions about the principal component is an obvious problem, which is commented upon in various parts of this volume.

Studies of experimental animals treated with commercially available hair dyes and laboratory mixtures of hair dyes were also considered. Because of the low levels of individual dyes in the mixtures, however, the results could not be used to evaluate the individual components. Furthermore, the lack of toxicity of the doses used in those studies made them inappropriate for evaluating the systemic toxicity or systemic carcinogenicity of the formulations.

Notes on the metabolism of aromatic amines and azo dyes

In order to be toxic, genotoxic or carcinogenic, aromatic amines usually require metabolic activation. The potential risk posed by structurally related compounds depends largely on their metabolic fate in the test sytem used and on the reactivity of the ultimate carcinogenic reactants. The Working Group noted the absence of pertinent data on the metabolism of a number of the compounds considered. Their metabolic activation and the subsequent formation of reactive metabolites capable of reacting with cellular macromolecules (e.g., nucleic acids and proteins) have not yet been adequately investigated.

Although specific information was not available on the absorption, distribution, metabolism and excretion of many of the aromatic amines in this volume, absorption may occur through the respiratory and gastrointestinal tracts and through the skin of humans. Aromatic amines and amides are readily metabolized enzymatically by oxidation of the ring carbon or of exocyclic nitrogen atoms in both experimental animals and in humans. The *C*-hydroxy metabolites are conjugated with glucuronide or sulfate and excreted in the urine. *N*-Oxidation is a necessary step in the formation of reactive intermediates that can form adducts with nucleic acid bases—mainly guanine and adenine.

Three of the hair dyes were studied within the US National Toxicology Program; these are HC Blue No. 1 (US National Toxicology Program, 1985a), HC Blue No. 2 (US National Toxicology Program, 1985b) and HC Red No. 3 (US National Toxicology Program, 1986), which were chosen because they are all derivatives of 1,4-diamino-2-nitrobenzene (2-nitro-*para*-phenylenediamine) (US National Cancer Institute, 1979) and structurally closely related. The *N*-hydroxyethyl groups on the nitrogens in positions 1 and 4 in HC Blue No. 2 may favour conjugation and urinary excretion, whereas the N-CH_3 group in position 4 of HC Blue No. 1 may favour *N*-oxidation and/or *N*-demethylation. In HC Red No. 3, the primary amino group in position 4 may undergo acetylation. These differences may affect the toxicological properties of the compounds. Comparisons of the results of experiments with HC Red No. 3 with those of the blue dyes are imbalanced, however, by the fact that the blue dyes were given in the diet but HC Red No. 3 was found to be unstable when mixed with feed and was administered by gavage in corn oil.

The route of administration of the dyes may have influenced the results of the studies. When they are administered orally, the dyes are exposed to the bacterial flora of the gastrointestinal tract and, since they are all nitro compounds, could undergo nitroreduction by the anaerobic flora, resulting in the formatiom of aromatic amines that might be absorbed and then subjected to *N*-acetylation and *N*-oxidation in the liver. HC Blue No. 1, for example, was not mutagenic *in vivo* in mice in the micronucleus test, in which compounds are

administered by intraperitoneal injection. That route of administration limits the amount of chemical available for reduction to an aromatic amine by the intestinal flora, a process which may be more efficient than that occurring at other sites, such as the liver.

The industrial dyes CI Acid Red 114 (US National Toxicology Program, 1991), a 3,3′-dimethylbenzidine-derived dye, and CI Direct Blue 15 (US National Toxicology Program, 1992), which is a 3,3′-dimethoxybenzidine-derived dye, are among the five chemicals that were selected for studies of toxicity and carcinogenicity as part of the US National Toxicology Program's Benzidine Dye Initiative, designed to evaluate representative benzidine congeners, benzidine congener-derived dyes and benzidine-derived dyes. The compounds were selected for consideration in this volume of *Monographs* because of the potential for human exposure during the production of bis-azobiphenyl dyes and because benzidine (see IARC, 1972a, 1987c), benzidine-based dyes (see IARC, 1987d) and the benzidine congeners 3,3′-dimethylbenzidine (*ortho*-tolidine) (see IARC, 1972b) and 3,3′-dimethoxybenzidine (see IARC, 1974c, 1987e) are known carcinogens. CI Direct Blue 218 was initially considered by the Working Group, but no monograph was included because of lack of data.

The benzidine congener-based dyes are metabolized to their parent congeners or to their *N*-acetyl derivatives and excreted in the urine. Studies with benzidine congener-based dyes have shown that the ultimate reactive metabolite is an activated form of benzidine congener produced *via* azo reduction. Reductive cleavage of the benzidine congener azo dyes is thought to occur primarily by bacterial action in the intestinal tract (Cerniglia *et al.*, 1982; Bos *et al.*, 1986). Following reductive cleavage, the less polar metabolites are subject to intestinal absorption and further metabolism in the liver. After dogs and rats had been exposed to CI Acid Red 114, 3,3′-dimethylbenzidine was found in their urine (Lynn *et al.*, 1980). The US National Institute for Occupational Safety and Health (1981) reported the presence of 3,3′-dimethylbenzidine in the urine of workers employed in a dye manufacturing plant, who had been in contact with 3,3′-dimethylbenzidine-based dyes but not with 3,3′-dimethylbenzidine itself.

The sequence of benzidine metabolism proceeds along the general known pathways for aromatic amines; it begins with *N*-acetylation, followed by *N*-oxidation to form *N*′-hydroxy-*N*-acetylbenzidine, which can be further activated by *O*-esterification, resulting in electrophilic intermediates that bind to DNA, RNA and proteins (Beland & Kadlubar, 1990).

Postulated association between erythrocytic toxicity and splenic sarcomas in rats

Two aromatic amines and one azo dye considered in this volume were associated with the induction of splenic sarcomas in rats. For several years, it has been known that splenic sarcoma, a rare spontaneous neoplasm, occurs in rats treated with aniline and aniline-related aromatic amines. Since these chemical agents produce haemolytic anaemia with methaemoglobinaemia, a causal relationship has been proposed between the haematotoxicity and the ultimate appearance of splenic sarcomas. Inflammatory fibrosis in the red pulp and capsule occurs prior to the development of sarcomas, and the sarcomas are frequently seen to arise within areas of splenic fibrosis. While several morphological variants of sarcomas have been documented, most of the splenic tumours are well-differentiated fibrosarcomas. A proposed explanation for the pathogenesis of splenic sarcomas is that enhanced splenic haemo-

siderosis or splenic vascular congestion and haemorrhage promotes a cascade of events leading to the development of sarcoma. No time-course or mechanistic studies have been reported formally, and the definitive pathogenesis of these chemically induced splenic sarcomas remains unknown. The observed non-neoplastic and neoplastic splenic changes are typically dose-related, and female rats are more resistant than males, despite a similar degree of induced methaemoglobinaemia. Fischer 344 rats are more sensitive than other strains or stocks, and mice are relatively resistant to the development of splenic sarcomas. Furthermore, many of the chemicals that have been shown to produce sarcomas in the spleen of male Fischer 344 rats have also been shown to produce tumours in other organs, probably by other mechanisms (Ward *et al.*, 1980; Goodman *et al.*, 1984; Weinberger *et al.*, 1985; Bus & Popp, 1987; Chhabra *et al.*, 1990; Stefanski *et al.*, 1990; Chhabra *et al.*, 1991).

While the induction of splenic sarcomas by aniline and aniline-related aromatic amines may be a consequence of erythrocytic toxicity and may depend upon the strain of rat employed, this end-point is nevertheless considered to be a valid indicator of carcinogenic potential.

References

Beland, F.A. & Kadlubar, F.F. (1990) Metabolic activation and DNA adducts of aromatic amines. In: Cooper, C.S. & Grover, P.L., eds, *Chemical Carcinogenesis and Mutagenesis I*, Berlin, Springer Verlag, pp. 267–325

Bos, R.P., van der Krieken, W., Smeijsters, L., Koopman, J.P., de Jonge, H.R., Theuws, J.L.G. & Henderson, P.T. (1986) Internal exposure of rats to benzidine derived from orally administered benzidine-based dyes after intestinal azo reduction. *Toxicology*, **40**, 207–213

Bus, J.S. & Popp, J.A. (1987) Perspectives on the mechanism of action of the splenic toxicity of aniline and structurally-related compounds. *Food Chem. Toxicol.*, **25**, 619–626

Cerniglia, C.E., Freeman, J.P., Franklin, W. & Pack, L.D. (1982) Metabolism of azo dyes derived from benzidine, 3,3′-dimethylbenzidine and 3,3′-dimethoxybenzidine to potentially carcinogenic aromatic amines by intestinal bacteria. *Carcinogenesis*, **3**, 1255–1260

Chhabra, R.S., Thompson, M., Elwell, M.R. & Gerken, D.K. (1990) Toxicity of *p*-chloroaniline in rats and mice. *Food Chem. Toxicol.*, **28**, 717–722

Chhabra, R.S., Huff, J.E., Haseman, J.K., Elwell, M.R. & Peters, A.C. (1991) Carcinogenicity of *p*-chloroaniline in rats and mice. *Food Chem. Toxicol.*, **29**, 119–124

Goodman, D.G., Ward, J.M. & Reichardt, W.D. (1984) Splenic fibrosis and sarcomas in F344 rats fed diets containing aniline hydrochloride, *p*-chloroaniline, azobenzene, *o*-toluidine hydrochloride, 4,4′-sulfonyldianiline, or D & C Red No. 9. *J. natl Cancer Inst.*, **73**, 265–273

IARC (1972a) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, Vol. 1, *Some Inorganic Substances, Chlorinated Hydrocarbons, Aromatic Amines,* N-*Nitroso Compounds and Natural Products*, Lyon, pp. 80–86

IARC (1972b) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, Vol. 1, *Some Inorganic Substances, Chlorinated Hydrocarbons, Aromatic Amines,* N-*Nitroso Compounds and Natural Products*, Lyon, pp. 87–91

IARC (1974a) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, Vol. 4, *Some Aromatic Amines, Hydrazine and Related Substances,* N-*Nitroso Compounds and Miscellaneous Alkylating Agents*, Lyon, pp. 57–64

IARC (1974b) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, Vol. 4, *Some Aromatic Amines, Hydrazine and Related Substances, N-Nitroso Compounds and Miscellaneous Alkylating Agents*, Lyon, pp. 65–71

IARC (1974c) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, Vol. 4, *Some Aromatic Amines, Hydrazine and Related Substances, N-Nitroso Compounds and Miscellaneous Alkylating Agents*, Lyon, pp. 41–47

IARC (1975) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, Vol. 8, *Some Aromatic Azo Compounds*, Lyon, pp. 107–111

IARC (1978a) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man*, Vol. 16, *Some Aromatic Amines and Related Nitro Compounds—Hair Dyes, Colouring Agents and Miscellaneous Industrial Chemicals*, Lyon, pp. 27–37, 73–82

IARC (1978b) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 17, *Some N-Nitroso Compounds*, Lyon

IARC (1979) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 19, *Some Monomers, Plastics and Synthetic Elastomers, and Acrolein*, Lyon, pp. 187–211

IARC (1982a) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 27, *Some Aromatic Amines, Anthraquinones and Nitroso Compounds, and Inorganic Fluorides Used in Drinking-water and Dental Preparations*, Lyon, pp. 307–318

IARC (1982b) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 29, *Some Industrial Chemicals and Dyestuffs*, Lyon, pp. 345–389

IARC (1987a) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, pp. 238–239

IARC (1987b) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, pp. 246–247

IARC (1987c) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, pp. 123–125

IARC (1987d) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, pp. 125–126

IARC (1987e) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, pp. 198–199

Lynn, R.K., Donielson, D.W., Ilias, A.M., Kennish, J.M., Wong, K. & Matthews, H.B. (1980) Metabolism of bisazobiphenyl dyes derived from benzidine, 3,3′-dimethylbenzidine or 3,3′-dimethoxybenzidine to carcinogenic aromatic amines in the dog and rat. *Toxicol. appl. Pharmacol.*, **56**, 248–258

Stefanski, S.A., Elwell, M.R. & Stromberg, P.C. (1990) Spleen, lymph nodes, and thymus. In: Boorman, G.A., Eustis, S.L., Elwell, M.R., Montgomery, C.A., Jr & MacKenzie, W.F., eds, *Pathology of the Fischer Rat, Reference and Atlas*, New York, Academic Press, pp. 369–393

US National Cancer Institute (1979) *Bioassay of 2-Nitro-*p*-phenylenediamine for Possible Carcinogenicity (CAS No. 5307-14-2)* (Tech. Rep. Ser. No. 169; DHEW Publ. No. (NIH) 79-1725), Washington DC, US Government Printing Office

US National Institute of Occupational Safety and Health (1981) Health hazard alert. Benzidine-, *o*-toluidine, and *o*-dianisidine-based dyes. *Am. ind. Hyg. Assoc. J.*, **42**, A-36–A-60

US National Toxicology Program (1985a) *Toxicology and Carcinogenesis Studies of HC Blue No. 1 (CAS No. 2784-94-3) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)* (NTP Technical Report 271; NIH Publ. No. 85-2527), Research Triangle Park, NC

US National Toxicology Program (1985b) *Toxicology and Carcinogenesis Studies of HC Blue No. 2 (CAS No. 33229-34-4) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)* (NTP Technical Report 293; NIH Publ. No. 85-2549), Research Triangle Park, NC

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

US National Toxicology Program (1991) *Toxicology and Carcinogenesis Studies of C.I. Acid Red No. 114 (CAS No. 6459-94-5) in F344/N Rats (Drinking Water Studies)* (NTP Technical Report 405; NIH Publ. No. 92-3136), Research Triangle Park, NC

US National Toxicology Program (1992) *Toxicology and Carcinogenesis Studies of C.I. Direct Blue No. 15 (CAS No. 2429-74-5) in F344/N Rats (Drinking Water Studies)* (NTP Technical Report 397; NIH Publ. No. 92-2852), Research Triangle Park, NC

Ward, J.M., Reznik, G. & Garner, F.M. (1980) Proliferative lesions of the spleen in male F344 rats fed diets containing *p*-chloroaniline. *Vet. Pathol.*, **17**, 200–205

Weinberger, M.A., Albert, R.H. & Montgomery, S.B. (1985) Splenotoxicity associated with splenic sarcomas in rats fed high doses of D & C Red No. 9 or aniline hydrochloride. *J. natl Cancer Inst.*, **75**, 681–690