DIAZEPAM

This substance was considered by previous working groups in October 1976 (IARC, 1977) and March 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 evaluation.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 439-14-5

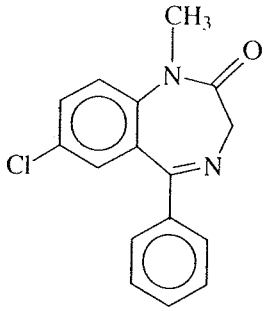
Deleted CAS Reg. No.: 11100-37-1; 53320-84-6

Chem. Abstr. Name: 7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one

IUPAC Systematic Name: 7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one

Synonym: Methyldiazepinone

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{16}H_{13}ClN_2O$ Relative molecular mass: 284.75

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Off-white to yellow, odourless, crystalline powder (Gennaro, 1995)

(*b*) *Melting-point*: 125–126 °C (Budavari, 1995)

(*c*) *Spectroscopy data*: Infrared, ultraviolet, nuclear magnetic resonance and mass spectral data have been reported (MacDonald *et al.*, 1972).

(*d*) *Solubility*: Slightly soluble in water (1 g/333 mL); soluble in acetone, benzene, chloroform (1 g/2 mL), diethyl ether (1 g/39 mL), dimethylformamide and ethanol (1 g/16 mL) (Gennaro, 1995)

(*e*) *Stability*: Stable in air (Gennaro, 1995)

(*f*) *Dissociation constant*: $pK_a = 3.4$ (American Hospital Formulary Service, 1995)

1.1.4 *Technical products and impurities*

Diazepam is available as 2-, 5- and 10-mg tablets, 15-mg extended release capsules, 2- and 5-mg/5 mL oral solutions, 5-mg/mL concentrated oral solution, 5-mg/mL parenteral injection, 5-mg/mL emulsion injection, 2- and 4-mg/mL rectal tube solutions and 10-mg suppositories. Preparations may also contain acetylated monoglycerides, anhydrous glucose, benzoic acid, benzyl alcohol, corn starch, ethanol, flavouring, fractionated egg phospholipids, fractionated soya bean oil, glycerol, lactose, magnesium stearate, methyl hydroxypropylcellulose, polyethylene glycol, propylene glycol, saccharin, sodium benzoate, sodium hydroxide, talc, D&C Yellow 10 (Quinoline Yellow), FD&C Blue 1 (Brilliant Blue FCF) or FD&C Yellow 6 (Sunset Yellow FCF). Sodium benzoate, benzoic acid and sodium hydroxide are added to the commercially available injection products to adjust pH (Thomas, 1991; Farmindustria, 1993; British Medical Association/Royal Pharmaceutical Society of Great Britain, 1994; American Hospital Formulary Service, 1995; Medical Economics, 1996).

Trade names and designations of the chemical and its pharmaceutical preparations include: Aliseum; Alupram; Amiprol; An-Ding; Anksiyolin; Ansiolin; Ansiolisina; Antenex; Apaurin; Apozepam; Armonil; Assival; Atensine; Atilen; Avex; Bensedin; Betapam; Bialzepam; Calmocitene; Calmpose; Canazepam; Cercine; Ceregulart; Condition; Deprestop; Diacepan; Diaceplex; Dialag; Dialar; Diapam; Diatran; Diaz; Diazem; Diazemuls; Diazepam-Lipuro; Diazidem; Dienpax; Dipam; Dizac; Dizam; Domalium; Doval; Drenian; Ducene; Duksen; Duxen; E-Pam; Eridan; Erital; Eurosan; Euphorin; Evacalm; Faustan; Gewacalm; Hexalid; Horizon; Kiatrium; LA 111; Lamra; Lembrol; Levium; Liberetas; Lizan; Lorinon; Mandrozep; Metil Gobanal; Méval; Morosan; Néo-Calme; Neosorex; Nervium; Neurolytril; Noan; Notense; Novazam; Novodipam; Paceum; Pacipam; Pacitran; Pax; Paxate; Paxel; Pro-Pam; Psychopax; Q-Pam; Quétinil; Quievita; Relaminal; Relanium; Relivan; Remedium; Renborin; Rival; Ro 5-2807; Saromet; Scriptopam; Sedapam; Sedipam; Seduxen; Serenak; Serenamin; Serenzin; Servizepam; Setonil; Sibazon; Sibazone; Sico Relax; Solis; Somasedan; Sonacon; Stesolid; Stesolin; Stress-Pam; Tensium; Tensopam; Tiromne; Tranimul; Tranquase; Tranquirit; Tranquo-Puren; Tranquo-Tablinen; Umbrium; Unisedil; Valaxona; ValCaps; Valclair; Valeo; Valibrin; Valiquid; Valitran; Valium; Valrelease; Vatran; Vival; Vivol; Wy 3467; Zepam; Zetran.

1.1.5 *Analysis*

Several international pharmacopoeias specify potentiometric titration with perchloric acid as the assay for purity of diazepam, and thin-layer chromatography for determining impurities and decomposition products. Assay methods for diazepam in capsules, tablets

and injection solutions include liquid chromatography or ultraviolet/visible absorption spectrometry using standards. Assays for heavy metal impurities are also specified (Society of Japanese Pharmacopoeia, 1992; British Pharmacopoeial Commission, 1993; United States Pharmacopeial Convention, 1994). Other spectrophotometric (Mañes *et al.*, 1987; El-Brashy *et al.*, 1993) and mass spectrometric (McCarley & Brodbelt, 1993) methods of analysis for diazepam in pharmaceutical preparations have been reported.

Diazepam and its metabolites (including oxazepam (see pp. 116–117) and temazepam (see pp. 162–163)) can be analysed in biological fluids and tissues by radioimmunoassay (Takatori *et al.*, 1991), gas chromatography (GC) (Löscher, 1982), GC–mass spectrometry (GC/MS) (Maurer & Pfleger, 1987), GC with electron capture detection (Peat & Kopjak, 1979; Beischlag & Inaba, 1992) and high-performance liquid chromatography (Peat & Kopjak, 1979; Lensmeyer *et al.*, 1982; Komiskey *et al.*, 1985; Mura *et al.*, 1987; Fernández *et al.*, 1991; Chiba *et al.*, 1995).

1.2 Production and use

1.2.1 *Production*

A method for preparing diazepam was first reported in 1961 (Sternbach & Reeder, 1961; Sternbach *et al.*, 1961); commercial production of diazepam in the United States of America was first reported in 1963 (United States Tariff Commission, 1964).

Diazepam is prepared by reacting 2-(methylamino)-5-chlorobenzophenone in ethereal solution with bromoacetyl bromide to form 2-(2-bromo-*N*-methylacetamido)-5-chlorobenzophenone. The latter is then reacted with ammonia in methanol solution to form the 2-amino-*N*-methylacetamido compound, which is cyclized with dehydration to produce diazepam. The crude diazepam may be purified by recrystallization from diethyl ether (Gennaro, 1995).

1.2.2 *Use*

Diazepam is a benzodiazepine with anxiolytic, sedative, muscle-relaxant and anticonvulsant properties. The active metabolite is *N*-desmethyldiazepam, which has a long duration of action (Reynolds, 1993). The therapeutic effects of the benzodiazepines are believed to be due to their binding to the protein receptor complex for the inhibitory neurotransmitter, γ-aminobutyric acid (GABA). This complex has binding sites for both phenobarbital and the benzodiazepines (Barnard *et al.*, 1984). Binding of benzodiazepines to the α subunit of the complex affects chloride conductance within long-fibre neurons and interneurons in the central nervous system and enhances the efficiency of GABAergic transmission (Richards *et al.*, 1986). Central benzodiazepine receptors have been found in human fetal brain tissues by 18 weeks of conceptual age (Brooksbank *et al.*, 1982). Besides this receptor in the central nervous system, there also appears to be a benzodiazepine receptor in peripheral organs (Krueger & Papadopoulos, 1992). This is a mitochondrial protein which may be involved in the regulation of steroid biosynthesis (see Section 4.2.2(*c*)).

Diazepam is used in the management of severe, disabling anxiety disorders, as a hypnotic in the short-term management of insomnia, in treating convulsions, particularly status epilepticus and febrile convulsions, and in controlling alcohol withdrawal symptoms. It is also used as a premedication and sedative before surgical and other procedures, and for the relief of muscle spasm as in cerebral palsy (Reynolds, 1993). Diazepam is a common adjunct in cancer therapy and may be provided as a pre-admission drug before cancer diagnosis (Derogatis *et al.*, 1979).

The oral dose for anxiety states usually ranges from 2 mg three times daily up to 30 mg daily in divided doses. Similar doses may be sufficient for control of mild to moderate symptoms of alcohol withdrawal. A single dose of 5–30 mg before retiring is given for insomnia associated with anxiety. In muscle spasm, 2–15 mg may be given daily in divided doses and increased, in severe spastic disorders, such as cerebral palsy, to up to 60 mg daily. A similar dosage range has been recommended for the adjunctive use of diazepam in some types of epilepsy. Diazepam at 5–20 mg may be given as a single oral dose or in divided oral doses as a premedication before dental, minor surgical or other procedures. A slow-release oral formulation of diazepam is available in some countries; a dose of 15 mg daily is considered to be equivalent to 5 mg three times daily of the conventional oral formulation. A suggested initial oral dose of diazepam for children is 100–200 μg/kg bw, but up to 800 μg/kg daily has been given. Dosage recommendations are not generally given for premature infants or infants 30 days of age or younger, since safety and efficacy have not been established for these groups (Reynolds, 1993; Medical Economics, 1996).

Diazepam may be given rectally as suppositories in doses similar to the oral doses. A rectal solution of 2–4 mg/mL diazepam may be particularly useful for the control of convulsions; the dose for adults and children over three years of age is 10 mg, and the dose for children aged one to three years is 5 mg. If there is no response after five minutes, the dose may be repeated (Reynolds, 1993).

Diazepam may be given by deep intramuscular injection, although absorption is erratic and gives rise to lower blood concentrations than those obtained after oral administration. It may also be given by intravenous injection, carried out slowly into a large vein of the antecubital fossa at a recommended rate of no more than 1 mL of a 0.5% solution (5 mg) per minute. In cases of severe anxiety or acute muscle spasm, diazepam (10 mg) may be given intramuscularly or intravenously and repeated after 4 h. Higher doses may be required for the treatment of delirium tremens. Patients with tetanus may be given 100–300 μg/kg bw intravenously, repeated every 1–4 h; alternatively, a continuous infusion of 3–10 mg/kg bw every 24 h may be used or similar doses may be given by nasoduodenal tube. Considerably higher doses have been used for extremely severe cases of tetanus. For premedication or sedation before dental, surgical or other procedures, 100–200 μg/kg bw (usually 10–20 mg for adults) may be given by injection. A suggested parenteral sedative or muscle-relaxant dose for children is up to 200 μg/kg bw (Reynolds, 1993).

Diazepam may be given parenterally, preferably by the intravenous route, for the control of status epilepticus or severe recurrent or febrile convulsions. In the United

Kingdom, the usual dose is 150–250 µg/kg bw (or 10–20 mg) for adults and 200–300 µg/kg bw or 1 mg per year of life for children. These doses may be repeated after 30–60 min if required. Once the seizures are controlled, their recurrence may be prevented by intravenous administration of phenytoin sodium (see monograph, pp. 178–179) or by a slow infusion of diazepam. For adults, the maximal total dose of diazepam is 3 mg/kg bw over 24 h (Reynolds, 1993). In the United States, the initial dose for adults is 5–10 mg, repeated if required at 10–15-min intervals up to a maximum of 30 mg. Doses for children are: infants over 30 days and under five years of age, 200–500 µg every 2–5 min up to a maximum of 5 mg; children five years and older, 1 mg every 2–5 min up to a maximum of 10 mg. The above dosage regimens may be repeated after a period of 2–4 h if necessary (Medical Economics, 1996). Elderly and debilitated patients should be given no more than one half of the usual adult dose. Reduction of dosage may also be required in patients with liver or kidney dysfunction (Reynolds, 1993).

Clinical uses of diazepam and other benzodiazepines have been reviewed (Hollister *et al.*, 1993). Diazepam has been used extensively in children (Goodman Gilman *et al.*, 1990).

Worldwide, diazepam is the most widely prescribed of the benzodiazepines. Comparative data on sales of diazepam in several countries are shown in Table 1. Overall, sales declined by approximately 16% from 1990 to 1995.

Table 2 compares the number of prescriptions written in the United States for several benzodiazepines, including diazepam, and for the anticonvulsant, phenytoin, in 1990 and 1995.

Table 3 compares the total sales for these same benzodiazepines and phenytoin in 1990 and 1995 in major markets worldwide.

1.3 Occurrence

1.3.1 *Natural occurrence*

Wildmann *et al.* (1987, 1988) reported the occurrence of trace amounts of diazepam in the brain and adrenals of rats and in wheat and potato samples.

Unseld *et al.* (1989) reported finding low concentrations of diazepam in brain tissue samples from several animal species and plants using GC/MS. Diazepam concentrations ranged from 0.005 to 0.019 ng/g wet weight in brain tissue from salmon, frog, monitor lizard, rat, cat and dog. Traces of diazepam were detected in deer, bovine, adult human and stillborn human brain tissue samples. Diazepam concentrations ranged from 0.002 to 0.010 ng/g in the plant samples (potato tuber, yellow soya beans, unpealed rice, mushrooms).

Unseld *et al.* (1990) reported on the 'natural' occurrence of diazepam in human brain samples. All brain samples were examined by GC/MS and the concentrations observed ranged from 0.15 to 0.34 ng/g wet weight tissue. The human brain tissue samples had been stored before diazepam was first synthesized in 1963.

Table 1. Sales of diazepam in various countries[a] (number of standard units[b], in thousands)

Country	1990	1991	1992	1993	1994	1995
Africa						
South Africa	8 227	7 843	7 903	6 977	7 657	7 456
North America						
Canada	119 159	104 306	100 400	80 016	84 831	82 060
Mexico	90 858	83 626	81 044	70 177	67 265	63 874
United States	775 409	711 049	667 798	678 466	697 750	764 904
South America						
Argentina	97 383	106 010	107 794	100 037	95 962	88 942
Brazil	387 216	388 859	327 549	282 044	259 621	209 231
Colombia	9 644	10 109	12 070	4 575	1 840	2 571
Venezuela	11 398	11 959	11 153	12 821	10 552	12 190
Asia						
Japan	533 690	520 677	511 530	484 280	482 458	474 676
Republic of Korea	22 647	22 903	23 253	22 990	22 356	21 310
Australia	94 309	85 418	82 738	81 212	80 203	80 653
Europe						
Belgium	21 044	21 379	21 748	21 496	21 309	20 544
France	102 252	96 216	88 013	82 141	77 222	68 051
Germany	152 300	141 334	118 695	113 702	113 156	115 477
Greece	28 691	22 454	20 476	18 719	17 557	14 291
Italy	265 153	252 443	242 370	221 544	194 035	190 047
Netherlands	45 821	48 460	48 497	46 615	45 161	44 898
Portugal	75 961	77 806	74 370	75 962	78 941	80 988
Spain	238 524	228 722	226 454	215 093	210 853	211 265
Sweden	51 830	46 781	47 085	46 296	46 255	45 552
Switzerland	11 364	11 007	10 616	7 947	7 588	7 232
Turkey	13 156	14 370	15 507	14 809	16 054	18 156
United Kingdom	238 198	235 807	227 345	230 963	224 861	232 365

[a] Data provided by IMS

[b] Standard dosage units, uncorrected for diazepam content

1.3.2 *Occupational exposure*

No quantitative data on occupational exposure levels were available to the Working Group.

The National Occupational Exposure Survey conducted between 1981 and 1983 in the United States by the National Institute of Occupational Safety and Health indicated that about 20 650 employees were potentially occupationally exposed to diazepam. The estimate is based on a survey of United States companies and did not involve measurements of actual exposure (United States National Library of Medicine, 1996).

Table 2. Use of some benzodiazepines and phenytoin in the United States[a] (numbers of prescriptions, in thousands)

Drug	1990	1995
Diazepam	13 056	12 475
Estazolam	0	598
Oxazepam	1 647	1 436
Prazepam	1 205	1
Temazepam	5 567	5 916
Phenytoin[b]	8 848	9 811

[a] Data provided by IMS. No sales of doxefazepam or ripazepam in the United States

[b] Dilantin® only

Table 3. Comparative sales of several benzodiazepines and phenytoin in major markets worldwide[a] (number of standard units[b], in millions)

	1990	1995	Countries with the highest use
Diazepam	3 394	2 857	USA, Japan, Brazil, Spain, UK
Phenytoin	2 423	2 218	USA, Japan, UK, Canada
Oxazepam	1 278	996	Germany, France, USA, Netherlands
Temazepam	706	756	UK, USA, Australia
Prazepam	361	276	France, USA, Italy
Estazolam	158	187	Japan, USA, Portugal

[a] Data provided by IMS

[b] Standard dosage units, uncorrected for content of active ingredient

1.4 Regulations and guidelines

Diazepam is listed in the following pharmacopoeias: Belgian, British, Brazilian, Chinese, Czech, Egyptian, European, French, Greek, Hungarian, Indian, International, Italian, Japanese, Mexican, Netherlands, Nordic, Portuguese, United States and former Yugoslavian (Reynolds, 1993).

2. Studies of Cancer in Humans

Worldwide, diazepam is the most widely used of the benzodiazepines. For this reason, most specific epidemiological information about potential carcinogenic effects of the benzodiazepines relates to this drug. In addition, reported use of unspecified sedatives or hypnotics probably implies principally use of diazepam. This section therefore reviews not only epidemiological studies which investigated diazepam specifically but also those which reported risk associated with unspecified psychotropics, tranquillizers or benzodiazepines.

Several potential biases in the epidemiological studies of benzodiazepines or diazepam in relation to cancer deserve mention. Control selection may be problematic in case–control studies, in that it may be difficult to select *a priori* a diagnostic category unrelated to the exposure, while general population controls may be less likely to self-report short- or long-term use. Indication for use of diazepam or other benzodiazepines has, in general, not been considered as a potential confounding factor. If anxiety or depression due to an underlying hormonal imbalance is involved in the etiological pathway of a particular cancer, the use of psychotropic drugs is merely a marker for the underlying condition, rather than indicating that the drug is the initiator or promoter of the cancer. Another, possibly most important, consideration is that diazepam is a common adjunct in cancer therapy and may be provided as a pre-admission drug before cancer diagnosis (Derogatis *et al.*, 1979). It is therefore essential to ascertain precisely the date of first use of the drug in relation to the date of diagnosis of the cancer, so that reasonable rules for censoring exposure history may be established.

2.1 Descriptive studies

A cross-sectional study examined use of psychotropic drugs for one month or more by 250 women who had been diagnosed with breast cancer at least one year previously and who were attending breast cancer clinics for follow-up visits at two general hospitals in the United Kingdom (Stoll, 1976). Hypnotics, minor tranquillizers, sedatives and antidepressants were used by 14% of the women during the 12 months before diagnosis and by 32% in the 12 months prior to the questionnaire. Among women with metastases at presentation or recurrence within 12 months, 22% had used such drugs before diagnosis, compared to 13% among women with local disease at presentation or recurrences later than 12 months ($p < 0.03$). [The Working Group noted that both the absence of comparably collected control data and a biological rationale for distinguishing the high-usage group were limitations of this descriptive study.]

2.2 Cohort studies

Since 1969, members of the Kaiser Permanente Medical Care Program (KPMCP) in northern California, United States, have been categorized according to their drug exposure, as identified from prescription records, and followed during their membership

in the KPMCP. The occurrence of cancer was identified from admission records or by cross-checking against the San Francisco Bay Area Tumor Registry. Expected numbers of cancers were based upon age- and sex-specific rates for the entire cohort. In the latest report from this study with follow-up through 1984 of 12 928 diazepam users, the standardized morbidity ratio for all cancers was 1.0 ([95% confidence interval (CI), 1.0–1.1]; 807 observed versus 784 expected); for breast cancer, 1.1 ([0.9–1.3]; 155 observed versus 144 expected); for Hodgkin's disease, 0.0 ([0.0–0.79]; 0 observed versus 4.7 expected) and for colon cancer, 0.7 ([0.5–0.9]; 57 observed versus 79.9 expected) (Selby *et al.*, 1989; Friedman & Selby, 1990). [The Working Group noted that dose response was not addressed in these data, and that age and sex were the only confounding factors considered.]

In a reconstructed retrospective cohort study, breast cancer in female members of a Group Health Cooperative (GHC) in Seattle, WA was investigated (Danielson *et al.*, 1982). During the period 1977–80, 302 women, aged 35–74 years, who had been members of the GHC for at least six months and who had a newly diagnosed breast cancer were identified. Age-specific incidence rates of breast cancer for users and non-users of diazepam were calculated. Women were classified as exposed to diazepam if at least one prescription for the drug had been filled in the six months before breast cancer diagnosis; drug taken only in the two weeks before mastectomy was not considered. Of 302 women with breast cancer, 27 had taken diazepam before their breast cancer diagnosis; on the basis of 184 438 women-years of observation in total, the age-adjusted risk ratio for breast cancer was 0.9 [95% CI, 0.6–1.3]. [The Working Group noted that this study could not address the effect of long-term use of diazepam and that no confounding factors had been considered.]

2.3 Case–control studies

Table 4 summarizes case–control data on use of benzodiazepines or diazepam that were available to the Working Group. By far the most numerous studies of diazepam in relation to human cancer are case–control studies of breast cancer.

2.3.1 *Breast cancer*

Wallace *et al.* (1982) compared diazepam use in 151 newly diagnosed breast cancer cases at hospitals of the University of Iowa, United States, between 1974 and 1978 with use in a similar number of women with non-cancer conditions selected from the general medical and surgical wards. Matching variables included age and hospital payment category. All subjects were white. The crude relative risk for breast cancer associated with diazepam use was 1.0 [95% CI, 0.5–1.8]. Adjustment for other potential confounders such as age at menarche, parity, type of menopause and family history of breast cancer did not affect the association. Details on refusal rates for controls were not provided. [The Working Group noted the small number of cases and insufficient data on refusal rates for controls.]

Table 4. Case–control studies of diazepam or benzodiazepine use

Study	Location, period	No. of cases/ controls	Source of controls	Exposure	Odds ratio	95% CI	Notes
Breast cancer							
Wallace *et al.* (1982)	Iowa, USA, 1974–78	151/151	Non-cancer patients	Diazepam, any use	1.0	[0.5–1.8]	Response rate not reported
Kleinerman *et al.* (1984)	USA, 1973–77	1075/1146	Participants in screening programme	Diazepam, any use	0.7 0.9 1.1	0.6–0.9 0.6–1.3 0.8–1.6	Invasive carcinoma > 1 cm Invasive carcinoma ≤ 1 cm In-situ carcinoma
Kaufman *et al.* (1982)	USA, Canada, Israel, 1976–80	1236/728	Cancer patients	Diazepam, 4 times/week, during ≥ 6 months, > 18 months before interview	0.9	0.5–1.6	Similar results with 'female' cancer or other cancer controls
Kaufman *et al.* (1990)	USA, 1981–87	3078/1259	Cancer and non-cancer patients	Diazepam, 4 times/week, during ≥ 6 months, > 18 months before interview	1.0	0.6–1.7	Similar results with non-cancer controls
	Toronto, Canada, 1982–86	607/1214	Census records	Diazepam, 4 times/week, during ≥ 6 months, > 18 months before interview	0.8	0.5–1.3	
Rosenberg *et al.* (1995)	USA, 1977–91	6056/1603	Cancer patients	Benzodiazepine, sustained use[a]	1.0	0.8–1.3	Overlap with Kaufman *et al.* (1982, 1990). Similar results with non-cancer controls. Odds ratio for ≥ 5 years of benzodiazepine use: 0.8 (0.5–1.4)
				Diazepam, sustained use[a]	1.0	0.7–1.4	

Table 4 (contd)

Study	Location, period	No. of cases/ controls	Source of controls	Exposure	Odds ratio	95% CI	Notes
Ovarian cancer							
Tzonou *et al.* (1993)	Athens, Greece, 1989–91	189/200	Visitors to hospitals	'Tranquillizers or hypnotics', any use	1.0	0.6–1.6	
Harlow & Cramer (1995)	Boston, MA, USA, 1978–81, 1984–87	450/454	General population	Benzodiazepine, any use	1.8	1.0–3.1	Higher risk for use before age 50 or ≥ 10 years before interview
Rosenberg *et al.* (1995)	USA, 1977–91	767/1603	Cancer patients	Benzodiazepine, sustained use[a]	0.9	0.6–1.4	Similar results with non-cancer controls. Odds ratio for ≥ 5 years of benzodiazepine use: 0.3 (0.1–0.9)
				Diazepam, sustained use[a]	1.0	0.6–1.6	
Malignant melanoma							
Adam & Vessey (1981)	England and Wales, 1971–76	150/496	General practitioners' patients	Diazepam, ≥ 1 month	1.2	0.6–2.2	General practitioners' records
		101/302	General practitioners' patients	Diazepam, ≥ 1 month	1.7	0.9–3.2	Self-reported use
Rosenberg *et al.* (1995)	USA, 1977–91	1457/3777	Cancer patients	Benzodiazepine, sustained use[a]	1.0	0.8–1.4	Similar results with non-cancer controls. Odds ratio for ≥ 5 years of benzodiazepine use: 0.9 (0.5–1.7)
				Diazepam, sustained use[a]	1.0	0.7–1.5	

Table 4 (contd)

Study	Location, period	No. of cases/ controls	Source of controls	Exposure	Odds ratio	95% CI	Notes
Multiple myeloma							
Linet *et al.* (1987)	Baltimore, MD, USA, 1975–82	100/100	Hospital patients	Diazepam, any use	2.0	0.4–12	
Lung cancer							
Rosenberg *et al.* (1995)	USA, 1977–91	1365/3777	Cancer patients	Benzodiazepine, sustained use[a]	1.0	0.7–1.4	Similar results with non-cancer controls. Odds ratio for ≥ 5 years of benzodiazepine use: 1.2 (0.6–2.6)
				Diazepam, sustained use[a]	0.8	0.5–1.2	
Colon cancer							
Rosenberg *et al.* (1995)	USA, 1977–91	2203/3777	Cancer patients	Benzodiazepine, sustained use[a]	0.8	0.6–1.1	Similar results with non-cancer controls. Odds ratio for ≥ 5 years of benzodiazepine use: 0.8 (0.5–1.2)
				Diazepam, sustained use[a]	0.7	0.5–1.0	
Non-Hodgkin's lymphoma							
Rosenberg *et al.* (1995)	USA, 1977–91	382/3777	Cancer patients	Benzodiazepine, sustained use[a]	0.8	0.5–1.4	Similar results with non-cancer controls. Odds ratio for ≥ 5 years of benzodiazepine use: 0.8 (0.3–2.1)
				Diazepam, sustained use[a]	0.8	0.4–1.6	
Hodgkin's disease							
Rosenberg *et al.* (1995)	USA, 1977–91	299/3777	Cancer patients	Benzodiazepine, sustained use[a]	0.6	0.3–1.4	Similar results with non-cancer controls. Odds ratio for ≥ 5 years of benzodiazepine use: 1.2 (0.3–4.5)
				Diazepam, sustained use[a]	0.9	0.4–2.0	

Table 4 (contd)

Study	Location, period	No. of cases/ controls	Source of controls	Exposure	Odds ratio	95% CI	Notes
Thyroid cancer							
Rosenberg *et al.* (1995)	USA, 1977–91	111/3777	Cancer patients	Benzodiazepine, sustained use[a]	0.9	0.4–2.4	Similar results with non-cancer controls. Odds ratio for ≥ 5 years of benzodiazepine use: 1.3 (0.3–6.0)
				Diazepam, sustained use[a]	0.8	0.2–2.7	
Liver cancer							
Rosenberg *et al.* (1995)	USA, 1977–91	37/3777	Cancer patients	Benzodiazepine, sustained use[a]	1.2	0.3–5.2	Similar results with non-cancer controls
				Diazepam, sustained use[a]	2.0	0.5–8.4	
Endometrial cancer							
Rosenberg *et al.* (1995)	USA, 1977–91	812/1603	Cancer patients	Benzodiazepine, sustained use[a]	1.2	0.8–1.9	Similar results with non-cancer controls. Odds ratio for ≥ 5 years of benzodiazepine use: 1.4 (0.6–2.9)
				Diazepam, sustained use[a]	1.4	0.8–2.3	
Testicular cancer							
Rosenberg *et al.* (1995)	USA, 1977–91	314/2174	Cancer patients	Benzodiazepine, sustained use[a]	1.2	0.5–3.1	Similar results with non-cancer controls
				Diazepam, sustained use[a]	1.4	0.4–4.7	

[a] ≥ 4 times/week, during ≥ 1 month, > 24 months before interview

Kleinerman *et al.* (1984) examined the association between breast cancer and diazepam use in white women participating in the Breast Cancer Detection Demonstration Project in the United States between 1973 and 1977. The study included 1075 prevalent cases who had a histologically confirmed breast cancer detected during the five-year period and 1146 controls selected from women with normal mammographic results that did not require biopsy. Controls were matched by screening centre, age and date at entry and length of continuation in the screening programme. Exposures were assessed by home interview, and participation rates were 86% for cases and 74% for controls. Only diazepam use begun at least six months before the date of the breast cancer diagnosis was considered. The relative risk associated with diazepam use for invasive tumours > 1 cm was 0.7 (95% CI, 0.6–0.9), that for invasive tumours ≤ 1 cm was 0.9 (0.6–1.3) and that for in-situ tumours was 1.1 (0.8–1.6). [The Working Group noted that some drug-exposed women with poor survival may not have been included in this study.]

Data on breast cancer in relation to diazepam use are available from a hospital-based case–control surveillance system (Kaufman *et al.*, 1982, 1990; Rosenberg *et al.*, 1995). [These three studies, although reported separately, may overlap in either study methodology or subjects included.] In the first report from this series, diazepam use was ascertained by personal interviews in 1236 women less than 70 years old diagnosed with primary breast cancer in the six months before admission and 728 controls admitted to metropolitan hospitals in the United States, Canada and Israel during 1976–80 (Kaufman *et al.*, 1982). Control women had other cancers including other 'female' cancers such as endometrial or ovarian cancer. Of patients approached, 5% refused to be interviewed. The principal analyses excluded use of diazepam in the 18 months before hospital admission, to avoid the possibility of recording use begun after a diagnosis of breast cancer or because of clinical symptoms preceding the diagnosis, and also focused on 'regular' use of diazepam (defined as use of the drug for at least four days per week) and 'sustained regular' use (for a total duration of at least six months). Potential confounding factors considered were age, geographical region, education, religion, parity, age at first pregnancy, menopausal status, age at menopause, family history of breast cancer and alcohol use. The relative risk for breast cancer associated with regular use of diazepam for six months or more was 0.9 (95% CI, 0.5–1.6), with all other cancers as the control group. The relative risk for breast cancer associated with regular use of diazepam for less than six months was 0.8 (0.4–1.4), with all other cancers as the control group. The relative risks were no greater for women with metastatic disease. Similar risk estimates were found when women with either other cancers or 'other female' cancers were used as the control group.

These investigators extended their study with data from 3078 breast cancer cases, 18–69 years old, with cancer diagnosed within six months before admission, interviewed between 1981 and 1987 in hospitals in the metropolitan United States and from three separate control groups interviewed over the same time period (Kaufman *et al.*, 1990). As in the previous study, women with other cancers (754) or with other 'female' cancers (505) were included as controls. A non-cancer control group (672) was also included, which was composed primarily of women with ectopic pregnancy (281), appendicitis

(230) or retinal detachment (100). Women admitted for trauma were not included because of the possibility that diazepam increases the risk for accidents. Of patients approached, 4% refused to be interviewed. The authors adjusted for age, geographical region, education, religion, parity, menopausal status, age at menarche, first birth and menopause, family history of breast cancer, alcohol, oral contraceptive and other benzodiazepine use. In this study, the percentage of white women varied from 64% in the non-cancer controls to 90% in the female cancer controls, and race was included as an adjustment variable. Regular use of diazepam, defined as in the previous study, was associated with a relative risk for breast cancer of 1.0 (95% CI, 0.6–1.7), compared with controls with any other cancer and 0.8 (0.4–1.8) compared with the non-cancer controls. Risks were similar for 'sporadic' use of diazepam, defined as use beginning at least 18 months before interview and lasting for less than six months or use involving fewer than four days per week. 'Recent' use of diazepam (beginning within 18 months of interview) was also associated with a significantly elevated risk for breast cancer of 1.9 (1.1–3.1), compared with controls with any other cancer and 5.6 (2.3–13.6) compared with the non-cancer controls. [With respect to 'recent use', see the comment below.]

In the same publication, results of a separate study of 607 cases of breast cancer identified between 1982 and 1986 through the Ontario Cancer Institute, Canada, were reported (Kaufman *et al.*, 1990). Controls in this study were 1214 women selected from municipal voting and census records matched for neighbourhood and decade of age. Refusal rates were 21% among potential cases and 35% among potential controls. The relative risks for breast cancer associated with categories of diazepam use, as defined above, were 0.8 (95% CI, 0.5–1.3) for regular use, 1.1 (0.8–1.5) for sporadic use and 3.1 (1.5–6.4) for recent use. [With respect to 'recent use', see the comment below.]

In the most recent and comprehensive report using this hospital surveillance system to investigate possible effects of benzodiazepine exposure, not only breast cancer but also other cancers were analysed (Rosenberg *et al.*, 1995). The 6056 breast cancer cases included some previously reported cases from 1977 to 1987 as well as new cases admitted between 1988 and 1991. The primary control group was 1603 women with other cancers excluding those of the endometrium or ovary. Participation rates were about 96% and adjustment was made for age at menarche, first pregnancy and menopause, parity, religion, education, race, family history of breast cancer and duration of use of oral contraceptives or oestrogen replacement therapy. In contrast to the previous reports from this series, 'recent' use was defined in relation to a two-year interval before hospital admission, rather than an 18-month interval. 'Sustained' use of diazepam for at least four days per week for one month initiated two or more years before admission was associated with a relative risk for breast cancer of 1.0 (95% CI, 0.7–1.4). For sustained use of all benzodiazepines combined, risk did not vary significantly with the number of years since last use or with a duration of use of five years or more.

[The Working Group noted that the elevated risks for breast cancer associated with benzodiazepine use within the 'recent' period before hospital admission observed in some of these studies could be attributed to drug use begun after the diagnosis of breast cancer. More precise information on the timing of the drug exposure in relation to the

date of diagnosis of breast cancer rather than the interview date might have clarified this potential bias.]

2.3.2 *Ovarian cancer*

Tzonou *et al.* (1984) studied 150 women with malignant epithelial ovarian tumours newly diagnosed during 1980 and 1981 in 10 hospitals in Athens, Greece, and compared them with 250 women in the Athens Hospital for Orthopedic Disorders. No controls approached were said to have refused, but participation rates for cases were not stated. 'Frequent' use of 'psychotropic' drugs was reported by eight cases and two controls, giving a crude odds ratio of 7.0 [95% CI, 1.8–27] for ovarian cancer associated with use of these drugs. [The Working Group noted that the indications given by the authors for 'frequent' use of 'psychotropic' drugs in this study suggest that benzodiazepines may have been used infrequently.]

In a subsequent report, the same investigators compared drug use in 189 women less than 75 years old with malignant epithelial ovarian cancer diagnosed during 1989–91 in two hospitals in greater Athens with that of 200 visitors to the same hospitals (Tzonou *et al.*, 1993). Participation rates were 90–94%. The relative risk associated with ever use of 'tranquillizers or hypnotics' over an 'extended' period was 1.0 (95% CI, 0.6–1.6) after adjustment for age, education, weight, age at menarche and menopause, parity, smoking and other study variables.

[The Working Group noted the small number of exposed subjects in both studies, a possible lack of appropriateness of the control selection and the lack of specificity of information on the agent and its duration of use.]

Benzodiazepine use was investigated in a study which combined two case–control studies conducted previously from ten hospitals in Boston, MA, United States (Harlow & Cramer, 1995). The study included 450 cases of malignant epithelial ovarian cancer diagnosed between 1978–81 and 1984–87 in women 18–80 years old and 454 controls identified from the general population during the same period and matched for age, race and precinct of residence. Participation rates for cases and controls were around 70%. Any use of a benzodiazepine tranquillizer was associated with a relative risk for ovarian cancer of 1.8 (95% CI, 1.0–3.1) after adjustment for parity, oral contraceptive use, religion, body mass, prior hysterectomy and therapeutic abortion. Risk appeared to be confined to women whose first use of the drug either was before the age of 50 years, where the relative risk was 2.7 (1.3–5.6), or occurred 10 or more years before the age at diagnosis (3.2; 1.4–7.6). [The Working Group noted that more specific data on risk for ovarian cancer by frequency or duration of drug use would have been helpful in establishing the validity of this association.]

Data on diazepam use in relation to ovarian cancer are also available from the study by Rosenberg *et al.* (1995), described previously. Among 767 women with ovarian cancer, sustained use of diazepam was reported by 25 (4.3%), giving a relative risk of 1.0 (95% CI, 0.6–1.6).

2.3.3 *Other cancers*

A case–control study of malignant melanoma in women, aged 15–49 years, in relation to diazepam use was conducted from a survey of general practitioners in southern England between 1971 and 1976 (Adam & Vessey, 1981). Controls were selected from the practice lists of the same doctors and matched by age and marital status. The relative risk for malignant melanoma associated with use of diazepam for more than one month, as assessed from the general practitioners' records, based on 150 cases and 496 controls was 1.2 (95% CI, 0.6–2.2). The risk was 1.7 (0.9–3.2) for diazepam use as assessed from postal questionnaires completed by 101 cases and 302 controls. [The Working Group noted that the poor response rates to the postal questionnaire may have accounted for the marginally greater risk for melanoma associated with diazepam use if a greater number of responding cases recalled short-term use.]

A hospital-based case–control study of multiple myeloma was conducted in Baltimore, MD, United States (Linet *et al.*, 1987). A total of 121 cases of multiple myeloma in white patients were ascertained from seven hospitals during the period 1 January 1975 to 31 December 1982. Controls were individually matched to cases on hospital, age (± 5 years), sex and year of diagnosis. The control group included patients randomly selected from 11 categories of disease which included digestive and nervous system diseases, accidents and poisoning but excluded diagnoses of cancer, diseases of blood-forming organs, mental disorders, obstetric conditions and congenital anomalies. Information about many factors was obtained from the study subjects with a questionnaire by telephone; if the individual concerned had died or could not be interviewed for any other reason, information was sought from the closest possible relative. The response rate was 83% among cases, leaving 100 cases for analysis. Data on drug use occurring before diagnosis were analysed. The crude odds ratio associated with diazepam use, based on nine discordant pairs, was 2.0 (95% CI, 0.4–12). [The Working Group noted that the diagnostic categories for controls could have been associated with exposure to diazepam. In addition, the greater number of proxy interviews for cases than controls could not be adjusted for with diazepam as the exposure.]

The study by Rosenberg *et al.* (1995), described previously, addressed risk for other cancers associated with diazepam use. For sustained use of diazepam, defined as use for at least four days per week for at least one month which had been initiated at least two years before admission, relative risks for selected sites were: 0.7 (95% CI, 0.5–1.0) for cancer of the large bowel, 0.8 (0.5–1.2) for lung cancer, 0.9 (0.4–2.0) for Hodgkin's disease, 0.8 (0.2–2.7) for cancer of the thyroid, 2.0 (0.5–8.4) for liver cancer, 1.4 (0.8–2.3) for endometrial cancer and 1.4 (0.4–4.7) for testicular cancer. [See Section 2.3.1 for the Working Group's comments on this study.]

Anthony *et al.* (1982) surveyed general practitioners in the United Kingdom and requested information on medications used by any new patients with cancer and by age- and sex-matched controls who were the 'next' patient with a 'new' complaint not related to cancer. Six (possibly seven) of 211 male patients with cancer (mostly lung) had taken barbiturates and benzodiazepines concomitantly, compared to none of 211 male controls [$p = 0.03$]. [The Working Group noted that participation by the general practitioners was

voluntary and quite variable and that the study may have included drug exposures postdating the cancer diagnosis.]

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

Diazepam was included as a reference compound in a study on the carcinogenicity of prazepam. Groups of 100 male and 100 female albino CF1 mice (control group) and 50 male and 50 female mice (diazepam group), eight weeks of age, were given 0 or 75 mg/kg bw diazepam (melting point, 131–135 °C) mixed in the diet for up to 80 weeks, when surviving animals were killed. The dose was chosen to match the high-dose level of prazepam. The diazepam concentration in the food was adjusted weekly for changes in body weight and food consumption. The diazepam/diet mixtures were prepared freshly each week. In treated mice, body-weight gains were similar to those of controls throughout the study. From graphic presentations, there appeared to be no significant effect on mortality in male mice, but the diazepam-treated females had lower survival (65–75% survival for control and diazepam-treated males, 70% for control females and 40% for diazepam-treated females) [statistics and exact numbers not given]. Major organs [not specified] and visually apparent lesions were examined histologically. By life table analysis, the incidences of benign hepatocellular tumours were (tumour-bearing mice/effective number of mice): control males, 1/93; diazepam-treated males, 2/43; control females, 1/91; and diazepam-treated females, 0/38. Those for malignant hepatocellular tumours were: control males, 7/93; diazepam-treated males, 9/43 ($p < 0.05$, chi-square test); control females, 1/91; and diazepam-treated females, 2/38 (de la Iglesia *et al.*, 1981). [The Working Group noted that diazepam was tested only as a reference chemical and that the study was terminated at 80 weeks.]

3.1.2 *Rat*

Diazepam was included as a reference compound in a study on the carcinogenicity of prazepam. Groups of 115 male and 115 female albino SPF Wistar rats (control group) and 65 male and 65 female rats (diazepam group), eight weeks of age, were given either 0 or 75 mg/kg bw diazepam (melting-point, 131–135 °C) daily mixed in the diet for up to 104 weeks, when surviving animals were killed. The diazepam concentration in the food was adjusted weekly for changes in body weight and food consumption. The diazepam/-diet mixtures were prepared freshly each week. In treated rats, body weight gains were similar to those of controls throughout the study. From graphic presentations, there appeared to be no significant effect on mortality (50–60% survival for males and about 60% for females) [statistics and exact numbers not given]. Major organs [not specified] and visually apparent lesions were examined histologically. No significant increase in the incidence of tumours at any site was seen for either male or female rats. The incidences of benign hepatocellular tumours were: control males, 1/115; diazepam-treated males,

0/65; control females, 1/115; and diazepam-treated females, 0/65. Those for malignant hepatocellular tumours were: control males, 0/115; diazepam-treated males, 3/65 [$p = 0.054$, Fisher's exact test]; control females, 0/115; and diazepam-treated females, 0/65 (de la Iglesia *et al.*, 1981).

To study initiating activity, groups of 10 male Fischer 344 rats weighing 150 g [age not specified] were given 7 and 70 mg/kg bw diazepam (purity, > 99%) suspended in 10% arabic gum solution daily by gastric instillation for 14 weeks. Forty rats served as untreated controls. Neoplastic nodules and iron-excluding foci were not found in the livers of diazepam-treated animals nor in those of the controls (Mazue *et al.*, 1982; Remandet *et al.*, 1984).

3.1.3 *Hamster*

Groups of 55–56 male and 55–56 female Syrian golden hamsters were given 120 mg/kg bw diazepam mixed in the diet for 57 weeks (females) or 79 weeks (males). Approximately 110 hamsters per sex served as controls. Some intercurrent deaths, mostly after week 30, occurred from severe enteritis. No significant increase in the incidence of tumours was found (Black *et al.*, 1987). [The Working Group noted the single dose, that diazepam was used as a reference compound for a study on quazepam and that specific data on survival were not given.]

3.1.4 *Gerbil*

Groups of 15 male and 12 female gerbils [strain and age not specified] were given 10 mg diazepam [purity not specified] per animal by gastric instillation weekly. Eleven males and ten females receiving saline served as vehicle controls. Male and female controls survived 80 and 69 weeks, respectively, while gerbils receiving diazepam survived 79–81 weeks. Complete histopathology was performed as animals died or became moribund, and two ovarian granulosa-cell tumours in control females were the only tumours reported (Green & Ketkar, 1978). [The Working Group noted the small numbers of animals and the weekly administration of diazepam.]

3.2 Administration with known carcinogens

3.2.1 *Mouse*

Groups of 40 male B6C3F1 mice, five weeks of age, were given a single intraperitoneal injection of either 0 or 90 mg/kg bw *N*-nitrosodiethylamine (NDEA) in tricaprylin. At seven weeks of age, the mice received 500 or 1500 mg/kg diet (ppm) diazepam [purity unspecified] in the diet. Eight mice per group were killed at 9, 21 and 33 weeks of exposure and the remainder were killed after 53 weeks of exposure. Complete necropsy was performed on each animal, and liver, lung, spleen, thyroid, kidney and visually apparent lesions in other organs were examined histologically. Between 33 and 53 weeks of exposure, there was an increase in the incidence of hepatocellular tumours in animals treated with NDEA and diazepam compared with those given NDEA alone (see Table 5) (Diwan *et al.*, 1986).

Table 5. Incidence of liver tumours in B6C3F1 mice

Treatment	Hepatocellular adenomas	Hepatocellular carcinomas
NDEA	10/16	0/16
NDEA + 500 ppm diazepam	13/14	5/14[a]
NDEA + 1500 ppm diazepam	15/15[a]	9/15[b]
500 ppm diazepam	0/16	0/16
1500 ppm diazepam	3/15	0/15

From Diwan *et al.* (1986)
[a] [$p < 0.05$, Fisher's exact test compared with NDEA controls]
[b] [$p < 0.01$, Fisher's exact test compared with NDEA controls]

3.2.2 *Rat*

Groups of 10 or 20 male Fischer 344 rats weighing 170 g [age unspecified] were fed basal diets or diets containing 200 mg/kg (ppm) 2-acetylaminofluorene (2-AAF) for eight weeks. The daily dose was estimated to be approximately 15 mg/kg bw. In one group of 10 rats, this was followed by 12 weeks' treatment with 70 mg/kg bw diazepam in 10% arabic gum solution (purity > 99%) daily by gastric instillation. There was no significant increase in the incidence of neoplastic nodules of the liver: untreated controls (20 rats), 0 neoplastic nodule/liver; 2-AAF alone (20 rats), 0.2 neoplastic nodule/liver; and 2-AAF plus diazepam (10 rats), 0.5 neoplastic nodule/liver (Mazue *et al.*, 1982; Remandet *et al.*, 1984). [The Working Group noted the small number of animals and the single dose level of diazepam.]

Groups of 10 male weanling Donryu rats, 21 days of age, were fed a diet containing 600 mg/kg (ppm) 3′-methyl-4-(dimethylamino)azobenzene for three weeks, then left for a week on basal diet followed by either basal diet or a diet with 500 mg/kg diazepam [purity unspecified] (daily intake, approximately 50 mg/kg bw) for a further 12 weeks. The rat livers were scored for adenosine triphosphatase (ATPase)-deficient islands greater than 50 μm in diameter. There was no significant difference between rats on basal diet and those given diazepam in the total number of enzyme-altered islands/cm^2 (control, 9.76 ± 1.32; diazepam-treated, 8.64 ± 0.80) or in the number of enzyme-altered islands > 400 μm (control, 1.03 ± 0.24; diazepam-treated, 1.33 ± 0.34) (Hino & Kitagawa, 1982).

3.2.3 *Gerbil*

Groups of 24 male and 16 female gerbils [strain and age not specified] were given 10 mg diazepam [purity not specified] per animal by gastric instillation weekly plus 30 min later weekly subcutaneous injections of 23 mg/kg bw NDEA for life. Groups of 20 male and 19 female gerbils receiving weekly subcutaneous administrations of NDEA only served as positive controls. Groups of 11 male and 10 female gerbils receiving weekly subcutaneous administrations of saline served as vehicle controls. Gerbils receiving NDEA plus diazepam tended to survive approximately 20 weeks longer than

gerbils receiving NDEA alone. The incidence of nasal cavity adenocarcinomas was high in both groups: 92–95% in males and 63–69% in females. In gerbils receiving NDEA alone, 85% males and 84% females had cholangiocarcinomas of the liver versus none in the NDEA plus diazepam group (75% females and 83% males had cholangiomas of the liver). Male gerbils receiving NDEA plus diazepam had three hepatocellular adenomas and one male and one female had a hepatocellular carcinoma versus none in the NDEA group (Green & Ketkar, 1978). [The Working Group noted the small number of animals and that diazepam was administered weekly.]

3.3 Carcinogenicity of metabolites

See the monographs on oxazepam (pp. 119–123) and temazepam (pp. 164–165).

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

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

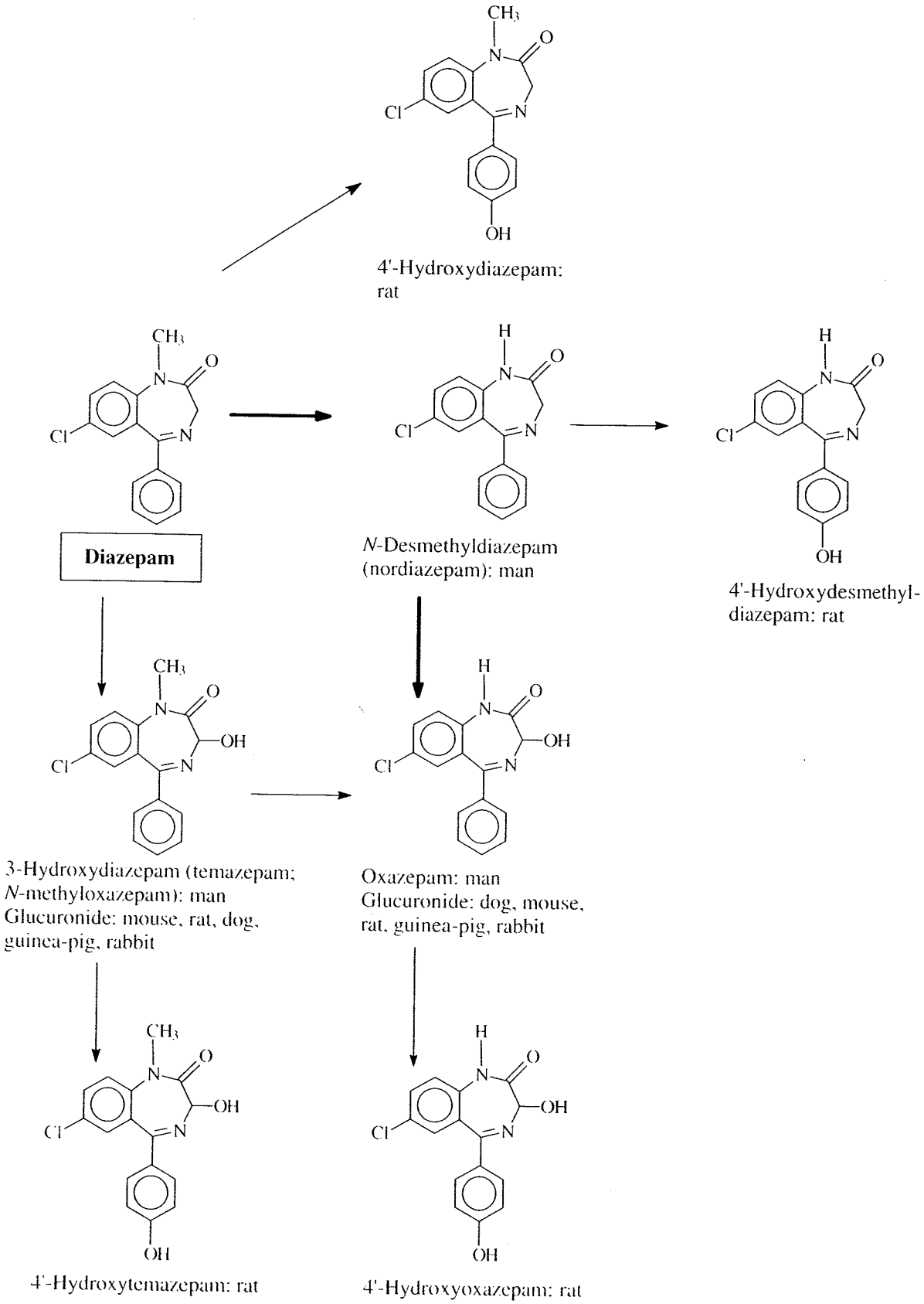
The disposition of diazepam has been reviewed (Mandelli *et al.*, 1978; Schmidt, 1995). Diazepam is rapidly and almost completely absorbed following oral doses of 5, 10 or 20 mg; peak plasma concentrations are usually obtained within 30–90 min and a secondary peak during the elimination phase has been observed in some studies (Baird & Hailey, 1972; Hillestad *et al.*, 1974; Gamble *et al.*, 1975; Kanto, 1975; Korttila & Linnoila, 1975; Schmidt, 1995). Peak plasma concentrations vary widely (30-fold range) in different subjects given the same dose of diazepam (Gamble *et al.*, 1973). Oral administration of two 5-mg tablets to 48 healthy male volunteers aged 18–44 years resulted in peak plasma concentrations after 0.9 h (range, 0.5–2.5 h) of 406 ng/mL (range, 253–586 ng/mL) (Greenblatt *et al.*, 1989). Results were similar among pregnant women receiving single 10-mg doses during the first trimester (Jørgensen *et al.*, 1988). Intravenous administration of 10 or 20 mg diazepam to volunteers gave peak plasma concentrations of 700–800 ng/mL and 1100–1607 ng/mL, respectively, within 3–15 min. Intramuscular administration is not clinically useful in adults, but, in newborn babies and children under 12 years, doses of 0.24–1 mg/kg bw give peak plasma concentrations of 206–1400 ng/mL in 10–60 min (reviewed in Schmidt, 1995). Diazepam has a low pK_a and is lipophilic and consequently distributes quickly into lipoid tissues, and rapidly crosses the blood–brain barrier. A distribution phase with a usual half-life of about 1 h following a single dose precedes the elimination phase (Kaplan *et al.*, 1973; Klotz *et al.*, 1976a,b; Mandelli *et al.*, 1978). Plasma protein binding of diazepam is about 97% (van der Kleijn *et al.*, 1971; Klotz *et al.*, 1976b). Irrespective of the route of administration, the terminal elimination half-life is usually within the range of 24–48 h, and mean values of about 32 h have been obtained in several single-dose studies (reviewed by Schmidt, 1995). Somewhat longer half-lives (mean of 44 h) were measured by Greenblatt *et al.* (1989) in

healthy male volunteers, and longer half-lives are also obtained following repeated administration (Kaplan *et al.*, 1973; Mandelli *et al.*, 1978). Elimination half-lives were longer in premature newborn babies (75 ± 35 h) than in full-term newborn babies (31 ± 2 h) (Morselli, 1977). Plasma clearance values (CL_E) are typically 15–35 mL/min for diazepam and 7–11 mL/min for *N*-desmethyldiazepam. The values tend to be lower in cases of liver disease and, at least for *N*-desmethyldiazepam, after the age of 60 years (Schmidt, 1995).

Diazepam has two major metabolic pathways in humans (see Figure 1), involving either the loss of the N_1-methyl group, yielding *N*-desmethyldiazepam, which is then oxidized at C_3 to oxazepam, or the direct oxidation at C_3, yielding temazepam. The elimination of oxazepam and temazepam is reviewed in sections 4.1.1 of the respective monographs in this volume. *N*-Desmethyldiazepam is the major circulating metabolite of diazepam, as some 50–60% of diazepam is demethylated (Bertilsson *et al.*, 1990). The plasma concentrations of *N*-desmethyldiazepam approach those of diazepam following a single dose, and typically exceed those of diazepam after multiple doses, since the elimination half-life of this metabolite is much longer (50–120 h) than that of diazepam. Thus, in the data tabulated by Schmidt (1995), the half-lives of *N*-desmethyldiazepam are longer than those of diazepam in every situation where both were measured (in volunteers, psychiatric patients, epileptic patients, the elderly and patients with liver disease). *N*-Desmethyldiazepam has a longer half-life (40–120 h) than diazepam (20–54 h) in adults (Mandelli *et al.*, 1978; Bertilsson *et al.*, 1990). There have been few studies of the excretion of diazepam and its metabolites, but it appears that conjugation is important before elimination. Schwartz *et al.* (1965) found that approximately 71% of orally administered diazepam and its metabolites is excreted in the urine and 10% in the faeces. It is not clear whether conjugated oxazepam or conjugated *N*-desmethyldiazepam is the more important urinary metabolite (Schwartz *et al.*, 1965; Morselli *et al.*, 1973; Kanto *et al.*, 1974; Arnold, 1975). In a recent, but preliminary, study (Chiba *et al.*, 1995), four male volunteers aged 24–40 years were given a single dose of 4 mg diazepam orally and urine was collected over 96 h. Following treatment of the urine with β-glucuronidase/sulfatase, diazepam was not detectable and the cumulative excretion of *N*-desmethyldiazepam, temazepam and oxazepam was 3.9 ± 0.4, 6.6 ± 1.4 and 2.8 ± 0.6% of the dose, respectively. Enterohepatic circulation might explain the long elimination half-life of diazepam and the secondary peak observed in some studies during the elimination phase, but most studies indicate that diazepam is not excreted in the bile in significant amounts (reviewed in Schmidt, 1995).

Diazepam is able to cross the placenta rapidly (deSilva *et al.*, 1964; Idänpään-Heikkila *et al.*, 1971a; Jørgensen *et al.*, 1988) and accumulates in the fetus with slow elimination of its active metabolite. The fetal plasma levels of diazepam are equal to or 1.2 times higher than the maternal plasma concentrations (Cavanagh & Condo, 1964; Erkkola *et al.*, 1974); the level of its active metabolite, *N*-desmethyldiazepam, is exceptionally high in fetal liver (Erkkola *et al.*, 1974). After the use of diazepam in labour, diazepam and *N*-desmethyldiazepam persist in the newborn for eight days postpartum (Cree *et al.*, 1973). Diazepam and its active metabolite also pass from the mother's blood into breast milk (Erkkola & Kanto, 1972; Cole & Hailey, 1975).

Figure 1. Postulated metabolic pathways of diazepam



Adapted from Schmidt (1995)
Major steps are indicated by thick arrows. Note that the chlorine at the 7 position remains intact.

Diazepam clearance shows marked interindividual differences. Factors which might contribute to this phenomenon include age, sex, smoking, liver disease, enzyme induction or inhibition and genetic factors affecting the regulation of metabolism (Klotz *et al.*, 1975, 1977; Greenblatt *et al.*, 1980; Ochs *et al.*, 1981a,b; Abernethy *et al.*, 1983; Ochs *et al.*, 1983; Alda *et al.*, 1987; Bertilsson *et al.*, 1989; Zhang *et al.*, 1990; Sohn *et al.*, 1992). With regard to the importance of the regulation of metabolism, a relationship was observed in studies of Swedish volunteers (Bertilsson *et al.*, 1989) and Korean volunteers (Sohn *et al.*, 1992) between more rapid elimination of diazepam and high *S*-mephenytoin hydroxylase activity (but see Section 4.1.2). There was no relationship with debrisoquin hydroxylation [CYP2D6] polymorphism among the Swedish subjects (Bertilsson *et al.*, 1989). No significant difference in the clearance of diazepam was found between Chinese subjects who were extensive or poor *S*-mephenytoin hydroxylators (Zhang *et al.*, 1990). Bertilsson and Kalow (1993) suggested that the racial differences can be explained in terms of the chance selection of different proportions of heterozygotes and homozygotes in these studies.

4.1.2 *Experimental systems*

In contrast to the extensive human pharmacokinetic investigations, equivalent in-vivo studies in experimental animals are rare. Garattini *et al.* (1973) gave rats, mice and guinea-pigs 5 mg/kg bw diazepam by intravenous injection and measured blood levels of diazepam, *N*-desmethyldiazepam and oxazepam at times from 1 min up to 40 h. Maximal diazepam concentrations of 2.33 ± 0.10, 1.36 ± 0.11 and 1.70 ± 0.10 μg/mL were found at 1 min in rats, mice and guinea-pigs, respectively. The blood levels were negligible by 5 h in rats and mice and by 10 h in guinea-pigs. In rats, *N*-desmethyldiazepam was detected only at 0.10 ± 0.01 μg/mL after 5 min, while oxazepam was not detected at all. However, in mice, *N*-desmethyldiazepam concentrations were significant from 1 min to 10 h, with a maximum at 30 min of 1.15 ± 0.04 μg/mL and significant oxazepam concentrations were found between 30 min and 10 h, with a maximum at 3 h of 0.22 ± 0.02 μg/mL. In guinea-pigs, concentrations of *N*-desmethyldiazepam were significant from 1 min to 20 h, with a maximum at 1 h of 0.37 ± 0.07 μg/mL, but no oxazepam was detected.

Lukey *et al.* (1991) administered a single dose of 100 μg/kg bw diazepam to six male rhesus monkeys by intramuscular injection. The maximal serum concentration of diazepam was 49.6 ± 13.9 ng/mL at 28.7 ± 3.6 min, while that of *N*-desmethyldiazepam was 38.4 ± 8.8 ng/mL at 169.8 ± 60.5 min. The volume of distribution and systemic clearance values were 1.5 L/kg and 19.4 mL/min/kg, respectively, assuming 100% bioavailability. Serum protein binding was about 95%.

Diazepam and *N*-desmethyldiazepam accumulate in adipose tissue. Garattini *et al.* (1973) found that adipose tissue/blood ratios in mice given 5 mg/kg bw diazepam intravenously varied from about 4 (at 5 min) to > 6 (at 5 h) while in mice given the metabolite (5 mg/kg bw), the corresponding ratios were 3 (at 5 min) and > 45 (at 5 h). Accumulation also occurred in the brain. Maximal diazepam concentrations in the brain after intravenous administration of 5 mg/kg bw were 7.04 ± 0.37 μg/g and 4.28 ± 0.14 μg/g at

1 min in mice and rats, respectively, and 6.28 ± 0.30 μg/g at 5 min in guinea-pigs. The brain : blood ratios varied from 2.6 (at 1 h) to 5.2 (at 1 min) in mice, from 1.8 (at 1 min) to 5.8 (at 3 h) in rats and from 2.8 (at 1 h) to 9.8 (at 5 min) in guinea-pigs.

The distribution of diazepam to certain tissues was examined in rats given 83 μg/kg bw by intraperitoneal injection (Takatori *et al.*, 1991). Serum concentrations were 3.75 ± 0.62 ng/mL at 1 h and 0.28 ± 0.05 ng/mL at 4 h. Diazepam was also found in the saliva, bone marrow and brain. From 1 h to 8 h after administration, the concentration in bone marrow was higher than that in serum by factors of about 1.2–8.0. Over the period 2–8 h after dosing, concentrations in the serum, saliva and brain were similar. Transplacental transfer of diazepam occurs in mice, hamsters and monkeys (Idänpään-Heikkilä *et al.*, 1971b).

Early studies of the metabolism of diazepam revealed substantial interspecies differences (see Figure 1), whether studied *in vivo* or by in-vitro methods using liver microsomes (Garattini *et al.*, 1973). N-Demethylation (yielding *N*-desmethyldiazepam) and C_3-oxidation (yielding temazepam) occur to various extents in all species studied, but hydroxylation at the 4′-position of the 5-phenyl substituent is a major pathway in the rat. The resulting phenolic derivatives of diazepam, *N*-desmethyldiazepam, oxazepam and temazepam are all found in rat urine. This pathway seems to be negligible in most other species, apart from rabbits (Jommi *et al.*, 1964). Oxazepam can be formed either from temazepam (by N-demethylation) or from *N*-desmethyldiazepam (by C_3-oxidation). The C_3-hydroxy compounds (oxazepam and temazepam) are eliminated in urine as glucuronides by mice (Marcucci *et al.*, 1968), rats (Schwartz *et al.*, 1967), guinea-pigs (Marcucci *et al.*, 1971), rabbits (Jommi *et al.*, 1964) and dogs (Ruelius *et al.*, 1965). The glucuronide and/or sulfate conjugates of several metabolites have also been identified in the intestinal contents of a rat dosed intraperitoneally with 100 mg/kg bw diazepam (Schwartz *et al.*, 1967).

In single-pass experiments with perfused male CD-1 mouse liver and an input concentration of 0.5 μM, diazepam was rapidly cleared, with a steady-state extraction ratio of 0.952 (St-Pierre & Pang, 1993). The mean hepatic clearance was 1.74 ± 0.21 mL/min/g, which was very close to the perfusate flow rate (1.82 mL/min/g). The metabolites recovered were (authors' terminology): nordiazepam (*N*-desmethyldiazepam) (47.7 ± 6.5%); nordiazepam conjugate (3.7 ± 2.1%); oxazepam (28.6 ± 7.1%); oxazepam glucuronide (1.7 ± 0.7%); 4′-hydroxynordiazepam (0.6 ± 0.2%); 4′-hydroxynordiazepam glucuronide (0.9 ± 0.3%); temazepam (0.7 ± 0.2%); temazepam glucuronide (0.3 ± 0.1%).

The metabolism of diazepam in cultured hepatocytes and subcellular preparations has also been studied extensively. An early study showed that diazepam undergoes both N-demethylation and C_3-hydroxylation in dog and rat liver *in vitro*, reactions that are NADPH-dependent and inducible by phenobarbital (Schwartz & Postma, 1968). Subsequently, Ackermann and Richter (1977) demonstrated that the oxidations are mediated by cytochrome P450, using human fetal liver preparations. The CYP2C11 isozyme catalyses the N-demethylation reaction, although other isozymes are also involved, while CYP3A2 is the major catalyst for C_3-hydroxylation (Reilly *et al.*, 1990;

Neville *et al.*, 1993; Yasumori *et al.*, 1993). *S*-Mephenytoin does not inhibit either N-demethylation or C_3-hydroxylation of diazepam by human liver microsomes (Hooper *et al.*, 1992). The human *S*-mephenytoin hydroxylases appear to be CYP2C9 or CYP2C18 enzymes (Nebert *et al.*, 1989; Wilkinson *et al.*, 1989; Romkes *et al.*, 1991), so the apparent relationship between rapid diazepam clearance and high *S*-mephenytoin hydroxylase activity observed in humans (see Section 4.1.1) remains to be explained.

4.2 Toxic effects

4.2.1 *Humans*

(*a*) *Acute toxicity*

In a recent report on 215 lethal intoxications due to self-poisoning with diazepam alone or in combination with other substances, diazepam was associated with a higher rate of death per million prescriptions than the average for benzodiazepine anxiolytics such as lorazepam and oxazepam. However, this may not reflect a higher toxicity of diazepam, since diazepam was used more frequently together with alcohol than the other tranquillizers investigated (Serfaty & Masterton, 1993). In non-lethal intoxications, the symptoms consist mainly of an enhancement of the therapeutic effects, with severe drowsiness, oversedation and ataxia, while in some cases, particularly in elderly persons, a paradoxical excitation may be induced. Rare but severe acute adverse effects after therapeutic intravenous administration include respiratory or cardiac arrest or both (reviewed by Dollery *et al.*, 1991).

(*b*) *Chronic toxicity*

In addition to the effects associated with psychological and physical dependence and rebound withdrawal phenomena, extremely rare but serious adverse reactions are increases in the levels of serum aminotransferase and alkaline phosphatase, jaundice (both hepatocellular and cholestatic), leukopenia, hypersensitivity reactions such as skin rashes, single cases of exfoliative dermatitis and, in predisposed persons, circulatory and respiratory depression (reviewed by Dollery *et al.*, 1991). Taking into account the widespread use of diazepam over a long period, the virtual lack of adverse effects reported in the literature suggests the absence of organ toxicity associated with chronic administration.

4.2.2 *Experimental systems*

(*a*) *Acute toxicity*

Average oral LD_{50} values of 1901 and 1517 mg/kg bw in mice and rats, respectively, have been reported. The average LD_{50} values after intraperitoneal administration were 774 mg/kg bw in mice and 661 mg/kg bw in rats (Owen *et al.*, 1970).

(*b*) *Subchronic and chronic toxicity*

Groups of 20 Charles River CD rats (10 male, 10 female) were administered 0, 600, 1250 or 2500 mg/kg diet (ppm) diazepam orally in the diet for 20–22 weeks. No deaths

occurred in either control or treated rats, and neither food consumption nor body weight differed significantly between the four groups. No haematological or ophthalmological effect of diazepam was found, but kidney and pancreas weights in males and liver weights in males and females were greater at all doses. In addition to the mild renal alterations observed in males of all treated and control groups, some males of the high-dose group had histopathological traces of brown, finely granular material within the epithelial cells of the renal proximal convoluted tubules in the absence of gross toxicity. Mild alteration of the thyroid architecture was observed in some animals in each treated group, the acini appearing condensed and containing less colloid than usual (Owen *et al.*, 1970).

Groups of four dogs were given 80, 127 or 200 mg/kg bw orally by capsule 30 min before feeding daily for four weeks (Owen *et al.*, 1970). One dog of the high-dose group died and marked losses in weight were observed in the mid- and high-dose groups associated with decreased food consumption due to severe sedation and somnolence; in addition, increased emesis was induced in the three treated groups. The authors suggested weakness and starvation as the cause of death. Haematological examination revealed elevated haemoglobin, haematocrit, total red cell count and blood viscosity in the mid- and high-dose groups; increased weights of the kidneys and adrenal gland were also observed in these two groups. Most dogs treated with diazepam displayed histopathological and biochemical signs of hepatobiliary dysfunction and some gonadal changes, namely testicular atrophy.

[The Working Group noted that, since the study of Owen *et al.* (1970) focused on evaluating oxazepam toxicity, with diazepam administered only for comparison purposes, the description of the effects of diazepam is incomplete on many points and the number of animals treated with diazepam was very small.]

(c) *Effects on cell proliferation and differentiation and on steroidogenesis: the role of peripheral benzodiazepine receptors*

The anxiolytic and hypnotic effects of diazepam are mediated via GABAergic receptors in the central nervous system. In addition, diazepam can affect peripheral organs, in particular the immune and endocrine system, directly through a second class of binding sites. Although the peripheral benzodiazepine receptors are ubiquitous in the organism, they are localized in very specific regions of the different organs and their density is strictly controlled by endocrine and neural mechanisms. In generalized anxiety disorders in humans and in chronically stressed or food-deprived experimental animals, the density of the peripheral benzodiazepine receptors is decreased in most organs, while diazepam administration has been reported to induce upregulation (Gavish *et al.*, 1992; Ferrarese *et al.*, 1993). These peripheral benzodiazepine receptors are mitochondrial proteins consisting of two subunits. The 'diazepam-binding inhibitor' peptide is a putative endogenous ligand for peripheral benzodiazepine receptors. This polypeptide has been purified from the brain and from a variety of other organs such as the liver, kidney and adrenal glands, and probably functions as a precursor of smaller biologically active neuropeptides that interact preferentially with either central or peripheral benzodiazepine receptors (Ferrarese *et al.*, 1993).

Experimental evidence suggests that the effects of diazepam on cell proliferation and differentiation observed *in vitro* are mediated via binding to benzodiazepine receptors. Such effects include blockage of mitogenesis in Swiss 3T3 cells, induction of differentiation in murine Friend erythroleukaemia cells, acceleration of melanogenesis in mouse melanoma cells, and inhibition of proliferation of human glioma cells, rat pituitary tumour cells and cultured mouse spleen lymphocytes *in vitro* (Wang *et al.*, 1984; Pawlikowski *et al.*, 1988a,b; Kunert-Radek *et al.*, 1994). In contrast, a single subcutaneous injection of diazepam induced increased thymic mitotic activity in rats *in vivo* (Stepien *et al.*, 1988). Inhibition of plasma membrane calcium influx through voltage-dependent channels has been repeatedly discussed as a mechanism possibly involved in the inhibitory effects on cell proliferation mediated via peripheral benzodiazepine receptors (Pawlikowski *et al.*, 1988b; Ferrarese *et al.*, 1993).

In adrenocortical and testicular Leydig cells and cultured cell lines, the 'diazepam-binding inhibitor' peptide, as well as other ligands including diazepam, stimulate hormone-induced steroid biosynthesis, probably by binding to peripheral benzodiazepine receptors and thus mediating the translocation of cholesterol from the outer to the inner mitochondrial membranes and regulating cholesterol side-chain cleavage to pregnenolone (Krueger & Papadopoulos, 1990; Ferrarese *et al.*, 1993). The extent of stimulation correlates with the binding affinity of the different ligands to these peripheral receptors (Mukhin *et al.*, 1989; Ferrarese *et al.*, 1993). 'Diazepam-binding inhibitor' has been also suggested to stimulate the release of corticotropin-releasing factor from neurons and to stimulate the synthesis of neurosteroids in glial cells (Ferrarese *et al.*, 1993). Earlier studies *in vivo* demonstrated that diazepam increases corticosterone and testosterone secretion in rats, as well as plasma levels of testosterone and 11-hydrocorticoids in humans (Marc & Morselli, 1969; Argüelles & Rosner, 1975). In contrast, flunitrazepam has been reported to antagonize the stimulatory effect of purified 'diazepam-binding inhibitor' on steroidogenesis *in vitro* (Papadopoulos *et al.*, 1991).

The presence of peripheral benzodiazepine receptors in both animal and human tumours has been explored extensively. Increased density was demonstrated in various tumours, such as rat gliomas, human gliomas or astrocytomas, human colon adenocarcinomas and human ovarian and prostatic carcinomas; in contrast, peripheral benzodiazepine receptors were absent in renal carcinomas (Katz *et al.*, 1988; Ferrarese *et al.*, 1989; Gorman *et al.*, 1989; Katz *et al.*, 1989, 1990). The localization of these binding sites in the mitochondria raises the possibility that they might be involved in intermediary metabolism and in respiratory control; hence quantitative and/or qualitative changes in their function might bring about important alterations in cellular biochemistry. However, the available data are fragmentary and do not allow assessment of the role of peripheral benzodiazepine receptors in tumour formation.

(*d*) *Effects on the immune system*

The effects of diazepam on the immune functions have been studied both *in vitro* and *in vivo* with conflicting results: both stimulatory and inhibitory effects have been demonstrated. Diazepam injected one day after immunization stimulated the humoral immune response of mice to sheep red blood cells, probably as a result of T cell-

dependent antigen binding to peripheral benzodiazepine receptors on macrophages (Ferrarese *et al.*, 1993). The presence of peripheral benzodiazepine receptors on the mitochondrial and plasma membranes of peripheral lymphocytes was demonstrated immunocytochemically. Compounds that act exclusively on central GABAergic receptors do not exert immune functions (Ferrarese *et al.*, 1992). Further studies *in vitro* demonstrated that binding of 'diazepam-binding inhibitor' and diazepam to the peripheral benzodiazepine receptors is involved in monocyte chemotaxis and enhances the production of interleukin-1 and tumour necrosis factor-α (Ruff *et al.*, 1985; Taupin *et al.*, 1991). In contrast, diazepam at micromolar concentrations, which can be achieved therapeutically in blood, inhibited phagocytosis and killing of *Candida albicans* cells by human polymorphonuclear cells and monocytes *in vitro* (Covelli *et al.*, 1989). At similar concentrations, diazepam also suppressed the activity of natural killer cells isolated from human peripheral blood against erythroleukaemia target cells, suggesting the possibility of impairment of antiviral and antitumour defence in humans taking diazepam (Stepien *et al.*, 1994). However, it is also possible that the demonstrated elevated levels of 'diazepam-binding inhibitor' in stress and anxiety are responsible for the reduced density of peripheral benzodiazepine receptors on lymphocytes and mediate, together with the increased levels of adrenal steroids, the immunosuppressive effects of stress (Ferrarese *et al.*, 1993). Hence, although the involvement of peripheral receptors in immunomodulation can be considered as proven, the role of diazepam versus 'diazepam-binding inhibitor' remains to be elucidated (Zavala & Lenfant, 1987).

4.3 Reproductive and prenatal effects

4.3.1 *Humans*

The maternal metabolism of diazepam during pregnancy is discussed in Section 4.1.1.

Three kinds of developmental consequences of diazepam treatment during pregnancy can be differentiated:

(i) Congenital abnormalities, i.e., the possible classical teratogenic effect of diazepam used mainly in the first trimester of gestation.

(ii) Short-term functional alterations that are manifested postnatally and are related mainly to diazepam treatment in the perinatal period.

(iii) Long-term postnatal developmental (including behavioural) effects which in general are connected with diazepam intake in the second and third trimesters of gestation, i.e., after the development of specific brain receptors for benzodiazepines.

(*a*) *Possible teratogenic effect*

The possible teratogenic effects are considered according to the circumstances of exposure of the mother.

Undefined exposure circumstances

The data of the Finnish Register of Congenital Malformations (1967–71) showed a significant association between oral clefts and maternal intake of antianxiety drugs

(Saxén, 1975). Extended analysis of the Finnish case–control material (Saxén & Saxén, 1975) indicated significantly greater use of benzodiazepines (diazepam, oxazepam, nitrazepam or chlordiazepoxide) in the first trimester by the mothers of affected children (14 versus 5) among 232 children with isolated cleft palate and 226 matched controls ($p < 0.05$). Benzodiazepine use was greater, but not significantly so, in 232 children with isolated cleft lip with or without cleft palate than in 230 matched controls (11 versus 4). [The Working Group noted that benzodiazepines were not differentiated and that confounding factors such as maternal illness and use of other drugs were not controlled.]

In the Metropolitan Atlanta Congenital Defects Program monitoring the incidence of birth defects since 1967, Safra and Oakley (1975) found, by interviews of 49 women who had infants with cleft lip with or without cleft palate, a history of diazepam ingestion in the first trimester in seven, and in nine of 229 mothers of children with other congenital abnormalities (relative risk, 4.1; 95% CI, 1.5–11.5). The other abnormalities included Down's syndrome, tracheo-oesophageal fistula and/or atresia, small-bowel, rectal and anal atresia, omphalocele, diaphragmatic hernia and limb reductions. The corresponding relative risk estimate for cleft palate alone was 0.9 based on one exposed infant. Later, Safra and Oakley (1976) considered the results of their previous study to be inconclusive because there was no association between secular trends in the prevalence at birth of children with cleft lip with or without cleft palate and in drug sales.

In a hospital-based study in Norway, Aarskog (1975) evaluated retrospectively 12 (1 exposed in the first trimester) cases with cleft palate and 99 (6 exposed in the first trimester) cases with cleft lip with or without cleft palate born in 1967–71 and 362 (9 exposed in the first trimester) controls born in 1972–75. The number of subjects exposed to diazepam in the first trimester was significantly higher in the combined group with oral clefts than that of controls. [The Working Group noted that the validity of the study is uncertain in view of the different study periods for cases and controls. Moreover, cleft lip with or without cleft palate is etiologically distinct from isolated cleft palate.]

Czeizel (1976) conducted an ad-hoc population-based study using data from the Hungarian Congenital Malformation Registry for the period 1970–75. Of 413 cases with cleft lip with or without cleft palate, 121 cases with cleft palate alone and a control series comprising 843 cases with neural-tube defects, 20 (4.8%), 2 (1.7%) and 37 (4.4%), respectively, had mothers who reported having received diazepam treatment in the first trimester of pregnancy. The differences were not statistically significant.

Subsequently, Czeizel (1988) analysed the data-set of the Hungarian Case–Control Surveillance System of Congenital Abnormalities for 1980–84. Approximately 15% of pregnant Hungarian women used diazepam in the 1980s (Czeizel & Rácz, 1990). Maternal diazepam use in the first, second and third months of pregnancy did not differ significantly between 355 cases with isolated cleft lip with or without cleft palate, 167 cases with isolated cleft palate and similar numbers of matched healthy controls. A prospective follow-up study was based on women who visited genetic counselling clinics between 1973 and 1980 following exposure to potentially hazardous environmental factors during early pregnancy. Of 546 women, 33 had ingested benzodiazepines, mainly diazepam; their 26 liveborn babies had no congenital abnormality.

Rosenberg *et al.* (1983) compared the use of diazepam during the first four lunar months of pregnancy in 445 infants with cleft lip with or without cleft palate, in 166 infants with cleft palate alone and in 2498 controls with congenital abnormalities other than oral clefts from the birth defect surveillance system in Boston, MA, and Philadelphia, PA, United States, and Toronto, Canada, in 1976–82. The relative risk, adjusted for several confounding factors, was 0.8 (95% CI, 0.4–1.7) for cleft lip with or without cleft palate and 0.8 (0.2–2.5) for cleft palate alone.

In a prospective study of 33 249 pregnant women, in the Birth Defects Study of the National Institute of Child Health and Human Development and Kaiser-Permanente in the United States, Shiono and Mills (1984) found no increase, based on 854 cases, in the relative risk for oral clefts associated with exposure to diazepam during the first trimester (relative risk, 1.2; 95% CI, 0.17–9.0).

Exposure by attempted suicide

Two retrospective studies were carried out in Budapest and the surrounding area in 1960–79 (Czeizel *et al.*, 1984, 1988) and 1980–84 (Czeizel & Lendvay, 1987; Lendvay & Czeizel, 1992) and one prospective study between 1985 and 1986 (Czeizel & Lendway, 1987) in self-poisoned pregnant women. [The Working Group calculated that, in these two studies, 46 pregnancies ended in births to mothers who had used diazepam for self-poisoning. Relatively low doses (25–45 mg) were used by 3 women, while higher doses of 50–95 mg were used by 11, 100–145 mg by 20 women, 150–195 mg by 3 women and more than 200 mg by 7 women. Of the children, only one was affected by congenital abnormality (Fallot tetralogy). Since the expected rate of congenital abnormalities was about 6.5% (Czeizel *et al.*, 1993), it appears that single, extremely high doses of diazepam did not cause an increase in the rate of detectable defects in the offspring.]

Gunnarskog and Källén (1993) observed that, of 70 infants born in Sweden to mothers exposed to psychoactive drugs as a result of suicide attempts during the organ-forming period, 20 of whom were exposed to benzodiazepines, none had a congenital abnormality. [Specific drugs were not mentioned.]

Psychotherapeutic exposure

Several studies relate to the offspring of women who had psychiatric disorders for which they received high doses of benzodiazepines throughout pregnancy.

Laegreid *et al.* (1987) reported seven cases exposed to benzodiazepines. All offspring had intra- and extrauterine growth retardation, facial dysmorphism and central nervous system dysfunctions. Of five cases with maternal exposure to diazepam, one had submucous cleft of the hard palate and secondary hydronephrosis, another was affected with submucous cleft hard palate and a third one with microcephaly and left renal aplasia. These cases resembled, but were not identical to, the fetal alcohol syndrome but abuse of alcohol was denied by all the mothers. All the mothers had received 30–75-mg daily doses of benzodiazepines throughout pregnancy, in some cases confirmed by the examination of stored blood samples for diazepam. Laegreid *et al.* (1989) reported two new cases with similar findings.

In addition, Laegreid *et al.* (1990) carried out a population-based study of surviving live births born in 1985–86 in Gothenburg, Sweden. Twenty-five children were identified with one or more of (a) embryopathy-fetopathy not otherwise specified, (b) oral clefts, (c) defects of the central nervous system and (d) urinary tract malformations. It was possible to analyse maternal plasma in 18 of these cases (three were reported in previous papers) and eight samples were found to be benzodiazepine-positive, including seven for diazepam. A control series of 109 children was selected using paired sampling. Of 60 controls for whom blood analysis could be carried out, two were positive, both for diazepam. The difference in the proportion exposed to diazepam was highly significant.

Laegreid *et al.* (1992a) studied psychotropic drug use in the mothers of all 73 perinatally dead infants in the city of Gothenburg, Sweden, in 1985–86 and in control mothers of 73 surviving infants. Serum samples obtained in early pregnancy were screened for benzodiazepines. Eighteen case-mothers had used psychotropic drugs (benzodiazepines in nine) during pregnancy as documented from case-notes, compared with seven control mothers (benzodiazepines in three). The association between benzodiazepine drug use and perinatal death was significant ($p = 0.03$), but confounding due to psychiatric disorders and other drug use could not be excluded.

Bergman *et al.* (1990) evaluated the follow-up of the children of 4640 mothers in 1971–86 for their exposure to diazepam, oxazepam or nitrazepam during pregnancy. Only six of the pregnant women appeared to be regular benzodiazepine users (two diazepam, two oxazepam, one nitrazepam and one diazepam plus nitrazepam) and none of their six children had abnormalities.

Later, Bergman *et al.* (1992) examined benzodiazepine use during pregnancy in 104 339 women whose deliveries were recorded by the United States public health insurance system, Medicaid, during 1980–83. Of 80 pregnant women who had received 10 or more benzodiazepine prescriptions (63 diazepam, 36 chlordiazepoxide, 8 lorazepam, 13 flurazepam), three experienced fetal death and two infants were found to have lethal congenital abnormalities. Records of 64 surviving children could be linked to these 80 pregnancies (11 survivors could not be located), and six children had congenital abnormalities of various types. These defects differed from the pattern described by Laegreid *et al.* (1987, 1989, 1990); in particular, there were no oral clefts.

(b) Short-term functional alterations

There are case reports of floppy infant syndrome (namely hypotonia, hyporeflexia, apnoeic spells, reluctance to feed, a risk for inhalation of feeds, impaired metabolic responses to cold, hypothermia, low Apgar rating at birth) associated with diazepam treatment of pregnant women at doses of 30 mg or more within the 15 hours before delivery (Owen *et al.*, 1972; Cree *et al.*, 1973).

Acute withdrawal effects (namely depressed respiration, hypothermia and feeding difficulties) have been documented in neonates exposed to diazepam *in utero* for long periods (Cree *et al.*, 1973). The time of onset of the symptoms, their severity and duration were related to the dosage and fetal kinetics (including elimination) of the drug (Mazzi, 1977; Mac New & Finnigan, 1980).

(*c*) *Human long-term postnatal studies*

The behavioural development of 101 children (and their 117 siblings) of pregnant women who attempted suicide has been studied (Lendvay & Czeizel, 1992). Forty-two of the mothers had used diazepam during pregnancy. Some of the children had behavioural alterations which could be explained mainly by their familial and social problems.

Laegreid *et al.* (1992b,c) examined the neurodevelopment of 17 children born to 16 mothers who used benzodiazepines throughout pregnancy: 15 used diazepam (5–30 mg daily) or oxazepam (15–60 mg daily) alone or in combination and one mother used lorazepam. The results were compared with those for 29 children born to mothers without any known use of psychotropic drugs. A neurological investigation was performed on the second day of life. Significant differences in the frequency of pre- and perinatal complications and in neurobehaviour were found between the two groups. The benzodiazepine-exposed children recovered from their lower mean birth weight at an early stage, whereas their slightly decreased head circumference at birth remained lower. Gross motor development was retarded at six and 10 months, but was nearly normal at 18 months. Impaired fine motor functions were found on all follow-up occasions (at six, 10 and 18 months of age).

The cases studied by Laegreid *et al.* (1990, 1992b,c) were followed up prospectively in late infancy and found to have a general delay in mental development up to 18 months of age associated with prenatal exposure to benzodiazepines (Viggedal *et al.*, 1993). [The Working Group noted that all the mothers in the benzodiazepine group had psychiatric disorders, and these have an important negative effect on children's development (Cox, 1988). In addition, neuropsychological symptoms are frequent among children of abusers of psychoactive substances (Deren, 1986; Van Baar *et al.*, 1989).]

4.3.2 *Experimental systems*

Few experimental data are available concerning the effect of diazepam on reproduction. The incidence of abnormal sperm heads was significantly higher in mice after a daily oral dose of 0.5 mg diazepam (Kar & Das, 1983; Šrám & Kocišová, 1984).

Guerriero and Fox (1977) found a significant decrease in mating performance, with depressed birth weights, among Swiss-Webster mice given a diet containing 500 mg/kg diazepam.

In A/J mice given a single intramuscular dose of 100 mg/kg diazepam on day 14 of pregnancy, the frequency in the offspring of both cleft lip with or without cleft palate and cleft palate only was 3.4% (Walker & Patterson, 1974). The authors observed that this was lower than the frequency of spontaneous occurrence of these defects reported in other series.

Miller and Becker (1975) treated Swiss-Webster mice with 50, 100, 140 or 500 mg/kg bw diazepam by gastric instillation once daily for three days on gestation days 8–10 or days 11–13 or for one day only between days 8 and 15 or with 280 or 400 mg/kg bw for one day only between days 11 and 14. The highest dose was associated with a maternal mortality rate of 50%. When 140 mg/kg bw diazepam was administered on day 13, there was 21% fetal resorption. The incidence of cleft palate was significantly increased in the

offspring of mice treated with 140 mg/kg bw diazepam on days 11, 12 and 13, and with single-day administrations of 400 mg/kg bw on days 11–14 and 500 mg/kg bw on days 9 and 11–15.

Tocco *et al.* (1987) reported an increase in the frequency of cleft palate in Swiss-Webster and AJ mice following two-day dosing with 400 mg/kg bw diazepam by gastric instillation on days 13.5 and 14.5. Maternal mortality was high (50% or more) but no increase in resorption was observed.

In rats, no abnormality was caused by oral administration of 20 or 80 mg/kg bw diazepam per day on days 6–15 of gestation (Beall, 1972). Saito *et al.* (1984) did not find a significant increase in any abnormalities after oral administration of 100 mg/kg bw diazepam on gestation days 8–14 in Sprague-Dawley rats.

In hamsters, exencephaly, cleft palate and limb defects were detected after a single oral dose of 30, 50, 70 or 100 mg on days 8 and 10 or single intravenous injections of 10 mg diazepam on day 11. There was no dose-related effect (Shah *et al.*, 1979). A single intraperitoneal injection of 120–980 mg/kg bw diazepam on day 8 of gestation induced a dose-related increase in the frequency of fetal malformations in hamsters, mainly exencephaly or cranioschisis, at doses of 280 mg/kg and above (Gill *et al.*, 1981).

No structural abnormality was observed in the offspring of two rhesus monkeys treated orally with diazepam (0.5–3.2 mg/kg bw) twice daily during the second and third trimesters, nor in those of three monkeys treated during the third trimester only (Jerome *et al.*, 1981). [The Working Group noted that there was no treatment during the first trimester.]

Specific receptors for benzodiazepines develop at the beginning of the fetal period in the central and peripheral nervous systems of rats (Braestrup & Nielsen, 1978). Behavioural studies have demonstrated pronounced effects in rodents following exposure to diazepam during late gestation. In rat models, prenatal exposure to diazepam and other benzodiazepines resulted in behavioural deficits in pups (Kellogg *et al.*, 1980), such as learning and memory disabilities (Gai & Grimm, 1982), the absence of acoustic startle reflexes and the impairment of conditioned avoidance response (Kellogg, 1992) which is dependent on the time of treatment (Frieder *et al.*, 1984). However, diazepam prevented the adverse effects of maternal restraint stress in postnatal development and learning in rats (Barlow *et al.*, 1979).

A chick embryotoxicity screening test did not show any teratogenic effect of diazepam (Peterka *et al.*, 1992).

4.4 Genetic and related effects (see also Table 6 for references and Appendices 1 and 2)

4.4.1 *Humans*

The first published report of a possible association between exposure to diazepam and chromosomal aberrations in the lymphocytes of patients using the drug as a tranquillizer (four patients) or for its muscle-relaxing properties (19 patients) was that of Stenchever *et al.* (1970). These patients were compared with eight controls. Although there was no

Table 6. Genetic and related effects of diazepam

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SAD, *Salmonella typhimurium*, DNA repair	–	NT	400	Waskell (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Waskell (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2500	Balbi *et al.* (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Preiss *et al.* (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NG	Matula & Downie (1983) (abstract)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2500	Balbi *et al.* (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	500	Preiss *et al.* (1982)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2500	Balbi *et al.* (1980)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	500	Preiss *et al.* (1982)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	500	Preiss *et al.* (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Waskell (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2500	Balbi *et al.* (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Preiss *et al.* (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Matula & Downie (1983) (abstract)
SCR, *Saccharomyces cerevisiae*, reverse mutation	–	NT	NG	Matula & Downie (1983) (abstract)
SCH, *Saccharomyces cerevisiae*, mitotic recombination and gene conversion	–	NT	NG	Matula & Downie (1983) (abstract)
SCN, *Saccharomyces cerevisiae*, aneuploidy	–	NT	250	Whittaker *et al.* (1990)
SCN, *Saccharomyces cerevisiae*, aneuploidy	–	NT	300	Albertini (1990)
ANN, *Aspergillus nidulans*, aneuploidy	–	NT	200	Crebelli *et al.* (1991)
URP, Unscheduled DNA synthesis, rat primary hepatocytes	–	NT	0.5	Swierenga *et al.* (1983) (abstract)
URP, Unscheduled DNA synthesis, rat primary hepatocytes	–	NT	1000	Williams *et al.* (1989)

Table 6 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	–	250	Röhrborn *et al.* (1984) (abstract)
GIA, Gene mutation, rat primary hepatocytes, *hprt* locus *in vitro*	–	–	50	Swierenga *et al.* (1983) (abstract)
MIA, Micronucleus test, Chinese hamster (Cl-1) cells *in vitro*	+[c]	NT	20	Antoccia *et al.* (1991)
MIA, Micronucleus test, Chinese hamster lung V79 cells *in vitro*	+[c]	NT	NG	Bonatti *et al.* (1992)
MIA, Micronucleus test, Chinese hamster pulmonary (Luc 2) cells *in vitro*	+	NT	10	Lynch & Parry (1993)
MIA, Micronucleus test, Chinese hamster lung V79 cells *in vitro*	+[c]	NT	100	Seelbach *et al.* (1993)
CIC, Chromosomal aberrations, Chinese hamster lung CHL cells *in vitro*	–	?	1000	Matsuoka *et al.* (1979)
CIC, Chromosomal aberrations, Chinese hamster lung CHL cells *in vitro*	–	NT	125	Ishidate *et al.* (1988)
CIC, Chromosomal aberrations, Chinese hamster (CHE-3N) cells *in vitro*	?	NT	100	Lafi & Parry (1988)
AIA, Polyploidy, Chinese hamster (Don) cells *in vitro*	+	NT	100	Satya-Prakash *et al.* (1984)
AIA, Aneuploidy, Chinese hamster (Don) cells *in vitro*	NT	+	100	Hsu *et al.* (1983)
AIA, Aneuploidy, Chinese hamster (CHE-3N) cells *in vitro* (hypodiploidy)	(+)	NT	100	Lafi & Parry (1988)
AIA, Aneuploidy, primary Chinese hamster embryonic cells *in vitro*	+[d]	NT	10	Natarajan *et al.* (1993)
AIA, Aneuploidy, Chinese hamster pulmonary (Luc 2p4) cells *in vitro* (hypodiploidy and polyploidy)	+	NT	10	Warr *et al.* (1993)
*, c-Mitoses, Chinese hamster (Cl-1) cells *in vitro*	+	NT	60	Antoccia *et al.* (1991)
TCL, Cell transformation, BHK 21-C13 cells *in vitro*	–	+	130	Röhrborn *et al.* (1984) (abstract)
SHF, Sister chromatid exchange, human fibroblast cell line *in vitro*	–	NT	28.5	Sasaki *et al.* (1980)
MIH, Micronucleus test, human lymphocytes *in vitro*	–	NT	75	Migliore & Nieri (1991)
MIH, Micronucleus test, human fibroblasts *in vitro*	+[c]	NT	25	Bonatti *et al.* (1992)
MIH, Micronucleus test, human lymphocytes *in vitro*	+[e]	NT	30	Ferguson *et al.* (1993)

Table 6 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
CHF, Chromosomal aberrations, human primary fetal fibroblasts *in vitro*	–	NT	50	Staiger (1969)
CHF, Chromosomal aberrations, human fibroblast cell line *in vitro*	–	NT	25	Staiger (1969)
CHF, Chromosomal aberrations, human fibroblast cell line *in vitro*	–	NT	28.5	Sasaki *et al.* (1980)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	50	Staiger (1970)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	–	–	NG	Röhrborn *et al.* (1984) (abstract)
AIH, Aneuploidy, human primary fibroblasts *in vitro*	–	NT	50	Staiger (1969)
AIH, Aneuploidy, human fibroblast cell line *in vitro*	–	NT	25	Staiger (1969)
AIH, Aneuploidy, human lymphocytes *in vitro* (hypodiploidy)	+[d]	NT	25	Sbrana *et al.* (1993)
*, c-Mitoses, human lymphocytes *in vitro*	+	NT	50	Sbrana *et al.* (1993)
DVA, DNA strand breaks, rat liver *in vivo*	–		285 po × 1	Carlo *et al.* (1989)
DVA, DNA strand breaks, rat liver *in vivo*	–		57 po × 15	Carlo *et al.* (1989)
MVM, Micronucleus test, mouse bone marrow *in vivo*	(+)		22 po × 1	Kar & Das (1979)
MVM, Micronucleus test, mouse bone marrow *in vivo*	+		20 po × 2	Das & Kar (1986)
MVM, Micronucleus test, mouse bone marrow *in vivo*	–		150 ip × 1	Adler *et al.* (1991)
MVM, Micronucleus test, mouse bone marrow *in vivo*	–		30 ip × 1	Leopardi *et al.* (1993)
MVM, Micronucleus test, mouse bone marrow *in vivo*	(+)		10 × 1[f]	Marrazzini *et al.* (1994)
CBA, Chromosomal aberrations, Chinese hamster bone marrow *in vivo*	–		300 po × 10	Schmid & Staiger (1969)
CBA, Chromosomal aberrations, rat bone marrow *in vivo*	–		500 po × 10	Neda *et al.* (1977)
CBA, Chromosomal aberrations, mouse bone marrow *in vivo*	(+)		75 ip × 7	Petersen *et al.* (1978) (abstract)
CBA, Chromosomal aberrations, mouse bone marrow *in vivo*	–		0.85 ip × 1	Degraeve *et al.* (1985) (abstract)

Table 6 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
CBA, Chromosomal aberrations, mouse bone marrow *in vivo*	–		0.85 ip × 22	Degraeve *et al.* (1985) (abstract)
CBA, Chromosomal aberrations, mouse bone marrow *in vivo*	(+)		1000 po × 28	Kocišová & Šrám (1985) (abstract)
CBA, Chromosomal aberrations, rat bone marrow *in vivo*	–		500 po × 10	Ishimura *et al.* (1975) (abstract)
CBA, Chromosome aberrations, mouse bone marrow *in vivo*	–		100 ip × 1	Xu & Adler (1990)
CBA, Chromosomal aberrations, mouse bone marrow *in vivo*	–		10 × 1[f]	Marrazzini *et al.* (1994)
CGG, Chromosomal aberrations, mouse spermatogonia treated *in vivo*, spermatagonia observed	–		0.85 ip × 22	Degraeve *et al.* (1985) (abstract)
CCC, Chromosomal aberrations, mouse spermatocytes treated *in vivo*, spermatocytes observed	–		0.85 ip × 22	Degraeve *et al.* (1985) (abstract)
DLM, Dominant lethal test, mouse *in vivo*	(+)		22 po × 15	Kar & Das (1979)
DLM, Dominant lethal test, mouse *in vivo*	–		0.85 ip × 40	Degraeve *et al.* (1985) (abstract)
DLM, Dominant lethal test, mouse *in vivo*	–		1000 po × 28	Šrám & Kocišová (1985)
*, c-Mitoses, mouse bone marrow *in vivo*	–		150 ip × 1	Miller & Adler (1989)
AVA, Polyploidy, mouse bone marrow *in vivo*	–		100 ip × 1	Xu & Adler (1990)
AVA, Aneuploidy, mouse secondary spermatocytes *in vivo*	(+)		150 ip × 1	Miller & Adler (1992)
AVA, Aneuploidy, mouse bone marrow *in vivo*	–		30 ip × 1	Leopardi *et al.* (1993)
AVA, Aneuploidy, mouse secondary spermatocytes *in vivo*	–		30 ip × 1	Leopardi *et al.* (1993)
AVA, Aneuploidy, mouse oocytes *in vivo*	–		150 ip × 1	Mailhes & Marchetti (1994)

Table 6 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
AVA, Aneuploidy, male mouse germ cells *in vivo*	+		150 ip × 1	Gassner & Adler (1995)
AVA, Aneuploidy, mouse bone marrow *in vivo*	(+)		10 × 1[f]	Marrazzini *et al.* (1994)
SLH, Sister chromatid exchange, human lymphocytes *in vivo*	–		0.2 po × 1	Husum *et al.* (1985)
SLH, Sister chromatid exchange, human lymphocytes *in vivo*	+		NG	Huong *et al.* (1988)
CLH, Chromosomal aberrations, human lymphocytes *in vivo*	–		0.5 po × 1[g]	Cohen *et al.* (1969)
CLH, Chromosomal aberrations, human lymphocytes *in vivo*	–		0.3 po × 1[h]	Stenchever *et al.* (1970)
CLH, Chromosomal aberrations, human lymphocytes *in vivo*	–		0.30 iv × 1	White *et al.* (1974)
CLH, Chromosomal aberrations, human lymphocytes *in vivo*	+		NG	Huong *et al.* (1988)
CLH, Chromosomal aberrations, human lymphocytes *in vivo*	–		2.4 po × 1[i]	van Bao *et al.* (1992)
MVH, Micronucleus test, human lymphocytes *in vivo*	–		2.4 po × 1[i]	van Bao *et al.* (1992)
AVH, Aneuploidy, human cells *in vivo* (hypodiploidy)	+		2.4 po × 1[i]	van Bao *et al.* (1992)
*, Inhibition of tubulin assembly *in vitro*	–		712	Brunner *et al.* (1991)
*, Inhibition of tubulin assembly *in vitro*	+		285	Wallin & Hartley-Asp (1993)
ICR, Inhibition of intercellular communication, Chinese hamster lung (V79) cells *in vitro*	+		1.25	Trosko *et al.* (1982)
ICR, Inhibition of intercellular communication, rat liver epithelial cells	–		10	Wälder & Lützelschwab (1984)
ICR, Inhibition of intercellular communication, mouse hepatocytes *in vitro*	+		25	Diwan *et al.* (1989)
ICR, Inhibition of intercellular communication, Chinese hamster lung (V79) cells *in vitro*	?		20	Toraason *et al.* (1992)
ICH, Inhibition of intercellular communication, human hepatoma cellular carcinoma cell line (SK-HEP-1)	–		10	Rolin-Limbosch *et al.* (1987)

Table 6 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SPM, Sperm morphology, mouse *in vivo*	+		20 po × 15	Kar & Dass (1983)
SPM, Sperm morphology, mouse *in vivo*	+		200 po × 28	Kocišová & Šrám (1985) (abstract)
BFA, Urine of mouse, Ames test, *Salmonella typhimurium* TA100	+	+	200 po × 1	Batzinger *et al.* (1978)
BFA, Urine of mouse, Ames test, *Salmonella typhimurium* TA98	+	+	200 po × 1	Batzinger *et al.* (1978)
BFA, Urine of mouse, Ames test, *Salmonella typhimurium* TA100	–		200	Matula & Downie (1983) (abstract)
BFA, Urine of mouse, Ames test, *Salmonella typhimurium* TA98	–		200	Matula & Downie (1983) (abstract)
*, Metabolites from canine gastric mucosa *in vitro*, Ames test, *Salmonella typhimurium* TA98	NT	+	NG	Rice *et al.* (1981)
*, Metabolites from human gastric mucosa *in vitro*, Ames test, *Salmonella typhimurium* TA98	NT	+	NG	Rice *et al.* (1981)

*Not shown on profile

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day; NG, dose not given

[c] Kinetochore-positive

[d] Negative for polyploidy

[e] Size ratio of micronuclei to main nucleus indicates aneuploidy induction

[f] Both intraperitoneal and oral routes were used when no useful indication concerning the most effective route was available from the literature.

[g] Average daily dose from six patients treated for 36–72 months

[h] Average daily dose from 23 patients teated for 0.5–36 months. One patient had a significant increase in cells with breaks following treatment (30 mg/day × 18 months)

[i] Average estimated dose from 25 self-poisoned individuals. Increase in hypodiploidy but not polyploidy 6–12 h after poisoning; no increase 3 and 30 days after poisoning

overall difference between the groups, three patients had elevated levels of chromosomal aberrations and one of these in particular showed chromosomal breakage in 15.3% of the cells examined, but, on re-examination six months after discontinuing the drug, only control levels of damage were found.

With regard to sister chromatid exchange, Torigoe (1979) studied 20 epileptic children (10 boys, 10 girls; age, 4–23 years) who had taken two to six anticonvulsant drugs for one to 18 years and 20 controls (10 boys, 10 girls; age, 6–15 years) who did not receive any drugs for at least six months; they found no significant difference between control subjects and epileptic patients. In the study of Husum *et al.* (1985), the peripheral lymphocytes of 34 persons (18 men and 16 women undergoing minor surgery) were examined before and 2–5 h after oral administration of a single 0.2 mg/kg bw dose of diazepam; possible effects of smoking were taken into account and no indication of the induction of sister chromatid exchange was found. In contrast with these observations, a cytogenetic investigation (Huong *et al.*, 1988) of 18 self-poisoned pregnant and 16 self-poisoned non-pregnant women and 31 controls (16 pregnant and 15 non-pregnant) found statistically significant differences in frequencies of sister chromatid exchange per cell between the third and seventh day after poisoning (pregnant: control, 8.55 ± 1.08; poisoned, 10.30 ± 1.63, $p < 0.01$; non-pregnant: control, 9.13 ± 1.32; poisoned, 11.26 ± 2.31, $p < 0.05$). In the same population, a very highly significant difference in the prevalence of chromosomal aberrations between self-poisoned women and controls (pregnant: control, 4.03%; poisoned, 9.56%, $p < 0.001$; non-pregnant: control, 5.99%; poisoned, 14.38%, $p < 0.001$) was also found; moreover, the frequency of chromatid aberrations was significantly lower in pregnant relative to non-pregnant women ($p < 0.05$). In contrast, the studies of White *et al.* (1974) on 20 patients given a single 20-mg intravenous injection of diazepam and of van Bao *et al.* (1992) on 25 patients 6–12 h, 3 days or 30 days after self-poisoning with diazepam failed to confirm the induction of chromosomal aberrations *in vivo* in human lymphocytes. The latter, however, reported that hypodiploidy (but neither hyperdiploidy nor polyploidy) was observed in the individuals studied 6–12 h after self-poisoning; the effects were not observed at later sampling times. [The Working Group noted that cytogenetic changes in lymphocytes disappeared six days after poisoning.]

4.4.2 *Experimental systems*

No studies have demonstrated bacterial DNA damage or mutagenicity due to diazepam itself.

No genetic effects were observed in single studies for mitotic recombination and gene conversion, in two studies for aneuploidy [no dose-dependent increase] with *Saccharomyces cerevisiae* or in a single study for chromosome malsegregation with *Aspergillus nidulans*.

Diazepam did not induce unscheduled DNA synthesis in primary cultures of rat hepatocytes in two studies or mutation at the *hprt* locus of Chinese hamster V79 cells or rat primary hepatocytes *in vitro*. Micronuclei were induced *in vitro* in Chinese hamster cell lines in four studies but the increase in chromosomal aberrations was judged to be

inconclusive in two studies (one in the presence of an exogenous metabolic activation system) and negative in another study.

All studies with Chinese hamster cells aimed at the detection of aneuploidy *in vitro* were positive: moreover, in all of the micronucleus tests in which they were examined, the micronuclei contained kinetochore(s). Two studies out of four which scored for chromosome numbers detected a significant increase in hypodiploidy but not in hyperdiploidy. One study which tested the induction of polyploidy in Chinese hamster cells was positive. Meiotic delay has been observed in mouse oocytes (Stenchever & Smith, 1981) and mitotic arrest has been demonstrated in Chinese hamster Don cells (Hsu *et al.*, 1983) and human fibroblasts (Andersson *et al.*, 1981).

In a study at very low doses with human fibroblasts *in vitro*, diazepam did not induce sister chromatid exchange, whereas a significant increase in micronuclei was observed at higher dose levels. Two studies in human lymphocytes gave contradictory results. All the studies in either human fibroblasts or lymphocytes aimed at the detection of chromosomal aberrations *in vitro* were negative. c-Mitosis and hypodiploidy (but not polyploidy) were observed in single studies with human lymphocytes treated *in vitro* with diazepam. One study reported the induction of large-sized micronuclei in human lymphocytes *in vitro*.

Diazepam caused inhibition of gap-junctional intercellular communication in two out of five studies.

Urine of mice exposed *in vivo* to diazepam induced gene mutations in *Salmonella typhimurium* TA100 or TA98 in one study but not in another; metabolites from dog and human gastric mucosa incubated *in vitro* with diazepam induced a significant increase in gene mutations in *S. typhimurium* TA98.

Two mammalian studies indicated a lack of micronucleus induction in the bone marrow of mice *in vivo*; however, three other similar studies were positive at lower dose levels. [The Working Group noted that the authors reported induction of larger micronuclei taking into account neither interanimal variation nor objective criteria for micronucleus scoring.]

No increase in DNA single-strand breaks and/or alkali-labile sites was observed in the liver of rats given a single dose (1 mmol/kg) or 15 successive daily doses (0.2 mmol/kg) orally. However, predominantly negative results (seven negative studies and two inconclusive) for the induction of chromosomal aberrations have been obtained in studies of mouse or rat bone marrow. Aneuploidy, c-mitoses or polyploidy were not observed in mouse bone marrow [the Working Group noted the inadequate presentation of the results]. In mouse secondary spermatocytes, meiotic delay and aneuploidy were found at higher concentrations (150 mg/mL; Miller & Adler, 1992) but not at a lower concentration (30 mg/mL; Leopardi *et al.*, 1993); the positive effect was due to an increase in hyperploidy. At the same higher concentration, abnormalities of chromosome/spindle segregation were also reported in male mouse germ cells.

As reported in abstracts, treatment of mice with diazepam increases the proportion of sperm with abnormal head morphology.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Diazepam is the most widely used of the benzodiazepine pharmaceuticals. Produced since the 1960s, it is prescribed for the treatment of anxiety and as a sedative, muscle relaxant, and anticonvulsant.

5.2 Human carcinogenicity data

Studies investigating unspecified hypnotics or tranquillizers as well as diazepam specifically have been included in this monograph because of the dominance of this benzodiazepine among those prescribed. The risk for a variety of cancers, especially of the breast, associated with diazepam use has been investigated in two cohort studies and in six distinct and three related case–control studies.

In none of the two cohort or five case–control studies on benzodiazepine or diazepam use in relation to breast cancer was a positive association found. One case–control study of ovarian cancer reported an increased risk for diazepam use, that was not confirmed by another study. This latter study reported no association between diazepam use and the risk of several other types of cancer.

5.3 Animal carcinogenicity data

Diazepam was tested for carcinogenicity in one experiment in mice, in one experiment in rats and in one experiment in hamsters by oral administration in the diet and also in one limited study in gerbils. An increase in the incidence of hepatocellular tumours occurred in male mice. No significant increase in the incidence of tumours was observed in rats, hamsters or gerbils.

In one study in mice, oral administration of diazepam enhanced the occurrence of hepatocellular tumours induced by *N*-nitrosodiethylamine. In two studies in rats initiated with 2-acetylaminofluorene or 3′-methyl-4-(dimethylamino)azobenzene, there was no promoting effect of diazepam. In gerbils initiated with *N*-nitrosodiethylamine, simultaneous administration of diazepam decreased the incidence of cholangiocarcinomas.

5.4 Other relevant data

Diazepam is absorbed rapidly and extensively in humans. A 30-fold range of peak plasma concentrations is obtained when the same dose is given to different subjects. Diazepam is metabolized initially to *N*-desmethyldiazepam (nordiazepam) and temazepam, both of which may be converted to oxazepam. Diazepam clearance shows marked inter-subject variability. The mean elimination half-life is about 32 h.

There is wide inter-species variability in diazepam metabolism. While formation of *N*-desmethyldiazepam and temazepam occurs to some extent in all species studied, hydroxylation in the 5-phenyl ring is the major pathway in rats.

Diazepam has low acute and chronic toxicity for humans at therapeutic concentrations. The main adverse effects of chronic administration are psychological and physical dependence and withdrawal phenomena. Specific organ toxicity of diazepam to humans has not been observed.

The acute toxicity of diazepam to experimental animals can be considered as low. In subchronic toxicity assays in dogs, high doses of diazepam induced mild toxic effects in the blood, liver and gonads, while in rats, slight chemical-related histopathological changes were observed in the kidneys and thyroid gland.

The effects of diazepam on the immune system have been investigated mainly in in-vitro experiments with conflicting results: both stimulatory and inhibitory effects have been demonstrated. There are no data on immunosuppressing or immunomodulating effects in humans.

In several cultured cell systems, diazepam inhibits cell proliferation.

No consistent association between orofacial clefts and diazepam has been identified in humans. No increase in the prevalence at birth of congenital abnormalities has been found associated with attempted maternal suicide using high doses of diazepam, in some instances during the first trimester. While excesses of anomalies associated with regular psychotherapeutic benzodiazepine use have been observed, the types of developmental defects involved have not been consistent between studies.

High doses of diazepam induce cleft palate in mice, but not in rats. In hamsters, exencephaly and limb defects are seen, as well as cleft palate.

In general, diazepam did not induce gene or chromosome mutations in bacteria, yeast or cultured mammalian cells. In cultured mammalian cells, it induced micronuclei and aneuploidy, and inhibited gap-junctional intercellular communication. There are contradictory results on the induction of gene mutation in bacteria by the urinary metabolites of treated mice.

In general, diazepam did not induce micronuclei, chromosomal aberrations, aneuploidy, c-mitoses or polyploidy in bone marrow of mice *in vivo*. In rats *in vivo*, neither chromosomal aberrations in bone marrow, nor DNA strand breaks or alkali-labile sites in liver were found. In mouse spermatocytes, but not in oocytes, diazepam induced aneuploidy.

Mechanistic considerations

Diazepam does not cause gene mutations or chromosomal aberrations. One of its metabolites, oxazepam, increased the incidence of liver tumours (benign and malignant) (see Monograph on oxazepam, pp. 119–123). However, it is not clear that levels of oxazepam sufficient to induce hepatic effects are achieved in mice treated with diazepam.

5.5 Evaluation[1]

There is *evidence suggesting lack of carcinogenicity* of diazepam to the breast and *inadequate evidence* for carcinogenicity at other sites in humans.

There is *inadequate evidence* in experimental animals for the carcinogenicity of diazepam.

Overall evaluation

Diazepam is *not classifiable as to its carcinogenicity to humans (Group 3).*

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[1]For definition of the italicized terms, see Preamble, pp. 22–25.

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