ESTAZOLAM

1. Exposure Data

1.1 Chemical and physical data

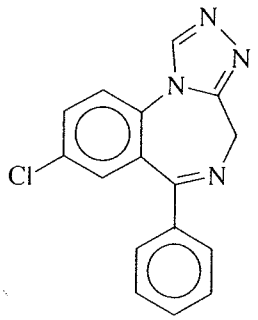
1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 29975-16-4

Chem. Abstr. Name: 8-Chloro-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine

IUPAC Systematic Name: 8-Chloro-6-phenyl-4*H*-*s*-triazolo[4,3-*a*][1,4]benzodiazepine

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{16}H_{11}ClN_4$ Relative molecular mass: 294.74

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: White crystals (Gennaro, 1995)

(*b*) *Melting-point*: 228–229 °C (Budavari, 1995)

(*c*) *Solubility*: Practically insoluble in water; soluble in ethanol (American Hospital Formulary Service, 1995)

1.1.4 *Technical products and impurities*

Estazolam is available as 1- or 2-mg tablets which also may contain corn starch, hydroxypropylcellulose, iron oxide, lactose, magnesium stearate or stearic acid (Farmindustria, 1993; Medical Economics, 1996).

Trade names and designations of the chemical and its pharmaceutical preparations include: A 47631; Abbott 47631; Bay k 4200; Cannoc; D 40TA; Deprinocte; Domnamid; Esilgan; Eurodin; Hypnomat; Julodin; Kainever; Nemurel; Noctal; Nuctalon; ProSom; Sedarest; Somnatrol; Tasedan; U 33737.

1.1.5 *Analysis*

The Pharmacopoeia of Japan specifies potentiometric titration with perchloric acid as the assay for purity of estazolam, and thin-layer chromatography for determining impurities and decomposition products. An assay for heavy metal impurities is also specified (Society of Japanese Pharmacopoeia, 1992). Other methods of analysis in pharmaceutical preparations include polarography (Li & Ji, 1990) and spectrophotometry (Gallo *et al.*, 1985).

Estazolam can be analysed in biological fluids by gas chromatography with electron capture detection (Kelly & Greenblatt, 1993) and high-performance liquid chromatography (di Tella *et al.*, 1986; Mura *et al.*, 1987; Boukhabza *et al.*, 1991).

1.2 Production and use

1.2.1 *Production*

Estazolam is prepared by reacting 7-chloro-1,3-dihydro-5-phenyl-2*H*-benzo[1,4]-diazepine-2-thione with formylhydrazine in boiling *n*-butyl alcohol (Gennaro, 1995). It was first marketed in Japan in 1975 and is currently available in at least 21 countries worldwide (Abbott Laboratories, 1996).

1.2.2 *Use*

Estazolam is a triazolobenzodiazepine derivative used for the short-term management of insomnia (see monograph on diazepam, pp. 39–41, for a brief overview of the pharmacology of therapeutic action for this class of drugs). The usual oral dose is 1–2 mg at night; for severe insomnia, up to 4 mg has been given. In debilitated elderly patients, an initial dose of 0.5 mg is recommended (Reynolds, 1993; Medical Economics, 1996).

Clinical uses of estazolam and other benzodiazepines have been reviewed (Hollister *et al.*, 1993).

Comparative data on sales of estazolam in several countries are shown in Table 1. Overall, sales increased by approximately 16% from 1990 to 1995.

1.3 Occurrence

Estazolam is not known to occur as a natural product.

1.4 Regulations and guidelines

Estazolam is listed in the French and Japanese pharmacopoeias (Reynolds, 1993; Vidal, 1995).

Table 1. Sales of estazolam in various countries[a] (no. of standard units[b], in thousands)

Country	1990	1995	Country	1990	1995
North America			Asia		
Canada	0	245	Japan	111 670	116 623
Mexico	1 195	1 446	Europe		
United States	0	19 372	France	15 213	9 617
South America			Italy	12 846	14 581
Argentina	837	599	Portugal	8 642	17 424
Brazil	7 958	7 253			
Colombia	0	76			

[a] Data provided by IMS

[b] Standard dosage units, uncorrected for estazolam content

2. Studies of Cancer in Humans

No data were available to the Working Group (see the monograph on diazepam, pp. 44–54, for a discussion of benzodiazepines).

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male and 50 female B6C3F1 mice, five to six weeks of age, were given 0.8, 3 or 10 mg/kg bw estazolam [purity not specified] mixed in the diet for up to 104 weeks, when surviving animals were killed. The estazolam concentration in the food was adjusted weekly for changes in body weight and food consumption. The estazolam/diet mixtures were prepared freshly each week. Controls were 100 male and 100 female B6C3F1 mice. Increased body weights were seen in the treated animals as compared to the controls. This was more prominent for the females. Food consumption was also increased in the treated mice, by 4–17% above control values over the two-year treatment period. Convulsions and hyperactivity were associated with exposure to estazolam. An increase in mortality was observed in male mice receiving 10 mg/kg bw (deaths — males: control, 13/100; low-dose, 11/50; mid-dose, 7/50; and high-dose, 21/50; females: control, 28/100; low-dose, 12/50; mid-dose, 12/50; and high-dose, 23/50). All major organs and visually apparent lesions were examined histologically. No increase in tumour incidence was found. The incidences of hepatocellular carcinomas were: males: control, 23/100; low-dose, 11/50; mid-dose, 12/50; and high-dose, 9/50;

females: control, 2/100; low-dose, 4/50; mid-dose, 4/50; and high-dose, 2/50 (Kimura *et al.*, 1984).

3.1.2 *Rat*

Groups of 50 male and 50 female Sprague-Dawley rats, five to six weeks of age, were given 0.5, 2 or 10 mg/kg bw estazolam [purity not specified] mixed in the diet for up to 104 weeks, when surviving animals were killed. The estazolam concentration in the food was adjusted weekly for changes in body weight and food consumption. The estazolam/-diet mixtures were prepared freshly each week. Controls were 100 male and 100 female Sprague-Dawley rats. No significant change in body weights was seen in the treated male rats as compared to the controls. Female rats exposed to 10 mg/kg bw estazolam had depressed body weights (approximately 13%) compared to controls. There was no significant difference in mortality between control and treated rats. All major organs and visually apparent lesions were examined histologically. There was no significant increase in tumour incidence. The incidences of neoplastic nodules in the liver were: males: control, 3/100; low-dose, 2/50; mid-dose, 1/50 and high-dose, 2/50; females: control, 5/100; low-dose, 0/50; mid-dose, 2/50 and high-dose, 1/50; those of hepatocellular carcinomas were: males: control, 4/100; low-dose, 0/50; mid-dose, 1/50 and high-dose, 1/50; females: control, 0/100; low-dose, 1/50; mid-dose, 1/50 and high-dose, 0/50 (Kimura *et al.*, 1984).

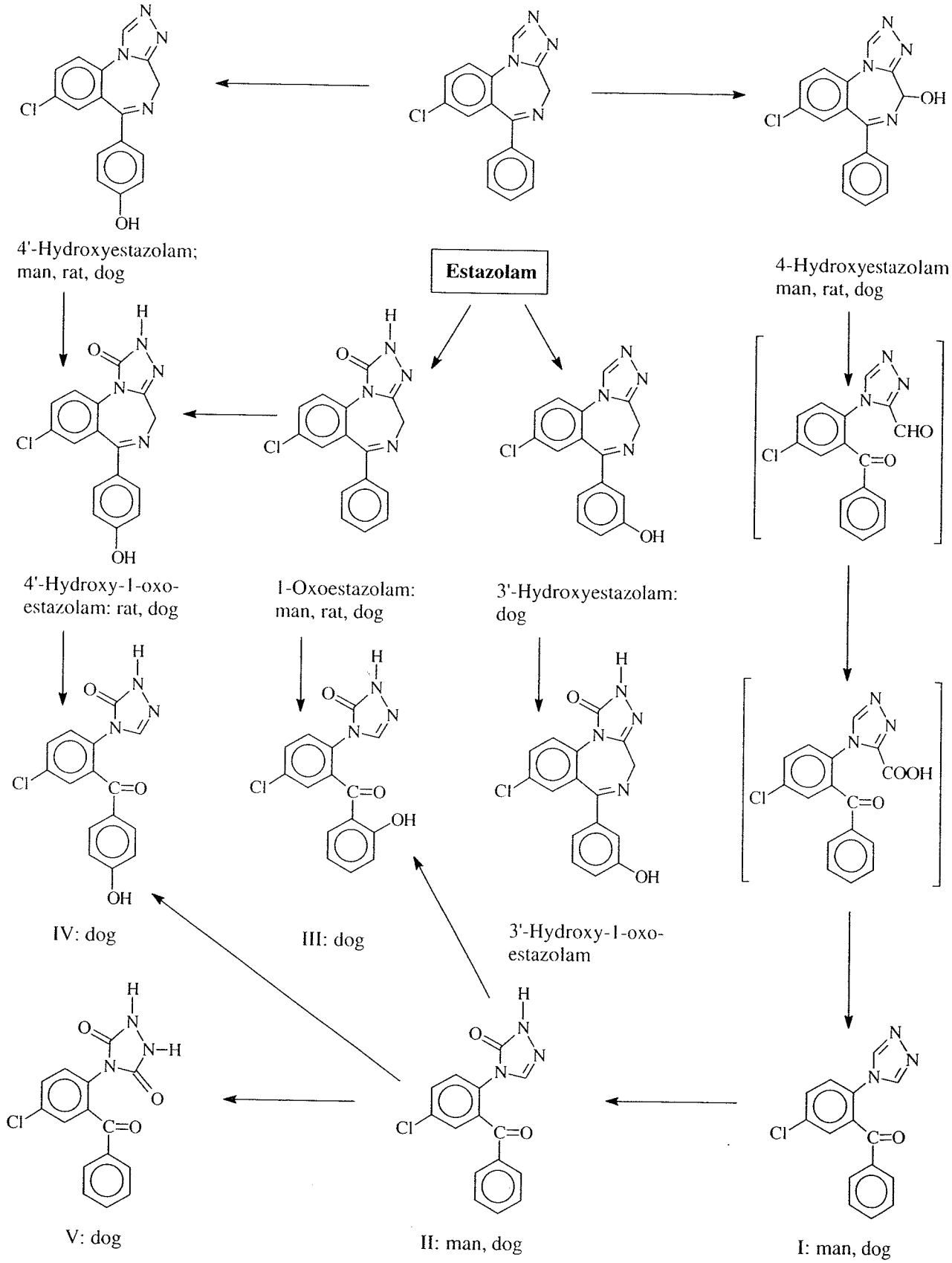
4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Estazolam is rapidly and almost completely absorbed after oral doses. Peak plasma concentrations of 103 ± 18 ng/mL were achieved about 0.5 h after oral dosing with 2 mg in aqueous solution (Machinist *et al.*, 1986), while 4-mg tablets gave peak plasma concentrations of 194 ± 3.5 ng/mL within 1–3 h (Mancinelli *et al.*, 1985). In an earlier study, single doses ranging from 2 to 16 mg resulted in peak plasma concentrations proportional with the dose within 6 h. Mean elimination half-lives were 14 h, 19 h and 17 h in these three studies, respectively. During three weeks of therapy with daily doses rising each week from 2 to 4 to 6 mg, plasma concentrations increased in proportion to the dose and accumulation was essentially complete within three days of each dose change. The drug is eliminated predominantly as metabolites in the urine (Allen *et al.*, 1979). Machinist *et al.* (1986) found that urinary and faecal excretion accounted for 87% and 4%, respectively, of a 2-mg [^{14}C]estazolam dose over five days. Kanai (1974) identified five metabolites in humans, 1-oxoestazolam, 4-hydroxyestazolam, 4′-hydroxy-estazolam and two benzophenones (I and II) (Figure 1). Machinist *et al.* (1986) found evidence of 11 metabolites in human urine, including those identified previously; the

Figure 1. Postulated metabolic pathways of estazolam



From Kanai (1974)

I, 5-Chloro-2(4*H*-1,2,4-triazol-4-yl)benzophenone; II, 5-Chloro-2-(2,3-dihydro-3-oxo-4*H*-1,2,4-triazol-4-yl)benzophenone; III, 5-Chloro-2-(2,3-dihydro-3-oxo-4*H*-1,2,4-triazol-4-yl)-2′-hydroxybenzophenone; IV, 5-Chloro-2-(2,3-dihydro-3-oxo-4*H*-1,2,4-triazol-4-yl)-4′-hydroxybenzophenone; V, 5-Chloro-2-(3,5-dioxo-2,3,4,5-tetrahydro-1*H*-1,2,4-triazol-4-yl)-benzophenone

major metabolite was not fully characterized but was believed to be a metabolite of 4-hydroxyestazolam.

4.1.2 *Experimental systems*

In mice given an intraperitoneal injection of 5 mg/kg bw estazolam, the plasma concentration was about 1300 ng/mL at 0.5 h, the earliest time investigated. The elimination half-life was 1.99 h, volume of distribution 3.12 L/kg and clearance 18.1 mL/min/kg (Kelly & Greenblatt, 1993).

After intravenous injection of 5 mg/kg bw into pregnant rats (Tanayama *et al.*, 1974), the fetal/maternal blood concentration ratio one hour after dosing was approximately unity and concentrations in fetal tissues declined in parallel with the concentrations in maternal blood. Autoradiography of the fetuses showed higher concentrations in the adrenal glands, adipose tissue, liver and gastrointestinal tract wall. The compound or its metabolites also enters rat milk.

A number of studies have compared the metabolism of estazolam in different species. Tanayama and Kanai (1974) observed extensive metabolism in mice, rats, guinea-pigs, rabbits and dogs following oral administration of ^{14}C-labelled drug. The percentages of dose recovered as metabolites in the urine plus faeces were: mice, 78%; rats, 51%; guinea-pigs, 44%; rabbits, 48%; and dogs, 78%. As in humans (see Section 4.1.1), rabbits and dogs excreted more radioactivity in the urine than in faeces; in contrast, mice, rats and guinea-pigs excreted more in the faeces. The patterns of metabolites differed between species (Figure 1), but some of the metabolites were identified mainly in rats (4′-hydroxy- and 1-oxo-4′-hydroxy-) and only in dogs (two benzophenones, I and II). Kanai (1974) presented more detailed findings on rats and dogs (as well as humans): 11 metabolites identified in dog urine included six hydroxylation products and five benzophenones. The benzophenones were not observed in rats. The findings of Kanai (1974) for dogs were largely confirmed by Machinist *et al.* (1986).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Oral LD_{50}s in male mice, rats and rabbits of 740, 3200 and 300 mg/kg bw have been reported (Budavari, 1995).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the working group.

4.3.2 *Experimental systems*

Estazolam crossed the rat placenta (Tanayama *et al.*, 1974) (see Section 4.1.2).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see also Table 2 for references and Appendices 1 and 2)

The results of the few available studies of induction of mutations in bacteria are negative. No increase in DNA single-strand breaks and/or alkali-labile sites was observed in the liver of rats receiving single or multiple doses of estazolam. In addition, estazolam does not induce chromosomal aberrations or aneuploidy in the bone-marrow cells of either rats or mice *in vivo*.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Estazolam is a triazolobenzodiazepine used since the 1970s for short-term management of insomnia.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Estazolam was tested for carcinogenicity in one experiment in mice and one experiment in rats by oral administration in the diet. No increase in the incidence of tumours was found.

5.4 Other relevant data

Estazolam is rapidly and almost completely absorbed in humans. It is extensively metabolized to at least 11 metabolites and excreted mainly in the urine. The elimination half-life is 14–19 h. Metabolism is extensive in various animal species. Rabbits and dogs excrete the metabolites principally in urine, while in mice, rats and guinea-pigs the excretion is mainly in faeces. Some metabolites are species-specific.

There were no data available on reproductive effects of estazolam.

The data available on genetic effects were negative.

Table 2. Genetic and related effects of estazolam

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2500	Wakisaka & Nishimoto (1987)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2500	Wakisaka & Nishimoto (1987)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2500	Wakisaka & Nishimoto (1987)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2500	Wakisaka & Nishimoto (1987)
SA2, *Salmonella typhimurium* TA102, reverse mutation	–	–	2500	Wakisaka & Nishimoto (1987)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	–	–	2500	Wakisaka & Nishimoto (1987)
DVA, DNA strand breaks, rat liver *in vivo*	–		285 po × 1	Carlo *et al.* (1989)
DVA, DNA strand breaks, rat liver *in vivo*	–		59 po × 15	Carlo *et al.* (1989)
CBA, Chromosomal aberrations, rat (Sprague-Dawley) bone marrow *in vivo*	–		100 po × 1	Kikuchi *et al.* (1973)
CBA, Chromosomal aberrations, rat (Sprague-Dawley) bone marrow *in vivo*	–		100 po × 5	Kikuchi *et al.* (1973)
CBA, Chromosomal aberrations, mouse (CF1) bone marrow *in vivo*	–		200 po × 1	Kikuchi *et al.* (1973)
CBA, Chromosomal aberrations, mouse (CF1) bone marrow *in vivo*	–		200 po × 1	Kikuchi *et al.* (1973)
AVA, Aneuploidy, rat (Sprague-Dawley) bone marrow *in vivo*	–		100 po × 1	Kikuchi *et al.* (1973)
AVA, Aneuploidy, rat (Sprague-Dawley) bone marrow *in vivo*	–		100 po × 5	Kikuchi *et al.* (1973)
AVA, Aneuploidy, mouse (CF1) bone marrow *in vivo*	–		200 po × 1	Kikuchi *et al.* (1973)
AVA, Aneuploidy, mouse (CF1) bone marrow *in vivo*	–		200 po × 5	Kikuchi *et al.* (1973)

[a] +, positive; (+), weak positive; –, negative; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day

5.5 Evaluation[1]

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

There is *evidence suggesting a lack of carcinogenicity* in experimental animals for estazolam.

Overall evaluation

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

6. References

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

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