

## 2,4-DIAMINOANISOLE AND ITS SALTS

This substance was considered by previous working groups, in 1977 (IARC, 1978), 1981 (IARC, 1982) and 1987 (IARC, 1987). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

### 1. Exposure Data

#### 1.1 Chemical and physical data

##### 1.1.1 Nomenclature

#### 2,4-Diaminoanisole

*Chem. Abstr. Serv. Reg. No.:* 615-05-4

*Chem. Abstr. Name:* 4-Methoxy-1,3-benzenediamine

*IUPAC Systematic Name:* 4-Methoxy-*meta*-phenylenediamine

*Synonyms:* 3-Amino-4-methoxyaniline; C.I. 76050; C.I. Oxidation Base 12; 1,3-diamino-4-methoxybenzene; 4-methoxy-1,3-phenylenediamine; *para*-methoxy-*meta*-phenylenediamine

#### 2,4-Diaminoanisole sulfate

*Chem. Abstr. Serv. Reg. No.:* 39156-41-7

*Chem. Abstr. Name:* 4-Methoxy-1,3-benzenediamine, sulfate

*IUPAC Systematic Name:* 4-Methoxy-*meta*-phenylenediamine, sulfate

*Synonyms:* CI 76051; CI oxidation base 12A; 2,4-DAA sulfate; 2,4-diaminoanisole, hydrogen sulfate; 2,4-diaminoanisole sulphate; 1,3-diamino-4-methoxybenzene sulphate; 2,4-diamino-1-methoxybenzene sulphate; 4-methoxy-1,3-benzenediamine sulfate (1:1); 4-methoxy-1,3-benzenediamine sulphate; 4-methoxy-*meta*-phenylenediamine sulfate; *para*-methoxy-*meta*-phenylenediamine sulphate; 4-methoxy-*meta*-phenylenediammonium sulphate

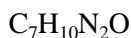
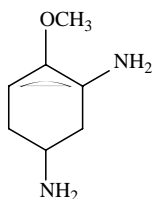
**2,4-Diaminoanisole dihydrochloride**

*Chem. Abstr. Serv. Reg. No.:* 614-94-8

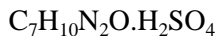
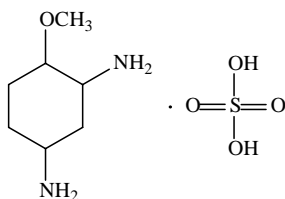
*Chem. Abstr. Name:* 4-Methoxy-1,3-benzenediamine, dihydrochloride

*IUPAC Systematic Name:* 4-Methoxy-*meta*-phenylenediamine, dihydrochloride

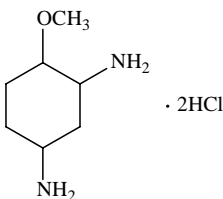
*Synonyms:* 2,4-Diaminoanisole hydrochloride

1.1.2 *Structural and molecular formulae and relative molecular masses*

Relative molecular mass: 138.17



Relative molecular mass: 236.25



Relative molecular mass: 211.07

1.1.3 *Chemical and physical properties of the pure substances***2,4-Diaminoanisole**

(a) *Description:* Needles from diethyl ether (Budavari, 2000)

(b) *Melting-point:* 67.5 °C (Lide & Milne, 1996)

(c) *Spectroscopy data:* Infrared [prism (2971), grating (15399)], ultraviolet (855) and nuclear magnetic resonance [proton (10617)], and mass spectral data have been reported (Sadler Research Laboratories, 1980; Lide & Milne, 1996).

- (d) *Solubility*: Soluble in diethyl ether and ethanol (Lide & Milne, 1996)
- (e) *Stability*: Darkens on exposure to light (Budavari, 2000)

### **2,4-Diaminoanisole sulfate**

- (a) *Description*: Off-white to violet powder (Budavari, 2000)
- (b) *Solubility*: Soluble in water and ethanol (Budavari, 2000)
- (c) *Spectroscopy data*: Infrared [prism/grating (51021)], ultraviolet (26381) and nuclear magnetic resonance [proton (23809)] data have been reported (Sadtler Research Laboratories, 1980).

### **2,4-Diaminoanisole dihydrochloride**

- (a) *Description*: Crystalline powder (TCI America, 2000)
- (b) *Solubility*: Soluble in water (TCI America, 2000)

#### 1.1.4 *Technical products and impurities*

Trade names for 2,4-diaminoanisole include Furro L, Pelagol DA, Pelagol Grey L and Pelagol L.

Trade names for 2,4-diaminoanisole sulfate include BASF Ursol SLA, Durafur Brown MN, Fouramine BA, Fournine 76, Fournine SLA, Furro SLA, Nako TSA, Pelagol BA, Pelagol Grey, Pelagol Grey SLA, Pelagol SLA, Renal SLA, Ursol SLA and Zoba SLE.

#### 1.1.5 *Analysis*

Methods for the analysis of aromatic amines, including 2,4-diaminoanisole, in inks of ball-point and fibre-tip pens and watercolour paints, oxidative hair dyes, dyestuff mixtures and in paper, coloured textiles and leather products have been reported. These methods include differential pulse voltammetry, gas chromatography–mass spectrometry with mass ion detection, thin-layer chromatography, high-performance thin-layer chromatography and high-performance liquid chromatography with ultraviolet, diode-array or mass spectrometry detection (Bernabei *et al.*, 1980; Johansson *et al.*, 1981; Liem & Rooselaar, 1981; Mancini *et al.*, 1981; Gottschalck & Machens, 1982; Ohshima *et al.*, 1982; Hoogewijs & Massart, 1983; Sardas *et al.*, 1985; Andrisano *et al.*, 1994, 1995; Friedrichs *et al.*, 1995; Verdú *et al.*, 1996; Winkeler, 1996; Bürgi *et al.*, 1997; Verdú *et al.*, 1997; Anon., 1998a,b; Bürgi *et al.*, 1998; Chen *et al.*, 1998; Mayer *et al.*, 1998; Planelles *et al.*, 1998; Štancer & Jeretin, 1998; Tomaselli *et al.*, 1998; Wang & Chen, 1998; Cioni *et al.*, 1999; Kellert *et al.*, 1999; Planelles *et al.*, 1999; Sinha & Kumar, 1999; Xiao *et al.*, 1999; Yang *et al.*, 2000).

## 1.2 Production

2,4-Diaminoanisole was first prepared in 1913 by the reduction of 2,4-dinitroanisole with iron and acetic acid (Richter, 1933; Budavari, 2000).

Information available in 2000 indicated that 2,4-diaminoanisole sulfate was manufactured by one company in China (CIS Information Services, 2000).

## 1.3 Use

2,4-Diaminoanisole and its sulfate salt have been used in the preparation of dyes, especially hair and fur dyes, as an intermediate in the production of C.I. Basic Brown 2 and as a corrosion inhibitor for steel (Budavari, 2000). It was used extensively in permanent, oxidative hair dyes until the late 1970s (IARC, 1993).

## 1.4 Occurrence

### 1.4.1 Occupational exposure

According to the 1981–83 National Occupational Exposure Survey (National Institute for Occupational Safety and Health, 2000), about 23 000 workers in the USA were potentially exposed to 2,4-diaminoanisole or its sulfate salt. They were all hairdressers or cosmetologists. The National Institute for Occupational Safety and Health (1978) estimated in the 1970s that as many as 400 000 workers were potentially exposed to 2,4-diaminoanisole in the USA. Hairdressers and cosmetologists comprised most of this group; a relatively small number of fur dyers were probably exposed to higher concentrations. According to the Finnish Register of Employees Exposed to Carcinogens, no workers were exposed to 2,4-diaminoanisole in Finland in 1997 (Savela *et al.*, 1999).

### 1.4.2 Environmental occurrence

No data were available to the Working Group.

## 1.5 Regulations and guidelines

No occupational exposure limits for 2,4-diaminoanisole have been established. It is classified as a carcinogen in several countries including Finland, Germany, Sweden, Switzerland and the USA (American Conference of Governmental Industrial Hygienists, 2000; Deutsche Forschungsgemeinschaft, 2000; UNEP, 2000).

The former European Community (now the European Union) stated that, effective in 1978, ‘2,4-diaminoanisole (and its salts) must not form part of the composition of cosmetic products; and Member States should prohibit the marketing of cosmetic products containing 2,4-diaminoanisole (and its salts)’ (UNEP, 2000).

## 2. Studies of Cancer in Humans

Although hairdressers and barbers and similar occupational groups as well as users of hair dyes may be exposed to 2,4-diaminoanisole, exposure to this compound itself has not been evaluated in epidemiological studies of cancer risk. The evidence for a carcinogenic risk of occupational and personal exposure to hair dyes was reviewed in a previous *IARC Monographs* volume (IARC, 1993). The risks for haematopoietic neoplasms and lymphomas were addressed in two recent papers (Correa *et al.*, 2000a,b).

## 3. Studies of Cancer in Experimental Animals

### 3.1 Oral administration

*Mouse:* Groups of 50 male and 50 female B6C3F<sub>1</sub> mice, 6 weeks of age, were fed diets containing 1200 or 2400 mg/kg diet technical-grade 2,4-diaminoanisole sulfate (of indeterminate purity, with at least one impurity detected by thin-layer chromatography) for 78 weeks and were observed for a further 18–19 weeks. Groups of 50 animals of each sex served as matched controls for each concentration group. The mean body-weight gains of treated and control animals were similar throughout the study, and the survival rates were comparable among treated and control mice: by the end of the study, 84, 78, 92 and 82% of males and 74, 76, 76 and 78% of females were still alive in the low-concentration control, high-concentration control, low-concentration and high-concentration groups, respectively. Among the males, follicular-cell adenomas of the thyroid were seen in 1/47 low-concentration controls, 0/40 high-concentration controls, 0/46 at the low concentration and 11/45 at the high concentration ( $p < 0.001$ ); one male at the low concentration had a follicular-cell carcinoma. Follicular-cell hyperplasia was found in 12/45 males at the high concentration. Among the females, follicular-cell adenomas were found in 0/43 low-concentration controls, 0/41 high-concentration controls, 0/42 at the low concentration and 6/45 at the high concentration ( $p = 0.017$ ); follicular-cell carcinomas were found in 2/45 at the high concentration; and follicular-cell adenomas and carcinomas combined were found in 8/45 at the high concentration ( $p = 0.004$ ). Thyroid hyperplasia occurred in 11/42 females at the low concentration (National Cancer Institute, 1978). [The Working Group noted that no explanation was provided for having matched controls for each concentration group.]

*Rat:* Groups of 50 male and 50 female Fischer 344 rats, 6 weeks of age, were fed diets containing technical-grade 2,4-diaminoanisole sulfate (same sample as used above) at a concentration of 5000 mg/kg for 78 weeks or 1250 mg/kg of diet for 10 weeks and 1200 mg/kg of diet for 68 weeks, followed by a 29-week observation period. Groups of 50 (49 for the high-concentration male controls) animals of each sex served as matched controls for each concentration group. The mean body-weight gains of male

and female rats at the high concentration were lower than those of controls throughout most of the study. The mortality rates of the treated and control male rats were similar by the end of the study: 54, 61, 60 and 54% of the animals were still alive in the low- and high-concentration control and treated groups, respectively. The female rats showed a significantly accelerated mortality rate, in particular at the high dietary concentration of the chemical, with 46, 74, 58 and 44% of the animals alive in low- and high-concentration control and low- and high-concentration treated groups, respectively. Malignant thyroid follicular-cell tumours were found in 2/35 low-concentration male controls, 0/48 high-concentration male controls, 2/47 at the low concentration and 17/49 at the high concentration ( $p = 0.001$ ) and in 2/38 low-concentration female controls, 1/45 high-concentration female controls, 1/46 at the low concentration and 10/49 at the high concentration ( $p = 0.006$ ). Eight males and three females at the high concentration but none of the controls had multiple follicular-cell tumours. The incidence of tumours of thyroid C-cell origin (adenomas or carcinomas) was significantly increased in male rats, with 1/35 in low-concentration male controls, 1/48 in high-concentration male controls, 4/47 in males at the low concentration and 10/49 at the high concentration ( $p = 0.004$ ), but not in female rats. In males, squamous-cell carcinomas, basal-cell carcinomas or sebaceous adenocarcinomas of the skin were found in 0/36 low-concentration controls, 0/48 high-concentration controls, 2/48 at the low concentration and 7/49 at the high concentration ( $p = 0.007$ ). Preputial or clitoral gland adenomas, papillomas or carcinomas were found in 0/36 low-concentration control males, 0/48 high-concentration control males, 2/48 at the low concentration, 8/49 at the high concentration ( $p < 0.003$ ) and in 0/39 low-concentration female controls, 3/50 high-concentration female controls, 5/49 at the low concentration ( $p = 0.049$ ) and 8/49 at the high concentration. In the Zymbal gland, squamous-cell carcinomas or sebaceous adenocarcinomas were found in 0/36 low-concentration male controls, 0/48 high-concentration male controls, 1/48 at the low concentration and 8/49 at the high concentration ( $p = 0.003$ ); and sebaceous adenocarcinomas were found in 0/39 low-concentration female controls, 0/50 high-concentration female controls, 0/49 at the low concentration and 7/49 at the high concentration ( $p = 0.006$ ) (National Cancer Institute, 1978). [The Working Group noted that no explanation was provided for having matched controls for each concentration group.]

Groups of 40–60 female Fischer 344 rats, 6 weeks of age, were fed a diet containing 2,4-diaminoanisole sulfate [purity not stated] at a concentration of 0 (control), 1200, 2400 or 5000 mg/kg for up to 82–86 weeks. Another 15 rats were fed a diet containing 5000 mg/kg for 10 weeks and were observed up to about 87 weeks. The mean body weights of rats at the high concentration were lower than those of controls. By 87–94 weeks, follicular-cell adenomas or carcinomas of the thyroid were found in 0/37 controls, 0/47 at the low concentration, 2/33 at the intermediate concentration and 28/40 at the high concentration (21 with adenomas and seven with carcinomas) and in 1/12 rats treated for 10 weeks. Mammary adenocarcinomas were found in 0/37 controls, 0/47 at the low concentration, 5/33 at the intermediate concentration and 3/40 at the high concentration; mammary adenomas were found in only 1/33 rats at the intermediate

concentration and 1/47 at the low concentration. Carcinomas (squamous- or sebaceous-cell or mixed) of the clitoral gland were found in 0/37 controls, 8/47 at the low concentration, 15/33 at the intermediate concentration, 9/40 at the high concentration and 1/12 rats treated for 10 weeks (Evarts & Brown, 1980). [The Working Group noted that no statistical analysis was provided.]

### 3.2 Skin application

Two studies, one in mice (Burnett *et al.*, 1975) and one in rats (Kinkel & Holzmann, 1973), in which 2,4-diaminoanisole sulfate was applied by skin painting, could not be evaluated because the test agent represented a mixture of compounds as a hair-dye formulation.

### 3.3 Administration with known carcinogens or modifying factors

*Rat:* In an evaluation of the promoting effect of 2,4-diaminoanisole sulfate on thyroid carcinogenesis, groups of 21 male Wistar rats, 7 weeks of age, were given an intraperitoneal injection of 2.1 g/kg bw *N*-nitrosobis(2-hydroxypropyl)amine (NBHPA) in water at the start of the study followed by a diet containing 0.5% 2,4-diaminoanisole sulfate for 19 weeks; other groups either received the intraperitoneal injection of NBHPA with no 2,4-diaminoanisole sulfate, the diet containing 2,4-diaminoanisole sulfate with no NBHPA or a control diet. The total observation period was 20 weeks. 2,4-Diaminoanisole sulfate alone did not cause thyroid tumours, but NBHPA alone caused thyroid follicular-cell adenomas in 6/21 (28%) rats and carcinomas in 1/21 (4%) rats. The combination of NBHPA and 2,4-diaminoanisole sulfate increased the incidence of thyroid adenomas to 20/21 (95%) and that of carcinomas to 9/21 (42%) ( $p < 0.05$ ;  $\chi^2$  test). The incidence of hyperplasia of the thyroid follicular epithelium was also increased in the group given the combination (Kitahori *et al.*, 1989).

In a study to assess the synergistic effect of three thyroid carcinogens, 2,4-diaminoanisole sulfate, *N,N'*-diethylthiourea (see monograph in this volume) and 4,4'-thiodianiline, groups of 20–21 male Fischer 344/Crj rats, 6 weeks of age, were fed a diet containing 610 mg/kg 2,4-diaminoanisole sulfate for 52 weeks alone or in combination with 200 mg/kg *N,N'*-diethylthiourea and 46 mg/kg 4,4'-thiodianiline. After 52 weeks of treatment, the rats were killed, necropsied and evaluated for tumour incidences. 2,4-Diaminoanisole sulfate alone did not induce thyroid tumours but significantly ( $p < 0.01$ ) increased the incidences of thyroid follicular-cell tumours caused by the combination of the other two agents. 2,4-Diaminoanisole sulfate did not induce liver tumours or lung tumours but may have increased the incidences of these tumours produced by 4,4'-thiodianiline (Hasegawa *et al.*, 1991). [From the study design, the Working Group concluded that it was not possible to assess the synergistic effect of 2,4-diaminoanisole

sulfate, if any, on the incidence of thyroid gland tumours induced by *N,N'*-diethylthiourea and/or 4,4'-thiodianiline.]

## 4. Other Data Relevant to an Evaluation of Carcinogenicity and Its Mechanisms

### 4.1 Absorption, distribution, metabolism and excretion

#### 4.1.1 *Humans*

After application of [<sup>14</sup>C]2,4-diaminoanisole at 4 µg/cm<sup>2</sup> (3–15 cm<sup>2</sup> per individual [exact number of individuals not given]) to the ventral forearm of male volunteers for 24 h, the skin penetration was estimated to be 3.9 ± 0.9%, as determined by excretion of radiolabel in the urine (Marzulli *et al.*, 1981).

#### 4.1.2 *Experimental systems*

After application of [<sup>14</sup>C]2,4-diaminoanisole at 4 µg/cm<sup>2</sup> (3–15 cm<sup>2</sup> per animal [exact number of animals not given]) to the abdomen of male and female rhesus monkeys (*Macaca mulatta*) for 24 h, the skin penetration was estimated to be 4.7 ± 4.3%, as determined by excretion of radiolabel in the urine (Marzulli *et al.*, 1981). Dermal absorption of [<sup>14</sup>C]2,4-diaminoanisole from three hair-dye formulations containing 0.6–1.8% by female Sprague-Dawley rats varied from 0.26 to 1.1% of the administered dose (Hofer & Hruby, 1983).

After intraperitoneal injection of 50 mg/kg bw [<sup>14</sup>C]2,4-diaminoanisole to rats, 85% of the radiolabel was excreted in the urine and 9% in the faeces after 48 h. The major metabolites were 4-acetylamino-2-aminoanisole, 2,4-diacetylaminoanisole and 2,4-diacetylaminophenol, and were excreted in the urine both free and as glucuronides and sulfates (Grantham *et al.*, 1979). After administration by gavage of [<sup>14</sup>C]2,4-diaminoanisole [dose not specified] to 18 male and female Sprague-Dawley rats, 49.9 ± 6.9 and 52.1 ± 4.8% of the applied dose were recovered in the urine and faeces, respectively, over 5 days. As 5.6 ± 1.7% of the orally administered radiolabel was eliminated in the bile within 3 h, the radiolabel found in the faeces might have originated from absorbed material (Hofer & Hruby, 1983).

The metabolism and covalent binding of [<sup>14</sup>C]2,4-diaminoanisole to cellular macromolecules *in vitro* and *in vivo* were shown to be cytochrome P450-dependent. Incubation of rat liver and kidney microsomes with radiolabelled 2,4-diaminoanisole in the presence of NADPH and oxygen led to the formation of products that were bound covalently to microsomal protein. Inhibitors of cytochrome P450 enzymes *in vivo* and *in vitro* decreased the binding; pretreatment with phenobarbital increased binding; and pretreatment with β-naphthoflavone had no effect. More [<sup>14</sup>C-ring]-labelled than [<sup>14</sup>C-



methyl]-labelled 2,4-diaminoanisole was bound; when the hydrogens in the methyl group were replaced by deuterium, both the binding and the mutagenicity of 2,4-diaminoanisole increased. Liver microsomes catalysed irreversible binding to endogenous microsomal RNA; no binding to purified calf thymus DNA was detected (Dybing *et al.*, 1979a). When 10–200 mg/kg bw [<sup>3</sup>H]2,4-diaminoanisole were injected into rats, the label was bound covalently to liver and kidney proteins. No covalent binding to hepatic RNA or DNA was detected (Dybing *et al.*, 1979b).

#### 4.1.3 *Comparison of animals and humans*

The amount of 2,4-diaminoanisole absorbed after dermal application is of the same order of magnitude in humans, monkeys and rats. No data were available on metabolism in humans to allow a comparison with data from experiments with rats.

## 4.2 **Toxic effects**

### 4.2.1 *Humans*

No data were available to the Working Group.

### 4.2.1 *Experimental systems*

The oral LD<sub>50</sub> of 2,4-diaminoanisole sulfate in an oil-in-water emulsion in rats was > 4000 mg/kg bw; the intraperitoneal LD<sub>50</sub> of a solution in dimethyl sulfoxide was 372 mg/kg bw (Burnett *et al.*, 1977).

Male and female B6C3F<sub>1</sub> mice and Fischer 344 rats were fed diets containing 0.075–0.58% 2,4-diaminoanisole sulfate for 4 weeks, followed by a 2-week observation period. One male rat each at 0.075, 0.125 and 0.58% and one male mouse at the highest dietary concentration died. No gross abnormalities were noted in either rats or mice (National Cancer Institute, 1978).

In a study of the effects of 2,4-diaminoanisole sulfate on thyroid function, groups of 15 male Wistar rats were given the compound in the diet at a concentration of 0.5% or were painted daily with a 5% solution on a 5 × 4-cm area of dorsal skin for up to 6 weeks. Five rats from each group were killed at weeks 1, 3 and 6. The mean thyroid weight of rats fed 2,4-diaminoanisole sulfate was significantly increased (60%) from week 1, and was markedly greater (2.5-fold) than the control value at week 6. In the group treated in the diet, the mean serum concentration of thyroid-stimulating hormone was markedly higher than that in the control group at weeks 1 and 3 (10- and 16-fold, respectively), but was only slightly elevated (2.5-fold) at week 6. Similarly, reductions in the serum concentrations of thyroxine and triiodothyronine noted at earlier times were less pronounced at week 6. In contrast to dietary administration, cutaneous application of 2,4-diaminoanisole sulfate did not have a significant effect on thyroid organ weight or function (Kitahori *et al.*, 1989).

In an experiment in which groups of five male Iva:Siv50 rats were treated with 2,4-diaminoanisoole sulfate at a dietary concentration of 0.25% for up to 8 weeks, the serum concentration of thyroid-stimulating hormone was increased by 68% after 1 week and that of triiodothyronine was decreased at weeks 1, 2, 4 and 8 (Zbinden, 1988).

### **4.3 Reproductive and prenatal effects**

#### *4.3.1 Humans*

No information was available on persons exposed to 2,4-diaminoanisoole alone. The evidence for reproductive disorders due to exposure of hairdressers to chemicals was evaluated from a literature review for the years 1985–93. Associations with menstrual disorders and spontaneous abortions were found in some epidemiological studies on hairdressers, but no association was found in other studies. It was concluded that there is little evidence for an increased incidence of reproductive disorders among hairdressers. None of the evidence related specifically to 2,4-diaminoanisoole (Kersemaekers *et al.*, 1995).

#### *4.3.2 Experimental systems*

No studies were available in which 2,4-diaminoanisoole was tested alone.

Three commercially available hair-dye formulations, containing 0.02, 2 or 4% 2,4-diaminoanisoole sulfate and several aromatic amine derivatives among their constituents, were tested for teratogenicity in groups of 20 mated female Charles River CD rats. Each formulation was mixed with an equal volume of hydrogen peroxide and applied topically to a shaved site on the dorsoscapular region at a dose of 2 mL/kg bw on days 1, 4, 7, 10, 13, 16 and 19 of gestation. The dams were killed on day 20 of gestation. There was no significant increase in the incidence of soft-tissue anomalies in the living fetuses, but minor skeletal changes were seen in nine of 169 live fetuses in three of 20 litters of dams given the formulation containing the highest concentration of 2,4-diaminoanisoole sulfate (4%). On comparison with the three negative control groups, this finding was found to be statistically significant ( $p < 0.05$  to  $p < 0.01$ ) but was considered by the authors not to be biologically significant (Burnett *et al.*, 1976).

### **4.4 Effects on enzyme induction/inhibition and gene expression**

No data were available to the Working Group.

### **4.5 Genetic and related effects**

#### *4.5.1 Humans*

No data were available to the Working Group.

#### 4.5.2 *Experimental systems* (see Table 1 for references)

The results obtained with 2,4-diaminoanisole sulfate trihydrate and with 2,4-diaminoanisole dihydrochloride are listed separately, but this distinction is not made in the summary of the results given below.

##### (a) *DNA damage*

2,4-Diaminoanisole induced DNA double-strand breaks in primary rat hepatocytes in culture and in liver cells of rats. No change in DNA viscosity was seen in liver cells of rats that received 2,4-diaminoanisole by intraperitoneal injection. DNA damage was produced in liver and brain, but not stomach, colon, kidney, bladder, lung or bone-marrow cells of mice as measured in the Comet assay.

2,4-Diaminoanisole induced unscheduled DNA synthesis in HeLa cells with and without S9.

##### (b) *Mutation and allied effects*

2,4-Diaminoanisole caused frameshift mutations in *Salmonella typhimurium* in the presence of exogenous metabolic activation from liver microsomes from uninduced and induced mice, rats and rabbits and from humans. It produced gene mutations and chromosomal aberrations in rodent cells *in vitro*.

2,4-Diaminoanisole was metabolized to mutagenic products by ram seminal vesicle microsomes or purified prostaglandin H synthase (Robertson *et al.*, 1983; Sarkar *et al.*, 1992). The reaction product of 2,4-diaminoanisole and hydrogen peroxide was mutagenic in *Salmonella* in the presence and absence of an exogenous metabolic activation system (Watanabe *et al.*, 1989).

2,4-Diaminoanisole induced mutation and mitotic recombination in *Saccharomyces cerevisiae*. It was not mutagenic to *Neurospora crassa* in a single study.

2,4-Diaminoanisole did not transform Syrian hamster embryo cells in culture in a single study.

Urine of phenobarbital-induced rats treated with 2,4-diaminoanisole was mutagenic in the presence, but not the absence, of rat liver microsomes. If rats were treated with  $\beta$ -naphthoflavone, methylcholanthrene, or 2,3,7,8-tetrachlorodibenzo-*para*-dioxin, the mutagenicity of their urine in the presence of microsomes was decreased. Treatment of the urine with  $\beta$ -glucuronidase increased mutagenic activity in the presence and absence of liver microsomes (Reddy *et al.*, 1980).

2,4-Diaminoanisole produced sex-linked recessive lethal mutations, but not somatic cell recombination, in *Drosophila melanogaster*. It produced sister chromatid exchanges, but not micronuclei, in mouse bone-marrow cells. It did not produce dominant lethal mutations in rats or altered sperm morphology in mice.

**Table 1. Genetic and related effects of 2,4-diaminoanisole, its sulfate trihydrate and its dihydrochloride**

Test system	Result <sup>a</sup>		Dose <sup>b</sup> (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
<b>2,4-Diaminoanisole</b>				
<i>Salmonella typhimurium</i> TA100, TA1535, TA1537, reverse mutation	–	–	500 µg/plate	Bruce & Heddle (1979)
<i>Salmonella typhimurium</i> TA100, reverse mutation	–	–	60	de Giovanni-Donnelly (1981)
<i>Salmonella typhimurium</i> TA100, reverse mutation	NT	+	10 µg/plate	Parodi <i>et al.</i> (1981)
<i>Salmonella typhimurium</i> TA100, TA102, TA98, reverse mutation	NT	(+) <sup>c</sup>	13.26	Sarkar <i>et al.</i> (1992)
<i>Salmonella typhimurium</i> TA1537, reverse mutation	–	+	3	Prival <i>et al.</i> (1980)
<i>Salmonella typhimurium</i> TA98, reverse mutation	(+)	+	3	Prival <i>et al.</i> (1980)
<i>Salmonella typhimurium</i> TA1538, reverse mutation	NT	+	10	Ames <i>et al.</i> (1975); Dybing & Aune (1977); Dybing <i>et al.</i> (1979a)
<i>Salmonella typhimurium</i> TA1538, reverse mutation	NT	+	5	Dybing & Thorgeirsson (1977); Aune & Dybing (1979)
<i>Salmonella typhimurium</i> TA1538, reverse mutation	NT	+	1.4	Aune <i>et al.</i> (1980a)
<i>Salmonella typhimurium</i> TA1538, TA98, reverse mutation	–	+	10	de Giovanni-Donnelly (1981)
<i>Salmonella typhimurium</i> TA1538, reverse mutation	NT	+	60 µg/plate	Mohn <i>et al.</i> (1982)
<i>Salmonella typhimurium</i> TA98, reverse mutation	–	+	19 µg/plate	Yoshikawa <i>et al.</i> (1977)
<i>Salmonella typhimurium</i> TA98, reverse mutation	–	+	20 µg/plate	Bruce & Heddle (1979)
<i>Salmonella typhimurium</i> TA98, reverse mutation	NT	+	10 µg/plate	Aune <i>et al.</i> (1980b)
<i>Salmonella typhimurium</i> TA98, reverse mutation	NT	+	1.25 µg/plate	Parodi <i>et al.</i> (1981)
<i>Salmonella typhimurium</i> TA98, reverse mutation	–	+ <sup>d</sup>	50 µg/plate	Maack <i>et al.</i> (1986)
<i>Neurospora crassa</i> , forward mutation, spot test, <i>ad-3</i> locus	–	NT	400 µg/plate	Ong (1978)
Gene mutation, Chinese hamster V79 cells, <i>Hprt</i> locus <i>in vitro</i>	–	–	552	Fassina <i>et al.</i> (1990)

Table 1 (contd)

Test system	Result <sup>b</sup>		Dose <sup>c</sup> (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Cell transformation, Syrian hamster embryo cells, focus assay	–	NT	50	Pienta & Kawalek (1981)
Unscheduled DNA synthesis, human HeLa cells <i>in vitro</i>	+	+	138	Loprieno <i>et al.</i> (1983)
Gene mutation, mouse lymphoma L5178Y cells <i>in vitro</i> , <i>Tk</i> locus	+	NT	19	Palmer <i>et al.</i> (1977)
DNA damage, Sprague-Dawley rat liver cells treated <i>in vivo</i> (alkaline elution assay)	+ <sup>e</sup>		91.2 ip × 1	Parodi <i>et al.</i> (1981)
DNA damage, Sprague-Dawley rat liver cells treated <i>in vivo</i> (DNA viscosity)	–		50 ip × 1	Brambilla <i>et al.</i> (1985)
Sister chromatid exchange, male mouse bone-marrow cells <i>in vivo</i>	+		12 ip × 1	Parodi <i>et al.</i> (1983)
Micronucleus formation, rat bone-marrow cells <i>in vivo</i>	–		500 po × 2	Hossack & Richardson (1977)
Micronucleus formation, (C57BL/6 × C3H/He)F <sub>1</sub> mouse bone- marrow cells <i>in vivo</i>	–		500 ip × 5	Bruce & Heddle (1979)
Dominant lethal mutation, rats <i>in vivo</i>	–		20 ip × 3/week; 8 weeks	Burnett <i>et al.</i> (1977)
Sperm morphology, (C57BL/6 × C3H/He)F <sub>1</sub> mice <i>in vivo</i>	–		500 ip × 5	Bruce & Heddle (1979)
<b>2,4-Diaminoanisole sulfate trihydrate</b>				
<i>Salmonella typhimurium</i> TA100, reverse mutation	–	(+)	333 µg/plate	Dunkel <i>et al.</i> (1985)
<i>Salmonella typhimurium</i> TA100, reverse mutation	–	+	100 µg/plate	Zeiger <i>et al.</i> (1988)
<i>Salmonella typhimurium</i> TA97, reverse mutation	(+)	+	33 µg/plate	Zeiger <i>et al.</i> (1988)
<i>Salmonella typhimurium</i> TA1535, reverse mutation	–	–	10 000 µg/plate	Dunkel <i>et al.</i> (1985)
<i>Salmonella typhimurium</i> TA1535, reverse mutation	–	–	3333 µg/plate	Zeiger <i>et al.</i> (1988)
<i>Salmonella typhimurium</i> TA1537, reverse mutation	–	+	10 µg/plate	Dunkel <i>et al.</i> (1985)
<i>Salmonella typhimurium</i> TA1538, reverse mutation	NT	+ <sup>f</sup>	0.87 µg/plate	Robertson <i>et al.</i> (1983)

Table 1 (contd)

Test system	Result <sup>b</sup>		Dose <sup>c</sup> (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
<i>Salmonella typhimurium</i> TA1538, reverse mutation	–	+	1 µg/plate	Dunkel <i>et al.</i> (1985)
<i>Salmonella typhimurium</i> TA98, reverse mutation	–	+	3.3 µg/plate	Dunkel <i>et al.</i> (1985)
<i>Salmonella typhimurium</i> TA98, reverse mutation	–	+	10 µg/plate	Reddy <i>et al.</i> (1980)
<i>Salmonella typhimurium</i> TA98, reverse mutation	+	+	1 µg/plate	Zeiger <i>et al.</i> (1988)
<i>Escherichia coli</i> WP2 <i>uvrA</i> , reverse mutation	–	–	10 000 µg/plate	Dunkel <i>et al.</i> (1985)
<i>Saccharomyces cerevisiae</i> , mitotic recombination (growing cells)	+	NT	500	Mayer & Goin (1980)
<i>Drosophila melanogaster</i> , somatic recombination ( <i>w/w</i> <sup>+</sup> locus)	–		118 feed	Rodriguez-Arnaiz & Hernández Aranda (1994)
<i>Drosophila melanogaster</i> , sex-linked recessive lethal mutations	+		1180 feed	Blijleven (1977)
DNA strand breaks, rat hepatocytes <i>in vitro</i> (alkaline elution)	+	NT	708	Storer <i>et al.</i> (1996)
Gene mutation, mouse lymphoma L5178Y cells, <i>Tk</i> locus <i>in vitro</i>	+	+	2	Mitchell <i>et al.</i> (1988)
Gene mutation, mouse lymphoma L5178Y cells, <i>Tk</i> locus <i>in vitro</i>	+	+	3.9	Myhr & Caspary (1988)
Chromosomal aberrations, Chinese hamster lung fibroblasts <i>in vitro</i>	+	NT	60	Ishidate (1988)
Urine from rats (50 mg/kg ip × 1), <i>Salmonella typhimurium</i> TA98 mutagenicity <sup>§</sup>	–	+	25 µL urine/ plate	Reddy <i>et al.</i> (1980)
Dominant lethal mutation, Holtzman albino rats <i>in vivo</i>	–		40 ip × 3/week; 10 weeks	Sheu & Green (1979)
<b>2,4-Diaminoanisole dihydrochloride</b>				
<i>Salmonella typhimurium</i> TA100, TA1535, reverse mutation	NT	–	1000 µg/plate	Shahin <i>et al.</i> (1980)
<i>Salmonella typhimurium</i> TA1537, TA97, reverse mutation	NT	+	50 µg/plate	Shahin <i>et al.</i> (1980, 1983, 1985)
<i>Salmonella typhimurium</i> TA1538, TA98, reverse mutation	NT	+	10 µg/plate	Shahin <i>et al.</i> (1980)

**Table 1 (contd)**

Test system	Result <sup>b</sup>		Dose <sup>c</sup> (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
<i>Salmonella typhimurium</i> TA98, reverse mutation	–	+	10 µg/plate	Venitt <i>et al.</i> (1984)
<i>Salmonella typhimurium</i> TA1538, reverse mutation	–	+	10 µg/plate	Loprieno <i>et al.</i> (1982)
<i>Salmonella typhimurium</i> TA98, reverse mutation (fluctuation test)	NT	+	0.33	Venitt <i>et al.</i> (1984)
<i>Salmonella typhimurium</i> TA98, reverse mutation	NT	+	2.5 µg/plate	Loprieno <i>et al.</i> (1982)
<i>Salmonella typhimurium</i> TA98, reverse mutation	–	+	10 µg/plate	Watanabe <i>et al.</i> (1989)
<i>Salmonella typhimurium</i> TA98, reverse mutation (in the presence of H <sub>2</sub> O <sub>2</sub> )	+	+	0.03 µg/plate	Watanabe <i>et al.</i> (1989)
<i>Saccharomyces cerevisiae</i> D4, mitotic gene conversion	–	+	1055 <sup>b</sup>	Loprieno <i>et al.</i> (1982)
<i>Schizosaccharomyces pombe</i> , forward mutation <i>ade</i> locus	+	+	528	Loprieno <i>et al.</i> (1982)
<i>Drosophila melanogaster</i> , sex-linked recessive lethal mutations	+	–	3165 feed	Blijleven (1982)
Gene mutation, Chinese hamster V79 cells, <i>Hprt</i> locus <i>in vitro</i>	+	–	844 <sup>i</sup>	Loprieno <i>et al.</i> (1982)
Chromosomal aberrations, Chinese hamster ovary (CHO) cells <i>in vitro</i>	+	+	50	Darroudi <i>et al.</i> (1982)
Unscheduled DNA synthesis, human HeLa cells <i>in vitro</i>	+	+	176	Loprieno <i>et al.</i> (1983)
DNA strand breaks, ddY mice (liver and brain) <i>in vivo</i> (Comet assay)	+	–	200 po × 1	Sasaki <i>et al.</i> (1999)
Micronucleus formation, male mouse bone-marrow cells <i>in vivo</i>	–	–	60 ip × 1	Morita <i>et al.</i> (1997)
Urine from rats (100 mg/kg bw po or ip), mutation in <i>Salmonella typhimurium</i> TA1538, TA98	–	+	100 µL urine/ plate	Shahin <i>et al.</i> (1980)
Urine from rats (120 mg on skin), mutation in <i>Salmonella typhimurium</i> TA1538, TA98	–	+	100 µL urine/ plate	Shahin <i>et al.</i> (1980)

**Table 1 (contd)**

Test system	Result <sup>b</sup>		Dose <sup>c</sup> (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Urine from rats (100 mg/kg bw po or ip), mutation in <i>Salmonella typhimurium</i> TA100	–	–	300 µL urine/ plate	Shahin <i>et al.</i> (1980)
Urine from rats (120 mg on skin), mutation in <i>Salmonella typhimurium</i> TA100	–	–	300 µL urine/ plate	Shahin <i>et al.</i> (1980)

<sup>a</sup> +, positive; (+), weak positive; –, negative; NT, not tested

<sup>b</sup> LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw per day; ip, intraperitoneal; po, oral

<sup>c</sup> Activation with prostaglandin H synthase

<sup>d</sup> Extracts from mammary tissue of lactating rats and human mammary tumour cell lines

<sup>e</sup> At 4 h after treatment; increase not significant at 24 h

<sup>f</sup> Positive with an exogenous metabolic activation system from a 9000 × g supernatant of rat liver (S9) and, at 10-fold higher dose, with ram seminal vesicle microsomes

<sup>g</sup> Test carried out with 2,4-diaminoanisoie disulfate

<sup>h</sup> A threefold higher concentration was negative in the absence of metabolic activation.

<sup>i</sup> 1055 µg/mL was negative in the presence of metabolic activation.



#### **4.6 Mechanistic considerations**

2,4-Diaminoanisole is metabolically activated to covalently protein-bound products in rat liver and kidney, but no covalent binding to hepatic DNA was detected. The data on genotoxicity indicate that 2,4-diaminoanisole is genotoxic.

Short-term oral treatment of rats with high doses of diaminoanisole sulfate leads to the development of thyroid tumours and concomitant alterations in thyroid hormone function. The serum concentrations of thyroid-stimulating hormone were elevated and those of thyroxine and triiodothyronine were lowered during the first few weeks after the beginning of treatment. These alterations in hormone concentrations tended to normalize after 6 weeks. The alterations in thyroid hormone homeostasis are presumed to be involved in the induction of thyroid tumours by 2,4-diaminoanisole, but a genotoxic mechanism cannot be excluded.

### **5. Summary of Data Reported and Evaluation**

#### **5.1 Exposure data**

2,4-Diaminoanisole is an aromatic amine which was used extensively in hair dyes and in the dyeing of furs until the late 1970s.

#### **5.2 Human carcinogenicity data**

Although epidemiological studies have been conducted on professional and personal users of hair dyes, none made specific mention of 2,4-diaminoanisole.

#### **5.3 Animal carcinogenicity data**

2,4-Diaminoanisole sulfate was tested by dietary administration in one experiment in mice and in two experiments in one strain of rats. Thyroid gland adenomas or carcinomas were induced in mice and rats. Tumours of the skin and of the preputial, clitoral and Zymbal glands were also induced in rats. 2,4-Diaminoanisole sulfate was tested for its promoting effects by dietary administration in two strains of rats. In one study in rats, it promoted thyroid gland tumours induced by *N*-nitrosobis(2-hydroxypropyl)amine.

#### **5.4 Other relevant data**

About 2–4% of a dermal dose of 2,4-diaminoanisole is absorbed by humans, monkeys and rats. The compound is completely absorbed after oral administration to rats, extensively metabolized to free and conjugated acetylated and oxidized products

and thereafter excreted in equal percentages of the applied dose in urine and faeces. The substance is metabolically activated to covalently protein-bound products in rat liver and kidney, but no covalent binding to hepatic DNA was detected. Short-term administration of high oral doses to rats induced thyroid enlargement (goitre) and alterations in thyroid hormone homeostasis.

2,4-Diaminoanisole is genotoxic *in vitro*, producing gene mutations and chromosomal damage. It was mutagenic in bacteria in the presence or absence of a microsomal fraction from the livers of uninduced rats, mice, rabbits or humans. It produced chromosomal aberrations and sister chromatid exchange in rodent cells *in vitro*, mitotic recombination in yeast and mutations in insects. The results of most tests in mammals *in vivo* were negative.

### 5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of 2,4-diaminoanisole.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 2,4-diaminoanisole.

### Overall evaluation

2,4-Diaminoanisole is *possibly carcinogenic to humans (Group 2B)*.

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