DOXEFAZEPAM

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

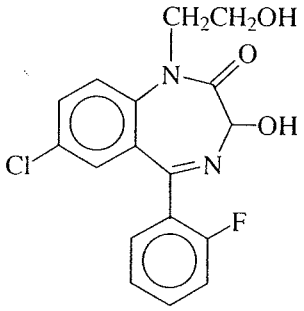
Chem. Abstr. Serv. Reg. No.: 40762-15-0

Chem. Abstr. Name: 7-Chloro-5-(2-fluorophenyl)-1,3-dihydro-3-hydroxy-1-(2-hydroxyethyl)-2*H*-1,4-benzodiazepin-2-one

IUPAC Systematic Name: 7-Chloro-5-(*ortho*-fluorophenyl)-1,3-dihydro-3-hydroxy-1-(2-hydroxyethyl)-2*H*-1,4-benzodiazepin-2-one

Synonym: *N*-1-Hydroxyethyl-3-hydroxyflurazepam

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{17}H_{14}ClFN_2O_3$ Relative molecular mass: 348.76

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Crystals (Budavari, 1995)

(*b*) *Melting-point*: 138–140 °C (Budavari, 1995)

(*c*) *Spectroscopy data*: Infrared, ultraviolet, nuclear magnetic resonance and mass spectral data have been determined (Schiapparelli Farmaceutici S.P.A., 1983).

(*d*) *Solubility*: Practically insoluble in water; soluble in acetone and chloroform; moderately soluble in ethanol; slightly soluble in diethyl ether (Schiapparelli Farmaceutici S.P.A., 1983)

1.1.4 *Technical products and impurities*

There are two enantiomeric forms of the doxefazepam structure (asymmetric centre at C_3); doxefazepam in pharmaceutical preparations was the racemic mixture (Schiapparelli Farmaceutici S.P.A., 1983).

Doxefazepam was available as 20-mg capsules, which also contained magnesium stearate, mannitol, microgranular cellulose, precipitated silica, starch, and colourants E 127, E 132, E 171 and E 172. Impurities included 7-chloro-3-hydroxy-1,3-dihydro-5-(2-fluorophenyl)-2*H*-1,4-benzodiazepin-2-one (≤ 0.5%), 1-(2-hydroxyethyl)-7-chloro-1,3-dihydro-5-(2-fluorophenyl)-2*H*-1,4-benzodiazepin-2-one-4-oxide (≤ 0.5%) and 1-(2-hydroxyethyl)-7-chloro-4,5-dihydro-5-(2-fluorophenyl)-2*H*-1,4-benzodiazepin-2,3-(1*H*)-dione (≤ 0.2%) (Schiapparelli Farmaceutici S.P.A., 1983).

A trade name and a designation for the chemical and its pharmaceutical preparations were available: Doxans and SAS 643.

1.1.5 *Analysis*

Doxefazepam can be analysed in biological fluids and tissues by gas chromatography with electron capture detection (Marcucci *et al.*, 1980; Mardente *et al.*, 1981) and reverse-phase high-performance liquid chromatography (Mascher *et al.*, 1984; Carlucci, 1988).

1.2 Production and use

1.2.1 *Production*

Doxefazepam can be prepared by alkylation of 7-chloro-1,3-dihydro-5-(2-fluorophenyl)-2*H*-1,4-benzodiazepin-2-one-4-oxide with 2-bromoethyl acetate and sodium hydride in dimethylformamide. The product is treated with acetic anhydride to form a diester. Ammonolysis of this diester, using methanolic ammonia, yields crude doxefazepam, which can be purified by crystallizing from dichloromethane/light petroleum (Tamagnone *et al.*, 1974).

1.2.2 *Use*

Doxefazepam is a benzodiazepine hypnotic, that has been used in the short-term management of insomnia at an oral dose of 20 mg before retiring at night (Reynolds, 1993) (see monograph on diazepam, pp. 39–41, for a brief overview of the pharmacology of therapeutic action for this class of drugs).

In 1990, approximately 184 000 standard units of doxefazepam (uncorrected for content of doxefazepam) were sold in Italy, the only country in which the drug was available. By 1995, it was no longer being sold in any country (information provided by IMS).

1.3 Occurrence

Doxefazepam is not known to occur as a natural product.

Doxefazepam is an active metabolite of flurazepam (Borelli *et al.*, 1990).

1.4 Regulations and guidelines

Doxefazepam was approved for use in Italy from the mid-1980s until 1995 (Searle Farmaceutici S.r.l., 1996).

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

Rat: Groups of 50 male and 50 female Sprague-Dawley rats, six weeks of age, were given 0 (control), 3, 10 or 30 mg/kg bw doxefazepam [purity not specified] mixed in the diet for up to 104 weeks, when surviving males and females of all groups were killed. The highest dose was set at 60 times the mean daily hypnotic dose level for an adult man. Body-weight gains and mortality rates were similar in all groups. From graphic presentations, survival appeared to be greater than 60% for all groups of males and 40–50% for the females. Complete histological examinations were performed on 47–50 males per group and 49–50 females per group. The incidences of hepatocellular adenomas in females were 1/49, 0/50, 3/49 and 5/50 [$p = 0.011$, trend test] and those for hepatocellular carcinomas were 0/49, 1/50, 1/49 and 1/50 in control, low-dose, mid-dose and high-dose animals, respectively. When the numbers of liver adenoma-bearing female rats were considered in relation to the numbers of females alive at the week of first tumour appearance (control, 1/18; low-dose, 0/17; mid-dose, 3/18; high-dose, 5/18), a significant trend by the Cochran-Armitage and Peto incidental tumour test ($p < 0.01$) was seen. In the male rats, the incidences of hepatocellular adenomas were 0/48, 0/50, 1/47 and 3/50 and those for hepatocellular carcinomas were 1/48, 3/50, 3/47 and 2/50 in control, low-dose, mid-dose and high-dose animals, respectively, showing no statistically significant increase. [When adenomas and carcinomas in male rats were combined, with the assumption that adenomas and carcinomas occurred in different animals, the cumulative incidence of tumours was not significantly increased (control, 1/48; low-dose, 3/50; mid-dose, 4/47; and high-dose, 5/50 ($p = 0.09$, trend test)] (Borelli *et al.*, 1990).

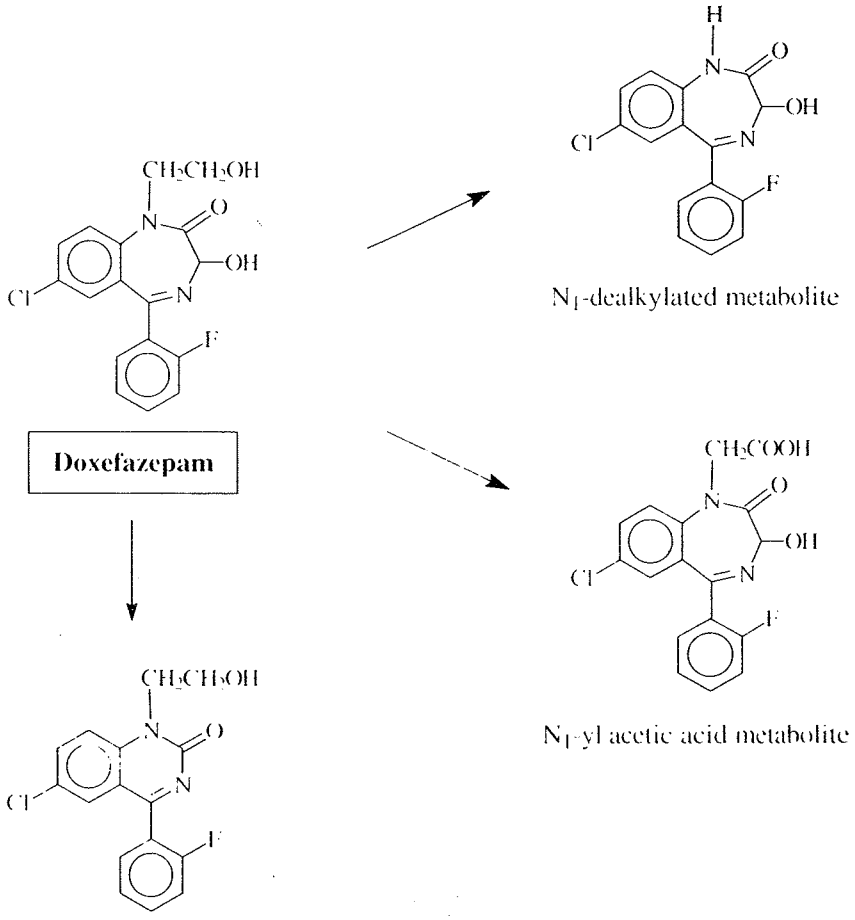
4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

The disposition of doxefazepam has received very little study. Mardente *et al.* (1981) reported mean plasma concentration–time profiles of total and non-conjugated doxefazepam in eight individuals given both 10-mg and 20-mg single oral doses. No pharmacokinetic data were presented, but concentrations had declined to about 10% of the peak values after 16 h [suggesting a half-life of around 3–4 h]. Means of 32% of the 10-mg dose and 50% of the 20-mg dose were recovered in urine as conjugated doxefazepam within 48 h. The N_1-dealkylated derivative and an oxidized derivative in which the N_1-substituent was $-CH_2COOH$ were identified as urinary metabolites (see Figure 1).

Figure 1. Metabolism of doxefazepam



Based upon Mardente *et al.* (1981)

4.1.2 *Experimental systems*

Very few data are available. One report of an analytical method (Marcucci *et al.*, 1980) included data showing that doxefazepam is rapidly absorbed and accumulates in adipose tissue after a relatively high oral dose (5 mg/kg) administered to rats. Brain levels were approximately double the plasma concentrations. After an intravenous dose of 5 mg/kg, the elimination half-lives in rats and mice were 0.29 h and 1.32 h respectively in the blood.

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

(*a*) *Acute toxicity*

Doxefazepam was generally well tolerated after oral and intraperitoneal administration to rodents and beagle dogs (Bertoli *et al.*, 1989). No difference between males and females in the response to the compound was apparent. The oral LD_{50} was > 2000 mg/kg bw in Swiss mice, Charles River rats and beagle dogs. The intraperitoneal LD_{50} was estimated to be 746, 544 and > 1000 mg/kg bw in mice, rats and dogs, respectively. Deaths and/or signs of toxicity, consisting mainly of dose-dependent dyspnoea and decreased motor activity (in all species), dose-dependent prostration (in rodents) and dose-dependent tachycardia (only in dogs), occurred within 72 h after treatment.

(*b*) *Subacute and chronic toxicity*

Male and female Sprague-Dawley rats were given doxefazepam by gastric instillation (0, 50 or 100 mg/kg bw per day for eight weeks or 0, 15, 30 or 60 mg/kg bw per day for 26 weeks) and male and female beagle dogs were similarly treated with 0 or 10 mg/kg bw per day for 26 weeks (Bertoli *et al.*, 1989). The only symptom observed in rats for several hours after administration was ataxia, which was dose-dependent in the eight-week study and occurred only at the highest dose in the 26-week study. Liver weights were increased in rats given the highest dose in the 26-week study. There was no other clinical, haematological or histopathological sign of toxicity in either rats or dogs.

4.3 Reproductive and prenatal effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Doxefazepam did not exert any teratogenic effect in offspring of Sprague-Dawley rats treated orally with 15 or 30 mg/kg bw at gestation days 6–16 or in those of New Zealand White rabbits treated orally with 10, 20 or 30 mg/kg bw at gestation days 6–18. More-

over, it did not alter the reproductive performance of Charles-River rats treated orally with 15, 30 or 45 mg/kg bw (Bertoli *et al.*, 1989).

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 1 for references and Appendices 1 and 2)

In one study, no significant response was observed in tests for mutation in *Salmonella typhimurium*, gene conversion in *Saccharomyces cerevisiae*, aneuploidy in *Aspergillus nidulans* or micronucleus induction in mouse bone-marrow cells *in vivo*. In another study, no increase in DNA strand breaks and/or alkali-labile sites was observed in the liver of rats given single or multiple oral doses of doxefazepam.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Doxefazepam is a benzodiazepine hypnotic that was used in the past to a limited extent in the short-term management of insomnia.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Doxefazepam was tested for carcinogenicity in one experiment in rats by oral administration in the diet. A slight dose-related increase in the incidence of hepatocellular adenomas was observed.

5.4 Other relevant data

Doxefazepam disposition has received little study. In humans, the drug was eliminated in urine mainly as a conjugate, and two oxidative metabolites were identified. The elimination half-life was 3–4 h. No satisfactory metabolism studies in animals were available.

Data on human toxicity were not available. In rats treated with 60 mg/kg bw per day for 26 weeks, increased liver weights were reported without other clinical, haematological or histopathological signs of toxicity.

In a single study, doxefazepam was not teratogenic in rats or rabbits.

The few data available on genetic effects were negative.

Table 1. Genetic and related effects of doxefazepam

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Bertoli *et al.* (1989)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	500	Bertoli *et al.* (1989)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	500	Bertoli *et al.* (1989)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Bertoli *et al.* (1989)
SCG, *Saccharomyces cerevisiae*, gene conversion	–	–	10	Bertoli *et al.* (1989)
ANG, *Aspergillus nidulans*, mitotic crossing-over	–	–	8000	Bertoli *et al.* (1989)
DVA, DNA strand breaks, rat liver *in vivo*	–		349 po × 1	Carlo *et al.* (1989)
DVA, DNA strand breaks, rat liver *in vivo*	–		70 po × 15	Carlo *et al.* (1989)
MVR, Micronucleus test, mouse bone marrow *in vivo*	–		155	Bertoli *et al.* (1989)

[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 doxefazepam.

There is *limited evidence* in experimental animals for the carcinogenicity of doxefazepam.

Overall evaluation

Doxefazepam 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.