IPCS International Programme on Chemical Safety

Environmental Health Criteria 74

Diaminotoluenes

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The **International Programme on Chemical Safety (IPCS)** is a joint venture of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. The main objective of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment. Supporting activities include the development of epidemiological, experimental laboratory, and risk-assessment methods that could produce internationally comparable results, and the development of manpower in the field of toxicology. Other activities carried out by IPCS include the development of know-how for coping with chemical accidents, coordination of laboratory testing and epidemiological studies, and promotion of research on the mechanisms of the biological action of chemicals.

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CONTENTS

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NOTE TO READERS OF THE CRITERIA DOCUMENTS

Every effort has been made to present information in the criteria documents as accurately as possible without unduly delaying their publication. In the interest of all users of the environmental health criteria documents, readers are kindly requested to communicate any errors that may have occurred to the Manager of the International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda, which will appear in subsequent volumes.

* * *

A detailed data profile and a legal file can be obtained from the International Register of Potentially Toxic Chemicals, Palais des Nations, 1211 Geneva 10, Switzerland (Telephone no. 988400 - 985850).

ENVIRONMENTAL HEALTH CRITERIA FOR DIAMINOTOLUENES

A WHO Task Group on Environmental Health Criteria for Diaminotoluenes met at the Monitoring and Assessment Research Centre, London, United Kingdom, from 20 to 25 October 1986. Professor P.J. Petersen welcomed the participants on behalf of the host Institution, and Dr G.C. Becking opened the meeting on behalf of the three co-sponsoring organizations of the IPCS (ILO/UNEP/WHO). The Task Group reviewed and revised the draft criteria document and made an evaluation of the health risks of exposure to diaminotoluenes.

The efforts of MS ANDREA BLASKA, US ENVIRONMENTAL PROTECTION AGENCY, Washington DC, USA, in the preparation of the draft, and of all others who helped in the preparation and finalization of the document are gratefully acknowledged.

* * *

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1. SUMMARY AND CONCLUSIONS

1.1 Summary

1.1.1 Identity and analytical methods

Diaminotoluenes are synthetic aromatic amines (total of 6 isomers). The isolated, purified isomers are colourless crystals, while the commercial isomeric mixtures are light yellow to tan (Meta-diaminotoluene), or light grey to purple (Ortho-diaminotoluene) solids. Diaminotoluenes are soluble in hot water, alcohol, ether, and hot benzene. When heated, they emit toxic fumes of nitrogen oxides.

Several qualitative and quantitative procedures for the determination of diaminotoluenes have been developed using thin-layer, high-performance-liquid, or gas chromatography, methods. Detection limits in air samples range from 0.1 to 10 m^3. The isomeric ratios in technical grade mixtures have been determined by nuclear magnetic resonance and infra-red spectrometry.

1.1.2 Production, uses, and sources of exposure

Diaminotoluenes are produced from dinitrotoluenes through a catalytic hydrogenation procedure, or by the reaction of iron and hydrochloric acid with dinitrotoluenes. Diaminotoluenes are large-volume intermediates used in the production of a wide variety of industrial and consumer products. The mixture of 2,4- and 2,6-isomers is used predominantly as an intermediate in the manufacture of toluene diisocyanate. Commercial mixtures of 2,3- and 3,4-isomers, as well as the 2,4- and 2,6-isomers, are used as co-reactants or as raw materials in the manufacture of urethane products, dyes, corrosion inhibitors, and rubber antioxidants. Diaminotoluene isomers have relatively limited use as epoxy curing agents and as photographic developers. The most commonly marketed isomers and isomer mixtures are 2,4-diaminotoluene (2,4-DAT), 3,4-DAT, Meta-DAT (an 80:20 or 65:35 mixture of the 2,4- and 2,6-isomers), and Ortho-DAT (3,4-, 2,3-isomers, as 60:40 mixture); 2,5-diaminotoluene is also marketed in small quantities. These isomers and their mixtures are reviewed together, because any single commercial product will contain various levels of the other isomers.

The major sources of environmental pollution are the manufacture of diaminotoluenes and their products. Over 50% of the losses into the environment are through industrial wastes deposited in landfills. Diaminotoluenes are soluble in water; therefore, leakage from landfills or storage sites, and

spillage during shipping and handling may also represent sources of surface and groundwater contamination.

Despite the wide use and the water solubility of diaminotoluenes, there is a lack of information concerning their levels in the environment, as well as data on their transport and their fate in the ecosystem.

Data are not available on the exposure of the general population to diaminotoluenes and there is a paucity of data on the exposure of workers to diaminotoluenes, though workplace air levels ranging up to 0.44 mg/m^3, with occasional excursions up to 11 mg/m^3, have been reported.

1.1.3 Kinetics

1.1.3.1 Animal studies

Diaminotoluenes have been absorbed via all exposure routes tested. Skin penetration by diaminotoluenes is affected by the type of vehicle and site of application. The greatest absorption of 2,4-diaminotoluene (approximately 50%) resulted when the test material was dissolved in acetone and applied to the abdominal skin of monkeys. Following intraperitoneal injection of [^{14}C]-2,4-diaminotoluene, absorption was rapid and peak concentrations in rat and mouse blood and plasma occurred within 1 h and decreased rapidly for 7 h.

Distribution varies with different species. However, data indicate that, in most species, the organs with the highest concentrations are the liver, kidneys, and adrenal glands. High concentrations are also observed in the gastrointestinal tract, while the lowest levels are found in the heart, gonads, brain, and blood. A dose-dependent binding of the 2,4-isomer to hepatic and renal proteins has been demonstrated.

The acetylation of amino groups, oxidation of methyl groups, and ring hydroxylation appear to be the major metabolic steps. Phenolic metabolites and trace amounts of unchanged diaminotoluenes are excreted in the urine of experimental animals. Elimination of diaminotoluene metabolites takes place via both urine and faeces. However, the primary route and rate of elimination varies with different species, e.g., urinary elimination is faster and more complete in mice (2 days) than in rats (6 days).

1.1.3.2 Human studies

Data are not available on the kinetics and metabolism of diaminotoluenes after oral or inhalation exposure. The results of skin penetration studies correspond with those from experimental animal studies. After 40 min of dermal contact, the highest rate of urinary excretion occurred 4 - 8 h after

exposure. During 24 h of dermal contact, the highest absorption of 2,4-diaminotoluene resulted when test material was dissolved in acetone and applied to the skin of the forearm (23.7%). Data from studies on human volunteers showed that, after subcutaneous injection of 5.5 mg 2,5-diaminotoluene, 47.6% of the dose was excreted in the urine as N,N'-diacetyl-2,5-diaminotoluene.

1.1.4 Effects on organisms in the environment

Diaminotoluenes are toxic for aquatic species. Daphnia, the most sensitive species of those tested, was adversely affected at concentrations of 2 - 5 mg/litre. At higher concentrations, diaminotoluenes were toxic for ostracods, fish, and algae, the algal species tested being the most tolerant. No data are available on other non-mammalian species in the environment.

1.1.5 Effects on experimental animals

2,4- and 2,5-Diaminotoluenes are ocular and dermal irritants. Instillation of 100 µg 2,4-diaminotoluene in the rabbit eye caused severe eye irritation within 24 h. In rabbits, irritation and blisters developed after 24-h dermal contact with 500 mg 2,4-diaminotoluene or 12.5 mg 2,5-diaminotoluene.

Dermal contact with 1 - 10% solutions of 2,5-diaminotoluene resulted in the development of severe irritation and leukocyte infiltration in 25 - 50% of exposed guinea-pigs. In addition, 35% of the exposed animals were sensitized to the test compound. Dermal contact was for 24 h/day for 2 periods of 5 days, separated by 2 days free of exposure.

Diaminotoluenes are mild cumulative poisons, and their toxicity in different species varies considerably. The acute oral LD_{50} of Meta-diaminotoluene for the mouse was 350 mg/kg body weight; for the rat, it ranged from 270 to 300 mg/kg body weight. The acute oral LD_{50} of Ortho-diaminotoluene for the rat was 810 mg/kg (range, 590 - 1120 mg/kg body weight). The dermal LD_{50} of Meta-diaminotoluene for the rat was 1200 mg/kg body weight, while the dermal LD_{50} of Ortho-diaminotoluene for the rabbit was 1120 mg/kg (range, 650 - 2040 mg/kg body weight).

At extremely high exposure levels, diaminotoluenes are toxic for the central nervous system, produce jaundice, and induce anaemia by destruction of the red blood cells after methaemoglobin formation.

In short-term studies, the toxic effects of 2,4-diaminotoluene are characterized by a decrease in body weight and an increase in the liver:body weight ratio. Following a 5-day

oral treatment of male F-344 rats with 70 mg 2,4-diaminotoluene/kg body weight, per day, the activities of microsomal cytochrome P-450-dependent enzymes were depressed, while that of epoxide hydrolase was markedly elevated (3 - 8 times that in controls). 2,4-Diaminotoluene or one of its metabolites has been shown to bind irreversibly to hepatic and renal proteins and to liver ribosomal RNA. Oral ingestion of 2,4-diaminotoluene at 50 or 100 mg/kg for 2 years accelerated the development of chronic renal disease in F-344 rats, an effect that contributed to a marked decrease in survival.

The reproductive and teratogenic effects of diaminotoluenes depend on the route of administration, the isomer studied, and the species of the experimental animal. Results of recent studies have shown that 2,4-diaminotoluene (98% pure) is a potent reproductive toxin in the male rat. At a level of 0.3 g/kg diet for 10 weeks (≈ 15 mg/kg body weight per day), this agent produced marked toxic effects on spermatogenesis (66% reduction) associated with a significant reduction in the weights of the seminal vesicles and epididymides, as well as a diminished level of circulating testosterone, and an elevation of serum-luteinizing hormone.

The 2,6-isomer, but not the 2,4-isomer, is embryotoxic in the rat and rabbit and has been reported to cause malformation in the rat. The no-observed-adverse-effect level for 2,6-diaminotoluene was 10 mg/kg body weight in the rat, and 30 mg/kg body weight in the rabbit. Ortho-diaminotoluene (2,3-, 3,4-isomer mixture) is toxic for the treated dams, their embryos, and fetuses. The no-observed-adverse-effect level is 30 mg/kg body weight in both the rat and rabbit.

Diaminotoluenes have been shown to be mutagenic in several *in vitro* assays and in *Drosophila*, but the results in several *in vivo* mammalian assays were negative.

2,4-Diaminotoluene is the only isomer that has been reported to produce an increased incidence of tumours in rodents. This isomer produces hepatocellular, subcutaneous, and mammary gland tumours in rats and hepatocellular and vascular tumours in mice, when present in the diet at levels $\geq$ 79 mg/kg. On the other hand, it was reported that the 2,6-isomer was not carcinogenic for rodents. Tumours in the same organs as those affected by 2,4-diaminotoluene were found after administration of 2,6-diaminotoluene at $\geq$ 250 mg/kg for 103 weeks, but they were considered not significant after extensive statistical evaluation.

1.1.6 Effects on human beings

Diaminotoluenes are irritant to the eyes and the skin. Local actions include severe dermatitis, blistering, and urticaria, and, in the eye, lachrymation, corneal opacities,

and permanent blindness, if untreated. In the case of inhalation of fumes, coughing, dyspnoea, and respiratory distress may result.

The epidemiological assessment of the reproductive hazards for males exposed to DAT (in most cases, together with dinitrotoluene) revealed inconclusive findings suggesting adverse effects on sperm production and on the viability of pregnancies in women whose husbands have been exposed. Sperm samples from workers in 3 DAT production plants showed a reduced sperm count in one plant (with the smallest study group and an unusually high sperm count in the control group), but also a reduced proportion of large morphological sperm. Studies of the reproductive history of the wives of workers in 3 plants (in 2 of which semen analysis was also carried out) revealed excess miscarriage rates, which are related to DAT exposure in two populations, though both suffered from limited size and the risk of some self selection of volunteers who participated in the study. Given the animal evidence of adverse effects on spermatogenesis, these findings are of concern.

1.2 Conclusions

Diaminotoluenes are highly irritating to the skin and eyes, and the fumes are irritating to the respiratory tract. Diaminotoluenes are readily absorbed through the skin, and exposure may result in methaemoglobinaemia. Renal toxicity after oral administration of 2,4-diaminotoluene has been reported in experimental animals. 2,4-Diaminotoluene has been shown to be carcinogenic for animals, but there is inadequate evidence to evaluate the carcinogenic potential of 2,5- and 2,6-diaminotoluene. All three of these isomers have been shown to be mutagenic. They are reproductive toxins in experimental animals, but human reproduction data are limited.

Diaminotoluenes should be handled as hazardous chemicals. Preventive measures should be taken to avoid exposure of workers and to prevent environmental pollution.

2. IDENTITY, PHYSICAL AND CHEMICAL PROPERTIES, ANALYTICAL METHODS

2.1 Identity

Diaminotoluenes are synthetic aromatic amines (total of 6 isomers) with two amino groups and a methyl group attached to a benzene ring (Table 1). The molecular formula is $C_7H_{10}N_2$ and the relative molecular mass, 122.17.

Commercial grades of diaminotoluenes are available; however, the most commonly marketed diaminotoluenes are: (a) "crude" diaminotoluenes-mixture, containing all 6 isomers (Table 1); (b) Meta-diaminotoluene (Meta-DAT), containing approximately 80% 2,4- and 20% 2,6-isomers (also produced in smaller amounts as 65:35 mixture); and (c) Ortho-diaminotoluene (Ortho-DAT), consisting of approximately 40% 2,3- and 60% 3,4-isomers. All commercial grades contain traces of the other isomers; therefore, diaminotoluenes and their mixtures are reviewed together in this document.

Most of the common and trade names for commercial diaminotoluenes are listed in Table 2.

2.2 Physical and Chemical Properties

Diaminotoluenes are colourless crystals that are freely soluble in hot water, alcohol, ether, and hot benzene. Some of the physical properties of the 6 isomers are listed in Table 3 (Buist, 1970; CRC, 1975). Diaminotoluenes are oxidized readily in neutral or alkaline solution to form dark-coloured products and tars. The oxidation products have not been fully characterized. When heated, diaminotoluenes emit toxic fumes of nitrogen oxides.

The composition and physical properties of the commercial mixtures vary considerably. Some of the physical properties of the 2 most widely-used commercial mixtures are summarized in Table 4.

Meta- and Ortho-diaminotoluenes are weakly basic and react with mineral acids to form water-soluble amine salts. These salts are more resistant to oxidation than the parent amine.

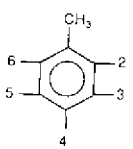
2.3 Conversion Factors

1 ppm in air = 5 mg/m^3 at 25 °C and 760 mmHg.

Table 1. Identity of diaminotoluene isomers[a]

Diaminotoluenes	CAS registry number	RTECS accession number index
Isomers		
2,3-DAT	2687-25-4	---
2,4-DAT	95-80-7	XS9625000
2,5-DAT	95-70-5	XS9700000
2,6-DAT	823-40-5	XS9750000
3,4-DAT	496-72-0	XS9820000
3,5-DAT	108-71-4	---
Commercial mixture		
Meta-DAT (2,4-, 2,6- isomers mix) (80:20)	95-80-7 823-40-5	---
Ortho-DAT (2,3-, 3,4- isomer mix) (40:60)	26787-25-4 496-72-0	---

[a] Structural formula:



Numbers show alternative positions for 2 $-NH_2$ groups

2.4 Analytical Methods

Analytical methods for the determination of diaminotoluenes in water, air, different consumer products, and biological fluids are listed in Table 5.

Diaminotoluenes may be analysed as free bases by reversed phase high-performance liquid chromatography using both

Table 2. Diaminotoluenes synonyms and trade names

A. Commercial mixtures	
I. Meta-Diaminotoluenes	
Chemical abstract name	benzenediamine,ar-methyl- (9CI)
Other chemical names	benzenediamine,ar-methyl (RTECS: TDB); diaminotoluene (RTECS: TDB); phenylenediamine,ar-methyl- (TDB); Meta-diaminotoluene; Meta-toluene-diamine (MTD); toluene-ar,ar-diamine (8CI) (CAS: TDB); toluenediamine (RTECS: TDB, DOT); tolylenediamine (RTECS, TDB)
Common name	diaminotoluene; toluenediamine; TDA
II. Ortho-Diaminotoluene	
Chemical abstract name	benzenediamine,ar-methyl- (9CI)
Other chemical names	o-TDA
Common name	Ortho-toluenediamine; OTD
B. Diaminotoluene isomers	
I. 2,3-isomer	
Chemical abstract name	1,2-benzenediamine,3-methyl- (9CI)
Other chemical names	toluene-2,3-diamine (8CI) (CAS: TDB); 1-methyl-1,2,3-phenylenediamine (TDB); 1,2-diamino-3-methylbenzene (TDB); 2,3-diaminotoluene (TDB*); 2,3-toluylenediamine (TDB); 2,3-tolylenediamine (TDB); 3-methyl-o-phenylenediamine (TDB); 3-methyl-1,2-phenylenediamine (TDB)
Common names	2,3-TDA
II. 2,4-isomer	
Chemical abstract name	1,3-benzenediamine,4-methyl- (9CI)
Other chemical names	m-toluenediamine (RTECS); m-toluylendiamin (Czech, RTECS: TDB); m-toluylenediamine (RTECS: TDB); m-tolyenediamine (RTECS: TDB); m-tolylenediamine (RTECS); Meta-toluylene diamine (RTECS: TDB); toluene-2,4-diamine (8CI) (CAS, RTECS: TDB*); tolylene-2,4-diamine (RTECS: TDB); 1,3-diamino-4-methylbenzene (RTECS: TDB);

Table 2 (contd).

B. Diaminotoluene isomers (contd).

II. 2,4-Isomer (contd).

Other chemical names (contd).	2,4-diamino-1-methylbenzene (RTECS: TDB); 2,4-diamino-1-toluene (RTECS: TDB); 2,4-diaminotoluen (Czech, RTECS: TDB); 2,4-diaminotoluene (MESH, RTECS: TDB); 2,4-diaminotoluol (RTECS: TDB); 2,4-tolamine (RTECS: TDB); 2,4-toluenediamine (MESH, RTECS: TDB); 2,4-toluylenediamine (DOT, RTECS: TDB); 2,4-tolylenediamine (RTECS: TDB); 3-amino-p-toluidine (RTECS: TDB); 4-m-tolylenediamine (RTECS: TDB); 4-methyl-m-phenylenediamine (RTECS: TDB); 4-methyl-1,3-benzenediamine (RTECS: TDB); 5-amino-o-toluidine (RTECS: TDB)
Common names	TDA; MTD; 2,4-TDA (CAS, RTECS: TDB)
Trade names	Azogen Developer H; Benzofur MT; C.I. Oxidation Base (RTECS); C.I. Oxidation Base 20; C.I. Oxidation Base 35 (RTECS); C.I. Oxidation Base 200; Developer B (RTECS: TDB); Developer DB (RTECS: TDB); Developer DBJ (RTECS: TDB); Developer H; Developer MC (RTECS: TDB); Developer MT (RTECS: TDB); Developer MT-CF (RTECS: TDB); Developer MTD (RTECS; TDB); Developer T (RTECS: TDB); Developer 14; Eucanine GB (RTECS: TDB); Fouramine; Fouramine J (RTECS: TDB); Fourrine M (RTECS: TDB); Fourrine 94 (RTECS: TDB); Lekutherm-Haerter VP-KU 6546; Nako TMT (RTECS: TDB); NCI-C02302 (RTECS: TDB); Pelagol J (RTECS: TDB); Pelagol Grey J (RTECS: TDB); Pontamine Developer TN (RTECS: TDB); Renel MD (RTECS: TDB); Tertral G; Zoba GKE (RTECS: TDB); Zogen Developer H (RTECS: TDB).
Colour index number	76035

Table 2 (contd).

	III. 2,5-isomer
Chemical abstract name	1,4-benzenediamine,2-methyl-(9CI)
Other chemical names	p-toluenediamine (MESH, RTECS: TDB); p-toluylendiamine (RTECS: TDB); P,m-tolylenediamine (RTECS: TDB); para-meta-tolylenediamine; para-toluenediamine; para-toluylenediamine; para-tolylenediamine; toluene-2,5-diamine (8CI) (CAS, RTECS: TDB); toluylene-2,5-diamine (RTECS: TDB); 2-methyl-p-phenylenediamine (RTECS: TDB); 2-methyl-1,4-benzenediamine (RTECS: TDB*); 2,5-diaminotoluene (MESH, RTECS: TDB); 2,5-toluenediamine; 4-amino-2-methylaniline (RTECS: TDB)
Common name	2,5-TDA
Trade names	Oxidation Base 4 (as sulfate)
Colour index number	C.I. 76042 (RTECS); 76043 (as sulfate)
	IV. 2,6-isomer
Chemical abstract name	1,3-benzenediamine,2-methyl- (9CI)
Other chemical names	m-phenylenediamine-2-methyl; toluene-2,6-diamine (8CI) (CAS, RTECS: TDB); tolylene-2,6-diamine; 1,3-benzenediamine; 2-methyl-m-phenylenediamine (TDB); 2-methyl-1,3-benzenediamine (TDB); 2-methyl-1,3-phenylenediamine (TDB); 2,6-diamino-1-methylbenzene (TDB); 2,6-diaminotoluene (MESH, RTECS: TDB*); 2,6-toluenediamine; 2,6-toluylenediamine (RTECS: TDB); 2,6-tolylenediamine (RTECS: TDB)
Common names	2,6-TDA
	V. 3,4-isomer
Chemical abstract names	1,2-benzenediamine,4-methyl- (9CI)
Other chemical names	o-toluenediamine; toluene-3,4-diamine (8CI) (CAS, RTECS: TDB); 1,2-benzenediamine,4-methyl- (RTECS: TDB);

Table 2 (contd).

B. Diaminotoluene isomers (contd).	
V. 3,4-isomer (contd).	
Other chemical names (contd).	1,2-diamino-4-methylbenzene (TDB); 3,4-diamino-1-methylbenzene (TDB); 3,4-diaminotoluene (RTECS: TDB); 3,4-toluenediamine; 3,4-toluylenediamine (RTECS); 3,4-tolylenediamine (RTECS: TDB); 4-methyl-o-phenylenediamine (TDB); 4-methyl-1,2-benzenediamine (TDB); 4-methyl-1,2-diaminobenzene (TDB); 4-methyl-1,2-phenylenediamine (TDB)
Common name	3,4-TDA
VI. 3,5-isomer	
Chemical abstract name	1,3-benzenediamine,5-methyl- (9CI)
Other chemical names	3,5-diaminotoluene; 3,5-toluenediamine
Common name	3,5-TDA

Table 3

Table 3. Physical properties of the diaminotoluene isomers

Property	Diaminotoluene isomers					
	2,3-	2,4-	2,5-	2,6-	3,4-	3,5-
Melting point (°C)	63-64	99	64	105	88.5	-
Boiling point (°C)	255	280	273-274	289[b]	265 (subl)	283-285
Vapour pressure[a] (kPa)						
at 150 °C	1.20	1.47	-	2.13	-	-
at 160 °C	1.87	2.27	-	3.33	-	-
at 180 °C	2.67	4.80	-	7.60	-	-

[a] To convert kPa to mmHg, divide by 0.133.
[b] Obtained by extrapolation from vapour pressure-temperature data and Antoine constants. From: Willeboordse et al. (1968).

Table 4. Physical and chemical properties of commercial grades of diaminotoluene

	Meta-DAT (80:20, 2,4-/2,6-isomers)	Ortho-DAT (60:40, 3,4-/2,3-isomers)
Appearance	solid, light yellow to tan; darkens on storage and exposure to air	light grey to purple solid
Odour	slight ammonia-like	slight ammonia-like
Melting range	80 - 90 °C (176 - 194 °F)	40 - 50 °C (104 - 122 °F)
Boiling point	283 °C (541 °F) at 760 mmHg	> 250 °C (> 480 °F)
Flash point	140 °C (284 °F)	> 110 °C (> 230 °F)
Autoignition temperature	450 °C (842 °F)	540 °C (1005 °F)
Vapour pressure	0.34 x 10^{-3} mmHg at 37.8 °C 1 mmHg at 106.5 °C 100 mmHg at 212 °C	2.23 mmHg at 100 °C 27.8 mmHg at 140 °C 43.5 mmHg at 160 °C
Specific gravity	-	1.045 at 100 °C
Density	0.086 kg/litre at 105 °C	-
Solubility	in hot water, alcohol, ether and many polar organic solvents	in hot water, alcohol, ether and many polar organic solvents

Table 5. Analytical methods for the determination of diaminotoluenes

Matrix	Analytical procedure	Determination	Detection limit	Reference
Water	high-performance liquid chromatography with ultraviolet and electrochemical detection	2,4-, 2,5-, 2,6-, 3,4-isomers	0.2 - 0.7 ng	Riggin & Howard (1983)
Air	gas-liquid chromatography/ nitrogen-phosphorus detector on glass capillary columns	2,4-isomer	3 μg/m³	Becher (1981)
	high-performance liquid chromatography with ultraviolet and electrochemical detection		0.1 - 10 μg/m³	Purnell et al. (1982)
	high-performance liquid chromatography with ultraviolet detection	2,4-isomer	-	Nieminen et al. (1983)
	gas-liquid chromatography with electron capture detection on glass capillary column	2,4- and 2,6-isomers	≈ 0.1 - 0.4 pg	Skarping et al. (1983a)
	gas-liquid chromatography/ nitrogen-phosphorus detector on glass capillary columns	2,4- and 2,6-isomers	10 - 20 pg amine	Skarping et al. (1983b)
Hair dyes	gas-liquid chromatography/ flame ionization detector	2,5-isomer	5 mg/litre	Choudhary (1980)
	thin-layer chromatography	2,4-, 2,5-, and 3,4-isomer	0.2 mg/litre	Kottemann (1966)
	high-performance liquid chromatography/ultraviolet detection	2,4-, 2,5-, and 2,6-isomers	0.5 mg/litre	Johansson et al. (1981)

Table 5 (contd).

Matrix	Analytical procedure	Determination	Detection limit	Reference
Hair dyes (contd)	high-performance liquid chromatography/ultraviolet detection	2,4-, 2,5-, and 3,4-isomers	-	Liem & Rooselaar (1981)
	high-performance liquid chromatography/ultraviolet detection gas-liquid chromatography/ mass spectrometry	2,4- and 2,6-isomer	0.1 µg/litre	Snyder et al. (1982)
Polyurethane foams	thin-layer chromatography/ fluorimetry	2,4- and 2,6-isomers	1 µg/g	Guthrie & McKinney (1977)
Biological tissues and fluids	high-performance liquid chromatography/ultraviolet detection	2,4- and 2,6-isomers	2 mg/litre	Unger & Friedman (1979)
Isomeric mixtures	nuclear magnetic resonance spectrometry	all isomers	-	Mathias (1966)
	thin-layer chromatography	all isomers	-	Macke (1968)
	gas-liquid chromatography/ flame ionization detector	all isomers	-	Boufford (1968)
	gas-liquid chromatography/ thermal detector	all isomers	-	Willeboordse et al. (1968)
	infra-red spectroscopy	2,4- and 2,6-isomers	± 11%	Biernacka et al. (1974)

ultraviolet (UV) and electrochemical detection (Purnell & Warwick, 1981; Purnell et al., 1982; Nieminen et al., 1983). Gas chromatographic methods usually involve derivatization to facilitate separation and increase sensitivity (Olufsen, 1979; Skarping et al., 1983a,b). Detection limits in air samples range from 0.1 to 10 $\mu g/m^3$.

Diaminotoluenes and their derivatives have been studied in blood, urine, and liver cytosol preparations using thin-layer chromatography and gas chromatography/mass spectrometry (GC/MS) (Kiese & Rauscher, 1968; Kiese et al., 1968; Glinsukon et al., 1975; Waring & Pheasant, 1976). A high-performance liquid chromatographic method for the determination of diaminotoluenes in urine and plasma has been described by Unger & Friedman (1979).

3. SOURCES IN THE ENVIRONMENT, ENVIRONMENTAL TRANSPORT AND DISTRIBUTION

3.1 Natural Occurrence

Diaminotoluenes are not known to occur as natural products.

3.2 Production

Currently, diaminotoluenes are produced commercially through the catalytic hydrogenation of dinitrotoluenes. This procedure, economic only for large-scale production, is used in the manufacture of toluene diisocyanates. At dye plants, diaminotoluenes are produced by the reaction of hydrochloric acid on dinitrotoluenes, in the presence of an iron catalyst (Austin, 1974).

Most diaminotoluenes produced are used on site by the manufacturer; therefore, published production figures do not adequately reflect the true world production of diaminotoluenes.

Between 1972 and 1976, the average annual production of diaminotoluenes in the USA was 89 x 10^6 kg, ranging from 76 x 10^6 kg in 1972 to 105 x 10^6 kg in 1976 (US ITC, 1977). Thereafter, the production was estimated from the known production of toluene diisocyanates ranging between 305 x 10^6 kg annually in the period 1977-81, and 360 x 10^6 kg in 1984 (US ITC, 1982, 1985).

Up to 1978, an estimated 180 - 200 x 10^6 kg of 2,4-DAT was produced annually in western Europe (IARC, 1978). During 1971-75, the the annual production of 2,4-DAT in Japan was approximately 210 x 10^3 kg; the compound was neither imported nor exported (IARC, 1978). However, in 1981, the production of 2,4-DAT in Japan was estimated to have declined to 50 x 10^3 kg (CIC Japan, 1983).

3.3 Uses

Diaminotoluenes are used extensively within the chemical industry as intermediates in the manufacture of widely different commercial products (Table 6). Minor applications of diaminotoluene isomers include their use as raw materials, co-reactants, and curing agents. Toluene diisocyanates represent the largest end-use accounting for more than 90% of the total annual production of diaminotoluenes, largely a mixture of 2,4- and 2,6-isomers (Backus, 1974; Milligan & Gilbert, 1978).

Diaminotoluenes are intermediates in the synthesis of dyes used for textiles, furs, leathers, biological stains and

Table 6. End-use application(s) of individual diaminotoluene isomers

Application	2,3-	2,6-	2,3-	3,4-	2,5-
Toluene diisocyanate (> 90% of total use of diaminotoluenes)	X	X			
Urethane co-reactants (DAT-initiated polyols)	X	X	X	X	
DETDA[a]	X	X			
DYES[b]	X[d]	X			[d]
Tolyltriazole			X	X	
Epoxy curing	X	X		X	
Mercaptotoluimidazole			X	X	
Photographic developer		X			

[a] DETDA = Diethyltoluenediamine.
[b] DYES = Fur, leather, biological stains, indicators, textiles, hair, spirit varnishes, wood stains, and pigments.
[c] Use in hair dyes and cosmetics prohibited in USA since 1971.
[d] Forbidden in Italy - 1978.

indicators, spirit varnishes and wood stains, and pigments. Previously used in hair-dyes, 2,4-DAT was removed from use by many countries after it was found to be a hepatocarcinogen in rats (Ito et al., 1969). Meta-DAT is used to produce diethyltoluenediamine (DETDA) for the manufacture of certain urethane elastomers (Milligan & Gilbert, 1978). Ortho-DAT is used to produce mercaptotoluimidazole (MTI) and its zinc salt, both of which are used primarily as specialty antioxidants in nitrile rubber elastomers (Gan et al., 1975).

3.4 Release into the Environment, Distribution, and Transformation

Data are lacking on the extent of the global release of diaminotoluenes, as well as their transport, distribution, and degradation within the environment.

Releases of 2,4-DAT into the environment have been estimated in the USA, the largest contribution being over 6 x 10^6 kg dumped in authorized landfills. Releases of 1.4 x 10^6 kg were estimated to occur from the production of diaminotoluenes, and 0.3 x 10^6 kg during dye production and

usage; unknown quantities of DAT may derive from the hydrolysis of TDI released into the environment.

Information on the transport, distribution, and degradation of DAT isomers under conditions approaching those found in natural bodies of water have not been reported in the literature. However, a bench-scale treatability study for 2,4-DAT using acclimated sludge from a treatment plant showed that the isomers are degradable. The observed total organic carbon removal was 45% in 4 h (Matsui et al., 1975).

4. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

4.1 Environmental Levels

No information was available in the literature reviewed from which environmental levels could be calculated. Two properties of the diaminotoluenes are relevant to this problem. Since the vapour pressure is low (Tables 4 and 5), the risk of contaminating the environment through evaporation is minimal. However, air emissions from inappropriately operated plants may pose a hazard. Since the chemical is soluble in water, the potential for exposure through water contamination is of concern. No data are available on levels of diaminotoluenes in surface and groundwater, in soil, and/or air.

4.2 General Population Exposure

No information is available on the exposure of the general population to diaminotoluenes.

4.3 Occupational Exposure

Filatova et al. (1970) reported concentrations of diaminotoluenes in manufacturing plants of up to 0.2 mg/m^3, with occasional excursions up to 11 mg/m^3.

The results of studies conducted in 3 plants manufacturing diaminotoluenes in the USA showed that the work-place ambient air levels ranged from 0.005 to 0.44 mg/m^3 (NIOSH, 1980, 1981, 1982). The highest level of diaminotoluenes (0.44 mg/m^3) was found in the filter room at one plant (NIOSH, 1980). A level of 0.39 mg diaminotoluenes/m^3 was measured in a sample taken at the breathing zone of an operator in a second plant (NIOSH, 1981). All values were calculated as time-weighted averages.

5. KINETICS AND METABOLISM

5.1 Studies on Experimental Animals

5.1.1 Absorption and retention

Skin penetration by test materials varied amoung species (monkeys, swine), and was affected by vehicle and site of application. In one study, [^{14}C]-2,4-DAT (4 µg/cm^2), dissolved in acetone, methanol, or a skin lotion, was applied to 3 - 15 cm^2 of the ventral forearm, abdomen, or back of 3 - 6 monkeys/group (9 groups). The material was removed after 24 h by washing with soap and water. The greatest absorption (53.8 ± 15.4%) resulted when [^{14}C]-2,4-DAT in acetone was applied to the abdominal skin of monkeys (Marzulli et al., 1981). The permeability of diaminotoluenes across the epidermis was highly dependent on the formulation used. When 1.4 g of 2,5-DAT was applied in a gel to the abdominal skin of dogs for a contact period of 3 h, 2.9% (40 mg) was absorbed. Addition of hydrogen peroxide, similar to the formulation used in hair dye, reduced the amount absorbed to < 0.21% (Kiese et al., 1968) or < 0.13% (Hruby, 1977). The hair in the exposed area retained 4% of the ^{14}C activity, 5 days after application (Hruby, 1977).

Hruby (1977) studied the absorption of [^{14}C]-2,5-DAT following oral and subcutaneous single-dose administrations to rats. Five days following subcutaneous injection of 3 - 5 mg [^{14}C]-2,5-DAT (in water), 6.9% of the dose was found in the total-body homogenate and 1.7% remained at the injection site. Five days after oral (gavage) administration of 10 mg [^{14}C]-2,5-DAT (in water), the rat gastrointestinal tract retained 1.4% of the applied radioactivity and 1.2% was found in the total body homogenate (Hruby, 1977).

No studies on uptake after inhalation were found.

5.1.2 Distribution and reaction with body components

The distribution of diaminotoluenes and their reaction with body components have been investigated, mainly after intraperitoneal injection of radioactive labelled compounds. No data on the distribution of diaminotoluenes and reaction with tissues after inhalation or oral ingestion were found in published reports.

Distribution of [^{14}C]-2,4-DAT after intraperitoneal injection was rapid, and the peak concentration in rat and mouse blood and plasma occurred in 1 h, then decreased rapidly for 7 h. On a comparative basis, all tissue concentrations of

bound ^{14}C were considerably lower in the male NIH-Swiss mice than in the male Fischer rats (Grantham et al., 1980).

Tissue distribution of [Me-^{14}C]-2,4-DAT hydrochloride was studied in male B6C3F1 mice given a single intraperitoneal injection (1 µCi, 0.667 mg/kg body weight) (Unger et al., 1980). The highest concentrations, 1/2 h after dosing, were found in the kidneys, gonads, epididymis, lungs, muscle, and blood. One hour after dosing, the liver contained the greatest amount, accounting for nearly 12% of the dose. The concentration in the adrenal glands exceeded that in the kidney, 1 and 2 h after dosing. High concentrations of radioactivity were also observed in the gastrointestinal tract.

Four hours after an intraperitoneal injection of 100 mg (0.8 mmol/kg, ring-labelled [^{3}H]-2,4-DAT) in male Wistar rats, 0.3 nmol was found covalently bound per mg liver protein. A similar degree of binding was seen in the kidneys. Subcellular fractionation of the liver showed that most of the bound material was in the microsomal fraction (Dybing et al., 1978). No significant binding to DNA *in vitro* or *in vivo* could be demonstrated using [^{3}H]-2,4-DAT, whereas it was found to bind covalently to hepatic RNA *in vivo*. These findings were confirmed by Aune et al. (1979).

5.1.3 Metabolism

Glinsukon et al. (1975, 1976) found that the 2,4-isomer was selectively N-acetylated at the p-amino group by liver cytosol prepared from hamsters, guinea-pigs, rabbits, mice, and rats. The cytosol from liver, kidney, intestinal mucosa, and lung of hamsters and rabbits was studied for N-acetyl transferase activity using 2,4-DAT and 4-acetylamino-2-aminotoluene as substrates. All tissues showed marked species differences in enzyme activity. Tissues with high N-acetyl transferase levels, such as liver, could produce both 4-acetylamino-2-aminotoluene and 2,4-diacetylaminotoluene (Glinsukon et al., 1975). There were also sex differences in the N-acetylation capacity of the liver cytosol.

After a single ip injection of 2,4-DAT (77 mg/kg body weight) in male rats, 69.4% of the dose was eliminated in the urine and faeces after 24 h as a complex mixture of metabolites, indicating both free and conjugated derivatives. The major urinary metabolites identified were 4-acetylamino-2-aminotoluene, 2,4-diacetylaminotoluene, and 4-acetylamino-2-aminobenzoic acid. In mice, oxidation of the methyl group to a benzoic acid was the major reaction and the major urinary metabolites in mice were 4-acetylamino-2-aminobenzoic, 4-acetylamino-2-aminotoluene, and 2,4-diacetylaminobenzoic acid (Grantham et al., 1980). Waring & Pheasant (1976) investigated the metabolism of 2,4-DAT in female rabbits, rats, and

guinea-pigs to determine whether the isomer gave rise to hydroxylamines or aminophenols, which might account for the observed toxic and carcinogenic effects. After oral administration (gavage) of 2,4-DAT (50 mg/kg body weight), phenolic metabolites were excreted in the urine. When free and conjugated metabolites were combined, 5-hydroxy-2,4-DAT was the major metabolite in all 3 species (Table 7).

Table 7. Excretion of metabolites after dosing with 2,4-diaminotoluene

Metabolite	Percentage dose excreted[a]		
	Rabbit	Rat	Guinea-pig
2,4-DAT	trace	1.3	trace
3-hydroxy-2,4-DAT	10	8	trace
5-hydroxy-2,4-DAT	22	12	9
6-hydroxy-2,4-DAT (m-aminophenol)	trace	5	trace
3-hydroxy-4-acetylamino-2-aminotoluene	10	18	trace
5-hydroxy-4-acetylamino-2-aminotoluene	6	14	17
glucuronide I, 3-hydroxy-DAT	10	16	15
glucuronide II, 5 hydroxy-DAT	32	12	46
glucuronide III, 6-hydroxy-DAT	2	6	4
unidentified phenolic compounds	0	trace	trace

[a] Results are given as percentage dose, average of 10 studies, standard deviation 6.4% for metabolites 1 - 5, and 12.8% for metabolites 6 - 8. Animals were dosed orally at 50 mg/kg; urine was collected for 48 h. From: Waring & Pheasant (1976).

The levels of methaemoglobin found in the rabbit, rat, and guinea-pig correlated well with the total urinary excretion of aminophenol. The methaemoglobin levels reached a peak 6 - 12 h after the administration of 2,4-DAT and then slowly declined. The highest levels of aminophenols and of methaemoglobin were found in the rabbit (Waring & Pheasant, 1976).

5.1.4 Excretion

During a 24-h period of dermal contact with [^{14}C]-2,4-DAT, ^{14}C urinary excretion in monkeys reached a peak at 8 - 12 h (Marzulli et al., 1981).

Data from studies on the rat, rabbit, mouse, guinea-pig, and dog exposed to diaminotoluenes (cutaneous, subcutaneous, intravenous, intraperitoneal, or oral by gavage) showed fast elimination rates (Kiese et al., 1968; Waring & Pheasant, 1976; Hruby, 1977; Grantham et al., 1980; Unger et al., 1980). The elimination of radioactivity from various tissues in rodents followed a well-defined biphasic pattern. Rapid elimination over 7 h was followed by a rather slow decline in the isotopic contents of tissues (Grantham et al., 1980; Unger et al., 1980). The half-lives of tissue elimination during the fast phase were 0.89, 0.43, and 1.51 h for male mouse liver, kidneys, and blood, respectively. During the slow phase of elimination, the half-lives for liver, kidneys, and blood in male mice were 11.7, 9.1, and 12.6 h, respectively. The half-lives of elimination of radioactivity, during the slow phase, were greater for muscle (23.9 h) and skin (29.2 h) than for any other tissue (Wagner, 1975; Unger et al., 1980).

The primary route of elimination in rodents was via the kidneys during the first hour after exposure. However, after 2 h, the predominant route shifted from urinary to faecal, probably a reflection of biliary excretion. Only 1.25% of the administered radioactivity had been exhaled after 24 h (Unger et al., 1980). On a comparative basis, faecal elimination was greater in rats than in mice, but the rate of urinary excretion was more rapid in mice than in rats. Approximately 90% of a dose was eliminated in the urine of mice in 24 h compared with 74% in the urine of rats (Grantham et al., 1980). Complete elimination was accomplished in 2 days in mice, while rats required 6 days.

Male and female rats, injected subcutaneously with 3 - 5 mg [^{14}C]-2,5-DAT hydrochloride, eliminated 65% of the dose in the urine and 5% in faeces after 24 h. The same pattern of elimination was found after oral administration of 10 mg of the labelled compound (Hruby, 1977).

When beagle dogs were intravenously injected with a dose of 224 mg, infused over 3 h, the total amounts of radioactivity eliminated in the urine and faeces were 60% and 19%, respectively. After 4 days, elimination mainly occurred within the first 24 h. After a skin application of 1.4 g [^{14}C]-2,4-DAT for 3 h (in 50 ml of a dye formulation), only 0.092 and 0.84% of the dose were eliminated, respectively, in the urine and faeces of beagle dogs over 4 days, reflecting the inhibitory effect of the dye formulation on the absorption of the 2,4-DAT (Hruby, 1977). In another study on dogs, about 40 mg 2,5-diaminotoluene was absorbed through the skin from a gel containing 1.4 g of the material. The addition of hydrogen peroxide to the gel reduced the amount absorbed to less than 3 mg. The amount excreted unchanged in the urine was 60 - 70 µg (Kiese et al., 1968).

5.2 Human Studies

As only limited information is available on the absorption, distribution, metabolism, and excretion of diaminotoluenes in human beings, these aspects are discussed together, rather than in separate sections.

Although the high boiling point of diaminotoluenes makes absorption through the lungs unlikely under normal working conditions, inhalation may occur when hot vapours escape from stills. Possible inhalation and dermal exposure to dusts may occur if diaminotoluenes are handled in a less than optimal manner. Since diaminotoluenes are soluble in water, absorption from the gastrointestinal tract could occur following ingestion. However, no data were found on the kinetics and metabolism of diaminotoluenes after oral or inhalation exposures.

Skin penetration by [^{14}C]-2,4-DAT was measured in human beings (Marzulli et al., 1981). When 4 µg [^{14}C]-2,4-DAT in acetone/cm^2 was applied to the skin of the forearm, the highest absorption of the chemical (23.7 ± 16.1% of the applied dose) resulted after 24 h of dermal contact. Urinary excretion reached a peak after 4 - 8 h of skin contact. In a study by Kiese & Rauscher (1968), the hair of 5 human subjects was dyed (40 min) with a formula containing 2.5 g 2,5-DAT; absorption of approximately 0.2% of the applied material occurred. No data were given on the retention and distribution of diaminotoluenes after this dermal contact.

Data from studies on 6 volunteers (3 males and 3 females) showed that, after subcutaneous injection of 5.54 mg 2,5-DAT, 47.6% of the dose was excreted in the urine as N,N'-diacetyl-2,5-DAT. The rate of excretion was highest during the first 24 h, and only a trace appeared in the urine excreted on the third day, in one study. When the compound was applied as a hair dye (40 min), the highest rate of excretion was observed during a period of 5 - 8 h after application. On average, a total amount of 3.7 mg N,N'-diacetyl-2,5-DAT (i.e., 0.09% of the applied dose) was calculated to have been excreted in urine taken over 2 days from 5 subjects (Kiese & Rauscher, 1968).

6. EFFECTS ON ORGANISMS IN THE ENVIRONMENT

Little information is available on the effects of diaminotoluenes on animal populations found in the environment. The effects of 2,4-DAT at concentrations ranging from 1 to 1000 mg/litre were observed for Daphnia (Daphnia magna Straus), ostracoda, guppies (Lebistes reticulatus Peters), and channel seaweed (Scenedesmus obliquus). Daphnia was the most sensitive species; 5 mg/litre was lethal in 5 - 10 days, and prolonged exposure to 2 mg/litre caused a reduction in the number of offspring produced. A concentration of 20 mg/litre was not lethal for ostracods after 10 days, but 50 mg/litre was lethal in 5 - 8 days. Fish survived for 10 days at 200 mg/litre, but a concentration of 500 mg/litre was lethal in 2 - 3 days. The algae tested were the most resistant, surviving for 10 days at a concentration of 1000 mg/litre (Smirnova et al., 1967).

7. EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO TEST SYSTEMS

The early literature (1881-1939) contains several reports on toxicological manifestations associated with the administration of diaminotoluenes in experimental animals. Most of the papers are difficult to interpret and to use in the assessment of chemical hazards, because massive doses of chemicals of unknown purity and isomeric composition were used, and because of the experimental designs chosen. The toxic effects were characterized by icterus, haemoglobinuria, disposition of haemosiderin in the spleen, bone marrow, and liver, respiratory and generalized central nervous system (CNS) depression, pulmonary and cerebral oedema, and increased bile acids in the liver, blood, and/or urine of exposed animals (Von Oettingen, 1941).

7.1 Single Exposures

Diaminotoluenes are considered to be dermal and eye irritants. In studies on rabbits, 12.5 mg 2,5-DAT or 500 mg 2,4-DAT caused skin irritation, defined as erythema and oedema, after 24 h of dermal contact. Instillation of 100 µg of the 2,4-isomer into the rabbit eye caused severe eye irritation within 24 h. Data showing the extent of the acute toxicity of diaminotoluenes in various laboratory animals are summarized in Tables 8 and 9.

The acute toxic effects of diaminotoluenes were characterized by marked central nervous system depression during exposure (e.g., decreased locomotor activity, piloerection, ptosis, ataxia, tremors) and production of methaemoglobin, 6 - 8 h after exposure.

Duodenal and glandular mucosal damage in the stomach were observed in fed, unrestrained rats, 24 h following a single subcutaneous dose of 3,4-DAT. The optimal ulcerogenic dose of 3,4-DAT (i.e., the dose causing a low mortality and a maximal incidence of duodenal damage within 24 h), was 350 mg/kg body weight (Perkins & Green, 1975).

7.2 Short-Term Exposures

When guinea-pigs were treated with 1 - 10% 2,5-DAT (24 h/day for 5 days, 2 days without treatment, followed by exposure for another 5 days), sensitization was obtained in 35% of treated animals (Schäfer et al., 1978).

Both the 2,4- and 3,4-isomers caused severe icterus in rats. In male and female rats, 3,4-DAT (unlike the 2,4-isomer), given orally or parenterally, produced a high incidence of perforating duodenal ulcers within a few days

Table 8. Lethality of diaminotoluenes

Species	Exposure route	LD_{50} (mg/kg body weight)	Reference
Ortho-DAT (2,3-, 3,4- mix)			
Rat	oral	810	Carpenter et al. (1974)
Rabbit	dermal	1120	Carpenter et al. (1974)
Meta-DAT (2,4-, 2,6- mix)			
Rat	oral	300	Izmerov et al. (1982)
Rat (male)	oral	270	Weisbrod & Stephan (1983)
Mouse (male)	oral	350	Weisbrod & Stephan (1983)
Rat (male)	ip	230[a]	Weisbrod & Stephan (1983)
Mouse (male)	ip	240[b]	Weisbrod & Stephan (1983)
Rat (male)	iv	350	Weisbrod & Stephan (1983)
Mouse (male)	iv	90-105	Weisbrod & Stephan (1983)
Rat	dermal	1200	Izmerov et al. (1982)
2,4-DAT (technical grade)			
Fischer rat (male)	ip	325	Grantham et al. (1980)
NIH-Swiss mouse (male)	ip	480	Grantham et al. (1980)
HaM/ICR mouse (male)	ip	80	Weisburger et al. (1978)
HaM/ICR mouse (female)	ip	90	Weisburger et al. (1978)

[a] A methaemoglobin level of 8.4% was observed, 6 h after ip administration.

[b] A methaemoglobin level of 7.8% was observed, 6 h after ip treatment.

Note: No published data on the acute toxicity of the 2,3-isomer were available.

(Selye, 1973). These effects were obtained in animals allowed to move freely with access to food and water during the period of observation. A dose of 500 mg diaminotoluenes/kg body weight was administered in 2 ml water, twice daily.

The effects of 2,4-DAT on the liver microsomal mixed-function oxidase system, DT-diaphorase, and epoxide hydrolase were reported by Dent & Graichen (1982). Following oral treatment with 2,4-DAT at 70 mg/kg body weight per day for 5 days, the activities of microsomal cytochrome P-450-dependent enzymes were depressed, while epoxide hydrolase activity was markedly elevated (3 - 8 times control) in male F-344 rats. Under these experimental conditions, an increase in the liver to body weight ratio (3.2 - 4%), and in the liver microsomal protein concentration (19.3 - 27.4 g/kg) were induced by 2,4-DAT.

Table 9. Summary of some single-dose studies

Species	Route of exposure	Dose (mg/kg body weight)	Effects	Reference
2,4-DAT (technical grade)				
Wistar rat (male)	oral	50	produced metHb[a]; highest amounts (5 - 6%) were found 6 - 8 h after exposure	Waring & Pheasant (1976)
		> 50	toxic	
Sprague Dawley rat (male and female)	oral	500	developed icterus and death	Selye (1973)
NZW rabbit	oral	50	MetHb reached 18 - 20% level 6 - 8 h after application	Waring & Pheasant (1976)
		> 50	toxic	
Dunkin-Harvey guinea-pig	oral	50	MetHb reached 3 - 4% level 6 - 8 h after application	Waring & Pheasant (1976)
		> 50	toxic	
3,4-DAT (97% pure)				
Sprague Dawley rat (female)	subcutaneous	125-500	discrete, non-perforated duodenal lesions were observed immediately distal to the gastroduodenal junction 24 h following the administration of a single dose	Perkins & Green (1975)

[a] metHb = methaemoglobin.

7.3 Long-Term Exposure

Oral administration of 2,4-DAT (see Table 11, section 7.6, for doses) for 79 - 103 weeks accelerated the appearance of renal toxicity in male F-344 rats, associated with a high incidence of secondary hyperparathyroidism (NCI, 1979). The chronic renal disease reported was believed to have decreased the longevity of the treated rats, either directly or through inhibition of the clearance of toxic metabolites (Cardy, 1979).

No studies were found on the effects of diaminotoluenes on the nervous system or the immune system after long-term exposure.

7.4 Reproduction and Teratogenicity

7.4.1 Reproduction

There are 2 studies on experimental animals that evaluate the reproductive toxicity of diaminotoluenes. Soares & Lock (1980) administered 2,4-DAT orally or ip at 40 mg/kg body weight for 2 days to DBA/2J male mice. Forty-eight hours after treatment, mating trials were conducted for 8 weeks. There were no treatment-related effects on sperm morphology or fertility, as measured by this dominant lethal assay. However, in male Sprague Dawley rats, long-term exposure to 2,4-DAT in the feed impaired reproductive performance and capacity (Thysen et al., 1985a,b). Dietary levels of 0.03% 2,4-DAT for 10 weeks (≈ 15 mg/kg body weight per day) decreased fertility and exerted an inhibitory effect on sperm production in male rats. Eleven weeks after treatment, the sperm count remained significantly depressed ($P < 0.001$), suggesting irreversible damage to the germinal components in the testes. Data from hormone analyses at the end of the 10 weeks of exposure, and at the end of 11 weeks after treatment, showed a significant decrease in serum-testosterone and an elevation of serum-luteinizing hormone concentrations, which were associated with a reduction in seminal vesicle weight. Histological changes found in the reproductive organs from treated males were correlated with these physiological changes. At a lower dose (0.01% or ≈ 5 mg/kg body weight), 2,4-DAT did not cause any of these toxic responses.

7.4.2 Teratogenicity

Studies on the teratogenic potential of diaminotoluenes are summarized in Table 10. Skin application of 2,4-DAT induced a low incidence of skeletal changes in rats (Burnett et al., 1976). Oral or intraperitoneal administration of this isomer did not produce any effects on the fertility or reproductive performance of male mice (Soares & Lock, 1980). Subcutaneous or intraperitoneal injection of 2,5-DAT in mice on day 8 of gestation, at levels of 50 or 75 mg/kg body weight, caused craniofacial malformation and fused or distorted thoracic vertebrae associated with the absence of, or fused, ribs (Inouye & Murakami, 1976, 1977). However, 2,5-DAT sulfate, at levels of 16 - 64 mg/kg body weight per day administered subcutaneously on days 6 - 15 of gestation, did not cause any malformations in mice or rats (Marks et al., 1981).

The results of oral administration of 2,6-DAT to rats and rabbits, at doses of between 10 and 300 mg/kg body weight (Knickerbocker et al., 1980), showed that, in rats, doses of

Table 10. Teratogenicity studies with diaminotoluenes

DAT isomer	Species	Route of administration	Dose and duration	Effects	Reference
2,4- (3% in hair-dye formula)	Charles River/CD rat (female)	skin	2 ml/kg body weight on days 1, 4, 7, 10, 13, 16, and 19 of gestation	skeletal changes seen in 6/169 live fetuses ($P > 0.05$)	Burnett et al. (1976)
2,5- (sulfate) (3% in hair-dye formula)	Charles River/CD rat	skin	2 ml/kg body weight on days 1, 4, 7, 10, 13, 16, and 19 of gestation	no increase in abnormalities in treated groups	Burnett et al. (1976)
2,5- dihydro-chloride	JCL:ddn mice (female)	subcutaneous or intraperitoneal single dose	50 mg/kg body weight on one day of days 7 - 14 of gestation or 75 mg/kg on day 8 of gestation or 50 mg/kg on day 8 of gestation	In groups treated sc or ip on day 8 of gestation, there was evidence of craniofacial malformation: exencephaly, prosoposchisis, and hair lip with cleft palate, and high incidence of skeletal malformation: fused or distorted thoracic vertebrae associated with absence of, or fused, ribs; no such malformed fetuses were found in groups treated on days 10 - 14 of gestation; only a very low incidence of vertebral and rib anomalies followed treatment on day 7 or 9; maternal toxicity was reported at 75 mg/kg but not at 50 mg/kg	Inouye & Murakami (1976, 1977)

Table 10 (contd).

2,5- (sulfate)	CD-1 mice	subcutaneous	16, 32, 48, or 64 mg/kg body weight per day on days 6 - 15 of gestation	no teratogenic effects were noted; maternal toxicity was evident at 48 and 64 mg/kg; reduced fetal weight was noted at $\geq$ 32 mg/kg	Marks et al. (1981)
2,5- (sulfate)	rat	oral (gavage)	10, 50, or 80 mg/kg body weight per day on day 15 of gestation	maternal toxicity and embryotoxicity evident at 80 mg/kg; no effects observed at lower doses	Spengler et al. (1986)
	rabbit	oral (gavage)	10, 25, or 50 mg/kg body weight per day on days 6 - 18 of gestation	no effects observed	
2,6-	Sprague Dawley rat	oral (gavage)	10, 30, 100, or 300 mg/kg body weight per day on days 6 - 15 of gestation	no effects on pregnancy, number of live fetuses, and resorption sites/dam; 300 mg/kg produced smaller body weight gain in the dams; 30 - 300 mg/kg produced increased haemorrhagic abdomens in the fetuses; 100 and 300 mg/kg increased the occurrence of incomplete vertebrae, and 300 mg/kg showed missing sternebrae and incomplete skull closure in the fetuses; the no-observed-adverse-effect dose was 10 mg/kg per day	Knickerbocker et al. (1980)
2,6	Dutch belted rabbit (female)	oral (gavage)	3, 10, 30, or 100 mg/kg body weight per day on days 6 - 18 of gestation	100 mg/kg per day reduced dam weights, increased resorptions, decreased fetal weights, and neonatal survival;	Knickerbocker et al. (1980)

Table 10 (contd).

2,6 (contd)	Dutch belted rabbit (female)	oral (gavage)	3, 10, 30, or 100 mg/kg body weight per day on days 6 - 18 of gestation	there were no differences in in skeletal or soft-tissue abnormalities between treated animals and controls; the no-observed-adverse-effect dose was 30 mg/kg per day	Knickerbocker et al. (1980)
o-DAT (2,3-, 3,4- isomer mix)	Sprague Dawley rat	oral	10, 30, 100, or 300 mg/kg body weight per day on days 6 - 15 of gestation	maternal toxicity was indicated at 300 mg/kg per day by reduced body weight gain during gestation; No significant differences in numbers of live fetuses, implantation or resorption sites; fetal body weight was reduced at the highest dose ($P \leq 0.05$); no evidence of teratogenic effects or effects on dams at doses $\leq$ 30 mg/kg; no skeletal or soft-tissue malformations that could be related to treatment; however, increased incidence of missing sternebrae at 300 mg/kg per day and incomplete ossified vertebrae at 100 and 300 mg/kg per day were noted compared with controls	Becci et al. (1983)
o-DAT (2,3-, 3,4- isomer mix)	Dutch belted rabbit	oral	3, 10, 30, or 100 mg/kg body weight per day on days 6 - 18 of gestation	maternal toxicity at 100 mg/kg per day elicited by reduced body weight gain during pregnancy; no significant difference in the number of implantations; at 100 mg/kg per day, fetal body weight was reduced and the number of resorption sites was increased; no skeletal or soft-tissue malformations that could be related to treatment were noted	Becci et al. (1983)

between 100 and 300 mg/kg body weight increased the occurrence of incomplete vertebrae and that the highest dose resulted in missing sternebrae and incomplete closure of the skull. A no-observed-adverse-effect level of 10 mg/kg body weight was reported in rats. No skeletal or soft-tissue abnormalities were observed in the offspring of rabbits, but, using fetal toxicity indices, a no-observed-adverse-effect level of 30 mg/kg body weight was reported.

Becci et al. (1983) administered Ortho-DAT (2,3-, 3,4-isomer mixture) by gavage to rats and rabbits (Table 10). Reduced body weight during gestation was noted at 300 mg/kg body weight in rats and 100 mg/kg body weight in rabbits. An increased incidence of several skeletal variations in the fetuses was noted, probably due, in part, to the maternal toxicity. The no-observed-adverse-effect level in both rats and rabbits was 30 mg/kg body weight.

7.5 Mutagenicity and Related End-Points

7.5.1 DNA damage

At concentrations of 1×10^{-4} mol and below, 2,4-DAT, but not 2,6-DAT, induced unscheduled DNA synthesis in primary cultures of rat hepatocytes (Bermudez et al., 1979). 2,4-DAT produced a significant elevation in unscheduled DNA synthesis at 2 and 12 h in the *in vivo/in vitro* hepatocyte DNA repair assay (Mirsalis & Butterworth, 1982; Mirsalis et al., 1982). 2,5-DAT produced a positive response in a DNA-repair assay in rat hepatocytes and a weak positive response in hamster hepatocytes at 10^{-5} mol, the highest concentration that was not toxic to the cells that were tested (Kornbrust & Barfknecht, 1984).

Shooter & Venitt (1979) used continuous administration of 2,4-DAT in the drinking-water to determine whether phosphotriesters could be detected in the DNA of the liver of treated rats. Positive results were obtained at 10 mg/litre. A low, but significant, level of these lesions was produced. Shooter & Venitt's studies on rodents indicated that methyl- and ethylphosphotriesters persist for many weeks in the DNA of certain organs (notably liver, kidney, and lung) and that such lesions are not eliminated by DNA repair.

The results of studies by Greene et al. (1981) showed that 2,4-, 2,5-, 2,6-, and 3,4-isomers significantly inhibited the incorporation of [^{125}I]-iododeoxyuridine into mouse testicular DNA and demonstrated dose-response characteristics. The 2,4-, 2,5-, and 3,4-isomers were capable of reaching the testes and of passing target cell membranes at this site. They concluded that the 3 isomers may present a genetic health hazard for an intact animal. The inhibition induced by

2,6-DAT might have been caused by a chemically-induced decrease in body temperature (Greene et al., 1981).

DNA damage was not found in human cultured fibroblasts after exposure to 100 µmol 2,4-DAT alone (3.5 ± 1.5 increase in percent single-strand DNA). When the cells were incubated in the presence of 1 mg ram seminal vesicle microsomes/ml and 100 µmol arachidonic acid, a significant increase in the fraction of single-strand DNA (21.3 ± 3.7, $P < 0.001$) was found in cells exposed to 2,4-DAT. DNA strand breaks were not induced when prostaglandin synthase (PGS) was inhibited by adding indomethacin (100 µmol) or acetylsalicylic acid (1 mmol) (Nordenskjöld et al., 1984). These results, which suggest that 2,4-DAT may be activated by PGS to form products that cause DNA damage in cultured human fibroblasts, are in agreement with the findings of Rahimtula et al. (1982).

7.5.2 Mutation

Several studies have shown that 2,4-, 2,6-, and 2,5-isomers can induce reverse mutations in Salmonella typhimurium strains TA 1538 and TA 98, in the presence of various metabolic activation systems (McCann et al., 1975; Dybing & Thorgeirsson, 1977; Dybing et al., 1977; Pienta et al., 1977b; Cinkotai et al., 1978; Aune et al., 1979; Shahin et al., 1980). However, Mori et al. (1982) showed that 2,4-DAT was inactive for strains TA 98 and TA 100 at doses ranging from 5 to 1000 µg/plate. While 2,3-DAT is inactive in S. typhimurium (Florin et al., 1980), its homologue 3,4-DAT showed a marginal response in strains TA 98 and TA 1538 (Greene et al., 1979).

2,4-DAT was shown to be a weak mutagen in Drosophila melanogaster, inducing sex-linked recessive lethals when fed to adult males at a concentration of 15.2 mmol (Blijleven, 1977; Venitt, 1978). In a study reported by Fahmy & Fahmy (1977), 2,4-DAT was injected around the testes of adult male Drosophila at doses ranging from 5 to 20 mmol. Mutagenicity was measured at the various stages of spermatogenesis, both on the X-chromosome and RNA genes. The overall induced frequency of X-recessives was extremely low. It was also observed that mutation yield was not dose-related and that it was maximal in the earliest progeny fraction, suggesting a greater toxicity for mature sperm.

2,4-DAT was mutagenic in L5178Y mouse lymphoma cells and CHO-AT3-2 cells (Matheson & Creasy, 1976; Coppinger et al., 1984). Mutagenic activity was observed in L5178Y cells, only in the absence of exogenous metabolic activation, but was observed in CHO-AT3-2 cells both with and without activation.

The in vivo mutagenic activity of 2,4-DAT was studied in DBA/2J male mice by the dominant lethal assay, sperm abnormality assay, and the recessive spot test. Mice were administered the compound by intraperitoneal injection and orally by gavage (2 daily doses of 40 mg/kg body weight), just before mating (Soares & Lock, 1980). No induction of dominant lethals was noted, nor was any increase in abnormal sperm or recessive spots reported.

No dominant lethals were induced in Charles River rats injected intraperitoneally, 3 times weekly for 8 weeks, with 20 mg 2,5-DAT/kg body weight, before mating (Burnett et al., 1977).

7.5.3 Cell transformation

Several studies have shown that the 2,4-, 2,5-, 2,6-, and 3,4-isomers can induce morphological transformations in Syrian golden hamster embryo cells (Pienta et al., 1977a; Greene & Friedman, 1980). Each isomer chemically transformed secondary hamster embryo cells, but none were active in more than 50% of the 5 or 6 separate tests performed on each isomer (Greene & Freidman, 1980).

7.5.4 Chromosomal effects

Cytogenetic preparations were made from the bone marrow of male mice, 30 and 48 h after intraperitoneal injection of 2 daily doses of 2,4-DAT at 40 mg/kg body weight. The treatment did not induce any obvious chromosome breaks (Soares & Lock, 1980).

2,5-DAT did not induce micronucleated cells in bone marrow after oral administration of 120 mg/kg body weight to male and female rats, in 2 doses separated by an interval of 24 h (Hossack & Richardson, 1977).

7.6 Carcinogenicity

Several long-term studies on the carcinogenic potential of the 2,4-, 2,5-, and 2,6-DAT isomers have been published. The experimental designs used in these studies are summarized in Table 11. The experimental designs used in 2 studies on the carcinogenic effects of hair dye formulations are also given in Table 11.

Table 11. Experimental designs of carcinogenicity studies on diaminotoluenes

Isomer	Species (sex)	Initial size of high-/low-dose groups (control)	Route of administration/ dose and duration	Reference
2,4-	rat (male and female)	20	subcutaneous; 2 mg in 0.5 ml propylene glycol weekly; total of 28 injections; 452 days	Umeda (1955)
2,4-	Wistar rat (male)	12/12 (6)	oral (diet); 0.6 and 1 g/kg; 36 weeks	Ito et al. (1969)
2,4-	Charles River/CD rat (male)	25/25 (25)	oral (diet); 500 and 1000 mg/kg for 4 months and 250 and 500 mg/kg for 14 months; oral (diet); 500 and 1000 mg/kg for 18 months	Weisburger et al. (1978)
	Charles River mouse (male)	25/25 (25)		
	Charles River mouse (female)	25/25 (25)		
2,5-	Fisher 344 rat (male)	50/50 (50/25)	oral (diet); 600 and 2000 mg/kg for 78 weeks + 31 weeks observation	NCI (1978)
	Fisher 344 rat (female)	50/50 (50/25)		
	B6C3F1 mouse (male)	50/50 (50/50)	oral (diet); 600 and 2000 mg/kg for 78 weeks + 19 weeks observation	
	B6C3F1 mouse (female)	50/50 (50/50)		
2,4-	Fisher 344 rat (male)	50/50 (20)	oral (diet); 125 and 250 mg/kg for 40 weeks reduced to 50 and 100 mg/kg for 63 weeks (time-weighted-average 79 and 176 mg/kg); high-dose males killed after 79 weeks and high-dose females after 84 weeks	NCI (1979)
	Fisher 344 rat (female)	50/50 (20)		

Table 11 (contd).

2,4	B6C3F1 mouse (male) B6C3F1 mouse (female)	50/50 (20) 50/50 (20)	oral (diet); 100 and 200 mg/kg for 101 weeks	NCI (1979)
2,6-	Fisher 344 rat (male) Fisher 344 rat (female)	50/50 (50) 50/50 (50)	oral (diet) 250 and 500 mg/kg for 103 weeks plus 1 week observation	NCI (1980)
	B6C3F1 mouse (male) B6C3F1 mouse (female)	50/50 (50) 50/50 (50)	oral (diet); 250 and 500 mg/kg for 103 weeks plus 1 week observation	
2,5- (formulations with 6% hydrogen peroxide added)	Sprague Dawley rat (male) Sprague Dawley rat (female)	50 (50) 50 (50)	dermal application twice weekly of 0.5 g of synthetic formulation containing 4% 2,5-DAT mixed with equal volume of 6% H_2O_2; treated 2 years	Kinkel & Holzmann (1973)
2,5-	Swiss Webster mouse (equal mixture male and female)	100 (250)	dermal application of 0.05 ml weekly of formulation to which equal volume of 6% H_2O_2 had been added; treatment period, 18 months	Burnett et al. (1975)
2,5- + 2,4- (formulations with 6% hydrogen peroxide added)	Swiss Webster mouse (equal mixture male and female)	100 (250)	dermal application of 0.05 ml weekly of formulation to which eqnal volume of 6% H_2O_2 had been added; treatment period, 18 months (same control group as above study)	Burnett et al. (1975)

Although the early studies of Umeda (1955) and Ito et al. (1969) used protocols that generated data of minimal use for a hazard evaluation, they did produce qualitative information showing that 2,4-DAT was carcinogenic for rats. These studies have been extended to the 2,5-, 2,6-, as well as 2,4-DATs using more animals per study and well-defined protocols (NCI, 1978, 1979, 1980; Weisburger et al., 1978).

Groups of 25 male Charles River/CD rats were administered 2,4-DAT in the diet at time-weighted levels of 300 and 625 mg/kg for 18 months. Similarly, groups of 25 male and female CD-1 mice were given diets containing 500 and 1000 mg 2,4-DAT/kg for 18 months (Weisburger et al., 1978). The rats and mice used in these studies had a high incidence of spontaneous tumours. However, there was a statistically significant increase in subcutaneous fibromas in male rats and hepatocellular carcinomas and vascular tumours in male and female mice compared with controls.

Studies by the US National Cancer Institute on the carcinogenicity of 2,4-DAT in rats and mice (NCI, 1979) confirmed the report by Weisburger et al. (1978). Administration of time-weighted average doses of 79 and 176 mg 2,4-DAT/kg diet to groups of 50 male and 50 female Fisher 344 rats, for 103 weeks, led to a severe depression in body weight gain, high mortality, and a dose-related development of hepatocellular carcinomas or neoplastic nodules in treated rats of both sexes. In addition, NCI reported that carcinomas and adenomas of the mammary gland occurred in female rats at incidences that were dose related and significantly greater than those in the controls in both the high- and low-dose groups. Groups of 50 male and 50 female B6C3F1 mice were similarly administered 2,4-DAT at 100 or 200 mg/kg diet, for 101 weeks. In male mice, tumour incidence was not significantly increased compared with that in the control animals. However, the incidence of hepatocellular carcinomas in female mice in both treated groups was dose related and significantly higher than that in the controls. Numbers of lymphomas were also higher in low-dose female mice. On the basis of these results, it was concluded that 2,4-DAT was carcinogenic for Fisher 344 rats of both sexes and for female B6C3F1 mice (NCI, 1979).

The carcinogenic potential of 2,5-DAT and 2,6-DAT for rats and mice was determined by the US National Cancer Institute. After administration of 2,5-DAT at 600 and 2000 mg/kg feed to groups of 50 male and 50 female Fisher 344 rats and 50 male and 50 female B6C3F1 mice, for 78 weeks, there was not sufficient evidence to demonstrate the carcinogenicity of 2,5-DAT (NCI, 1978). However, the study had been curtailed

and this reduced the potential of the test for detecting carcinogenicity.

Using a similar protocol, 2,6-DAT was incorporated at levels of 250 and 500 mg/kg into the diet of groups of 50 male and 50 female Fisher 344 rats, and B6C3F1 mice for 103 weeks (NCI, 1980). There was some question of whether mice of either sex received a maximum tolerated dose, but the dose given to rats appeared to be at the maximum tolerated level. As reported by NCI (1980), islet-cell adenomas of the pancreas and neoplastic nodules or carcinomas of the liver occurred in male rats in dose-related trends that were significant using the Cochran-Armitage test, but not using the Fisher exact test. In the low-dose male mice, NCI reported that the incidence of lymphomas was greater than that in the controls. However, the incidence was not significant when the Bonferroni criterion of multiple comparison was used. Similarly, the occurrence of hepatocellular carcinomas in female mice was dose related, but not significant by the Fisher exact test, when the incidence in the high-dose groups was compared with that in the controls. Under the conditions of the bioassay, it was concluded that 2,6-DAT was not carcinogenic for male and female F344 rats or for male and female B6C3F1 mice (NCI, 1980). Summaries of the 3 NCI bioassays have been published by Cardy (1979), Reuber (1979), and Sontag (1981).

Using the protocols outlined in Table 11, no evidence of carcinogenicity was obtained when hair-dye formulations containing 2,5- and 2,5- plus 2,4-DAT were painted on the skin of rats and mice (Kinkel & Holzmann, 1973; Burnett et al., 1975). Given the duration of exposure, the amounts of diaminotoluenes placed on the skin, and the use of hydrogen peroxide prior to administration, such negative results would be expected in studies on the formula containing 2,4-DAT (Burnett et al., 1975). These studies have shown a low order of dermal toxicity for these hair dyes, even after long-term exposure. However, no definitive statement can be made on the carcinogenic potential of 2,4- and 2,5-DAT after dermal administration.

8. EFFECTS ON MAN

8.1 Single and Short-Term Exposures

In human beings, as in animals, diaminotoluenes are considered to be irritants for the mucous membranes and skin, and to lead to conjunctivitis and corneal opacities. When solutions come into contact with skin, they can cause irritation, severe dermatitis, and blistering (Von Oettingen, 1941). In case of the inhalation of fumes, coughing, dyspnoea, and respiratory distress can result. No data are available for evaluating the sensitizing potential of diaminotoluenes. In the case of ingestion of massive amounts, nausea, vomiting, and diarrhoea would occur, with the possible production of methaemoglobinaemia. No cases of human poisoning from short-term exposures to 2,4-DAT have been documented in the published literature.

8.2 Long-Term Occupational Exposure - Epidemiological Studies

Filatova et al. (1970) investigated the physiological and biochemical status of workers in a plant manufacturing diaminotoluenes. Fifty-two of the 59 workers (58 males and 1 female) had worked in the plant for $\geq$ 2 1/2 years. Seventeen workers were between 30 and 45 years of age and 42 workers were under 30 years old. In general, all workers had equal exposure to the toxic chemicals used at the plant, namely, dinitrotoluene, diaminotoluenes, methanol, and o-dichlorobenzene. There were a few complaints of headache (2 cases), excess coughing (2 cases), stomach pains (2 cases), and chest pain (4 cases); however, the majority of the workers did not exhibit any exposure-related adverse effects. Nevertheless, the investigators concluded that the 10 workers exhibiting the symptoms listed were indeed affected by their exposure to the complex of chemicals at this factory. It is impossible to delineate the role played by the diaminotoluenes in the production of these adverse effects.

The US National Institute for Occupational Safety and Health (NIOSH) evaluated the reproductive health of workers in 3 plants manufacturing diaminotoluenes (NIOSH, 1980, 1981, 1982). Exposure usually involved both DAT and dinitrotoluene (DNT). All 3 surveys were conducted in response to requests from employees or their unions. The reason for the first request was the workers' belief that their wives were suffering increased rates of spontaneous abortion. The other 2 studies were prompted by the publicity given to the first study.

In 2 of the studies, environmental hygiene sampling took place (section 4.2) and workers were invited to volunteer for

a medical examination, to complete a reproductive history questionnaire, and to provide semen and blood samples. Semen samples were analysed for volume, sperm count, and sperm morphology. Blood samples were analysed for various markers of renal and hepatic function, neither of which showed any significant inter-group differences in any of the studies. Wives of workers were given a more detailed reproductive history questionnaire.

In the first study (NIOSH, 1980), there were 44 volunteers, 30 of whom provided usable semen specimens. The total potential study population was not given. Of the 30, 9 were exposed, 9 were controls, and 12 were in an intermediate category. The rate of spontaneous abortions was higher among the wives of exposed workers (6/18 pregnancies while the husband was exposed) compared with the controls (4/23 pregnancies), with 6/28 for the intermediate group. The small number of congenital malformations was not exposure related. The sperm count for the exposed (median = 49 million) was significantly ($P < 0.03$) lower than that for the control group (median = 121 million); however, the latter figure was unusually high. The exposed group showed a significant reduction in the proportion of the large morphological type. The second study involved only a reproductive history questionnaire and the reporting of hygiene data by the company. Thirty-five out of 41 workers in DAT- and DNT-production areas were interviewed. The rates of congenital abnormalities or spontaneous abortion did not significantly differ between exposure groups. Where the husband was employed in DNT production, 1 out of 9 pregnancies ended in a spontaneous abortion; the equivalent data for DAT was 1 miscarriage out of 14 pregnancies.

In the third study, 50 volunteers were examined, 41 of whom provided semen specimens. The total eligible population was not given, though it was reported that 25 workers were regularly exposed, 15 of whom participated in the study. There were no significant differences in sperm count morphology between exposure groups, but the miscarriage rate was reported to be significantly ($P < 0.05$) higher for the wives of workers in the DAT-exposed area of the plant, where 6 out of 15 pregnancies ended in miscarriage compared with 1 out of 7 for the wives of DNT-exposed workers, and 3 out of 38 for the wives of unexposed workers.

The ranges of levels of reported exposures overlapped between the 3 studies and were all within the OSHA recommended standard of 1.5 mg/m^3 for dinitrotoluenes (NIOSH, 1982). The first study might have been expected to show an excess, because it was provoked by a cluster of miscarriages. All the studies were of limited size and subject to some risk of selection bias, because the population was restricted to those

who volunteered. Also, in all 3 studies, crude figures, with no adjustment for age, were presented. However, in the 2 follow-up studies, where apparently neither of the populations held a prior belief that there was an excess miscarriage rate, it is significant that an apparent excess of miscarriages was found in the wives of DAT-exposed workers.

An epidemiological assessment of the reproduction hazards for males after occupational exposure to diaminotoluenes and dinitrotoluenes was carried out by Hamill et al. (1982). Reported occupational exposures were similar to those reported by NIOSH (1980, 1981, 1982) and were generally well within the OSHA recommended level of 1.5 mg/m^3 for dinitrotoluenes. Examination of 84 workers and 119 unexposed subjects consisted of semen analysis, blood testing, medical examination, and an interview. Seventy-two percent of non-vasectomized exposed workers provided semen samples. These groups of workers were defined by exposure intensity, frequency, and recency, and compared with controls. Although no significant differences in miscarriage rates were reported between exposure categories, the categories were as defined at the time of the study, not at the time of pregnancy. Fertility rates were reported to be unaffected by exposure, but no figures were given. There were no statistically significant differences in semen analysis, sperm count and morphology, and FSH levels, between the 3 exposure groups and unexposed workers. The authors concluded that the results of their study suggested that no detectable reproductive effects existed among male workers exposed to dinitrotoluenes and diaminotoluenes.

Levine et al. (1985) reported an analysis of the fertility of workers exposed to DNT and DAT. The approach taken consisted of workers completing reproductive history questionnaires and of observed births being compared with expected births for the married workers. Expected births were derived from US birth rates by age, calendar year, and parity, and the ratio of observed to expected was expressed as a standardized fertility ratio (SFR). Populations of 137, 207, and 235 persons, respectively, were studied and were largely, but not exclusively, male. It is not clear whether any of the plants were the same as those described above. Comparisons of SFR between different exposure categories, both for the whole population and also among individuals who spent at least part of their reproductive life exposed, did not reveal any significant effects on SFR between exposure groups. The authors estimated that the power of this study to detect a 50% reduction in fertility would have been 90%. In the third NIOSH study, the miscarriage rate for wives of DAT-exposed workers was 6.8 times higher than that for wives of unexposed workers (rates given as miscarriages per 100 person-years), but the fertility rate was only 0.8 times lower (ratio of

rates of live births per 100 person-years). Thus, overall fertility may not be a sensitive index of adverse reproductive outcome.

9. EVALUATION OF HUMAN HEALTH RISKS AND EFFECTS ON THE ENVIRONMENT

9.1 Evaluation of Human Health Risks

9.1.1 General considerations

There are insufficient data on the effects of diaminotoluenes on human beings to carry out a detailed hazard assessment or risk evaluation. However, absorption and metabolic studies in human beings have indicated that diaminotoluenes are rapidly absorbed, metabolized, and excreted in the urine in a manner similar to that found in experimental animals. Therefore, the risk evaluation that follows is based largely on data from animals, supported by data from human studies, where available.

9.1.2 Assessment of exposure

Diaminotoluenes can be absorbed through the skin and gastrointestinal tract, and by inhalation. Given the properties of this class of chemicals, the major route of human exposure is dermal, in the work-place, with a possibility of the inhalation of fumes during heating. Exposure through ingestion is minimal, except in case of accidents.

No data exist on general ambient levels of diaminotoluenes in air, water, and food. Bioaccumulation of diaminotoluenes in the food-chain should not occur. Levels in the work-place air of up to 0.44 mg/m^3, with occasional excursions up to 11 mg/m^3, have been reported.

9.1.3 Single and short-term exposures

Diaminotoluenes are classed as toxic, highly irritant chemicals. The oral LD_{50} for animals is between 270 and 350 mg/kg body weight. Dermal contact has been shown to cause irritation, severe dermatitis, blistering, and possible skin sensitization. Single oral doses of diaminotoluenes of 50 mg/kg body weight have led to methaemoglobinaemia in rats, rabbits, and guinea-pigs. Eye contact with diaminotoluenes has led to conjunctivitis and corneal opacities. In case of inhalation of fumes, coughing, dyspnoea, and respiratory distress can result.

9.1.4 Long-term exposure

9.1.4.1 Carcinogenicity and mutagenicity

No epidemiological data are available on the incidence of cancer in human beings after exposure to diaminotoluenes.

Several studies using 2,4-DAT have been carried out on experimental animals and, in each, the isomer was shown to be carcinogenic for rats and mice. In the most recent study, doses of, or greater than, 79 mg/kg diet led to an increase in hepatocellular carcinomas or neoplastic nodules in rats; there was an increase in hepatocellular carcinomas and lymphomas in female mice at doses exceeding 100 mg/kg diet.

Using a similar protocol, the US NCI concluded that 2,6-DAT was not carcinogenic for rats and mice after administration of up to 500 mg/kg diet for 103 weeks. It should be noted that hepatocellular carcinomas, neoplastic nodules, and lymphomas were detected, as in the bioassay for 2,4-DAT, however, they were considered not significant after detailed statistical analyses.

There was no evidence of carcinogenicity in mice and rats after administration of 2,5-DAT at levels of up to 2000 mg/kg diet, for 78 weeks. However, the short duration of the study reduced the potential of the test for detecting carcinogenicity. No evidence of carcinogenicity was noted after the dermal application of hair-dye formulations containing 2,5-DAT (following application of hydrogen peroxide) or a mixture of 2,5-DAT and 2,4-DAT with hydrogen peroxide.

Positive mutagenic activity was noted in *S. typhimurium* when 2,4-, 2,5-, 2,6-, and 3,4-DAT were tested. In addition, DAT isomers were mutagenic in mammalian cells *in vitro*. Significant DNA damage was produced by 2,4-DAT in human cultured fibroblasts, only after activation by prostoglandin synthase. The isomer 2,4-DAT was weakly mutagenic in *Drosophila melanogaster* and induced unscheduled DNA synthesis in primary rat hepatocytes *in vitro*.

The 2,4- and 2,5-isomers were inactive in *in vivo* mammalian mutagenicity assays. Micronuclei and dominant lethals were not produced by 2,5-DAT, and 2,4-DAT did not produce chromosomal breaks, dominant lethals, abnormal sperm morphology, or recessive spots.

It has been shown that 2,4-, 2,5-, and 2,6-DAT can inhibit DNA synthesis in the testes after ip injection of high doses. On the basis of this study, 2,4-DAT may pose a genetic hazard in addition to its potential to cause adverse effects on reproduction.

9.1.4.2 Reproduction and teratogenicity

The results of limited studies on the reproduction hazards for male workers exposed to diaminotoluenes are equivocal. In surveys of reproductive outcome in 3 plants, an excess of spontaneous abortions among the wives of male workers exposed to DAT and dinitrotoluene was reported in 2 surveys, though these excesses were based on small numbers, and not all workers in the plants participated in the studies. In 1 out of the 3 plants studied, some adverse effects on spermatogenesis were suggested. Analysis of the overall fertility of workers in 3 other production plants did not reveal any adverse effects from exposure to DAT.

In a study on animals fed 2,4-DAT, there was a significant and persistent decrease in the sperm count.

Embryotoxicity was observed in animal studies after oral and dermal doses exceeding 30 mg/kg body weight for the 2,3- and 3,4-isomers and 10 mg/kg body weight for 2,6-DAT.

Skeletal changes were noted after dermal application of a hair-dye formula containing 3% 2,4-DAT at 2 ml/kg body weight.

9.2 Evaluation of Effects on the Environment

Information is lacking concerning levels of diaminotoluenes in the environment, and their transport, bioconcentration, biotransformation, and biodegradation.

A few data indicate that diaminotoluenes may be hazardous for aquatic organisms. No data on the effects of diaminotoluenes on other non-mammalian targets in the environment could be found.

9.3 Conclusions

Diaminotoluenes are highly irritating to the skin and eyes and the fumes are irritating to the respiratory tract. They are readily absorbed through the skin. Methaemoglobinaemia may occur in exposed individuals. Renal toxicity after oral administration of 2,4-DAT has been reported in experimental animals. 2,4-DAT has been shown to be carcinogenic for animals, but there is inadequate evidence to evaluate the carcinogenic potential of 2,5- and 2,6-diaminotoluene. All 3 of these isomers have been shown to be mutagenic. Limited data are available concerning a reproductive hazard for male workers handling DATs. DATs have been shown to impair spermatogenesis in experimental animals and to be both embryotoxic and teratogenic.

10. RECOMMENDATIONS

1. Monitoring should be undertaken to determine the sources, levels, and fate of diaminotoluenes in the environment. Ecotoxicity data should be collected.

2. For a better evaluation of occupational exposure and effects, studies on the uptake, kinetics, and metabolism of DAT and the relevant routes of exposure are important to provide a sound basis for biological monitoring.

3. To assist in the development of appropriate health surveillance systems, a systematic evaluation of the toxicity of diaminotoluenes should be carried out to compliment available data on carcinogenicity and reproductive effects.

4. Additional data should be obtained on human morbidity and mortality related to exposure to diaminotoluenes, with particular emphasis on carcinogenic, teratogenic, and reproductive end-points.

11. PREVIOUS EVALUATIONS BY INTERNATIONAL BODIES

IARC (1978) evaluated the data on the carcinogenicity of diaminotoluenes and concluded that there was sufficient evidence of the carcinogenicity of 2,4-diaminotoluene in experimental animals. An evaluation of additional data by IARC (1986) further supported this conclusion.

In the absence of case reports or epidemiological studies, there was inadequate data to assess the carcinogenicity of diaminotoluenes for human beings (IARC, 1978).

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