AURAMINE AND AURAMINE PRODUCTION

1. Exposure Data

1.1 Chemical and Physical Data

1.1.1 *Nomenclature*

Auramine

Chem. Abstr. Serv. Reg. No.: 492–80–8
CAS Name: 4,4'-Carbonimidoylbis[*N*,*N*-dimethylbenzenamine]
Synonyms: C.I. 41000B; C.I. Solvent Yellow 34; 4,4'-dimethylaminobenzophenonimide; 4,4'-(imidocarbonyl)bis(*N*,*N*-dimethylaniline); glauramine; Solvent Yellow 34; yellow pyoctanine

Auramine hydrochloride

Chem. Abstr. Serv. Reg. No.: 2465–27–2
CAS Name: 4,4'-Carbonimidoylbis[*N*,*N*-dimethylbenzenamine], hydrochloride (1:1)
Synonyms: Auramine chloride; 4,4'-carbonimidoylbis[*N*,*N*-dimethylbenzenamine], monohydrochloride; C.I. 41000; C.I. Basic Yellow 2; C.I. Basic Yellow 2, monohydrochloride

Michler's base

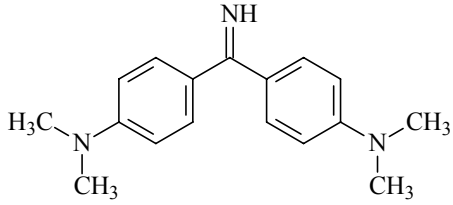
Chem. Abstr. Serv. Reg. No.: 101-6-1
CAS Name: 4,4'-Methylenebis(*N*,*N*-dimethyl)benzenamine
Synonyms: 4,4'-methylenebis(*N*,*N*-dimethyl) aniline; tetramethyldiaminodiphenylmethane; 4,4'-bis(dimethylamino) diphenylmethane; tetra base; methane base; Michler's hydride; Michler's methane
Use: 4,4'-Methylenebis(*N*,*N*-dimethyl)benzenamine (Michler's base) is an intermediate in the synthesis of several organic dyes, e.g., auramine.

Michler's ketone

Chem. Abstr. Serv. Reg. No.: 90-94-8
CAS Name: Bis[4-(dimethylamino)phenyl]methanone.
Synonyms: 4,4'-bis(dimethylamino)benzophenone; *p,p'*-bis(dimethylamino)benzophenone; bis[*p*-(*N,N'*-dimethylamino)phenyl]ketone; tetramethyldiaminobenzophenone
Use: Bis[4-(dimethylamino)phenyl]methanone (Michler's ketone) is a chemical intermediate used in the synthesis of a number of dyes and pigments, particularly auramine derivatives. It is a hydrolysis product of auramine.

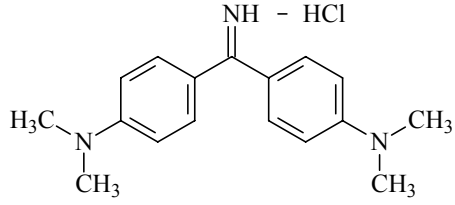
1.1.2 *Structural formula, molecular formula, and relative molecular mass*

Auramine



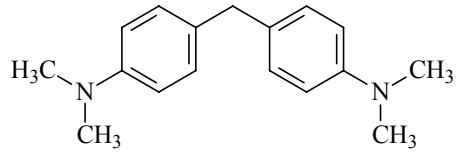
$C_{17}H_{21}N_3$ Rel. mol. mass: 267.37

Auramine hydrochloride



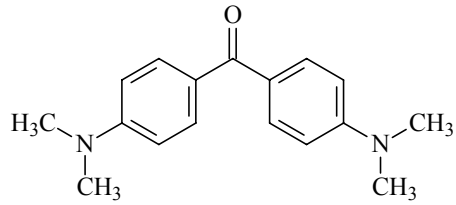
$C_{17}H_{21}N_3.HCl$ Rel. mol. mass: 303.83

Michler's base



$C_{17}H_{22}N_2$ Rel. mol. mass: 254.41

Michler's ketone



$C_{17}H_{20}N_2O$ Rel. mol. mass: 268.36

1.1.3 *Chemical and physical properties of the pure substance [hydrochloride salt]*

Description: Yellow needles from water (Lide, 2008)
Melting-point: 267 °C (Lide, 2008)
Solubility: Slightly soluble in water (Lide, 2008); 10 mg/ml in water; 20 mg/ml in ethanol; 60 mg/ml in ethylene glycol methyl ether (Green, 1990)

1.1.4 *Technical products and trade names*

Trade names for auramine include: Auramine Base; Auramine N Base; Auramine O Base; Auramine OAF; Auramine OO; Auramine SS; Baso Yellow 124; Brilliant Oil Yellow; Orient Oil Yellow 101; and Waxoline Yellow O.

Trade names for auramine hydrochloride include: ADC Auramine O; Aizen Auramine; Aizen Auramine Conc. SFA; Aizen Auramine OH; Aizen Auramine OW 100; Arazine Yellow; Auramine 0–100; Auramine A1; Auramine Extra; Auramine Extra 0–100; Auramine Extra 0–125; Auramine Extra Conc. A; Auramine FA; Auramine FWA; Auramine II; Auramine Lake Yellow O; Auramine N; Auramine O; Auramine ON; Auramine OO; Auramine OOO; Auramine OS; Auramine Pure; Auramine SP; Auramine Yellow; Basic Flavine Yellow O; Basic Light Yellow; Basic Light Yellow O; Basic Yellow 2; Basic Yellow O; Basonyl Yellow120; Calcozine Yellow OX; Flexo Yellow 110; and Mitsui Auramine O.

1.1.5 *Analysis*

Analytical studies on auramine began in the 1970s and continued during the 1980s, by use of high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) to assess auramine levels in shrimp tissue and biological dyes, respectively. Analyses conducted since 1990 use the more sensitive HPLC and mass-spectrometric methods. Table 1.1 presents selected methods of detection and quantification of auramine in various matrices.

Table 1.1. Selected methods of analysis of auramine in various matrices

Sample matrix	Sample preparation	Assay method	Detection limit	Reference
Shrimp tissue	Extract with hexane; remove hexane; extract residue with ethanol; remove ethanol; heat residue with ethanol/ammonia water; shake liquid with acetic acid and with Amberlite LA-2; shake with Amberlite in ethyl acetate; wash ethyl acetate with sodium chloride solution and with 5% ammonia water	HPLC	Not given	Tonogai *et al.* (1983)
Biological dyes	Dissolve dye; elute with n-butanol-acetic acid-water (4:1:5) or n-butanol-ethanol-water (9:1:1)	TLC	Not given	Allison & Garratt (1989)
Ball-point pen inks	A 1-mm section is cut from an ink line drawn on paper; extract with methanol, and analyse	FD-MS	Not given	Sakayanagi *et al.* (1999)
Soybean	Mill auramine-soybean mixture; extract with ethanol using ultrasound; filter; wash filtercake with ethanol; combine ethanol fractions; dilute further with ethanol, and analyse	HPLC	0.25 µg/mL	Luo *et al.* (2005)

FD, field desorption; HPLC, high-performance liquid chromatography; MS, mass spectrometry; TLC, thin-layer chromatography.

1.2 Production and use

1.2.1 *Production*

The diphenylmethane dyes are usually grouped with the triarylmethane dyes. The dyes of the diphenylmethane subclass are ketoimine derivatives, and include auramine O (hydrochloride salt). These dyes are still used extensively for the colouration of paper and in the preparation of pigment lakes (Thetford, 2000).

Auramine and its salts can be manufactured by first heating 4,4'-bis(dimethylaminodiphenyl)methane (Michler's base; CAS No. 101–61–1) with a mixture of urea, sulfamic acid, and sulfur in ammonia at 175°C. The auramine sulfate formed in the reaction may be used directly in the dyeing process or can be converted into auramine base or auramine hydrochloride (auramine O). Highly concentrated solutions for use in the paper industry can be prepared by dissolving auramine base in formamide containing sodium bisulfate. The nitrate and nitrite salts exhibit excellent solubility in alcohols, which facilitates their use in lacquers and flexographic printing colours (Kirsch *et al.*, 1978; Thetford, 2000).

Production of auramine took place first in Europe (Switzerland, Germany, United Kingdom, France), and later also in the USA. Production in these locations has generally been discontinued. Auramine manufacturing is currently mainly located in India and China.

In 1993, approximately 9000 tonnes of basic diphenylmethane and triphenylmethane dyes were sold. Crystal violet, methyl violet, malachite green, auramine, and Rhodamine B are suitable for many purposes and are among the economically most important dyes. Auramine O and its ethyl homologue, ethylauramine, are brilliant yellow dyes with high colour strength. Worldwide annual sales of these dyes are approximately 1000 tonnes (Gessner & Mayer, 2000).

Information was collected from 1996 to 1998 in Europe for the IUCLID database for substances with a production or import volume between 10 and 1000 tonnes/year (Low Production Volume Chemicals (LPVCs)). Auramine hydrochloride was included on the list of LPVCs (Allanou *et al.*, 1999; European Commission, 2008).

The USEPA (2003, 2007) Inventory Update Rule regulation requires manufacturers and importers of certain chemical substances listed in the TSCA Chemical Substance Inventory to report manufacturing information (aggregate production volumes) for chemicals manufactured (including those imported) in amounts of 10 000 pounds or greater (in 1986) or 25 000 pounds or greater (in 2003) at a single site. Table 1.2 presents the aggregate production volumes that were reported for auramine hydrochloride.

Available information indicates that auramine was produced and/or supplied in research quantities in the following countries: Hong Kong Special Administrative Region, India, the People's Republic of China, and the USA (Chemical Sources International, 2008).

Available information indicates that auramine hydrochloride was produced and/or supplied in research quantities in the following countries: Canada, Germany, Hong Kong Special Administrative Region, India, Japan, the Netherlands, Switzerland, the United Kingdom, and the USA (Chemical Sources International, 2008).

Table 1.2. Auramine hydrochloride production volumes

Year	Volume (in thousands of pounds)
1986	10–500
1990	NR
1994	10–500
1998	10–500
2002	NR
2006	NR

NR, not reported

1.2.2 *Use*

Auramine dyes are used for dyeing of leather, jute, tanned cotton, and paints, and as dye components in inking ribbons, ballpoint pastes, oils and waxes, and carbon paper. The most important areas of application are in dyeing paper and in flexographic printing. For the latter, a large number of salts are produced, which have a high solubility in ethanol and ethanol-water mixtures (e.g. nitrates, nitrites, bromides, iodides, salts of alkyl-, aralkyl-, or arylsulfonic acids, thiocyanates, and phosphates). For dyeing paper, solutions of the hydrochloride in organic solvents (e.g., thiodiglycol) are employed, and tartaric and citric acids have been used as stabilizers. The chlorides can also be converted into more easily soluble acetates or propionates to obtain highly concentrated solutions. The hydrogen sulfates of auramine are also readily soluble. Coloured salts based on auramine with tannic acid, phosphomolybdic acid, and preferably phosphotungsto-molybdic acid are used as yellow toner pigments for the development of latent electrostatic images (Fierz-David & Blagey, 1949; Gessner & Mayer, 2000; Thetford, 2000; Varella *et al.*, 2005).

Auramine was used as a fluorescent staining agent to stain acid-fast bacteria in sputum or infected tissue, and also in combination with the dye rhodamine in the Truant auramine-rhodamine stain for *Mycobacterium tuberculosis* (Silver *et al.*, 1966).

Auramine was historically used as a component in brilliantine, a grooming product intended to soften men's hair (Gubéran *et al.*, 1985), particularly in the 1930s, which could have resulted in occupational exposure as well as customer exposure to auramine.

Auramine has also been used in some countries as a food colourant (D'Aquino & Santini, 1977). Auramine has been detected in a small percentage of food samples from India (Tripathi *et al.*, 2007), including fresh peas (Rao & Bhat, 2003). In China, auramine has been detected in bean products (Lin, 2007).

Auramine has been used to colour smoke, in military applications (Department of the Army, 1990) and in firework displays (Shimizu, 1981).

1.3 Occurrence and exposure

1.3.1 *Natural and environmental occurrence*

Auramine is not known to occur as a natural product.

No data have been reported on levels of auramine in environmental matrices such as water and soil.

1.3.2 *Occupational exposure*

The US National Occupational exposure survey (1981–1983) estimated that about 19 000 workers were exposed to auramine. The industries with the largest numbers of exposed workers included the paper and allied products industry, and the health services industry (laboratory workers) (NIOSH, 1990).

The only well-described groups of workers exposed to auramine include British (Case & Pearson, 1954) and German auramine production workers (Kirsch *et al.*, 1978; Thiess *et al.*, 1982). Case reports of bladder cancer among Swiss auramine-production workers have also been published (Müller, 1933). Among a cohort of 4772 laboratory workers, 8% were reported to have been exposed to auramine and its salts (Kauppinen *et al.*, 2003). Exposure measurements in the workplace or biological samples of workers employed in the production of auramine are not available.

The manufacture of auramine involves potential exposure to its process chemicals (e.g., dimethylaniline, formaldehyde, sulfur, ammonium chloride, ammonia, Michler's base), as well as to other chemicals used and produced at the same location (e.g., benzidine, 1-naphthylamine, 2-naphthylamine, magenta, aniline) (Case & Pearson, 1954).

1.4 Regulations and guidelines

1.4.1 *Auramine*

(a) Europe

(i) *Directive 2004/37/EC*

The manufacture of auramine and auramine hydrochloride is regulated by Directive 2004/37/EC (European Commission, 2004a), which applies to activities in which workers are exposed to carcinogens or mutagens of Category 1 or 2. This Directive specifies rules

regarding the employers' obligations of reduction and replacement, prevention and reduction of exposure, unforeseen exposure, foreseeable exposure, access to risk areas, hygiene and individual protection, information for the competent authority, information and training of workers, consultation and participation of workers, health surveillance, record keeping and limit values.

(ii) *Directive 2004/93/EC*

The Commission Directive 2004/93/EC of 21 September 2004 amends the Council Directive 76/768/EEC for the purpose of adapting Annexes II and III thereto to technical progress (European Commission, 2004b). In this Directive, 4,4′-Carbonimidoylbis[*N,N*-dimethylaniline] (auramine) and its salts are listed in Annex II as substances that must not form part of the composition of cosmetic products.

(b) Germany

Deviating from the EU classification, auramine and auramine hydrochloride are classified as Category-2 carcinogens by the MAK Commission. The MAK Commission listed auramine and auramine hydrochloride as substances where percutaneous absorption may significantly contribute to systemic exposure. A MAK value was not set (MAK, 2007).

(c) Japan

The Japan Society for Occupational Health (2007) follows the classification by IARC of auramine in Group 2B.

(d) Other

(i) *GESTIS*

Table 1.3 presents some international limit values for auramine (GESTIS, 2007).

Table 1.3. International limit values (2007) for auramine

Country	Limit value – Eight hours (mg/m^3)	Limit value – Short-term (mg/m^3)	Comments
Austria	0.08 inhalable aerosol	0.32 inhalable aerosol	Technical guidance concentration (based on technical feasibility)
Switzerland	0.08		

1.4.2 *Michler's Base* [CAS No. 101–61–1]

(a) Europe

(i) *Directive 2004/37/EC*

N,N,N′,N′-Tetramethyl-4,4′-methylenedianiline [Michler's base] is regulated by Directive 2004/37/EC (European Commission, 2004a), which applies to activities in which workers are exposed to carcinogens or mutagens of Category 1 and 2. This Directive specifies rules regarding the employers' obligations of reduction and replacement, prevention and reduction of exposure, unforeseen exposure, foreseeable exposure, access to risk areas, hygiene and individual protection, information for the competent authority, information and training of workers, consultation and participation of workers, health surveillance, record keeping and limit values.

(ii) *Directive 2005/90/EC*

In Directive 2005/90/EC, the list of substances classified as carcinogenic, mutagenic or toxic to reproduction (c/m/r) of Directive 76/769/EEC was amended to include *N,N,N′,N′*-tetramethyl-4,4′-methylenedianiline [Michler's base] (European Commission, 2005a).

(iii) *Directive 2005/80/EC*

The Commission Directive 2005/80/EC of 21 November 2005 amends Council Directive 76/768/EEC, concerning cosmetic products, for the purposes of adapting Annexes II and III thereto to technical progress (European Commission, 2005b). In this Directive, *N,N,N′,N′*-tetramethyl-4,4′-methylenedianiline [Michler's base] is listed in Annex II as a substance that must not form part of the composition of cosmetic products.

(b) Germany

4,4'-Methylenebis(*N,N*-dimethylaniline) [Michler's base] is classified as a Category-2 carcinogen by the MAK Commission. The MAK Commission listed 4,4'-methylenebis(*N,N*-dimethylaniline) as a substance for which percutaneous absorption may significantly contribute to systemic exposure. A MAK value was not set (MAK, 2007).

(c) Japan

The Japan Society for Occupational Health (2007) follows the classification by IARC of 4,4'-methylenebis(2-methylaniline) [Michler's base] in Group 2B.

(d) USA

4,4′-Methylenebis(*N,N*-dimethylbenzenamine) [Michler's base] is listed in the NTP *Report on Carcinogens* as *reasonably anticipated to be a human carcinogen* (NTP, 2005).

(e) Other

(i) GESTIS

Table 1.4 presents some international limit values for Michler's base (GESTIS, 2007).

Table 1.4. International limit values (2007) for Michler's base

Country	Limit value – Eight hours (mg/m^3)	Limit value – Short-term (mg/m^3)	Comments
Austria	0.1 inhalable aerosol	0.4 inhalable aerosol	Technical guidance concentration (based on technical feasibility)
Switzerland	0.1 inhalable aerosol		

2. Studies of Cancer in Humans

2.1 Case report

Müller (1933) described two cases of bladder cancer in men occupied in auramine manufacture.

2.2 Cohort studies

Case & Pearson (1954) showed a relatively high incidence of bladder tumours in 238 workers engaged in the manufacture of auramine, with a latent period ranging between 9 and 28 years. Care had been taken to eliminate workers who were recorded as also having been in contact with benzidine, 1-naphthylamine, or 2-naphthylamine. Overall, there were six death certificates mentioning bladder tumours, where only 0.45 would have been expected from the overall male population of England and Wales (SMR 13.3, 95% CI: 4.9–29.0).

A mortality study was conducted among 191 workers who had been employed for > 1 month in auramine production at the Badische Anilin- und Sodafabrik (BASF) in Ludwigshafen (Federal Republic of Germany, FRG) during the period 1932–1976. Subjects were followed-up until death or until 15–08–1976, whichever came first. A total of 20 workers were lost to follow-up. The mortality in the group was compared with those in the populations of Ludwigshafen (1970–1973), in the district of Rheinhessen-Pfalz (1970–1975), and in the FRG (1971–1974). A comparison with another group of workers in the plant could not be made. Among a total of 45 deaths, ten were the result of malignant tumours (proportional mortality ratio 1.35, compared with FRG). The tumours occurred in the bladder ($n = 2$), lung ($n = 2$), prostate ($n = 3$), and stomach ($n = 3$). All ten

cases had been employed before 1950, and six of them before 1940. Co-exposure to 1- and 2-naphthylamine could not be excluded (Kirsch *et al.*, 1978).

A cohort of 703 male and 677 female hairdressers born in or after 1880 who started to run salons in Geneva between 1900 and 1964 was followed-up to the end of 1982. Cause-specific mortality was analysed for the period 1942–1982 using sex-, age- and year-specific death rates for Switzerland as the reference; a significant excess mortality from bladder cancer (observed deaths, 10; expected, 3.9) was found among males. Cancer incidence recorded for the years 1970–1980 showed a significant increase among males for all neoplasms (obs, 65; exp, 51.4), for cancer of the buccal cavity and pharynx (obs, 6; exp, 2.5), for cancer of the prostate (obs, 12; exp, 6.1), and for bladder cancer (obs, 11; exp, 5.3). Among female hairdressers, bladder cancer was observed in two cases, where 1.5 would be expected. It was suggested that the excess in male hairdressers might be related to some colouring agent(s) in brilliantines, which were widely used in men's hairdressing salons in Geneva until about 1950. Auramine was one of the commonly used dyes in brilliantines during the 1930s. However, the impurities of dyes raise the concern that the extra deaths from bladder cancer could have been caused by other agents, e.g., 2-naphthylamine, a known human bladder carcinogen (Gubéran *et al.*, 1985).

3. Studies of Cancer in Experimental Animals

3.1 Auramine

3.1.1 *Oral administration*

(a) Mouse

Thirty mice (15 males, 15 females; strain and age unspecified) were given a diet containing 0.1% of commercial auramine (BDH; purity unspecified) in arachis oil for 52 weeks (estimated total intake, 728 mg per animal), and kept for their life-span. Nineteen mice died before termination of the experiment (90 weeks). In the treated group, seven mice (23%) developed hepatomas and 11 (37%) developed lymphomas, compared with none and five (8%), respectively, in 60 control animals treated with arachis oil only. One subcutaneous sarcoma was also reported in the treated group (Bonser *et al.*, 1956).

A group of thirty stock mice (15 males, 15 females) was given 0.1% auramine, and a group of 27 CBA mice (12 males, 15 females) was given 0.2% auramine (BDH; purity not specified) dissolved in acetone in the diet for 52 weeks (approximate total estimated intake, 1820 mg and 3640 mg per mouse, respectively). In stock mice, four of the seven (57%) males and three of the 10 (30%) females that survived to tumour-bearing age showed hepatomas. No hepatomas were seen in 16 stock control mice. In CBA mice, seven of 12 males (58%) and 11 of 15 females (73%) that survived to tumour-bearing age showed hepatomas. Seven of 90 CBA control mice developed hepatomas (Walpole, 1963).

(b) Rat

Twelve male Wilmslow-Wistar rats were given a diet containing 0.1% of commercial auramine (ICI Ltd; purity not specified) for 87 weeks (estimated total intake, 10 g per rat), followed by normal diet until death. Eleven animals (92%) developed hepatomas between the 91st and the 122nd week after the start of treatment. Twelve control rats were tumour-free at death between 90 and 120 weeks (Williams & Bonser, 1962; Walpole, 1963).

Female Sprague-Dawley rats (age 50–55 days) received a single dose of 150 mg auramine O (source not clear) in sesame oil by oral gavage and were autopsied after six months. No tumours were seen in 19 rats observed. Another group received an oral dose of 80 mg per animal every three days between 40 and 70 days of age (total dose, 800 mg/rat), followed by autopsy after nine months. No tumours were seen in 15 animals observed (Griswold *et al.*, 1966, 1968).

Groups of 40 Sprague-Dawley rats (20 females, 20 males) received technical grade auramine (BASF; purity, 87%) at 0, 50, 100 and 200 ppm in the diet for 24 months. In these four dose groups, tumours (benign and malignant combined) were seen in 6, 13, 8 and 10 male rats, respectively, and in 19, 18, 15 and 19 female rats. The corresponding total numbers of tumours were 12 (one malignant), 15 (none malignant), nine (none malignant), and 10 (two malignant) in male rats, respectively, and 34 (four malignant), 41 (6), 36 (4), and 53 (6) in females, respectively. The tumour induction was not statistically significant (Kirsch *et al.*, 1978).

(c) Other animal species

(i) *Rabbit*

In a preliminary comparative experiment, nine rabbits (strain unspecified) were given auramine (source and purity not stated) orally (dose and dose regimen not given) to the limit of tolerance, and the treatment was continued until the onset of the final illness. Six animals were sacrificed in the first two years, and three between three and four years after the start of treatment. Metaplasia of the urinary tract epithelium, suggestive of pre-cancerous change, was seen in two of five (40%) rabbits examined. No tumours were seen in control and treated animals (Bonser, 1962). [The Working group noted the lack of experimental details.]

(ii) *Dog*

No abnormalities were detected in dogs (strain not specified) given auramine (source and purity not stated) orally (dose not given) daily for about seven years (total ingested amount, 66 g per animal) (Walpole, 1963). [The Working Group noted the lack of description of experimental design and lack of use of controls.]

3.1.2 *Subcutaneous administration*

(a) Rat

Twenty-four male Wilmslow Wistar rats, 8–10 weeks of age, were given subcutaneous injections (0.1 mL per 100 g bw) of a 2.5% suspension of commercial auramine (purity not specified) in arachis oil on five days per week for 21 weeks (estimated total dose, 110–120 mg per animal). In 20 surviving animals, 11 fibrosarcomas (tumour yield: 55%) and three hepatomas (tumour yield: 15%) were observed. Three intestinal carcinomas were also reported (Williams & Bonser, 1962). [The Working Group noted that no control data were given.]

3.2 Michler's base

3.2.1 *Oral administration*

(a) Mouse

A bioassay for the possible carcinogenicity of technical-grade Michler's base was conducted with B6C3F1 mice. Michler's base was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals. The high and low dietary concentrations of Michler's base were, respectively, 2500 and 1250 ppm for the two sexes. Twenty animals of each sex were placed on test as controls. The compound was administered for 78 weeks, followed by an observation period of 13 weeks. There were no significantly positive associations between the concentrations of Michler's base administered and mortality among mice of either sex. Adequate numbers of animals survived sufficiently long to be at risk from late-developing tumours. The mean body weights of dosed mice were significantly lower than those of the controls. There were elevated incidences of hepatocellular adenomas in dosed mice when compared with controls (i.e., 2/20 (10%), 3/50 (6%), and 16/48 (33%) in control, low-dose, and high-dose males, respectively; and 1/19 (5%), 18/49 (37%), and 22/48 (46%) in control, low-dose, and high-dose females, respectively). The incidences of hepatocellular carcinomas in dosed mice did not differ greatly from those in controls. Among both sexes of mice, there was a significant positive association between the concentrations of the chemical administered and the incidences of a combination of hepatocellular adenomas and hepatocellular carcinomas. For male mice, the Fisher exact-test comparisons were not significant; however, for females, both the comparisons of high-dose with control and low-dose with control were significant. Under the conditions of this bioassay, Michler's base was carcinogenic in female B6C3F1 mice, inducing liver neoplasms (National Cancer Institute, 1979a).

(b) Rat

A bioassay for the possible carcinogenicity of technical-grade Michler's base was conducted with Fisher 344 rats. Michler's base was administered in the feed, at either of

two concentrations, to groups of 50 male and 50 female animals. The high and low dietary concentrations of Michler's base were, respectively, 750 and 375 ppm for the two sexes. Twenty animals of each sex were placed on test as controls. The compound was administered for 59 weeks, followed by an observation period of 45 weeks. There were no significantly positive associations between the concentrations of Michler's base administered and mortality among rats of either sex. Adequate numbers of animals survived sufficiently long to be at risk from late-developing tumours. There was slight dose-related mean body-weight depression among female rats, the mean body weight of high-dose male rats was slightly less than that for controls. For both male and female rats, there was a significant positive association between the concentrations of Michler's base administered and the incidences of follicular-cell carcinomas of the thyroid (i.e., 1/18 (6%), 4/50 (8%), and 21/46 (46%) in the control, low-dose, and high-dose males, respectively; and 0/20, 3/46 (7%), and 23/45 (51%) in the control, low-dose, and high-dose females, respectively). The high-dose to control Fisher exact-test comparisons were also significant for each sex. Under the conditions of this bioassay, Michler's base was carcinogenic in Fisher 344 rats, inducing thyroid follicular-cell carcinomas in both males and females (National Cancer Institute, 1979a).

3.3 Michler's ketone

3.3.1 *Oral administration*

(a) Mouse

A bioassay for the possible carcinogenicity of technical-grade Michler's ketone was conducted with B6C3F1 mice. Michler's ketone was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals. The high and low dietary concentrations of Michler's ketone were 2500 and 1250 ppm, respectively, for mice of both sexes. Twenty animals of each sex were placed on test as controls. The compound was administered for 78 weeks. The period of compound administration was followed by an observation period of 13 weeks. There were significant positive associations between the concentrations of Michler's ketone administered and mortality in mice of both sexes. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumours. There was distinct dose-related mean body-weight depression in mice of both sexes. There were significant positive associations between the concentrations of Michler's ketone administered and the incidences of hepatocellular carcinomas in female mice and haemangiosarcomas in male mice. Incidences in hepatocellular carcinomas in females were: 0/19 (P for trend < 0.001), 16/49 (33%), and 38/50 (56%), for increasing doses, respectively. Incidences in haemangiosarcomas in males were: 0/19 (P for trend < 0.001), 5/50 (10%), and 20/50 (40%), for increasing doses, respectively. In all of these cases the high-dose to control Fisher exact-test comparison of incidences was also significant ($P < 0.001$) (National Cancer Institute, 1979b).

(b) Rat

A bioassay for the possible carcinogenicity of technical-grade Michler's ketone was conducted with Fischer 344 rats. Michler's ketone was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals. The high and low dietary concentrations of Michler's ketone were, respectively, 500 and 250 ppm for male rats, and 1000 and 500 ppm for female rats. Twenty animals of each sex were placed on test as controls. The compound was administered for 78 weeks. The period of compound administration was followed by an observation period of 28 weeks for male and high-dose female rats, and 29 weeks for low-dose female rats. There were significant positive associations between the concentrations of Michler's ketone administered and mortality in rats of both sexes. Adequate numbers of animals in all groups survived sufficiently long to be at risk of late-developing tumours. There was distinct dose-related mean body-weight depression in female rats, and the mean body weight among dosed male rats was slightly lower than that in controls. There were significant positive associations between the concentrations of Michler's ketone administered and the incidences of hepatocellular carcinomas in both sexes of rats. Incidences in males were: 0/20 (p for trend < 0.001), 9/50 (18%), and 40/50 (80%) for increasing doses, respectively. Incidences in females were: 0/20 (p for trend < 0.001), 41/47 (87%), and 44/49 (90%), for increasing doses, respectively. In all of these cases the high-dose to control Fisher exact-test comparison of incidences was also significant ($P < 0.001$) (National Cancer Institute, 1979b).

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, elimination

No data were available to the Working Group.

4.2 Genetic and related effects (see Table 4.1 for details)

[Some general comments on the data in Table 4.1: a) results were obtained with commercial ("technical grade") auramine, the grade of purity presumably varying from 65% to 85%; b) purified auramine was tested only in the study by Parodi *et al.* (1982), together with the technical-grade product.]

Commercial preparations of auramine gave a positive outcome in the prophage-induction test in the presence of metabolic activation (Ho & Ho, 1981). They were mutagenic to *Salmonella typhimurium* strains TA98, TA1535, TA1538, and YG10, but only in the presence of metabolic activation. The effect was observed in four of nine studies with TA98 (Parodi *et al.*, 1981; Nagao & Takahashi, 1981; Zeiger *et al.*, 1992; Varella *et al.*, 2005), in one of four studies with TA1535 (Richold & Jones, 1981), in four of six studies with TA1538 (Rowland & Severn, 1981; Richold & Jones, 1981; Simmon

Table 4.1. Genetic and related effects of auramine

Test system	Result[a]		Dose (HID/LED)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Prophage, induct/sos/strand breaks/x-links	NT	+	300 µg/ml	Ho & Ho (1981)
S. typhimurium, forward mutation	NT	+	100 µg/plate	Skopek *et al.* (1981)
S. typhimurium TA100, TA98, TA97, reverse mutation	–	–	100 µg/plate	Brams *et al.* (1987)
S. typhimurium TA100, reverse mutation	NT	–	2000 µg/plate	Parodi *et al.* (1981)
S. typhimurium TA98, reverse mutation	NT	(+)	2000 µg/plate	Parodi *et al.* (1981)
S. typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	NR	Kier *et al.* (1986)
S. typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	125 µg/plate	Simmon (1979a)
S. typhimurium TA100, TA98, reverse mutation	–	NT	NR	Ichinotsubo *et al.* (1981)
S. typhimurium TA100, TA1537, TA98, reverse mutation	–	–	500 µg/plate	Richold & Jones (1981)
S. typhimurium TA100, TA98, reverse mutation	–	–	NR	Venitt & Crofton-Sleigh (1981)
S. typhimurium TA100, reverse mutation	–	–	100/1000 µg/plate[b]	Zeiger *et al.* (1992)

Table 4.1 (contd)

Test system	Result[a]		Dose (HID/LED)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
S.typhimurium TA100, reverse mutation	–	–	1200 µg/plate	Varella *et al.* (2004)
S.typhimurium TA100, reverse mutation	–	–	500 µg/plate	Hakura *et al.* (2005)
S.typhimurium TA1535,TA1538, reverse mutation	–	NT	125 µg/plate	Rosenkranz *et al.* (1976)
S. typhimurium TA1535, TA1538, reverse mutation	–	–	125 µg/plate	Rosenkranz & Poirier (1979)
S. typhimurium TA1535, reverse mutation	–	+	250 µg/plate	Richold & Jones (1981)
S. typhimurium TA1538, reverse mutation	?	+	50 µg/plate	Rowland & Severn (1981)
S. typhimurium TA1538, reverse mutation	–	+	50 µg/plate	Richold & Jones (1981)
S. typhimurium TA1538, reverse mutation	–	+	250 µg/plate	Simmon & Shepherd (1981)
S. typhimurium TA1538, reverse mutation	NT	+	33–667 µg/plate	Zeiger *et al.* (1992)
S. typhimurium TA98, reverse mutation	–	+	250 µg/plate	Nagao & Takahashi (1981)
S. typhimurium TA98, reverse mutation	–	+	100/33 µg/plate[b]	Zeiger *et al.* (1992)
S. typhimurium TA98, reverse mutation	–	+	300 µg/plate	Varella *et al.* (2004)
S. typhimurium YG10, reverse mutation	–	+	150 µg/plate	Varella *et al.* (2004)

Table 4.1 (contd)

Test system	Result[a]		Dose (HID/LED)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
S. typhimurium (other), reverse mutation	–	–	50 µg/plate	Gatehouse (1981)
E. coli (other), reverse mutation	–	–	40 µg/plate	Gatehouse (1981)
Bacillus subtilis, multigene test	?	?	NR	Macgregor & Sacks (1976)
E. coli WP2 *uvrA*, reverse mutation	–	–	NR	Venitt & Crofton-Sleigh (1981)
Saccharomyces, differential tox	+	+	100 µg/ml	Sharp & Parry (1981b)
S. cerevisiae, intrachromosomal recombination	–	NT	1200 µg/ml	Schiestl *et al.* (1989)
S. cerevisiae, deletion assay	+	NT	1200 µg/ml	Schiestl *et al.* (1989)
S. cerevisiae, gene conversion	+	NT	75 µg/ml	Sharp & Parry (1981a)
S. cerevisiae, gene conversion	+	NT	78 µg/ml	Zimmermann & Scheel (1981)
S. cerevisiae, homozygosis	+	+	500 µg/ml	Simmon (1979b)
S. cerevisiae, reverse mutation	–	?	889 µg/ml	Mehta & von Borstel (1981)
D. melanogaster, intrachromosomal recombination	–		134 µg/ml	Consuegra *et al.* (1996)

Table 4.1 (contd)

Test system	Result[a]		Dose (HID/LED)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Wheat, aneuploidy	+	NT	NR	Rédei & Sandhu (1988)
S. cerevisiae, aneuploidy	+	NT	200 µg/ml	Parry & Sharp (1981)
DNA strand breaks/x-links, rat primary hepatocytes	+	NT	0.8 µg/ml	Sina *et al.* (1983)
DNA strand breaks/x-links, rat primary hepatocytes	+	NT	2.67 µg/ml	Martelli *et al.* (1998)
Gene mutation, Chinese hamster lung V79 cells, *Hprt* locus	–	+	401/1069 µg/ml	Fassina *et al.* (1990)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus	NT	(–)	45 µg/ml	Amacher *et al.* (1980)
SCE, Chinese hamster cells *in vitro*	–	+	100 µg/ml	Perry & Thomson (1981)
Micronucleus test, rat hepatocytes *in vitro*	?	NT	8.54 µg/ml	Martelli *et al.* (1998)
Micronucleus test, Syrian hamster embryo cells *in vitro*	+	NT	NR	Fritzenschaf *et al.* (1993)
Cell tranformation, SHE, clonal assay	NT	+	2 µg/ml	Pienta & Kawalek (1981)
Cell tranformation, SHE, clonal assay	–	NT	1 µg/ml	Pienta *et al.* (1977)
DNA strand breaks/x-links, human cell line HuF22 *in vitro*	+	NT	80 µg/ml	Parodi *et al.* (1982)

Table 4.1 (contd)

Test system	Result[a]		Dose (HID/LED)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DNA strand breaks/x-links, human primary hepatocytes	+	NT	4.81 µg/ml	Martelli *et al.* (1998)
Micronucleus test, human hepatocytes *in vitro*	?	NT	8.54 µg/ml	Martelli *et al.* (1998)
Host-mediated assay, microbial cells	–		660 mg/kg	Simmon *et al.* (1979)
DNA strand breaks/x-links, rat liver cells, *in vivo*	+		29.4 mg/kg	Parodi *et al.* (1981)
DNA strand breaks/x-links, rat liver cells *in vivo*	+		9 mg/kg	Brambilla *et al.* (1985)
DNA strand breaks/x-links, rat liver cells *in vivo*	+		125 mg/kg	Martelli *et al.* (1998)
DNA strand breaks/x-links, rat urinary bladder cells *in vivo*	+		125 mg/kg	Martelli *et al.* (1998)
DNA strand breaks/x-links, mouse liver cells *in vivo*	+		80 mg/kg	Sasaki *et al.* (1997)
DNA strand breaks/x-links, mouse kidney cells *in vivo*	(+)		80 mg/kg	Sasaki *et al.* (1997)
DNA strand breaks/x-links, mouse lung cells *in vivo*	(+)		80 mg/kg	Sasaki *et al.* (1997)
DNA strand breaks/x-links, mouse spleen cells *in vivo*	–		80 mg/kg	Sasaki *et al.* (1997)
DNA strand breaks/x-links, mouse bone-marrow cells *in vivo*	–		80 mg/kg	Sasaki *et al.* (1997)
DNA strand breaks/x-links, mouse bone-marrow cells *in vivo*	+		30 mg/kg	Parodi *et al.* (1982)

Table 4.1 (contd)

Test system	Result[a]		Dose (HID/LED)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DNA strand breaks/x-links, rat liver cells *in vivo*	+		7.5 mg/kg	Parodi *et al.* (1982)
DNA strand breaks/X-links, rat liver cells *in vivo*	–		30 mg/kg	Parodi *et al.* (1982)[c]
DNA strand breaks/x-links, rat liver cells *in vivo*	+		300 mg/kg	Kitchin and Brown (1994)
DNA strand breaks/x-links, rat kidney cells *in vivo*	+		15 mg/kg	Parodi *et al.* (1982)
SCE, mouse bone-marrow cells *in vivo*	–		15 mg/kg	Parodi *et al.* (1983)
SCE, mouse bone-marrow cells *in vivo*	–		15 mg/kg	Parodi *et al.* (1982)[c]
SCE, mouse bone-marrow cells *in vivo*	–		15 mg/kg	Parodi *et al.* (1982)
Micronucleus test, mice *in vivo*	–		82 mg/kg	Salamone *et al.* (1981)

In-vitro tests, µg/ml; in-vivo tests, mg/kg bw
[a] +, positive; (+) weakly positive; –, negative; ?, inconclusive; NT, not tested
[b] In the absence/in the presence of an exogenous metabolic system
[c] These data were obtained with purified auramine
HID, highest ineffective dose; LED, lowest effective dose; NR, not reported

& Shepherd, 1981; Zeiger *et al.*, 1992), and in one study with YG10 (Varella *et al.*, 2004). Commercial auramine gave a negative outcome in both the absence and the presence of metabolic activation in eight studies, and in one study in the absence of metabolic activation (Ichinotsubo *et al.*, 1981), with TA100 (Brams *et al.*, 1987; Kier *et al.*, 1986; Simmon, 1979a; Richold & Jones, 1981; Venitt & Crofton-Sleigh, 1981; Zeiger *et al.*, 1992; Varella *et al.*, 2004; Hakura *et al.*, 2005), in three studies with TA1537 (Simmon, 1979a; Kier *et al.*, 1986; Richold & Jones, 1981). It also gave a negative outcome in both the absence and the presence of metabolic activation (Rosenkranz & Poirier, 1979) and in the absence of metabolic activation (Rosenkranz *et al.*, 1976) with TA1535 and TA1538. Auramine induced forward mutation in *S. typhimurium* in the presence of metabolic activation (Skopek *et al.*, 1981). In both the absence and the presence of metabolic activation it was non-mutagenic in two studies with *E. coli* (Venitt & Crofton-Sleigh, 1981; Gatehouse, 1981), and gave equivocal results in the *Bacillus subtilis* multigene test (Macgregor & Sacks, 1976).

In studies with *Saccharomyces cereviasiae*, auramine (technical grade) induced deletions (Schiestl *et al.*, 1989), gene conversion (Sharp & Parry, 1981a; Zimmermann & Scheel, 1981), homozygosis (Simmon, 1979b) and aneuploidy (Parry & Sharp, 1981) in the absence of metabolic activation, but it did not produce intrachromosomal recombination (Schiestl *et al.*, 1989) and reverse mutation (Mehta & von Borstel, 1981). Auramine (technical grade) gave a positive result in the differential toxicity assay, both in the absence and the presence of metabolic activation (Sharp & Parry, 1981b). It induced aneuploidy in wheat (Rédei & Sandhu, 1988). Auramine did not induce intra-chromosomal mitotic recombination in *D. melanogaster* (Consuegra *et al.*, 1996).

In cultured, non-human mammalian cells, both positive and negative results were obtained. Commercial auramine induced DNA strand breaks in primary cultures of rat hepatocytes (Sina *et al.*, 1983; Martelli *et al.*, 1998). In the presence of metabolic activation, mutations were induced at the *Hprt* (Fassina *et al.*, 1990) but not at the *Tk* (Amacher *et al.*, 1980) locus, sister chromatid exchange was induced in Chinese hamster cells (Perry & Thomson, 1981), and morphological transformation in Syrian hamster embryo cells (Pienta & Kawalek, 1981). Cell transformation was also observed in the absence of metabolic activation (Pienta *et al.*, 1977). Micronucleus formation was observed in Syrian hamster embryo cells in the absence of metabolic activation (Fritzenschaf *et al.*, 1993), but not in primary rat hepatocytes (Martelli *et al.*, 1998). Auramine induced DNA strand-breaks in the human cell line HuF22 in the absence of metabolic activation (Parodi *et al.*, 1982), and it caused DNA fragmentation but not micronucleus formation in primary human hepatocytes (Martelli *et al.*, 1998).

In vivo, auramine gave negative results in a host-mediated assay with microbial cells (Simmon *et al.*, 1979). It induced DNA fragmentation in the liver (Parodi *et al.*, 1981; Brambilla *et al.*, 1985; Martelli *et al.*, 1998; Parodi *et al.*, 1982; Kitchin & Brown, 1994), in the kidney (Parodi *et al.*, 1982) and in the urinary bladder of rats (Martelli *et al.*, 1998). In mice, DNA fragmentation was induced in the liver, kidney and lung, but not in the spleen (Sasaki *et al.*, 1997); in the bone marrow, contrasting results were obtained in two

studies (Sasaki *et al.*, 1997; Parodi *et al.*, 1982). In mice, auramine did not induce sister chromatid exchange in bone-marrow cells (Parodi *et al.*, 1982, 1983) and it gave a negative outcome in the micronucleus test (Salamone *et al.*, 1981).

Taken as a whole, the results listed in Table 4.1, obtained with commercial auramine, show that this compound is potentially mutagenic and genotoxic, but only in the presence of an appropriate system of metabolic activation. Importantly, purified auramine was inactive in tests for induction of DNA fragmentation in rat liver and induction of sister chromatid exchange in bone-marrow cells of mice.

5. Summary of Data Reported

5.1 Exposure data

Auramine is manufactured in two steps by reaction of *N*,*N*-dimethylaniline with formaldehyde to form Michler's base, followed by reaction of this intermediate with ammonium chloride and sulfur in the presence of ammonia. This process can lead to auramine that contains Michler's ketone, probably from the hydrolysis of the target dye. Auramine is used as a colourant for paper and inks, to a lesser degree for textiles and leather, in laboratories as a biological stain, and to colour smoke. Auramine is not known to occur in nature. Occupational exposure to auramine can occur during its production or during its use in paper and allied products industries. Auramine has been detected in food samples from India, including fresh peas, and in bean products in China. The production of auramine is prohibited in Europe and the USA; its manufacture continues mainly in India and China.

5.2 Human carcinogenicity data

A single landmark study in the United Kingdom has shown a marked excess of bladder cancer in workers engaged in the manufacture of auramine. Workers exposed to benzidine and β-naphthylamine were excluded. Studies are not available to evaluate the role of pure auramine in carcinogenicity.

5.3 Animal carcinogenicity data

Auramine (technical-grade) was tested for carcinogenicity by oral administration in mice, rats, rabbits and dogs, and by subcutaneous injection in rats. Following its oral administration, it induced hepatomas and lymphomas in mice and hepatomas in rats. The studies in rabbits and dogs were inadequate for evaluation. After subcutaneous injection in one study in rats, it induced local sarcomas. In a well-designed study, dietary administration of Michler's ketone caused an increased incidence of hepatocellular

carcinomas in male and female rats and female mice, and of hemangiosarcomas in male mice. In a well-designed study, dietary administration of Michler's base caused increased incidence of hepatocellular carcinoma and adenomas in mice and follicular-cell carcinomas of the thyroid in rats.

5.4 Other relevant data

There are no data on the toxicokinetics of auramine.

In genotoxicity tests, purified auramine was used in one study only, along with the technical-grade product. The purified sample [composition not analysed] was inactive in inducing in-vivo DNA fragmentation in rat liver and sister chromatid exchange in mouse bone-marrow cells. All other data on mutagenicity and genotoxicity of auramine were obtained with commercial preparations of the compound with varying degrees of purity, which presumably was never higher than 85%. This may explain the variability of the results reported.

Taken as a whole, the results show that commercial auramine is potentially mutagenic and genotoxic both in bacteria and cultured cells in the presence of metabolic activation. In one study, purified auramine was inactive in a test to assess DNA fragmentation.

In in-vivo experiments in rats and mice, the technical-grade auramine induced liver-DNA fragmentation in four of five experiments; it was also positive in this assay in urinary bladder cells of the rat, weakly positive in kidney and lung cells of the mouse, but negative in mouse spleen and bone-marrow cells.

6. Evaluation

6.1 Cancer in humans

There is *sufficient evidence* in humans for the carcinogenicity of auramine production. Auramine production causes bladder cancer in humans.

There is *inadequate evidence* in humans for the carcinogenicity of auramine.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of auramine, technical grade.

There is *sufficient evidence* in experimental animals for the carcinogenicity of Michler's ketone.

There is *sufficient evidence* in experimental animals for the carcinogenicity of Michler's base.

6.3 Overall evaluation

Auramine production is *carcinogenic to humans (Group 1)*.
Auramine is *possibly carcinogenic to humans (Group 2B)*.
Michler's ketone is *possibly carcinogenic to humans (Group 2B)*.

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