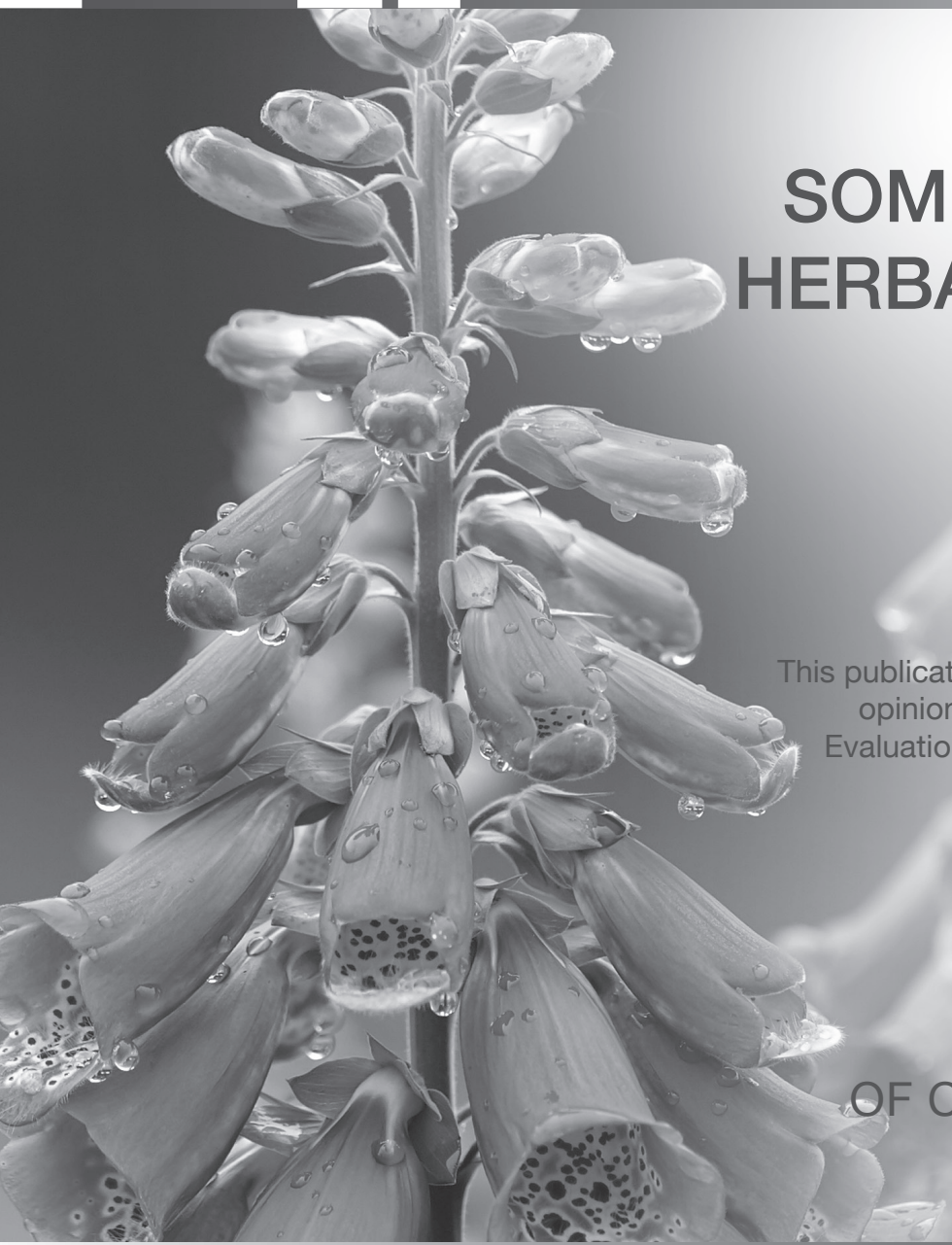
IARC MONOGRAPHS



SOME DRUGS AND HERBAL PRODUCTS

VOLUME 108

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 4–11 June 2013

Lyon, France - 2016

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

PRIMIDONE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 125-33-7 (O'Neil, 2006)

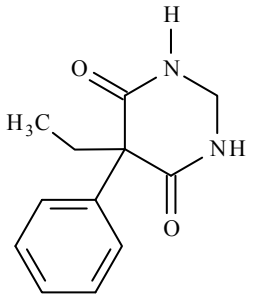
Chem. Abstr. Serv. Name: 4,6(1*H*,5*H*)-Pyrimidinedione, 5-ethyldihydro-5-phenyl- (O'Neil, 2006; US Pharmacopeia, 2007)

IUPAC Systematic Name: 5-Ethyl-5-phenyl-1,3-diazinane-4,6-dione (DrugBank, 2013)

Synonym: 2-Deoxyphenobarbital

See WHO (2007) for names in other languages.

1.1.2 Structural and molecular formulae and relative molecular mass



$C_{12}H_{14}N_2O_2$
Relative molecular mass: 218.25

1.1.3 Chemical and physical properties of the pure substance

Description: White or almost white, crystalline powder (European Pharmacopoeia, 2008); white crystalline powder, odourless, very slight bitter taste, with no acidic properties (Japanese Pharmacopoeia, 2007; US Pharmacopeia, 2009)

Density: 1.138 ± 0.06 g/cm^3 (predicted) (SciFinder, 2013)

Melting-point: 279–284 °C (Japanese Pharmacopoeia, 2007; US Pharmacopeia, 2007); 281–282 °C (O'Neil, 2006)

Spectroscopy Data: Data from infrared spectroscopy have been reported (Daley, 1973)

Solubility: Very slightly soluble in water, slightly soluble in ethanol (96%). It dissolves in alkaline solution (O'Neil, 2006; European Pharmacopoeia, 2008; US Pharmacopeia, 2009); soluble to 100 mM in dimethylsulfoxide (Tocris, 2013); soluble in dimethylformamide, sparingly soluble in pyridine, and practically insoluble in diethyl ether (Japanese Pharmacopoeia, 2007)

Stability data: Stable; finished product has shelf-life of 5 years (US Pharmacopeia, 2009; MHRA, 2013)

Octanol/water partition coefficient (log P): 0.91 (US Pharmacopeia, 2009)

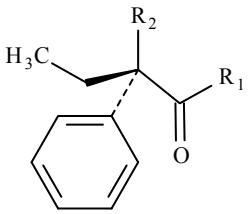
1.1.4 Technical products and impurities

(a) Trade names

Mysoline; Cyral; Liskantin; Majsolin; Midone; Mylepsinum; Mysedon; Primoline; Primron; Prysoline; Resimatil; Sertan (NTP, 2000; O'Neil, 2006)

(b) Specified impurities and enantiomer

Several impurities have been detected in the technical product (European Pharmacopoeia, 2008), including:



- R1 = NH_2, R2 = CO-NH_2: 2-ethyl-2-phenyl-propanediamide (ethylphenylmalonamide)
- R1 = NH_2, R2 = H: (2*RS*)-2-phenylbutanamide
- R1 = NH_2, R2 = CN: (2*RS*)-2-cyano-2-phenylbutanamide
- R1 = OH, R2 = H: (2*RS*)-2-phenylbutanoic acid
- Phenobarbital
- 5-Ethyl-5-phenyl-2-[(1*RS*)-1-phenylpropyl] dihydropyrimidine-4,6(1*H*,5*H*)-dione

1.2 Analysis

Selected compendial and noncompendial methods are presented in Table 1.1. Primidone can be quantitatively determined using ultraviolet spectroscopy, liquid chromatography using ultraviolet detection, and gas chromatography using flame ionization detection.

Primidone can be analysed in human plasma by extraction followed by protein precipitation, centrifugation and finally subjecting to ultra-performance liquid chromatography with electrospray ionization mass spectrometry, with a limit of detection of < 0.05 mg/mL (Kuhn & Knabbe, 2013).

The physical properties of the substance (spectroscopy, melting point) are used for the identification of the substance.

1.3 Production and use

1.3.1 Production and consumption volume

The synthetic dug primidone is not used frequently, with around 250 000 uses in the USA per year in 2005–2012 mentioned by office-based physicians in visits with patients. Based on the same source, approximately 80 000 patients in the USA were exposed to primidone in 2012 (IMS Health, 2012a). According to the National Prescription Audit Plus (IMS Health, 2012b), there were a total of 1.5 million prescriptions for primidone dispensed in the USA in 2012, similar to the 1.4 million prescriptions dispensed in 2008. [The Working Group recognized that these prescription figures were larger than expected based on the drug uses reported by office-based physicians.]

Total worldwide sales of primidone in 2012 were US$ 41 million (IMS Health, 2012c), with 60% occurring in the USA. The only other country with appreciable use was Germany, with sales of US$ 3 million.

1.3.2 Use

(a) Indications

Primidone is an anticonvulsant that metabolizes to phenobarbital and phenylethylmalonamide. All three compounds are thought to be biologically active. Primidone is used in the treatment of a range of conditions, including seizure disorders, tremor, neuropathic pain, trigeminal neuralgia, tinnitus, and migraine headache. Its use for seizure disorders has declined substantially with a shift to newer medications with

Table 1.1 Analytical methods for primidone

Sample matrix	Sample preparation	Assay method	Detection limit	Reference
Compendial methods				
Assay	–	UV-visible Wavelength: 257 nm	–	European Pharmacopoeia (2008)
Assay	–	UV-visible Wavelength: minima 254 nm, 261 nm, and maxima 257 nm	–	US Pharmacopeia (2013)
Assay (tablet and suspension)	–	GC Column: glass column packed with acid-washed, silanized diatomaceous support coated with phenyl methyl silicone fluid	–	British Pharmacopoeia (2013)
Assay (tablet and suspension)	–	GC Column: 10% liquid phase G3 on support S1AB Flow rate: 40 mL/min	–	US Pharmacopeia (2013)
Non-compendial methods				
Human plasma or serum	Protein precipitation, vortex-mixing, centrifugation, analysis of clear organic supernatant	UPLC-ESI-MS-MS Column: C_{18} Mobile phase: solvent A, 0.1% formic acid in water containing 2 mmol/L ammonium acetate; and solvent B, 0.1% formic acid in methanol containing 2 mmol/L ammonium acetate Flow rate: 0.5 mL/min MRM: 219.0 *m/z* reducing to 162.0 *m/z*	< 0.05 mg/L	Kuhn & Knabbe (2013)
Human serum	Centrifugation, supernatant injected onto the anti-primidone column, washing with methanol and water, elution with methanol and acetic acid, evaporation, sonication	MIP-ESI-MS Needle voltage: 11.40 kV Target electrode voltage: 9.00 kV Liquid flow rate: 6 µL/min Drift field: 600 V/cm Desolvation field: 600 V/cm Drift gas flow (N_2): 500 mL/min Desolvation gas flow (N_2): 900 mL/min Drift tube length: 11 cm Shutter grid pulse: 0.3 ms	0.0051 µg/mL	Rezaei *et al.* (2009)
Human plasma	Extraction by a liquid-liquid extraction system, vortex mixing and centrifugation, organic layer evaporated, reconstituted with methanol in water for injection on to the MECC system	MECC Capillary: fused-silica Wavelength: 210 and 285 nm Buffer: 10 mM monobasic sodium phosphate, with 6 mM tetraborate, and 75 mM SDS pH 9.0	0.7 µg/mL	Lançaas *et al.* (2003)

Table 1.1 (continued)

Sample matrix	Sample preparation	Assay method	Detection limit	Reference
Tablet	Nitration of primidone with sulfuric-nitric acid mixture to form 3-nitrophenyl derivative	Polarography Electrode: dropping mercury electrode	Observed half-wave potential was −0.17 V vs the saturated calomel electrode	Daley (1973)
Human serum	Acidified and extracted with $CHCl_3$ and isopropanol (70 : 30)	LC-UV Column: C_{18} Mobile phase: acetonitrile and water (12 : 88)	50–1000 ng/mL (LOQ)	Sato *et al.* (1986)
Tablet	Dissolved in DMSO-d_6 using maleic acid as internal standard	Proton NMR	Chemical shift value: primidone, 7.53 ppm; and maleic acid, 6.50 ppm	Özden *et al.* (1989)
Serum or plasma	Serum or plasma + anticoagulants, centrifugation	Immunoassay The enzyme activity is determined spectrophotometrically at 340 nm	0.5 µg/mL	Thermoscientific (2004)
Rat urine	Extraction by LRC column	LC-UV Column: C_{18} Mobile phase: 0.01 M potassium phosphate buffer, methanol and acetonitrile (270 : 30 : 30) pH: 4.0 Flow rate: 1.0 mL/min Wavelength: 227 nm	0.5 mg/mL	Ferranti *et al.* (1998)
Rat plasma	Solid phase extraction Bond-Elut C-18 cartridge column	LC-UV Column: C_{18} Mobile phase: acetonitrile and 0.01 M KH_2PO_4 (25 : 75) Flow rate: 0.8 mL/min Wavelength: 210 nm	0.1 µg/mL	Moriyama *et al.* (1994)

DMSO, dimethylsulfoxide; GC, gas chromatography; LC, liquid chromatography; LOQ, limit of quantitation; LRC, large reserve capacity; MECC, micellar electrokinetic capillary chromatography; MIP-ESI-IMS, molecular imprinted polymer electrospray ionization ion mobility spectrometry; MRM, multiple reaction monitoring; NMR, nuclear magnetic resonance; SDS, sodium dodecyl sulfate; UPLC-ESI-MS-MS, ultra-performance liquid chromatography with electrospray ionization and tandem mass spectrometry; UV, ultraviolet spectroscopy; vs, versus

Table 1.2 Most commonly reported clinical indications for primidone in the USA, 2011–2012

Diagnosis	ICD-9 code[a]	Drug uses (in thousands)	Percentage of total
Benign essential tremor	333.101	263	52.5
Tremor, NOS	781.005	139	27.8
Convulsion, NOS	780.301	39	7.8
Familial tremor	333.102	14	2.8
Parkinson disease	332.005	9	1.7
Migraine, NOS	346.903	7	1.5
All other diagnoses	–	28	5.8
Total with reported diagnoses	–	502	100.0

[a] The ICD-9 code listed is a more detailed, proprietary version developed by IMS Health
NOS, not otherwise specified
From IMS Health (2012a)

fewer adverse effects, fewer drug interactions, and less potential for addiction and abuse. Once a key medication in the management of seizure disorders, primidone is now considered at best a third-line medication for partial and tonic-clonic seizures (MicroMedex, 2013).

In the USA, primidone is currently labelled for use as a treatment for epilepsy in children and adults, either alone or as an adjunct to other anticonvulsants (FDA, 2013; MicroMedex, 2013). Most prescriptions in the USA are for off-label indications (Table 1.2).

Primidone is used relatively infrequently as anticonvulsant, accounting for 0.4% of all medications reported as therapies for seizure disorders (whether alone or in combination with other agents) (IMS Health, 2012a). There are numerous other anticonvulsants with overlapping clinical indications that have largely replaced primidone, even in cases of non-responsiveness to multiple medications. In contrast, there are few comparatively effective treatments for essential tremor (Zesiewicz *et al.*, 2011). As a result, primidone comprises the largest fraction (35%) of all medications reported as therapies for essential tremor (IMS Health, 2012a).

In the European Union, primidone is indicated for essential tremor, and in the management of grand mal and psychomotor (temporal lobe) epilepsy (eMC, 2013).

[Given its use for chronic conditions, primidone therapy would be expected to be long-term in the absence of short- or long-term adverse effects.]

(b) Dosage

Primidone is available in tablets of 50 mg and 250 mg, with a tablet of 125 mg and an oral suspension formulation being available in some countries (MicroMedex, 2013; eMC, 2013). Therapy is initiated at lower doses and then increased, although lower doses may be taken when primidone is employed as an adjunct (MicroMedex, 2013). There is a wide range of dosing regimens, varying from 50 mg once per day to 500 mg twice per day; 50 mg once or twice daily are the most common regimens, each representing 21% of all uses. The mean daily dosage for primidone is 183 mg per day (IMS Health, 2012a).

1.4 Occurrence and exposure

Primidone has been reported in groundwater, spring water and well-water (Morasch, 2013). Primidone, and its metabolite phenobarbital, were detected in groundwater within the catchment area of a drinking-water treatment plant located downstream of a former sewage farm in Berlin, Germany. The age of shallow groundwater samples ranged from years to a decade,

whereas the age of groundwater was up to four decades. Concentrations of the compounds in groundwater increased with age. This indicated a strong persistence of these compounds in the environment under anoxic aquifer conditions (Hass *et al.*, 2012).

Human exposure is largely limited to use as a medication. Workers in pharmaceutical manufacturing plants may be exposed, but no specific data were available to the Working Group.

1.5 Regulations and guidelines

Primidone has been widely approved by drug regulatory agencies. Primidone was approved by the United States Food and Drug Administration in 1954 (FDA, 2013).

There were no extraordinary regulatory restrictions on use. Primidone was listed in 1999 as a "chemical known to the State to cause cancer" by the Office of Environmental Health Hazard Assessment of the State of California, requiring public notice of potential environmental exposures (OEHHA, 2013). The basis of this listing was an evaluation by the United States National Toxicology Program (NTP, 2000).

2. Cancer in Humans

Primidone has been used to treat grand seizures in epilepsy patients. Elevated risks of several types of cancers, mainly tumours of the brain and central nervous system, lymphoma, myeloma, and cancers of the lung, liver, pancreas, and gastrointestinal tract have been seen in some but not all studies of epilepsy patients, suggesting that epilepsy and long-term use of anti-epileptic drugs may be risk factors for cancer (Lamminpää *et al.*, 2002; Olsen *et al.*, 1989). The evaluation of causality was complicated because epileptic seizures can be early symptoms of tumours of the brain, or can prompt clinical examinations, thus the observed associations between anti-epileptic drugs and cancer may be attributable to detection bias (Adelöw *et al.*, 2006).

Few studies have conducted analysis specific for individual anti-epileptic drugs such as primidone. The epidemiological studies available for evaluating exposure to primidone were limited to two case–control studies nested in a cohort of epileptic patients conducted by Olsen and colleagues in Denmark (Olsen *et al.*, 1993, 1995). The cohort study (Olsen *et al.*, 1989) provided information on the source population for the case–control studies; several anti-epileptic drugs were used in this cohort. A cohort study of offspring of mothers from the Danish cohort, which provided limited information on exposure to primidone, is also briefly discussed (Olsen *et al.*, 1990).

2.1 Cohort studies

A cohort study of patients at the Filadelfia epilepsy treatment community, in Dianalund, Denmark, was the only cohort study to report on incidence of cancer after treatment with primidone (Olsen *et al.*, 1989). The cohort consisted of 8004 patients admitted between 1933 and 1962, who had not died before 1943, and who had hospital stays of 4 weeks or greater and traceable records. Patients were treated primarily with phenobarbital, phenytoin, and primidone (500–1500 mg per day starting in mid-1950). Newer drugs became more common in the 1960s. The cohort was followed for cancer incidence until 1984, with cases identified by linkage to the Danish cancer registry. In the analysis, hospitalization was used as a proxy for drug use, and analyses were not conducted for anti-epileptic drugs, either specifically or as a class. Standardized incidence rates were adjusted for age, sex, and calendar year. Among patients who were not known to have received Thorotrast (a radioactive compound used as a contrast medium for radiology),

statistically significant excesses were observed in the incidence of all malignant neoplasms, and cancers of the brain and central nervous system, lung, and secondary and unspecified sites (combined). Non-statistically significant elevations (≥ 20%) were found for non-Hodgkin lymphoma, and cancers of the buccal cavity and pharynx, oesophagus, larynx, liver, biliary tract, thyroid, testes, and unspecified sites. The risk of cancer of the liver or biliary tract increased with increasing time since first admission, while no clear pattern was observed for cancer of the lung. Findings for malignant lymphoma, and cancers of the liver and biliary tract, urinary bladder, and lung were explored in subsequent nested case–control studies. A statistically significant decrease in incidence was observed for cancer of the urinary bladder. [The strengths of this study were adequate follow-up and case ascertainment. The study population consisted mainly of severe cases of epilepsy and thus it was not known whether severity of disease modified the risk of cancer. The major limitation was the lack of information on exposure to specific drugs and potential confounders at an individual level.]

Olsen *et al.* (1990) also conducted a record-linkage study among 3727 offspring of women from the Filadelfia cohort who were alive as of 1968. No increased risk of any malignant cancer was found among 2579 children born after the mother's first hospital admission and presumably exposed to anti-epileptic drugs in utero (relative risk, RR, 1.0; 95% CI, 0.6–1.7). Mothers of 2 of the 14 children with cancer had taken primidone and phenytoin during pregnancy. [Although the size of the cohort was relatively large and case ascertainment and follow-up were adequate, this study was not considered to be informative because the findings were not reported specifically for primidone, and few cancers were observed in the cohort.]

2.2 Nested case–control studies

See Table 2.1

The nested case–control studies on four types of cancer were reported in two publications: cancer of the lung and urinary bladder were reported by Olsen *et al.* (1993), and malignant lymphoma and cancer of the liver and biliary tract were reported by Olsen *et al.* (1995). The studies had similar methodologies and designs. Cancer cases identified in follow-up until 1984 were matched with two controls each from the cohort by sex, birth year, and survival time. Detailed drug information was extracted from medical records: between 23% and 27% of recorded prescriptions were for primidone, but 25% of patients had no records of prescriptions for any anticonvulsive drugs. Smoking information was surveyed among living controls, but not among cases.

Among patients who had ever used primidone, non-statistically elevated relative risks were observed for malignant lymphoma (odds ratio, OR, 1.3; Olsen *et al.*, 1995) and cancers of the lung (OR, 1.3; 95% CI, 0.7–2.3) and urinary bladder (OR, 1.6; 95% CI, 0.4–6.3) (Olsen *et al.*, 1993). The relative risk was close to unity for use of primadone and cancer of the liver and biliary tract (Olsen *et al.*, 1995). Patients exposed to Thorotrast were excluded from the reported analyses of lymphoma and cancer of the liver and biliary tract, while analyses of cancers of the lung and bladder reportedly gave similar results when repeated excluding Thorotrast-exposed patients. [The strengths of these studies were the same as those of the cohort studies. Limitations included incomplete information on exposure to primidone (with respect to duration of use; drug exposure information was collected only during the patient's stay in hospital) and on potential confounders, and small numbers of exposed cases, especially for cancers of the urinary bladder, lymphoma, and liver and biliary tract.]

Table 2.1 Nested case–control studies of cancer and exposure to primidone

Reference Study location, period	Total No. cases Total No. controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Olsen *et al.* (1993) Denmark, 1932–84	104 cases 200 controls	Nested case–control; cohort of 8004 patients with epilepsy	Medical records from epilepsy centre; smoking information (living controls only) collected via mail survey	Lung	Ever-exposed	29	1.3 (0.7–2.3)	Adjusted for other anticonvulsant treatments
	18 cases 33 controls			Urinary bladder	Ever-exposed	5	1.6 (0.4–6.3)	Controls matched to cases on sex, yr of birth and survival time; analyses excluding patients given Thorotrast were also conducted; cohort smoked more than the general population
Olsen *et al.* (1995) Denmark, 1932–84	39 cases 73 controls	Nested case–control; cohort of 8004 patients with epilepsy	Medical records from epilepsy centre	Liver and biliary tract	Ever-exposed (> 10 g, 40 tablets)	NR	0.9 (0.4–2.3)	Adjusted for other anticonvulsant treatments
	21 cases 98 controls			Malignant lymphoma [non-Hodgkin lymphoma and Hodgkin lymphoma]		NR	1.3 (0.3–5.0)	Controls matched to cases on sex, year of birth and survival time; analyses excluding patients given Thorotrast were also conducted; cohort smoked more than the general population

NR, not reported; yr, year

Table 3.1 Studies of carcinogenicity in mice and rats given diets containing primidone

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M, F) 104–105 wk NTP (2000)	Dietary concentrations of 0, 300, 600, or 1300 ppm, equivalent to daily doses of 0, 30, 65, or 150 mg/kg bw (M), or 0, 25, 50, or 100 mg/kg bw (F) 50 M and 50 F/group (age, 5–6 wk)	Hepatocellular adenoma: 22/50*, 41/50**, 39/50**, 32/50*** (M) 15/50****, 42/50**, 45/49**, 47/50** (F) Hepatocellular carcinoma: 12/50****, 31/50**, 35/50**, 38/50** (M) 3/50****, 11/50***, 19/49**, 38/50** (F) Hepatoblastoma: 0/50, 17/50**, 26/50**. 7/50***(M) 1/50, 4/50, 4/49, 4/50 (F) Hepatocellular adenoma, hepatocellular carcinoma or hepatoblastoma (combined): 31/50****, 49/50**, 49/50**, 46/50** (M) 16/50****, 42/50**, 46/49**, 50/50** (F) Thyroid follicular cell adenoma: 0/49*, 3/48, 3/50, 6/50*** (M)	*$P \leq 0.05$ (trend) **$P \leq 0.001$ ***$P \leq 0.05$ ****$P \leq 0.001$ (trend)	Purity, > 99%
Rat, F344/N (M, F) 104 wk NTP (2000)	Dietary concentrations of 0, 600, 1300, or 2500 ppm, equivalent to daily doses of 0, 25, 50, or 100 mg/kg bw 50 M and 50 F/group (age, 6 wk)	Thyroid follicular cell adenoma: 1/50, 1/50, 6/49*, 3/49 (M) Renal tubule adenoma or carcinoma (standard and extended evaluations combined): 4/50**, 2/50, 4/50, 7/50*** (M)	*$P = 0.047$ **$P = 0.025$ (trend) ***$P = 0.050$	Purity, > 99% No significant increase in the incidence of any neoplasm in females

bw, body weight; F, female; M, male; wk, week

3. Cancer in Experimental Animals

See Table 3.1

Primidone was tested for carcinogenicity by oral administration (feed) in one study in mice and one study in rats.

3.1 Mouse

In one study of carcinogenicity, groups of 50 male and 50 female B6C3F$_1$ mice (age, 5–6 weeks) were given diets containing primidone (purity, > 99%) at a concentration of 0 (control), 300, 600, or 1300 ppm for 104–105 weeks. Primidone intake was equivalent to average daily doses of approximately 0, 30, 65, or 150 mg/kg body weight (bw) in males, and 0, 25, 50 or 100 mg/kg bw in females (NTP, 2000). Survival in exposed groups was similar to that of controls, except for the group of males at the highest dose, in which survival was less than that of controls. Primidone caused significant increases in the incidence of hepatocellular adenoma, of hepatocellular carcinoma, and of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma (combined) in all dosed groups of males and females compared with controls. Primidone caused significant increases in the incidence of hepatoblastoma in all dosed groups of males. In males, there was also a significant positive trend in the incidence of follicular cell adenoma of the thyroid in mice receiving pyrimidone, with a significant increase in incidence in the group receiving the highest dose. There was an increased incidence in follicular cell hyperplasia of the thyroid in males and females receiving pyrimidone.

3.2 Rat

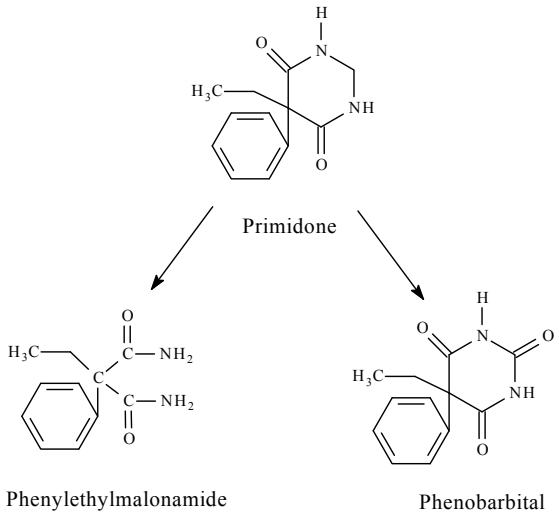
In one study of carcinogenicity, groups of 50 male and 50 female F344/N rats (age, 6 weeks) were given diet containing primidone (purity, > 99%) at a concentration of 0 (control), 600, 1300, or 2500 ppm for 104 weeks. Primidone intake was equivalent to average daily doses of approximately 0, 25, 50, or 100 mg/kg bw in males and females (NTP, 2000). Survival in exposed groups was similar to that in controls, except for males at the intermediate and highest doses, for which survival was less than that for controls. Primidone caused a significant increase in the incidence of follicular cell adenoma of the thyroid in males receiving the intermediate dose. In the extended evaluations involving additional step sections of the kidney in males, there was a small but significant increase in the incidence of renal tubule adenoma or carcinoma (single and extended evaluations combined) at the highest dose; these tumours also occurred with a small but significant positive trend. [The Working Group noted the unusually high incidence of these uncommon tumours in the controls.] The incidence of renal tubule hyperplasia was also increased in all groups of males receiving primidone. There was no significant increase in the incidence of any neoplasm in females.

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

The metabolism of primidone is shown in Fig. 4.1.

Fig. 4.1 Metabolism of primidone



Compiled by the Working Group

4.1.1 Humans

In humans, primidone is partly eliminated unchanged via urinary excretion, and partly metabolized by hepatic cytochrome P450 (CYP) isozymes, principally to phenylethylmalonamide (PEMA) by cleavage of the pyrimidine ring, and to phenobarbital by oxidation of the methylene group (Baumel *et al.*, 1972; Martines *et al.*, 1990; Sato *et al.*, 1992; Anderson, 1998; Tanaka, 1999). The CYP isoenzymes responsible for metabolizing primidone are presently uncertain (Anderson, 1998; Tanaka, 1999).

(a) Pharmacokinetics of single doses

Baumel *et al.* (1972) reported the pharmacokinetics of primidone in two subjects given a single oral dose of 500 mg of primidone. Peak plasma concentration of primidone, measured by gas-liquid chromatography, was reached at 0.5 hours in one subject, and 4.8 hours in the other. Estimated half-lives for primidone were 5.8 and 3.3 hours, respectively. Two hours after dosing, the metabolite PEMA was detected in the plasma of both subjects, reaching peak concentrations at 7 and 8 hours before gradually declining. Estimated half-lives were 29 and

36 hours, respectively. The second metabolite, phenobarbital, was not detectable in this study.

In a study of seven volunteers given a single oral dose of 500 mg of primidone , the mean peak plasma concentration (± standard deviation) of primidone was 41.4 ± 5.2 µmol/L, reached in approximately 2 ± 1 hours. The elimination half-life was 17 ± 2.4 hours. PEMA was detected in the serum, reaching peak concentrations (4.1 ± 0.7 µmol/L) at 2–24 hours in these subjects. Phenobarbital was below the level of detection (< 2 µmol/L) of gas-liquid chromatography (Pisani *et al.*, 1984).

Subsequent studies using high-performance liquid chromatography of samples from three healthy volunteers given a single oral dose of 600 mg, showed initial slow absorption of primidone; peak concentration of unchanged primidone (mean C_{max}, 41.2 ± 5.4 µmol/mL) was achieved at 12 hours in each subject. Mean elimination half-life was 19.4 ± 2.2 hours. The metabolite PEMA was detectable at 1.3 ± 0.3 hours, and reached peak concentration (1.7 ± 0.3 µmol/L) at 36 hours. Elimination half-life was 26.5 ± 1.0 hours. The metabolite phenobarbital was detectable at 5.3 ± 1.3 hours, and reached maximal concentration (1.3 ± 0.2 µmol/L) at 52 ± 11 hours, with a long (125 ± 20 hours) elimination half-life (Sato *et al.*, 1992).

In a study of the pharmacokinetics of PEMA given as a single oral dose of 400 mg to two groups of subjects (six patients aged 10–43 years receiving long-term treatment with various anti-epileptic drugs and six "drug-free" subjects aged 22–42 years), showed no statistically significant differences between the two groups; peak serum concentrations were normally reached within 2–4 hours after dosing in both groups. In the drug-free subjects, recovery of unchanged PEMA in the urine gave an estimated oral bioavailability of at least 80%. The elimination half-life ranged from 17 to 25 hours in drug-free subjects, and from 10 to 23 hours in patients. There was no evidence for a glucuronide conjugate. The study indicated that PEMA is readily absorbed from the gastrointestinal tract and eliminated predominantly unchanged in the urine (Cottrell *et al.*, 1982).

(b) Pharmacokinetics of repeated doses

Although phenobarbital was not detected after administration of single doses of primidone, long-term administration of primidone (at "various" doses) in 46 epilepsy patients showed serum accumulation of phenobarbital, and PEMA (Baumel *et al.*, 1972). Although there was significant inter-individual variability, concentrations of the two metabolites showed correlation with those of the parent drug, and concentrations of phenobarbital were consistently higher than those of PEMA. Two of the subjects had been on a daily dose of primidone (750 mg in divided doses) for more than 3 years. After a single dose of 750 mg in this study, peak serum concentrations of primidone were achieved rapidly (by 0.5 hour), and declined slowly (half-lives, 5.3 and 7.0 hours). In both subjects, peak concentrations of metabolites, PEMA (12 and 10 µg/mL) and phenobarbital (33 and 11 µg/mL), remained relatively constant. In the cerebrospinal fluid, binding to protein by PEMA and by primidone was negligible, and approximately 60% by phenobarbital (Baumel *et al.*, 1972).

In a subsequent study in eight epileptic patients (aged 18–26 years) receiving long-term treatment with primidone (mean daily dose, 422 ± 115 mg per day), the half-life for primidone was 14.7 ± 3.5 hours (Martines *et al.*, 1990).

(c) Absorption, distribution, and excretion under certain conditions

(i) Age-dependent effects

The pharmacokinetics and metabolism of primidone at steady-state were studied in 18 epileptic patients who had been receiving a constant dose of primidone for at least 2 months. Data were compared in two groups: 10 elderly

patients (age, 70–81 years), and 8 young patients (age, 18–26 years) (Martines *et al.*, 1990). The mean daily doses were moderately, but not significantly, higher in the elderly group, than the young (575 ± 206 mg/day and 422 ± 115 mg/day, respectively). In the elderly and young, respectively, the mean half-life of primidone was 12.1 ± 4.6 hours and 14.7 ± 3.5 hours, and the mean total clearance of primidone was 34.8 ± 9.0 mL/hour per kg and 33.2 ± 7.2 mL/hour per kg. Differences between the two groups were not statistically significant, indicating that half-life and total clearance of primidone were unaltered in elderly patients. However, some differences between the two groups were highlighted; serum concentrations of the metabolites PEMA and phenobarbital (relative to those of parent drug) were higher in the elderly than the young, significantly so in the case of PEMA ($P < 0.01$). Renal clearances of primidone, phenobarbital, and PEMA were moderately decreased (again, significantly for PEMA, $P < 0.05$) in the elderly (Martines *et al.*, 1990).

The results of this study supported previous suggestions that PEMA (unlike primidone and phenobarbital) is eliminated only by renal excretion (Cottrell *et al.*, 1982), and so its serum accumulation in the elderly probably results from moderately reduced renal elimination accompanied by an increase in the fraction of primidone metabolized (Cottrell *et al.*, 1982; Pisani *et al.*, 1984; Martines *et al.*, 1990).

The metabolism and excretion of orally administered primidone was studied in 12 children (age, 7–14 years) undergoing long-term (> 3 months) treatment for epilepsy, and were assumed to be in steady state. Four children were taking primidone only, and eight were also taking phenytoin. Plasma concentrations peaked at 4–6 hours and declined exponentially over 6–24 hours, with half-lives ranging from 4.5 to 11 hours. Mean recovery of the administered dose in the urine within 24 hours was 92% (range, 72–123%) as primidone and metabolites. Of the total daily dose administered, 42.3% was recovered as unchanged drug, 45.2% as PEMA, and 4.9% as phenobarbital. The rate of metabolism to phenobarbital showed wide variation (25-fold) among children, which, although not influencing the overall elimination rate constant for primidine, is an important determinant of the individual patient's steady-state concentration of phenobarbital. Concomitant use of phenytoin had no detectable effect on half-life or serum concentrations of phenobarbital. Of the total primidone daily dose, approximately equal amounts of parent drug (~40%) and PEMA (~45%) were excreted, with phenobarbital as approximately 5% (Kauffman *et al.*, 1977).

(ii) Pregnancy

The placental transfer of primidone and metabolites was investigated in 14 women treated for epilepsy with primidone (and additionally phenytoin, ethosuximide or valproate in 5 women) throughout pregnancy. Primidone, PEMA, phenobarbital, and polar metabolites (*p*-hydroxyphenobarbital and *p*-hydroxyphenobarbital glucuronide) were found in similar concentrations in maternal and cord blood at birth (Nau *et al.*, 1980).

In the same study, the pharmacokinetics of primidone were studied in seven of the newborns during the first weeks of life (Nau *et al.*, 1980). Mean elimination half-lives were longer than those found in children by Kauffman *et al.* (1977): 23 ± 10 hours for primidine; 113 ± 40 hours for phenobarbital; and 35 ± 6 hours for PEMA. The shortest half-lives for primidone (8–11 hours) were detected in two neonates whose mothers had been treated with phenytoin in addition to primidone. Serum concentrations and elimination rates varied among neonates, and during the period of study. For example, serum concentrations of phenobarbital and PEMA increased in some neonates during the first few days, due to neonatal metabolism of primidine, and rate of

elimination increased after a few days in some babies (Nau *et al.*, 1980).

Analyses of maternal milk of four of the mothers detected primidone and PEMA at approximately 75%, phenobarbital at approximately 50%, and total *p*-hydroxyphenobarbital (conjugated and non-conjugated) at approximately equal to concentrations measured in serum. Because of breastfeeding, all compounds were also detected in neonatal blood (Nau *et al.*, 1980).

(iii) Liver disease

The disposition of a single oral dose of 500 mg of primidone was studied in seven patients with acute viral hepatitis and in seven healthy subjects (controls). The elimination half-life and the apparent clearance of unchanged primidone in the patients did not differ significantly from that in the controls (mean elimination half-life, 18.0 ± 3.1 hours in patients, and 17.0 ± 2.4 hours in controls; mean apparent clearance of unchanged primidone, 42 ± 14 mL/hour per kg in patients, and 35 ± 8 mL/hour per kg in controls). The metabolite PEMA was detectable in serum of all healthy subjects within 2–24 hours, but undetectable (< 2 µmol/L) in sera of all except one patient. In all subjects, serum concentrations of phenobarbital remained below the limit of detection of gas-liquid chromatography. These findings indicated that accumulation of primidone is unlikely to occur in epilepsy patients who develop acute viral hepatitis (Pisani *et al.*, 1984).

(d) Pharmacokinetic and drug interactions

The CYP isozymes 1A2, 2C9, 2C19, and 3A4, and UDP-glucoronosyl transferase and epoxide hydrolases are induced by primidone and its metabolite phenobarbital [phenobarbital also induces CYP2A6] (Riva *et al.*, 1996; Anderson, 1998; Patsalos & Perucca, 2003). Thus pharmacokinetic interactions are likely to occur between primidone and other substrates for these enzymes, ultimately causing either an increase or decrease in pharmacologically active species. Primidone is frequently used in combination with such substrates (e.g. anticonvulsants such as carbamazepine, ethosuximide, valproic acid, and phenytoin). A study by Sato *et al.* (1992) showed that, in patients taking both primidone and phenytoin, metabolites of primidone in serum were detected earlier, elimination was faster, and total body clearance was increased, when compared with patients taking primidone only. In a study of seven neonates, whose mothers were treated for epilepsy throughout pregnancy, the shortest half-lives for primidone were reported in two neonates whose mothers had been treated with both phenytoin and primidone (Nau *et al.*, 1980). Conversely, Kauffman *et al.* (1977) reported that there were no effects on half-life or serum concentrations of phenobarbital in children being treated for epilepsy with phenytoin in addition to primidone.

4.1.2 Experimental systems

PEMA and phenobarbital have been identified as the major metabolites of primidone in mice (McElhatton *et al.*, 1977), rats (Baumel *et al.*, 1973; Moriyama *et al.*, 1994), rabbits (Fujimoto *et al.*, 1968; Hunt & Miller, 1978), and dogs (Frey & Löscher, 1985).

In a study by the NTP (2000), groups of B6C3F$_1$ mice were given a single dose of primidone (at 30, 80, or 200 mg/kg bw) by gavage and blood samples were collected at various timepoints (ranging from 0.25 hour to 48 hours) after administration. Plasma concentrations of primidone in mice were dependent on dose and time. Absorption was rapid, and for all dose groups, plasma concentrations were detectable within 15 minutes after dosing, and remained above the limit of detection for at least 30 hours (after a dose of 30 or 80 mg/kg bw) and for at least 48 hours (after a dose of 200 mg/kg bw). Slightly higher plasma concentrations of primidone were detected in males than females.

Plasma concentrations of phenobarbital were dose-, time- and sex-dependent; phenobarbital was detected within 15 minutes after dosing. Earlier and slightly higher peak concentrations were observed in males than in females, indicating that, in mice, primidone is more rapidly metabolized to phenobarbital in males than in females (NTP, 2000).

Studies in pregnant mice given repeated intragastric doses of primidone at 100 mg/kg bw [a known teratogenic dose] over several days demonstrated no accumulation of the parent compound, or of the metabolites PEMA or phenobarbital, and all were cleared rapidly from the plasma within 24 hours. The relatively long period of dosing with primidone resulted in its more rapid rate of metabolism, resulting in higher concentrations of metabolites, than after a single dose (McElhatton *et al.*, 1977).

Studies of single doses of primidone (given by gavage) in the mouse, showed a dissimilar trend in results. The plasma half-life of phenobarbital was reported to be twice that of primidone and PEMA, and plasma : brain ratios indicated poor penetration of primidone into the brain (Leal *et al.*, 1979).

In contrast, in rats given primidone by gavage, concentrations of the parent drug peaked in the plasma after 1 hour, and in the brain after 2 hours (Baumel *et al.*, 1973). This result was supported by a subsequent study in rats given primidone by intraperitoneal injection (at a dose of 50, 100 or 200 mg/kg bw), which suggested that primidone and metabolites were able to penetrate the blood–brain barrier. Primidone was first detected in the serum (mean T_{max} range, 1.5–2.5 hours) and in the cerebrospinal fluid (mean T_{max} range, 2.0–3.5 hours), followed by its metabolites, PEMA and phenobarbital (Nagaki *et al.*, 1999).

Moriyama *et al.* (1994) reported the pharmacokinetic parameters of primidone and its major metabolites in the rat. After oral administration of primidone (at a dose of 50 mg/kg bw), the plasma concentration of primidone rapidly increased achieving maximal levels by 1 hour, but by 12 hours had decreased to very low levels, and at 24 hours was undetectable. In contrast, concentrations of PEMA and phenobarbital gradually increased, reaching maximum levels after 4–8 hours, and these metabolites were still detected after 24 hours. T_{max} values for primidone, PEMA, and phenobarbital were 1.36, 5.70, and 6.55 hours, respectively, and C_{max} values were 18.15 μg/mL, 8.11 μg/mL, and 9.64 μg/mL, respectively. Thus concentrations of PEMA and phenobarbital were approximately 50% that of primidone. Half-lives were reported as 1.64, 4.29, and 4.96 hours for primidone, PEMA and phenobarbital, respectively.

In the study by Nagaki *et al.* (1999), the concentrations of primidone, PEMA, and phenobarbital rose in a linear and dose-dependent manner in serum and cerebrospinal fluid (mean free fraction in serum [free non-protein-bound/total concentration ratio], 0.86, 0.97, and 0.88, respectively). The respective mean values for the cerebrospinal fluid : serum ratio were 0.73, 1.06, and 0.65, suggesting rapid equilibration between blood and cerebrospinal-fluid compartments. Mean half-life values for primidone, PEMA and phenobarbital in the cerebrospinal fluid were similar to those reported in serum (Nagaki *et al.*, 1999).

In a study by the NTP (2000), groups of male and female F344/N rats were given a single dose of primidone (30, 80, or 130 mg/kg bw) by gavage, and blood samples were collected from all dose groups at various times (from 15 minutes to 30 hours) after administration (NTP, 2000). Plasma concentrations of primidone were dependent on dose and time; absorption was rapid at all doses, and primidone was detectable in the plasma within 15 minutes after dosing. Although the time-course and dose–response profiles were similar in male and female rats, plasma concentrations of primidone (at most doses and time points) were consistently higher

(approximately double) and half-lives greater (two- to fivefold) in females than in males. Plasma concentrations of the metabolite phenobarbital were also dependent on dose, time, and sex; although phenobarbital was detectable in the plasma of male rats within 15 minutes after dosing, phenobarbital was undetectable in the plasma of female rats at 15 and 30 minutes, and plasma concentrations of phenobarbital, for a given dose, were consistently higher in males than in females. [Thus, the metabolism of primidone in rats appeared to be dependent on sex, with males metabolizing primidone more rapidly than females.] Phenobarbital was still detectable in the plasma of male and female rats at 30 hours after dosing (NTP, 2000).

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

See Table 4.1

(a) Mutagenicity

Primidone (concentration range, 33–10 000 µg/plate) was mutagenic at concentrations of 3333 µg/plate and higher in *Salmonella typhimurium* strain TA1535 in the absence of metabolic activation; no mutagenic activity was detected in TA1535 in the presence of metabolic activation, or in strains TA100, TA1537, or TA98, with or without metabolic activation (Mortelmans *et al.*, 1986).

No increases in the frequencies of sex-linked recessive lethal mutations were detected in germ cells of male *Drosophila melanogaster* treated as larvae by feeding on primidone solutions of 6–12 mM (Zolotareva *et al.*, 1979).

(b) Chromosomal damage

No increases in sister-chromatid exchange or chromosomal aberration were noted in cultured Chinese hamster ovary cells treated with primidone at concentrations ranging from 125 to 1250 µg/mL, with or without metabolic activation (NTP, 2000). Additional in-vitro studies showing no induction of sister-chromatid exchange in Chinese hamster ovary cells, or chromosomal aberration in human lymphocytes or Chinese hamster ovary cells, have been reported (Stenchever & Allen, 1973; Bishun *et al.*, 1975; Riedel & Obe, 1984).

In vivo, no induction of dominant lethal mutation was observed in germ cells of male mice treated with a single intraperitoneal injection of primidone at doses of up to 90 mg/kg bw (Epstein *et al.*, 1972) or 400 mg/kg bw (Zolotareva *et al.*, 1979). No induction of chromosomal aberrations was reported in bone-marrow cells of male mice treated with primidone at doses of up to 400 mg/kg bw by a single intraperitoneal injection (Zolotareva *et al.*, 1979). There was one report of an increased frequency of micronucleated polychromatic erythrocytes in the bone marrow of mice given 13.11 mg of primidone [dose, approximately 500 mg/kg bw] twice with an interval of 24 hours (Rao *et al.*, 1986). [The Working Group noted that the mice were sampled 6 hours after the second dose, which was too brief an interval to measure the effects of the second treatment, and possibly too long to evaluate accurately the induction of micronuclei after the initial treatment. These protocol deficiencies hindered the interpretation of the data.] Contrasting results were seen in B6C3F_1 male mice, in which no significant increases in the frequency of micronucleated polychromatic erythrocytes were detected in bone marrow after administration of primidone (dose range, 87.5–350 mg/kg bw) by intraperitoneal injection, three times at 24-hour intervals, in each of two replicate trials (NTP, 2000).

Table 4.1 Genetic and related effects of primidone

Test system	Results[a]		Concentration/dose (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
In vitro				
Salmonella typhimurium TA100, TA1537, TA98, reverse mutation	–	–[b]	10 000 µg/plate	Mortelmans *et al.* (1986)
Salmonella typhimurium TA1535, reverse mutation	+	–[b]	3333 µg/plate	Mortelmans *et al.* (1986)
Drosophila melanogaster, sex-linked recessive lethal mutation in germ cells	–	NT	12 mM in food	Zolotareva *et al.* (1979)
Sister-chromatid exchange, Chinese hamster ovary cells	–	–	1250 µg/mL	NTP (2000)
Sister-chromatid exchange, Chinese hamster ovary cells	–	–	100 µg/mL	Riedel & Obe (1984)
Chromosomal aberration, Chinese hamster ovary cells	–	–	1250 µg/mL	NTP (2000)
Chromosomal aberration, Chinese hamster ovary cells	–	–	100 µg/mL	Riedel & Obe (1984)
Chromosomal aberration, human lymphocytes	–	NT	100 µg/mL	Stenchever & Allen (1973)
Chromosomal aberration, human lymphocytes	–	NT	70 µg/mL	Bishun *et al.* (1975)
In vivo				
Dominant lethal mutation, male ICR/Ha Swiss mouse, germ cells	–		90 mg/kg bw, ip × 1	Epstein *et al.* (1972)
Dominant lethal mutation, male mouse, germ cells	–		400 mg/kg bw, ip × 1	Zolotareva *et al.* (1979)
Chromosomal aberration, male mouse, bone-marrow cells	–		400 mg/kg bw, ip × 1	Zolotareva *et al.* (1979)
Micronucleus formation, Swiss mouse, bone-marrow cells	+		13.11 mg, po × 2[c]	Rao *et al.* (1986)
Micronucleus formation, male B6C3F$_1$ mouse, bone-marrow cells	–		350 mg/kg bw, ip × 3	NTP (2000)

[a] +, positive; –, negative

[b] S9 (9000 × *g* supernatant) from Sprague-Dawley rats and Syrian hamsters treated with Aroclor 1254

[c] Dose was approximately 500 mg/kg bw; four mice per treatment group. Mice were killed 6 hours after the second treatment; 3000 polychromatic erythrocytes were scored per mouse

bw, body weight; LED, lowest effective dose; HID, highest ineffective dose; ip, intraperitoneal; NR, not reported; NT, not tested; po, oral

4.2.3 Genetic and related effects of the metabolite phenobarbital

In contrast to the limited information on primidone, there was a significant body of literature describing the results of tests for genotoxicity with phenobarbital, a major metabolite of primidone. The extensive literature on the genetic and related effects of phenobarbital was reviewed by a previous Working Group (IARC, 2001), and is summarized briefly below.

Phenobarbital did not induce sister-chromatid exchange in patients with epilepsy receiving only this drug (IARC, 2001).

In studies in which rodents were exposed to phenobarbital in vivo, no covalent binding to mouse liver DNA was observed, but the frequency of alkali-labile damage in mouse liver cells was increased. Gene mutation was not induced in a transgenic mouse strain, and sister-chromatid exchange, micronucleus formation, and chromosomal aberrations were not induced in mouse bone-marrow cells. Phenobarbital did not increase the frequency of sperm-head abnormalities in mice, but spermatogonial germ-cell chromosomal aberrations were reported in male mice in one laboratory. Further increases in the frequency of chromosomal aberration were found in liver foci cells of mice treated with phenobarbital after prior treatment with a genotoxic agent (IARC, 2001).

Chromosomal aberrations, but not gene mutations, were induced in cultured human lymphocytes treated with phenobarbital (IARC, 2001).

The numerous types of test for the genetic effects of phenobarbital in vitro included assays for DNA damage, DNA repair induction, gene mutation, and chromosomal aberration in mammalian cells, tests for gene mutation and mitotic recombination in insects and fungi, and tests for gene mutation in bacteria. Although the majority of the test results were negative, the numerous positive results could not be ignored, although they did not present a consistent pattern of genotoxicity. The inconsistency of the results, the absence of any direct evidence for an interaction with DNA, and the generally negative data in vivo led to the conclusion that phenobarbital is not genotoxic (IARC, 2001).

Phenobarbital transformed hamster embryo cells. It inhibited gap-junctional intercellular communication in hepatocytes of rats treated in vivo, and in primary cultures of hepatocytes from rats and mice, but not (in a single study) in primary cultures of hepatocytes from humans or rhesus monkey (IARC, 2001).

4.3 Other mechanistic data relevant to carcinogenesis

4.3.1 Humans

Toxicity associated with primidone in humans has been documented with reference to side-effects after use of primidone as a drug. The side-effects included nausea, vomiting, dizziness, ataxia, and somnolence, and caused early discontinuation of treatment. Smith *et al.* (1987) reported that both carbamazepine and phenytoin were associated with statistically significantly lower incidences of intolerable side-effects than were primidone or phenobarbital. Patients receiving primidone experienced the highest incidence of toxicity.

Administration of anti-epileptic drugs, such as primidone, and also carbamazepine, gabapentin, oxcarbazepine, and phenytoin affected serum concentrations of folate, homocysteine, and vitamin B_{12}. In a study involving 2730 patients treated with various anti-epileptic drugs, 170 untreated patients, and 200 healthy controls, Linnebank *et al.* (2011) reported that primidone monotherapy (10 patients) was associated with a higher frequency of folate concentrations that were below the reference range when compared with untreated patients and controls. This association was dose-dependent. Primidone

monotherapy was also associated with plasma concentrations of homocysteine that were above the reference range when compared with controls (Linnebank *et al.*, 2011).

A review by Benedetti *et al.* (2005) of several studies in humans suggested that therapeutic levels of primidone or phenobarbital are not associated with an increase in thyroid-stimulating hormone levels.

4.3.2 Experimental systems

Carl *et al.* (1987a, b) studied the effects of treatment with primidone on one-carbon metabolism by measuring levels of methylene-tetrahydrofolate reductase and related parameters in the brain and liver of rats given primidone (100 mg/kg bw every 12 hours) by gastric gavage for up to 8 weeks. Primidone caused a decrease of pteroylpentaglutamates in the liver to less than half the control value within 1 week. Overall, the data suggested that primidone affects concentrations of folate in the tissue and plasma by interfering with folate-dependent metabolic processes, possibly through the interaction of primidone with the synthesis of folylpolyglutamates (Carl *et al.*, 1987a).

4.4 Susceptibility

No studies primarily addressing the susceptibility of humans to carcinogenesis induced by primidone were available to the Working Group. In a review, Singh *et al.* (2005) speculated that there might be a partly biological basis (e.g. genetic predisposition) for the association between epilepsy and cancer, possibly involving the tumour suppressor gene leucine-rich glioma inactivated 1 (*LGI1*). El-Masri & Portier (1998) have suggested that there is wide inter-individual variation in the metabolic profile of primidone, which may indicate the presence of people who produce greater amounts of primidone metabolites than the general population, and who are thus more sensitive to effects induced by primidone metabolites.

4.5 Mechanistic considerations

In humans, and in mice and rats, primidone is extensively, but not totally, metabolized to phenobarbital. Given the evidence for the carcinogenicity of primidone (see Section 3) and phenobarbital (IARC, 2001), the carcinogenic activity attributable to primidone in mice can be reasonably hypothesized to be the result of the metabolism of primidone to phenobarbital considering that both cause malignant hepatocellular tumours in this species.

The carcinogenicity of phenobarbital was evaluated by the Working Group in 2000 (IARC, 2001). Epidemiological data primarily comprised three large cohort studies of patients with epilepsy. On the basis of these and all other available studies, the Working Group concluded that there was *inadequate evidence* in humans for the carcinogenicity of phenobarbital (IARC, 2001). Singh *et al.* (2005) reviewed studies involving risk of cancer in people with epilepsy, with specific reference to the role of anti-epilepsy drugs, noting studies concerning cancer of the liver, lung, and brain. Despite considerable long-term pharmaco-epidemiological data being available for phenobarbital, evidence for carcinogenicity in humans was not consistent and phenobarbital was considered to be "possibly" carcinogenic to humans by the authors.

The Working Group in 2000 concluded that phenobarbital was *possibly carcinogenic* to humans (Group 2B) based solely on *sufficient evidence* in experimental animals (IARC, 2001).

Studies aiming to elucidate mechanisms of carcinogenesis attributable to phenobarbital in mice have been reported. Typically, these investigations exploited comparison between strains of mice that were variously sensitive and resistant to phenobarbital-induced hepatocarcinogenesis. Thus Watson & Goodman (2002) reported

that there was a clear indication of more extensive changes in methylation in GC-rich regions of DNA, primarily hypermethylation, in the tumour-sensitive mice in response to treatment with phenobarbital.

Phillips *et al.* (2009) reported the effects of treatment with phenobarbital on DNA methylation and gene expression that occurred only in liver tumour-prone $B6C3F_1$ mice but not in tumour-resistant C57BL/6 mice, after 2 or 4 weeks of treatment. Differences in epigenetic control (e.g. DNA methylation) between species could, in part, underlie the enhanced propensity of rodents, as compared with humans, to develop cancer.

5. Summary of Data Reported

5.1 Exposure data

Primidone is a synthetic drug that was used commonly as an oral anticonvulsant, beginning in the 1950s. It is now only in modest use, predominantly for the treatment of essential tremor, with stable use over the past decade. Exposure is likely to be predominantly through use as a medication. Environmental contamination in groundwater has been reported.

5.2 Human carcinogenicity data

The available epidemiological studies evaluating exposure specifically to primidone were limited to two case–control studies reporting on several types of cancer nested in a cohort of epileptic patients in Denmark. Small excesses of malignant lymphoma and cancers of the lung and urinary bladder were observed among patients who were ever treated with primidone; however, the findings were based on small numbers of exposed cases. Other limitations included incomplete information on exposure to primidone (with respect to duration and post-discharge drug use) and on potential confounders. The available studies were not informative on whether exposure to primidone is a cancer hazard.

5.3 Animal carcinogenicity data

Primidone was tested for carcinogenicity in one oral administration study in mice, and one oral administration study in rats. In male and female mice, feed containing primidone caused significant increases in the incidences of hepatocellular adenoma, of hepatocellular carcinoma, and of hepatocellular adenoma, hepatocellular carcinoma and hepatoblastoma (combined). Primidone also caused a significant increase in the incidence of hepatoblastoma and of thyroid follicular cell adenoma in males. In male rats, feed containing primidone caused a significant increase in the incidence of thyroid follicular cell adenoma. Primidone also caused a small but significant increase in the incidence of renal tubule adenoma or carcinoma (combined) in males. There was no significant increase in the incidence of any neoplasm in female rats.

5.4 Mechanistic and other relevant data

In humans, primidone is partly eliminated unchanged via urinary excretion, or metabolized, by hepatic cytochrome P450 isozymes principally to phenylethylmalonamide and to phenobarbital, a non-genotoxic agent. The data on genetic toxicity for primidone in traditional assays are limited in scope and amount, but suggest that any mutagenic action of the chemical is highly specific: clear demonstration of the mutagenic activity of primidone was limited to a single report of mutation induction in *Salmonella typhimurium* strain TA1535 in the absence of metabolic activation only, and at high concentrations. The majority of well-conducted

studies of chromosomal damage available for review suggested that primidone does not induce chromosomal changes in vitro or in vivo.

The reported carcinogenicity of primidone in mice is likely to be mediated through a non-genotoxic mechanism resulting from the metabolism of primidone to phenobarbital.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for carcinogenicity of primidone.

6.3 Overall evaluation

Primidone is *possibly carcinogenic to humans (Group 2B)*.

References

Adelöw C, Ahlbom A, Feychting M, Johnsson F, Schwartzbaum J, Tomson T (2006). Epilepsy as a risk factor for cancer. *J Neurol Neurosurg Psychiatry*, 77(6):784–6. doi:10.1136/jnnp.2005.083931 PMID:16705202

Anderson GD (1998). A mechanistic approach to antiepileptic drug interactions. *Ann Pharmacother*, 32(5):554–63. doi:10.1345/aph.17332 PMID:9606477

Baumel IP, Gallagher BB, DiMicco J, Goico H (1973). Metabolism and anticonvulsant properties of primidone in the rat. *J Pharmacol Exp Ther*, 186(2):305–14. PMID:4719783

Baumel IP, Gallagher BB, Mattson RH (1972). Phenylethylmalonamide (PEMA). An important metabolite of primidone. *Arch Neurol*, 27(1):34–41. doi:10.1001/archneur.1972.00490130036005 PMID:4626105

Benedetti MS, Whomsley R, Baltes E, Tonner F (2005). Alteration of thyroid hormone homeostasis by antiepileptic drugs in humans: involvement of glucuronosyltransferase induction. *Eur J Clin Pharmacol*, 61(12):863–72. doi:10.1007/s00228-005-0056-0 PMID:16307266

Bishun NP, Smith NS, Williams DC (1975). Chromosomes and anticonvulsant drugs. *Mutat Res*, 28(1):141–3. doi:10.1016/0027-5107(75)90327-9 PMID:1143294

British Pharmacopoeia (2013). Primidone. London, United Kingdom: Medicines and Healthcare products Regulatory Agency (MHRA).

Carl GF, Eto I, Krumdieck CL (1987a). Chronic treatment of rats with primidone causes depletion of pteroylpentaglutamates in liver. *J Nutr*, 117(5):970–5. PMID:3585552

Carl GF, Gill MW, Schatz RA (1987b). Effect of chronic primidone treatment on folate-dependent one-carbon metabolism in the rat. *Biochem Pharmacol*, 36(13):2139–44. doi:10.1016/0006-2952(87)90142-0 PMID:3606631

Cottrell PR, Streete JM, Berry DJ, Schäfer H, Pisani F, Perucca E *et al.* (1982). Pharmacokinetics of phenylethylmalonamide (PEMA) in normal subjects and in patients treated with antiepileptic drugs. *Epilepsia*, 23(3):307–13. doi:10.1111/j.1528-1157.1982.tb06196.x PMID:7084140

Daley RD (1973). Primidone. In: Florey K, editor. Analytical Profiles of Drug Substances. Academic Press; pp. 409–437.

DrugBank (2013). Primidone. DrugBank: Open Data Drug & Drug Target Database. Version 3.0. Available from: http://www.drugbank.ca/

El-Masri HA, Portier CJ (1998). Physiologically based pharmacokinetics model of primidone and its metabolites phenobarbital and phenylethylmalonamide in humans, rats, and mice. *Drug Metab Dispos*, 26(6):585–94. PMID:9616196

eMC (2013). Primidone. Datapharm Communications Ltd., electronic Medicines Compendium (eMC). Available from: http://www.medicines.org.uk/emc, accessed 4 September 2014.

Epstein SS, Arnold E, Andrea J, Bass W, Bishop Y (1972). Detection of chemical mutagens by the dominant lethal assay in the mouse. *Toxicol Appl Pharmacol*, 23(2):288–325. doi:10.1016/0041-008X(72)90192-5 PMID:5074577

European Pharmacopoeia (2008). Primidone. European Pharmacopoeia Work Programme; pp. 2752–53. Available from: http://www.edqm.eu/en/european-pharmacopoeia-publications-1401.html, accessed 4 September 2014.

FDA (2013). Primidone. Silver Spring (MD): United States Food and Drug Administration. Available from: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.DrugDetails, accessed 4 September 2014.

Ferranti V, Chabenat C, Ménager S, Lafont O (1998). Simultaneous determination of primidone and its three

major metabolites in rat urine by high-performance liquid chromatography using solid-phase extraction. *J Chromatogr B Biomed Sci Appl*, 718(1):199–204. doi:10.1016/S0378-4347(98)00356-9 PMID:9832377

Frey HH, Löscher W (1985). Pharmacokinetics of anti-epileptic drugs in the dog: a review. *J Vet Pharmacol Ther*, 8(3):219–33. doi:10.1111/j.1365-2885.1985.tb00951.x PMID:3932673

Fujimoto JM, Mason WH, Murphy M (1968). Urinary excretion of primidone and its metabolites in rabbits. *J Pharmacol Exp Ther*, 159(2):379–88. PMID:5638658

Hass U, Dünnbier U, Massmann G (2012). Occurrence of psychoactive compounds and their metabolites in groundwater downgradient of a decommissioned sewage farm in Berlin (Germany). *Environ Sci Pollut Res Int*, 19(6):2096–106. doi:10.1007/s11356-011-0707-x PMID:22227832

Hunt RJ, Miller KW (1978). Disposition of primidone, phenylethylmalonamide, and phenobarbital in the rabbit. *Drug Metab Dispos*, 6(1):75–81. PMID:23277

IARC (2001). Some thyrotropic agents. *IARC Monogr Eval Carcinog Risks Hum*, 79:i–iv, 1–725.http://monographs.iarc.fr/ENG/Monographs/vol79/index.php PMID:11766267

IMS Health (2012a). National Disease and Therapeutic Index (NDTI). Plymouth Meeting, Pennsylvania: IMS Health.

IMS Health (2012b). National Prescription Audit Plus (NPA). Plymouth Meeting, Pennsylvania: IMS Health.

IMS Health (2012c). Multinational Integrated Data Analysis (MIDAS). Plymouth Meeting, Pennsylvania: IMS Health

Japanese Pharmacopoeia (2007). Primidone. In: Hosokawa R editor. *The Japanese Pharmacopoeia*. 15th ed. Tokyo, Japan: Ministry of Health, Labour and Welfare; pp. 1026–7.

Kauffman RE, Habersang R, Lansky L (1977). Kinetics of primidone metabolism and excretion in children. *Clin Pharmacol Ther*, 22(2):200–5. PMID:884921

Kuhn J, Knabbe C (2013). Fully validated method for rapid and simultaneous measurement of six antiepileptic drugs in serum and plasma using ultra-performance liquid chromatography-electrospray ionization tandem mass spectrometry. *Talanta*, 110:71–80. doi:10.1016/j.talanta.2013.02.010 PMID:23618178

Lamminpää A, Pukkala E, Teppo L, Neuvonen PJ (2002). Cancer incidence among patients using antiepileptic drugs: a long-term follow-up of 28,000 patients. *Eur J Clin Pharmacol*, 58(2):137–41. doi:10.1007/s00228-002-0429-6 PMID:12012147

Lanças FM, Sozza MA, Queiroz ME (2003). Simultaneous plasma lamotrigine analysis with carbamazepine, carbamazepine 10,11 epoxide, primidone, phenytoin, phenobarbital, and PEMA by micellar electrokinetic capillary chromatography (MECC). *J Anal Toxicol*, 27(5):304–8. doi:10.1093/jat/27.5.304 PMID:12908944

Leal KW, Rapport RL, Wilensky AJ, Friel PN (1979). Single-dose pharmacokinetics and anticonvulsant efficacy of primidone in mice. *Ann Neurol*, 5(5):470–4. doi:10.1002/ana.410050512 PMID:464548

Linnebank M, Moskau S, Semmler A, Widman G, Stoffel-Wagner B, Weller M *et al.* (2011). Antiepileptic drugs interact with folate and vitamin B12 serum levels. *Ann Neurol*, 69(2):352–9. doi:10.1002/ana.22229 PMID:21246600

Martines C, Gatti G, Sasso E, Calzetti S, Perucca E (1990). The disposition of primidone in elderly patients. *Br J Clin Pharmacol*, 30(4):607–11. doi:10.1111/j.1365-2125.1990.tb03820.x PMID:2291873

McElhatton PR, Sullivan FM, Toseland PA (1977). Plasma level studies of primidone and its metabolites in the mouse at various stages of pregnancy. *Xenobiotica*, 7(10):617–22. doi:10.3109/00498257709038683 PMID:910462

MHRA (2013). UK Public Assessment Report. Mysoline 50mg tablets (primidone) PL 20132/0006. London, United Kingdom: Medicines and Heathcare Products Regulatory Agency. Available from: www.mhra.gov.uk/home/groups/par/documents/websiteresources/con088170.pdf, accessed 24 April 2015

MicroMedex (2013). Primidone. MicroMedex 2.0. Ann Arbor (MI): Truven Health Analytics Inc.

Morasch B (2013). Occurrence and dynamics of micropollutants in a karst aquifer. *Environ Pollut*, 173:133–7. doi:10.1016/j.envpol.2012.10.014 PMID:23202643

Moriyama M, Furuno K, Oishi R, Gomita Y (1994). Simultaneous determination of primidone and its active metabolites in rat plasma by high-performance liquid chromatography using a solid-phase extraction technique. *J Pharm Sci*, 83(12):1751–3. doi:10.1002/jps.2600831220 PMID:7891306

Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B, Zeiger E (1986). Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. *Environ Mutagen*, 8(S7):Suppl 7: 1–119. doi:10.1002/em.2860080802 PMID:3516675

Nagaki S, Ratnaraj N, Patsalos PN (1999). Blood and cerebrospinal fluid pharmacokinetics of primidone and its primary pharmacologically active metabolites, phenobarbital and phenylethylmalonamide in the rat. *Eur J Drug Metab Pharmacokinet*, 24(3):255–64. doi:10.1007/BF03190029 PMID:10716065

Nau H, Rating D, Häuser I, Jäger E, Koch S, Helge H (1980). Placental transfer and pharmacokinetics of primidone and its metabolites phenobarbital, PEMA and hydroxyphenobarbital in neonates and infants of epileptic mothers. *Eur J Clin Pharmacol*, 18(1):31–42. doi:10.1007/BF00561476 PMID:7398746

NTP (2000). NTP Toxicology and Carcinogenesis Studies of Primidone (CAS No. 125–33–7) in F344/N Rats and B6C3F1 Mice (Feed Studies). *Natl Toxicol Program Tech Rep Ser*, 476:1–290. PMID:12571687

O'Neil MJ (2006). The Merck Index. 14th Ed. Whitehouse Station (NJ): Merck & Co., Inc.

OEHHA (2013). Chemicals known to the state to cause cancer or reproductive toxicity. CA: Office of Environmental Health Hazard Assessment, State of California Environmental Protection Agency. Available from: http://oehha.ca.gov/prop65/prop65_list/files/P65single052413.pdf, accessed 30 June 2014.

Olsen JH, Boice JD Jr, Fraumeni JF Jr (1990). Cancer in children of epileptic mothers and the possible relation to maternal anticonvulsant therapy. *Br J Cancer*, 62(6):996–9. doi:10.1038/bjc.1990.424 PMID:2257233

Olsen JH, Boice JD Jr, Jensen JP, Fraumeni JF Jr (1989). Cancer among epileptic patients exposed to anticonvulsant drugs. *J Natl Cancer Inst*, 81(10):803–8. doi:10.1093/jnci/81.10.803 PMID:2716074

Olsen JH, Schulgen G, Boice JD Jr, Whysner J, Travis LB, Williams GM *et al.* (1995). Antiepileptic treatment and risk for hepatobiliary cancer and malignant lymphoma. *Cancer Res*, 55(2):294–7. PMID:7812960

Olsen JH, Wallin H, Boice JD Jr, Rask K, Schulgen G, Fraumeni JF Jr (1993). Phenobarbital, drug metabolism, and human cancer. *Cancer Epidemiol Biomarkers Prev*, 2(5):449–52. PMID:8220089

Özden S, Özden T, Gümül F, Tosun A (1989). Quantitative proton magnetic resonance analysis of primidone in solid dosage forms. *Spectrosc Lett*, 22(4):471–6. doi:10.1080/00387018908053896

Patsalos PN, Perucca E (2003). Clinically important drug interactions in epilepsy: general features and interactions between antiepileptic drugs. *Lancet Neurol*, 2(6):347–56. doi:10.1016/S1474-4422(03)00409-5 PMID:12849151

Phillips JM, Burgoon LD, Goodman JI (2009). The constitutive active/androstane receptor facilitates unique phenobarbital-induced expression changes of genes involved in key pathways in precancerous liver and liver tumors. *Toxicol Sci*, 110(2):319–33. doi:10.1093/toxsci/kfp108 PMID:19482888

Pisani F, Perucca E, Primerano G, D'Agostino AA, Petrelli RM, Fazio A *et al.* (1984). Single-dose kinetics of primidone in acute viral hepatitis. *Eur J Clin Pharmacol*, 27(4):465–9. doi:10.1007/BF00549596 PMID:6519155

Rao KP, Shaheen S, Usha Rani MV, Rao MS (1986). Effect of primidone in somatic and germ cells of mice. *Toxicol Lett*, 34(2-3):149–52. doi:10.1016/0378-4274(86)90204-3 PMID:3798475

Rezaei B, Jafari MT, Khademi R (2009). Selective separation and determination of primidone in pharmaceutical and human serum samples using molecular imprinted polymer-electrospray ionization ion mobility spectrometry (MIP-ESI-IMS). *Talanta*, 79(3):669–75. doi:10.1016/j.talanta.2009.04.046 PMID:19576428

Riedel L, Obe G (1984). Mutagenicity of antiepileptic drugs. II. Phenytoin, primidone and phenobarbital. *Mutat Res*, 138(1):71–4. doi:10.1016/0165-1218(84)90087-9 PMID:6541755

Riva R, Albani F, Contin M, Baruzzi A (1996). Pharmacokinetic interactions between antiepileptic drugs. Clinical considerations. *Clin Pharmacokinet*, 31(6):470–93. doi:10.2165/00003088-199631060-00005 PMID:8968658

Sato J, Sekizawa Y, Owada E, Ito K, Sakuta N, Yoshihara M *et al.* (1986). Sensitive analytical method for serum primidone and its active metabolites for single-dose pharmacokinetic analysis in human subjects. *Chem Pharm Bull (Tokyo)*, 34(7):3049–52. doi:10.1248/cpb.34.3049 PMID:3769107

Sato J, Sekizawa Y, Yoshida A, Owada E, Sakuta N, Yoshihara M *et al.* (1992). Single-dose kinetics of primidone in human subjects: effect of phenytoin on formation and elimination of active metabolites of primidone, phenobarbital and phenylethylmalonamide. *J Pharmacobiodyn*, 15(9):467–72. doi:10.1248/bpb1978.15.467 PMID:1287181

SciFinder (2013). SciFinder databases: a division of the American Chemical Society. Available from: https://www.cas.org/products/scifinder, accessed 30 June 2014.

Singh G, Driever PH, Sander JW (2005). Cancer risk in people with epilepsy: the role of antiepileptic drugs. *Brain*, 128(Pt 1):7–17. doi:10.1093/brain/awh363 PMID:15574465

Smith DB, Mattson RH, Cramer JA, Collins JF, Novelly RA, Craft B (1987). Results of a nationwide Veterans Administration Cooperative Study comparing the efficacy and toxicity of carbamazepine, phenobarbital, phenytoin, and primidone. *Epilepsia*, 28(s3):Suppl 3: S50–8. doi:10.1111/j.1528-1157.1987.tb05778.x PMID:3319543

Stenchever MA, Allen M (1973). The effect of selected antiepileptic drugs on the chromosomes of human lymphocytes in vitro. *Am J Obstet Gynecol*, 116(6):867–70. PMID:4736739

Tanaka E (1999). Clinically significant pharmacokinetic drug interactions between antiepileptic drugs. *J Clin Pharm Ther*, 24(2):87–92. doi:10.1046/j.1365-2710.1999.00201.x PMID:10380060

Thermoscientific (2004). Immonoassay products. Thermo Scientific. Available from: http://www.thermoscientific.com/en/search-results.html?keyword=primidone&matchDim=Y, accessed 30 June 2014.

Tocris (2013). Primidone safety data sheet. Tocris bioscience. Available from: http://www.tocris.com/literature/0830_sds.pdf?1432306358, accessed 4 September 2014.

US Pharmacopeia (2007). Primidone. Report No. USP30-NF25. Rockville (MD): The United States Pharmacopeial Convention; pp. 3016.

US Pharmacopeia (2009). Material Safety Data Sheet - Primidone. Rockville (MD): The United States Pharmacopeial Convention.

US Pharmacopeia (2013). Official Monographs – Primidone. Rockville (MD): The United States Pharmacopeial Convention.

Watson RE, Goodman JI (2002). Effects of phenobarbital on DNA methylation in GC-rich regions of hepatic DNA from mice that exhibit different levels of susceptibility to liver tumorigenesis. *Toxicol Sci*, 68(1):51–8. doi:10.1093/toxsci/68.1.51 PMID:12075110

WHO (2007). Cumulative List No. 14 of INN in Latin, English, French, Spanish, Arabic, Chinese and Russian (in alphabetical order of the Latin name), with supplementary information. Geneva, Switzerland: World Health Organization. Available from: http://www.vpb.gov.lt/files/539.pdf, accessed 30 June 2014.

Zesiewicz TA, Elble RJ, Louis ED, Gronseth GS, Ondo WG, Dewey RB Jr *et al.* (2011). Evidence-based guideline update: treatment of essential tremor: report of the Quality Standards subcommittee of the American Academy of Neurology. *Neurology*, 77(19):1752–5. doi:10.1212/WNL.0b013e318236f0fd PMID:22013182

Zolotareva GN, Akaeva EA, Goncharova RI (1979). Antimutagenic activity of the antispasmodic reparation hexamidine. Effect of hexamidine on level of spontaneous mutation in a number of subjects *Dokl. Acad. Nauk SSSR*, 246:469–471.[Russian]