# PHENYTOIN

This substance was considered by previous working groups, in October 1976 (IARC, 1977) and March 1987 (IARC. 1987). Since that time, new data have become available, and these have been incorporated in the monograph and taken into consideration in the evaluation.

## 1. Exposure Data

### 1.1 Chemical and physical data

#### 1.1.1 *Nomenclature*

**Phenytoin**

*Chem. Abstr. Serv. Reg. No.*: 57-41-0

*Deleted CAS Reg. No.*: 125-59-7

*Chem. Abstr. Name*: 5,5-Diphenyl-2,4-imidazolidinedione

*IUPAC Systematic Name*: 5,5-Diphenylhydantoin

*Synonyms*: Diphenylhydantoin; DPH

**Phenytoin sodium**

*Chem. Abstr. Serv. Reg. No.*: 630-93-3

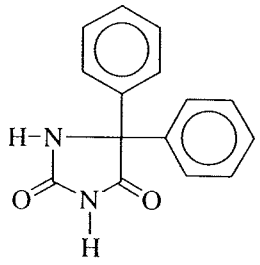
*Deleted CAS Reg. Nos*: 143-75-9; 1421-15-4; 8017-52-5

*Chem. Abstr. Name*: 5,5-Diphenyl-2,4-imidazolidinedione, monosodium salt

*IUPAC Systematic Name*: 5,5-Diphenylhydantoin sodium salt

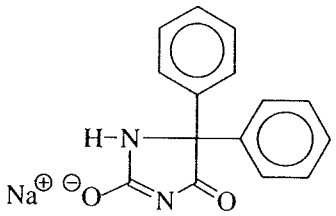
*Synonyms*: Diphenylhydantoin sodium; 5,5-diphenylhydantoin sodium; SDPH; sodium diphenylhydantoin; sodium 5,5-diphenylhydantoin; sodium diphenylhydantoinate; sodium 5,5-diphenyl-2,4-imidazolidinedione; sodium phenytoin; soluble phenytoin

### 1.1.2 *Structural and molecular formulae and relative molecular mass*



Phenytoin

$C_{15}H_{12}N_2O_2$ Relative molecular mass: 252.27



Phenytoin sodium

$C_{15}H_{11}N_2O_2.Na$ Relative molecular mass: 274.25

### 1.1.3 *Chemical and physical properties of the pure substances*

**Phenytoin**

(*a*) *Description*: White, odourless powder (Gennaro, 1995)

(*b*) *Melting-point*: 295–298 °C (Budavari, 1995)

(*c*) *Spectroscopy data*: Infrared, ultraviolet, nuclear magnetic resonance and mass spectral data have been reported (Philip *et al.*, 1984).

(*d*) *Solubility*: Practically insoluble in water; soluble in acetone (1 g/30 mL), ethanol (1 g/60 mL) and alkali hydroxides (Budavari, 1995)

(*e*) *Dissociation constant*: $pK_a$ = 8.06–8.33 (American Hospital Formulary Service, 1995)

**Phenytoin sodium**

(*a*) *Description*: White, odourless powder (Gennaro, 1995)

(*b*) *Spectroscopy data*: Infrared, ultraviolet, nuclear magnetic resonance and mass spectral data have been reported (Philip *et al.*, 1984).

(*c*) *Solubility*: Soluble in water (approx. 1 g/66 mL) and ethanol (1 g/10.5 mL); insoluble in chloroform and diethyl ether (Budavari, 1995); freely soluble in warm propylene glycol (American Hospital Formulary Service, 1995)

(*d*) *Stability*: Easily dissociated by weak acids (including carbon dioxide absorbed on exposure to air) regenerating phenytoin; somewhat hygroscopic (Budavari, 1995)

### 1.1.4 *Technical products and impurities*

Both phenytoin and phenytoin sodium are used in pharmaceutical preparations worldwide, although phenytoin sodium is the more common form.

Phenytoin is available as 50-mg tablets which may also contain flavour, saccharin sodium, sucrose, talc, aluminium lake, D&C Yellow 10 (Quinoline Yellow) or FD&C Yellow 6 (Sunset Yellow FCF). It is also available as 30-, 100- and 125-mg/5 mL oral suspensions, with a maximal alcohol content not greater than 0.6%, and may contain anhydrous citric acid, carboxymethylcellulose, flavours, glycerin, magnesium aluminium silicate, polysorbate 40, sodium benzoate, sucrose, vanillin, D&C Red 33, FD&C Red 40 (Allura Red AC) or FD&C Yellow 6 (Thomas, 1991; British Medical Association/Royal Pharmaceutical Society of Great Britain, 1994; Medical Economics, 1996).

Phenytoin sodium is available as 25-, 30-, 50-, 100- and 300-mg capsules, 30- and 100-mg prompt- and extended-release capsules, and 50- and 100-mg coated tablets, which may also contain citric acid, colloidal silicon dioxide, gelatin, glyceryl monooleate, hydrogen peroxide, lactose, polyethylene glycol 200, sodium benzoate, sodium lauryl sulfate, sucrose, talc, titanium dioxide, FD&C Blue 1 (Brilliant Blue FCF), FD&C Red 3 (Erythrosine) or FD&C Yellow 6. It is also available as a 50-mg/mL injection solution in a vehicle containing 40% propylene glycol (0.4 g/mL) and 10% ethanol (0.1 g/mL) in water, adjusted to pH 12 with sodium hydroxide (Thomas, 1991; British Medical Association/Royal Pharmaceutical Society of Great Britain, 1994; American Hospital Formulary Service, 1995; Medical Economics, 1996).

Trade names for phenytoin and its pharmaceutical preparations include: Aleviatin; Denyl; Difhydan; Dihycon; Di-Hydan; Dihydantoin; Dilabid; Di-Lan; Dilantin; Dilantin-125; Dilantin Infatabs; Dilantin-30 Pediatric; Dintoina; Diphantoin; Diphedan; Diphentyn; Ekko; Enkefal; Epanutin; Epdantoin Simple; Epelin; Epiland; Epinat; Eptoin; Fenantoin; Hidantal; Hydantin; Hydantol; Lehydan; Lepitoin; Novophenytoin; Phenhydan; Phenhydantin; Sodanton; Tacosal; Zentropil.

Trade names for phenytoin sodium and its pharmaceutical preparations include: Alepsin; Aleviatin; Aleviatin sodium; Antisacer; Citrullamon; Danten; Dantoin; Denyl; Difenin; Difetoin; Difhydan; Dilantin; Di-Len; Dintoina; Diphantoine; Di-Phen; Diphenin; Diphenine; Diphenylan; Ditoin; Enkefal; Epanutin; Epdantoin Simple; Epelin; Epilan D; Epilantin; Epsolin; Eptoin; Hidantal; Hydantin; Hydantoinal; Idantoin; Minetoin; Muldis; Neosidantoina; Novodiphenyl; Om-Hydantoïne; Phenhydan; Pyorédol; Solantyl; Tacosal; Thilophenyt; Zentropil.

### 1.1.5 *Analysis*

Methods for the analysis of phenytoin have been reviewed (Glazko, 1982; Philip *et al.*, 1984; Burke & Thénot, 1985).

Several international pharmacopoeias specify liquid chromatography (LC) or titration with sodium hydroxide or sodium methoxide as the assay for purity of phenytoin, and LC, gas chromatography (GC) with flame ionization detection (FID) or thin-layer chromatography (TLC) for determining levels of benzil, benzophenone and other impurities and decomposition products. Assays for determining clarity and colour, acid or

alkali, chloride, benzilic acid and heavy metals are also specified. The assay for phenytoin in powders, tablets and oral suspensions uses titration with sodium methoxide or sodium hydroxide or a gravimetric method following acidification and extraction (Society of Japanese Pharmacopoeia, 1992; British Pharmacopoeial Commission, 1993; United States Pharmacopeial Convention, 1994).

Several international pharmacopoeias specify LC or titration with sodium hydroxide as the assay for purity of phenytoin sodium, and LC, TLC or GC/FID for determining benzophenone levels and other impurities and decomposition products. Assays for determining clarity and colour, free phenytoin, water and heavy metals are also specified. The assay for phenytoin sodium in capsules, tablets and injectable solutions uses titration with tetrabutylammonium hydroxide, an LC method or a gravimetric method following acidification and extraction. Assays for determining bacterial endotoxins, clarity and colour, pH, alcohol and propylene glycol content, particulate matter and heavy metals for the injectable solution are also specified (Society of Japanese Pharmacopoeia, 1992; British Pharmacopoeial Commission, 1993; United States Pharmacopeial Convention, 1994).

Phenytoin and its metabolites can be analysed in biological fluids by spectrophotometry (colorimetry), radioimmunoassay, enzyme-mediated immunoassay techniques, differential pulse polarography, GC, GC–mass spectrometry and high-performance liquid chromatography (Glazko, 1982; Maya *et al.*, 1992).

## 1.2 Production and use

### 1.2.1 *Production*

A method for preparing phenytoin sodium was first reported by Biltz in 1908 (Budavari, 1995).

Phenytoin and phenytoin sodium are prepared by treating benzaldehyde with sodium cyanide to form benzoin, which is oxidized to benzil with nitric acid or cupric sulfate. The benzil is then heated with urea in the presence of sodium ethoxide or isopropoxide, forming phenytoin sodium. Phenytoin sodium yields the base (phenytoin) on acidification of its aqueous solution (Gennaro, 1995).

In the United States of America, commercial production of phenytoin and phenytoin sodium was first reported in 1946 and 1938, respectively (United States Tariff Commission, 1939; 1948). Phenytoin (and/or its sodium salt) is currently available as a pharmaceutical in over 65 countries (Parke-Davis, 1995).

### 1.2.2 *Use*

Phenytoin is an anticonvulsant given orally (as phenytoin or phenytoin sodium) or by slow intravenous injection (as phenytoin sodium) in the treatment of epilepsy. Phenytoin exerts a stabilizing effect on excitable membranes of a variety of cells, including neurons and cardiac myocytes. It can decrease resting fluxes of sodium as well as sodium currents that flow during action potentials or chemically induced depolarizations (Jones & Wimbish, 1985). It is therefore used to control tonic-clonic (grand mal) and partial

(focal) seizures; it has also been used for the prophylactic control of seizures developing during and after neurosurgery or following severe traumatic injury to the head. It is believed to stabilize rather than elevate the seizure threshold and to limit the spread of seizure activity. Phenytoin also has antiarrhythmic properties, which were discovered only in the 1950s (Goodman Gilman *et al.*, 1990; Reynolds, 1993).

The dose of phenytoin is typically adjusted to the needs of the individual patient to achieve adequate control of seizures, preferably with monitoring of plasma concentration; in many patients, control requires total plasma phenytoin concentrations of 10–20 µg/mL (40–80 µmol/L), but some are satisfactorily controlled at concentrations outside this range. A suggested initial oral dose of phenytoin or phenytoin sodium is 100 mg three times daily progressively increased with care to 600 mg daily if necessary; the suggested interval between increments ranges from about one week to about one month. Particular care is required at higher doses, where saturation of metabolism may mean that a small increment produces a large rise in plasma concentration. The usual maintenance dose is 300–400 mg daily (Reynolds, 1993).

A suggested initial dose for children is 5 mg/kg bw daily in two or three divided doses; a suggested maintenance dose is 4–8 mg/kg bw daily in divided doses. Young children may require a higher dose per kilogram body weight than adults, due to more rapid metabolism (Reynolds, 1993).

In the treatment of tonic-clonic status epilepticus, a benzodiazepine such as diazepam is usually given intravenously first, followed by intravenous administration of phenytoin sodium. For adults, a suggested dose of phenytoin sodium is 10–15 mg/kg bw, given by slow intravenous injection at a uniform rate of not more than 50 mg/min; thereafter maintenance doses of 100 mg are given orally or intravenously every 6–8 h. The suggested intravenous dose for children and neonates ranges from 10 to 20 mg/kg bw at a rate not exceeding 1–3 mg/kg bw/min (Reynolds, 1993).

Phenytoin sodium is absorbed only very slowly from an intramuscular site, and intramuscular administration is appropriate only in certain situations (e.g., prophylactic control of seizures during neurosurgery) (Reynolds, 1993; Medical Economics, 1996).

Phenytoin is also a class Ib antiarrhythmic agent (see Glossary, p. 447); it is used in the treatment of cardiac arrhythmias, particularly those associated with digitalis intoxication. The usual dose is 3.5–5 mg/kg bw administered by slow intravenous injection at a uniform rate of not more than 50 mg/min; this dose may be repeated once if necessary. Phenytoin has also been used in the treatment of trigeminal neuralgia refractory to carbamazepine or in patients intolerant of carbamazepine (Reynolds, 1993).

Comparative data on sales of phenytoin in several countries are shown in Table 1. Worldwide, sales decreased by approximately 8% from 1990 to 1995, while United States prescriptions increased by about 10% (see Table 2 in the monograph on diazepam, p. 43).

## 1.3 Occurrence

### 1.3.1 *Natural occurrence*

Phenytoin is not known to occur as a natural product.

### 1.3.2 *Occupational exposure*

In several hospital dispensaries in Japan, dust in the air and on surfaces was collected on several work days and analysed by microscopy, TLC and mass spectrometry. Drugs of several types, including phenytoin, were identified but exposure levels were not quantified (Ichiba *et al.*, 1984, 1986; Rikihisa *et al.*, 1984).

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

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

| Country | 1990 | 1995 | Country | 1990 | 1995 |
|---|---|---|---|---|---|
| Africa | | | Australia | 61 508 | 54 340 |
| South Africa | 10 175 | 9 290 | Europe | | |
| North America | | | Belgium | 18 705 | 17 735 |
| Canada | 101 748 | 101 085 | France | 36 149 | 27 006 |
| Mexico | 103 204 | 96 439 | Germany | 91 567 | 77 451 |
| United States | 1 093 250 | 984 527 | Greece | 14 511 | 13 012 |
| South America | | | Italy | 46 206 | 39 557 |
| Argentina | 47 786 | 49 865 | Netherlands | 30 816 | 26 914 |
| Brazil | 131 010 | 98 831 | Portugal | 15 015 | 15 426 |
| Colombia | 29 063 | 27 253 | Spain | 75 760 | 77 944 |
| Venezuela | 15 570 | 19 655 | Sweden | 21 792 | 18 084 |
| Asia | | | Switzerland | 11 872 | 10 229 |
| Japan | 249 944 | 238 584 | Turkey | 24 125 | 44 232 |
| Republic of Korea | 6 146 | 5 439 | United Kingdom | 187 566 | 165 014 |

[a] Data provided by IMS

[b] Standard dosage units, uncorrected for phenytoin content

The National Occupational Exposure Survey conducted between 1981 and 1983 in the United States by the National Institute for Occupational Safety and Health indicated that approximately 23 400 and 11 850 employees were potentially occupationally exposed to phenytoin and phenytoin sodium, respectively. The estimate was based on a survey of United States companies and did not involve measurements of actual exposure (United States National Library of Medicine, 1996).

### 1.4 Regulations and guidelines

Phenytoin is listed in the following pharmacopoeias: Australian, British, Brazilian, Czech, French, German, Hungarian, International, Japanese, Mexican, Nordic, Romanian, Swiss, Turkish and United States (Reynolds, 1993; Vidal, 1995).

Phenytoin sodium is listed in the following pharmacopoeias: Australian, British, Brazilian, Chinese, Egyptian, European, French, German, Greek, Indian, International, Italian, Japanese, Mexican, Netherlands, Portuguese, Swiss, United States and former Yugoslavian (Reynolds, 1993; Vidal, 1995).

## 2. Studies of Cancer in Humans

### 2.1 Case reports

#### 2.1.1 *Lymphomas and leukaemias*

In early years, numerous case reports described the occurrence of lymphomas and leukaemias in patients treated with phenytoin (IARC, 1977, 1987). Between 1962 and 1980, reports appeared of 79 cases of lymphoma worldwide in patients taking phenytoin, with or without other antiepileptic drugs (Scoville & White, 1981). Some difficulties were encountered in distinguishing benign lymph node changes from lymphoma; however, the hydantoin-induced lymph node reactions regress after drug withdrawal (see Section 4.2.1). Saltzstein and Ackerman (1959) introduced the term 'pseudolymphoma' for this phenomenon (Halevy & Feuerman, 1977). Some patients originally described as developing 'pseudolymphomas' following phenytoin (and in some cases also phenobarbital) treatment were later diagnosed with lymphoma (Gams *et al.*, 1968). In 1968, the term pseudo-pseudolymphoma was introduced by Gams *et al.* (1968) to underline the possibility that what is initially considered a non-malignant phenytoin reaction may, after long-term follow-up, turn out to be a true lymphoma (Scoville & White, 1981).

Most of the malignant lymphomas described in case reports were seen after long-term phenytoin therapy rather than early in therapy. An additional case appeared as an isolated malignant lymphoma of the jejunum (Rubinstein *et al.*, 1985).

#### 2.1.2 *Childhood cancers*

Several case reports have suggested an association between childhood cancer and prenatal exposure to phenytoin. From 1976 to 1981, five cases of neuroblastoma were reported in infants and young children (up to five years of age) exposed *in utero* to phenytoin (Pendergrass & Hanson, 1976; Sherman & Roizen, 1976; Ramilo *et al.*, 1979; Allen *et al.*, 1980; Ehrenbard & Chaganti, 1981). These cases were also adversely affected by the fetal hydantoin syndrome (see Section 4.3.1(*b*)). After four cases had been described since 1976 in United States, Ehrenbard and Chaganti (1981) calculated that, within the United States, it should take 45 years for four to develop by chance. However, no more cases were reported until 1989 (Koren *et al.*, 1989). [The Working

Group noted that this may reflect a reporting bias, with clinicians lacking motivation to report additional cases of an 'established' phenomenon.] The last two cases (reported in 1989 and 1992) did not have major anomalies consistent with the fetal hydantoin syndrome (Koren *et al.*, 1989; Al-Shammri *et al.*, 1992).

Four cases of malignancies other than neuroblastoma in children exposed *in utero* to phenytoin have been reported (Blattner *et al.*, 1977; Taylor *et al.*, 1980; Jimenez *et al.*, 1981; Bostrom & Nesbit, 1983). In six of the 10 recorded cases of cancer associated with prenatal maternal phenytoin ingestion, the drug had not been given alone, but in combination with primidone or phenobarbital. Also, in some cases, there was evidence of alcohol abuse in the mother, which is associated with the fetal alcohol syndrome.

Koren *et al.* (1989) also examined 188 cases of childhood neuroblastoma diagnosed between January 1969 and October 1986 at the Hospital for Sick Children in Toronto, Canada. A review of medical records showed that none of the mothers or fathers had had epilepsy or been treated with phenytoin. In North America, the prevalence of epilepsy in the general population is 0.5%, and about half of all epileptic patients receive phenytoin. This serial cohort of 188 children indicates that, statistically, phenytoin cannot be incriminated in more than two cases of this series or in 1.5% of children with this malignancy in general.

## 2.2 Cohort studies

In a cohort study of members of the Kaiser Permanente Medical Care Program, described in the monograph on diazepam (pp. 44–45), phenytoin was used by 954 subjects (0.7%) (Selby *et al.*, 1989). Incidence of all cancers was marginally increased among phenytoin users (standard incidence ratio (SIR), 1.2 [95% confidence interval (CI), 0.9–1.5]; 61 cases), as were the incidences of brain cancer (SIR, 8.2 [95% CI, 3.3–17]; 7 deaths) and of oesophageal cancer (SIR, 5.0 [95% CI, 1.0–15]; 3 deaths). No other site showed a significant increase or decrease in cancer incidence. In an earlier study (Friedman, 1986), one case of multiple myeloma was reported in these subjects, with 0.6 expected. A case–control study of multiple myeloma in this population is described in Section 2.3.1.

Olsen *et al.* (1989) selected 8004 patients from all patients admitted for treatment of epilepsy to the Filadelfia treatment community in Denmark between 1933 and 1962 to compare cancer incidence with that in the general population. The patients had received powerful and prolonged treatment with anticonvulsants. During the 1940s, phenytoin became popular and was used alone (at daily doses of 100–400 mg) or in combination with phenobarbital. Time since hospitalization was used as a surrogate for cumulative drug exposure. To trace patients, the Central Population Register and mortality files were used (completeness of follow-up was > 90%). The follow-up lasted from 1943 to 1984. For each cancer site, expected numbers of cases were based on incidence rates in the general population by sex, five-year age group and calendar year. Tumour morphology and behaviour comparisons were made with population samples from Danish Cancer Registry records. For the 8004 patients (4246 men and 3758 women), the total person-years of follow-up amounted to 207 798 (average, 23.5 years). A total of 789 cancers

(based on 7864 patients, excluding 140 patients known to have received Thorotrast) were reported, compared with 663.7 expected (relative risk (RR), 1.2; 95% CI, 1.1–1.3). Site-specific relative risks are presented in Table 2. Significant excesses were found for cancers of the lung and brain and central nervous system. Brain cancer risk was highest within one year after admission and declined markedly with time. No overall cancer risk was apparent after excluding the brain cancer cases (RR, 1.03). Nonsignificant risk elevations were also observed for cancers of the liver and biliary tract and for non-Hodgkin lymphoma. Significant deficits of urinary bladder cancer and melanoma were observed. [The Working Group noted that brain tumours may account for the seizure disorder and are unlikely to be due to exposure to the anticonvulsant treatment and, furthermore, that the excess of liver cancer may be due to unrecorded exposure to Thorotrast. The slight excess of lung cancer is difficult to interpret, because no information was available on smoking history.]

## 2.3 Case–control studies

### 2.3.1 *Multiple sites*

Four nested case–control studies based on the cohort of Olsen *et al.* (1989) were conducted to investigate the possible influence of anticonvulsant treatment on the risk of lung cancer and urinary bladder cancer (including cases of bladder papillomas), lymphoma and hepatobiliary cancer (Olsen *et al.*, 1993, 1995). The studies included 104 cases of lung cancer, 18 cases of urinary bladder cancer, 26 cases of primary liver cancer, 13 cases of biliary tract cancer, 15 cases of non-Hodgkin lymphoma and six cases of Hodgkin's disease. Cases were individually matched to controls on the basis of sex, year of birth (± 1 year) and survival time. The matching ratio was 2, except in the study of lymphoma, in which five controls were selected per case. Six percent of cases and 5% of controls were excluded because of missing medical records. Information about detailed drug use was abstracted from the medical records at the epilepsy centre. Cumulative doses were computed by assuming that treatment continued daily at the prescribed dose after each hospital discharge until the date of cancer diagnosis (or equivalent date for matched controls) or until the end of 1964, whichever occurred first. After the mid-1960s, many new anticonvulsants were released, and therefore no credible assumptions about continuation of previous treatment after discharge were possible for the years after 1964. The median cumulative dose of phenytoin was 750 g. Risk estimates were adjusted for other anticonvulsant treatments, but not for smoking. The prevalence of phenytoin use (alone or in combination with another drug) was around 50% among controls. Use of phenytoin was not associated with the risk for either lung (RR, 1.0; 95% CI, 0.6–1.7) or urinary bladder cancer (RR, 1.1; 95% CI, 0.4–3.5). Also, dose–response analyses revealed no consistent relationships between either lung cancer or bladder cancer and cumulative exposure to phenytoin. Data from a smoking survey among Danish epileptics indicated that the elevated risk in the cohort is probably attributable to confounding by smoking (Olsen *et al.*, 1993). Epileptic patients who had ever been given phenytoin had a slight, nonsignificantly increased risk for lymphoma

**Table 2. Numbers of incident cancers occurring between 1943 and 1984 among patients hospitalized for epilepsy during 1933–62 at Filadelfia, Denmark, excluding 140 patients known to have received Thorotrast**

| Site | Observed | Observed/expected ratio (95% CI) |
|---|---|---|
| All malignant neoplasms | 789 | 1.2 (1.1–1.3) |
| Buccal cavity and pharynx | 18 | 1.2 (0.7–1.9) |
| Oesophagus | 7 | 1.3 (0.5–2.8) |
| Stomach | 33 | 1.0 (0.7–1.4) |
| Colon | 48 | 1.1 (0.8–1.4) |
| Rectum | 28 | 0.9 (0.6–1.2) |
| Liver (primary) | 9 | 1.9 (0.9–3.6) |
| Liver (not otherwise specified) | 2 | 0.8 (0.1–3.1) |
| Biliary tract | 11 | 1.7 (0.9–3.1) |
| Pancreas | 20 | 1.1 (0.7–1.7) |
| Larynx | 10 | 1.6 (0.8–2.9) |
| Lung | 106 | 1.4 (1.2–1.7) |
| Breast | 80 | 1.0 (0.8–1.2) |
| Cervix uteri | 32 | 0.8 (0.5–1.1) |
| Corpus uteri | 19 | 1.0 (0.6–1.5) |
| Ovary | 18 | 0.8 (0.5–1.3) |
| Prostate | 19 | 0.8 (0.5–1.3) |
| Testis | 13 | 1.6 (0.8–2.7) |
| Kidney | 18 | 1.0 (0.6–1.6) |
| Bladder | 18 | 0.6 (0.3–0.9) |
| Melanoma of skin | 7 | 0.5 (0.2–1.0) |
| Other skin | 60 | 1.0 (0.8–1.3) |
| Brain and central nervous system | 118 | 5.7 (4.7–6.8) |
| Thyroid | 4 | 1.2 (0.3–3.2) |
| Non-Hodgkin lymphoma | 16 | 1.4 (0.8–2.3) |
| Hodgkin's disease | 6 | 0.9 (0.3–2.1) |
| Multiple myeloma | 3 | 0.5 (0.1–1.5) |
| Leukaemia | 13[a] | 0.8 (0.4–1.4) |
| Other specified sites | 31 | 1.2 (0.8–1.8) |
| Secondary and unspecified sites | 22[b] | 1.9 (1.2–2.8) |

From Olsen *et al.* (1989)

[a] Seven patients, chronic lymphocytic leukaemia; three, chronic myelogenous leukaemia; one, acute leukaemia; and two, leukaemia not otherwise specified

[b] Excluding liver

(RR, 1.6; 95% CI, 0.5–4.8). The risk was highest for the subgroup of non-Hodgkin lymphoma (RR, 1.8; 95% CI, 0.5–6.6). In this subgroup, patients who had received a cumulative dose of 750 g phenytoin or more had a nonsignificantly higher risk than patients who had been given less than 750 g (RR, 3.1; 95% CI, 0.6–15; versus RR, 1.0; 95% CI, 0.2–4.9). [The Working Group noted that the number of non-Hodgkin lymphoma patients in the study was very small.] The risk for hepatobiliary cancer was not increased among patients ever treated with phenytoin (RR, 1.2; 95% CI, 0.5–3.1) (Olsen *et al.*, 1995).

### 2.3.2 *Multiple myeloma*

A case–control study was conducted to investigate (among other factors) the relationship between multiple myeloma and medicinal drugs, such as propoxyphene and phenytoin (Friedman, 1986). A total of 327 cases of multiple myeloma occurring during 1969–82 were identified among Kaiser Foundation Health Plan subscribers in northern California (United States) (96.6% based on pathological examination and 3.4% on strong clinical evidence). Controls (one per case) were selected from the Health Plan membership file of the same year as the case's year of first hospitalization for multiple myeloma, matched to the case by sex, year of birth, area of residence and, when feasible, race. No association was found between phenytoin use and multiple myeloma risk. [The Working Group noted that RR and 95% CI were not reported].

In the hospital-based, case–control study of multiple myeloma in whites in Baltimore area, MD, United States, in 1975–82, reported in the monograph on diazepam (p. 53), a nonsignificantly elevated risk was found for prior use of phenytoin (odds ratio, ∞; 95% CI, 0.6–∞; discordant pairs ratio: 3/0) (Linet *et al.*, 1987). [The Working Group noted that the diagnostic categories for controls could have been associated with exposure to phenytoin. In addition, the greater number of proxy interviews for cases than for controls could not be adjusted for with phenytoin as the exposure.]

### 2.3.3 *Hodgkin's disease*

A case–control study was conducted to investigate a number of risk factors for Hodgkin's disease in Brazil (Kirchhoff *et al.*, 1980). Cases were patients diagnosed from 1963 to 1976 at one hospital and were divided into three groups: private, self-paying patients; patients whose care was paid for by the national health insurance programme; and patients who were treated without charge. Out of a total of 546 patients, 70 patients of the last two groups were included in this study. A control group was made up of 128 siblings of the Hodgkin's disease patients. All of the patients and controls whose cooperation was solicited agreed to participate in the study. Exposure to phenytoin was assessed by asking whether the subjects had ever taken medicine for epilepsy, convulsions, fits or migraine headaches. The mean age of the cases was 27 years. For the sibling control group, the mean age was 22 years. Phenytoin had been used by 4% of the cases and 3% of the sibling controls. [The crude odds ratio for phenytoin use was 1.4; 95% CI, 0.3–6.4.] [The Working Group noted that the criteria for selection of cases were unclear.]

#### 2.3.4 *Childhood cancer*

In a case–control study of 11 169 pairs of childhood cancer cases and matched controls in 1953–71 in the United Kingdom, use of drugs during pregnancy was assessed through hospital and general practitioners' records (Sanders & Draper, 1979). Phenytoin use was reported for mothers of 11 cases and 7 controls [crude odds ratio, 1.6; 95% CI, 0.6–4.0].

In a case–control study of incident cases of neuroblastoma diagnosed between 1970 and 1979 in the United States, drug use during pregnancy was investigated among mothers of 104 cases and 101 controls selected by telephone random digit dialling (Kramer *et al.*, 1987). No use of phenytoin during the index pregnancy was reported among mothers of cases or controls.

## 3. Studies of Cancer in Experimental Animals

### 3.1 Oral administration

#### 3.1.1 *Mouse*

Groups of 48 female C57Bl, C3H/F or SJL/J mice, two to three months of age, were given 0 or 60 mg/kg bw phenytoin sodium (Dilantin) (pharmaceutical grade) in a liquid diet for 168 days. Of those surviving to 10 months, 3/24 treated C57Bl mice as well as 3/24 C3H/F mice developed thymic lymphosarcomas, whereas no pathological lesion was found in 48 controls [$p = 0.03$]. Of the treated SJL/J mice, 6/42 had generalized lymphomas during the fourth to eight months, but no lymphoma was seen in 48 controls after eight months [$p = 0.008$]. After the eighth month, 90% of the treated SJL/J mice developed reticulum-cell sarcomas which appeared 2–3 months earlier than in the controls (Krüger *et al.*, 1972).

Groups of 30 and 23 female C3H/Sn mice, 3.5 months of age, were given 0 or 2 mg/animal phenytoin sodium (Diphenin) (pharmaceutical grade) in 0.2 ml tap-water by gastric instillation five times per week for life. The life span of treated mice was significantly increased (mean: controls, 450 ± 19 days; treated, 558 ± 28 days; $p < 0.05$, Student's $t$ test). The number of mice with mammary gland adenocarcinomas was significantly decreased (19/30 controls, 7/23 treated mice; $p < 0.05$, chi-square test); the numbers of mice with leukaemia were 5/30 controls and 2/23 treated mice; and the numbers of mice with polyps of the endometrium [not otherwise specified] were 4/30 controls and 0/23 treated mice (Dilman & Anisimov, 1980).

Groups of 50 male and 50 female B6C3F1 mice, six weeks old, were given 0, 60 or 120 mg/kg diet (ppm) phenytoin (purity, > 99%) in the diet for 78 weeks. The estimated mean total intake per mouse was 150 mg or 301 mg in males and 154 mg or 292 mg in females. The mice were kept for a further eight weeks, after which time all surviving animals were killed. Survival at week 86 was: males — 86% control, 72% low-dose and 82% high-dose; and females — 94% control, 88% low-dose and 86% high-dose. All major organs and visually apparent lesions were examined histologically. In male mice,

the incidence of hepatocellular adenomas was decreased (19/46 control, 12/45 low-dose and 11/45 high-dose); that of hepatocellular carcinomas was 7/46 control, 8/45 low-dose and 5/45 high-dose. The decreased incidence of combined hepatocellular adenomas and carcinomas was statistically significant (26/46 control, 20/45 low-dose and 16/45 high-dose; $p < 0.05$, chi-square test). There was no significant difference in the incidence of tumours at other sites between treated and control males. No increase in tumours was seen in females (Maeda *et al.*, 1988).

#### 3.1.2 *Rat*

In a number of early carcinogenicity studies of phenytoin of limited duration, negative results were reported (Griswold *et al.*, 1966, 1968; McDonald, 1969; Morris *et al.*, 1969; Peraino *et al.*, 1975). [The Working Group considered that these studies were inadequate for assessing carcinogenicity.]

Groups of 75 and 34 female rats [strain not specified], 3.5 months of age, were given 0 and 7.5 mg/rat phenytoin [purity not specified] in 5 mL tap-water by gastric instillation five times per week for life. Animals were killed when moribund. Neoplastic tissues were examined histologically. Mean life span was 681 ± 14 days in controls and 724 ± 36 days in treated animals. No treatment-related increase in tumour incidence was observed (Anisimov, 1980).

Groups of 50 male and 50 female Fischer 344/DuCrj rats, five weeks of age, were given 0, 250 or 500 (maximum tolerated dose) mg/kg diet (ppm) phenytoin (> 99% pure) in the diet for two years. After a further eight weeks, survival was greater than 60% in all groups, and all surviving rats were killed at that time. There was a dose-related decrease in mean body weight in treated females but not in males. All rats that died or were killed were autopsied. All lesions and most organs were examined histologically. There was no significant difference in tumour incidence between treated and control rats (Jang *et al.*, 1987).

### 3.2 Perinatal and/or adult administration

#### 3.2.1 *Mouse*

Groups of 60 female C57Bl/6N and 60 male C3H/HeN mice were exposed perinatally ($F_0$), as adults (B6C3F1 mice) ($F_1$) or both to various concentrations of phenytoin, as shown in Table 3. Four groups of 60 females aged 10–14 weeks were given 0, 21, 70 or 210 mg/kg diet (ppm) phenytoin (approximately 98% pure) in the diet for one week before breeding. After breeding with previously unexposed male mice, the females continued to receive the same diet throughout pregnancy and lactation. Weaning occurred on day 28 post-partum and dietary exposure of the pups continued at the same concentrations until they were approximately eight weeks of age. From eight weeks of age, groups of 60 male and 60 female pups were fed diets containing 0, 30, 100 or 300 ppm (males) and 0, 60, 200 or 600 ppm phenytoin (females), respectively, for up to two years (perinatal/adult exposure). The highest dose was set on the basis of body weight changes in prechronic studies. Additional groups of 60 male and 60 female adults, 10–14 weeks of age, were given diets containing 0, 100 or 300 ppm and 0, 200 or 600 ppm

**Table 3. Incidence of hepatocellular adenoma or carcinoma in mice exposed to phenytoin in the diet**

| Concentration (ppm) | | Liver tumour incidence | | |
|---|---|---|---|---|
| $F_0$ | $F_1$ | Adenoma | Carcinoma[a] | Adenoma or carcinoma[a] |
| **Males** | | | | |
| *Adult exposure groups* | | | | |
| 0 | 0[b] | 19/50 | 13/50 | 29/50 |
| 0 | 100 | 19/49 | 15/49 | 29/49 |
| 0 | 300 | 22/49 | 7/49 | 26/49 |
| *Perinatal/adult exposure group* | | | | |
| 21 | 30 | 16/50[c] | 13/50[c] | 25/50[c] |
| 70 | 100 | 20/50 | 18/50 | 31/50 |
| 210 | 100 | 23/49 | 18/49 | 35/49 |
| 210 | 300 | 31/50[c,d] | 20/50 | 41/50[c,d] |
| *Perinatal exposure group* | | | | |
| 210 | 0 | 23/50 | 14/50 | 33/50 |
| **Females** | | | | |
| *Adult exposure groups* | | | | |
| 0 | 0[b] | 5/48 | 0/48 | 5/48 |
| 0 | 200 | 13/49[d] | 1/49 | 14/49[d] |
| 0 | 600 | 22/50[c] | 12/50[c] | 30/50[c] |
| *Perinatal/adult exposure groups* | | | | |
| 21 | 60 | 11/50[c] | 4/50 | 13/50[c,d] |
| 70 | 200 | 25/50[c,f] | 3/50 | 26/50[c,f] |
| 210 | 200 | 12/50 | 4/50 | 16/50[c,f] |
| 210 | 600 | 26/50[c,f] | 10/50[b,e] | 34/50[c,f] |
| *Perinatal exposure group* | | | | |
| 210 | 0 | 11/49 | 1/49 | 12/49[g] |

From United States National Toxicology Program (1993)

[a] For adult exposure: carcinoma is hepatoblastoma or hepatocellular carcinoma

[b] Historical control rates at the laboratory: males — 167/410 (40%; range, 17–68%); females — 56/416 (13%; range, 3–26%) (Chhabra *et al.*, 1993)

[c] From Chhabra *et al.* (1993)

[d] Significantly different ($p < 0.05$) from the 0:0 group (logistic regression analysis)

[e] Significantly different ($p < 0.001$) from the 0:0 group (logistic regression analysis)

[f] Significantly different ($p \leq 0.01$) from the 0:0 group (logistic regression analysis)

[g] Not significantly different ($p = 0.055$) from the 0:0 group (logistic regression analysis)

phenytoin for two years. After nine months of phenytoin administration, 10 animals from each group were killed. Surviving animals were killed at the end of the two-year study period. Survival at that time was similar in control (males, 78%; females, 72%) and treated groups (66–88%). Incidences of hepatocellular tumours are summarized in Table 3. In male mice, perinatal exposure alone did not increase the incidence of liver tumours. However, there was a significant $F_0 : F_1$ interaction, which reflected the enhancing effects of combined 210 ppm perinatal treatment and 300 ppm adult treatment on the incidences of liver neoplasms. In female mice, both adult exposures and perinatal plus adult exposure resulted in increased incidence of liver tumours (United States National Toxicology Program, 1993).

#### 3.2.2 *Rat*

Groups of 60 female and 60 male Fischer 344/N rats were exposed perinatally ($F_0$), as adults ($F_1$) or both to various concentrations of phenytoin, as shown in Table 4. Four groups of 60 females aged 10–12 weeks were given 0, 63, 210 or 630 mg/kg diet (ppm) phenytoin (approximately 98% pure) in the diet for one week before breeding. After breeding with previously unexposed male rats, the females continued to receive the same diet throughout pregnancy and lactation. Weaning occurred on day 28 post-partum and dietary exposure of pups continued at the same concentrations until they were approximately eight weeks of age. From eight weeks of age, groups of 60 male and 60 female pups were fed diets containing 0, 240, 800 or 2400 ppm phenytoin for up to two years (perinatal/adult exposure). The highest dose was set on the basis of body weight changes in prechronic studies. Additional groups of 60 male and 60 female adults, 10–12 weeks of age, were given diets containing 0, 800 or 2400 ppm phenytoin for two years. After nine months of phenytoin administration, 10 animals from each group were killed. Surviving animals were killed at the end of the two-year study period. Survival at that time was similar in control (males, 52%; females, 62%) and treated groups (44–76%). Incidences of hepatocellular tumours (adenoma and carcinoma combined) are summarized in Table 4. In male rats, the incidence of liver tumours was slightly increased in the high-dose perinatal/adult treatment group ($p < 0.05$, logistic regression analysis) (US National Toxicology Program, 1993).

### 3.3 Intraperitoneal administration

*Mouse*: A group of 50 male and female random-bred albino mice, weighing 18–20 g [age not specified], was given 0.6 mg/animal phenytoin as phenytoin sodium [purity not specified] suspended in water or in saline daily by intraperitoneal injection over a 66-day period (total of 57 injections; 34.2 mg/animal). No weight gain was observed and 10 animals died during this period. The remaining 40 animals were observed for nine months. A group of 50 untreated controls was observed for 11 months. Ten treated mice developed tumours, comprising four thymic and two mesenteric lymphomas and four leukaemias. The leukaemias were found between 60 and 142 days and the lymphomas between 100 and 255 days. In controls, one thymic lymphoma and one lung adenoma were observed (Juhász *et al.*, 1968).

**Table 4. Incidence of hepatocellular adenoma and carcinoma combined in rats exposed to phenytoin in the diet**

| Concentration (ppm) | | Liver tumour incidence | |
|---|---|---|---|
| $F_0$ | $F_1$ | Male | Female |
| *Adult exposure groups* | | | |
| 0 | 0[a] | 0/50 | 0/50 |
| 0 | 800 | 2/50 (1 carcinoma) | 1/50 |
| 0 | 2400 | 4/50 | 1/50 |
| *Perinatal/adult exposure groups* | | | |
| 63 | 240 | 3/49 (1 carcinoma) | 0/50 |
| 210 | 800 | 2/49 | 1/50 |
| 630 | 800 | 1/49 | 0/50 |
| 630 | 2400 | 5/49[b] | 0/50 |
| *Perinatal exposure group* | | | |
| 630 | 0 | 1/50 | 0/49 |

From United States National Toxicology Program (1993)
[a] Historical control rates at the laboratory: males — 6/302 (2%; range 0–10%); females — 0/300 (Chhabra *et al.*, 1993)
[b] Significantly different ($p < 0.05$) from the 0:0 group (logistic regression analysis) (Chhabra *et al.*, 1993)

### 3.4 Administration with known carcinogens

*Mouse*: In a short-term assay based upon lung adenoma induction in mice, three groups of SWR inbred mice [age and sex not specified] were given 0.5 mg/g bw urethane [purity not specified] in water as a single intraperitoneal injection. Two groups also received seven daily subcutaneous injections either of 0.5 mg/animal phenytoin [purity and solvent not specified] or of the solvent only before and after the administration of urethane, while one group received no further treatment. Twelve weeks after the injection of urethane, all animals were killed and their lungs were examined macroscopically for the presence of adenomas. The incidence of lung adenomas was 100% in the urethane-treated controls (15 mice) and in animals treated with urethane plus solvent (15 mice) and was 85.7% in animals treated with urethane plus phenytoin (14 mice). The number of adenomas per mouse was reduced in the phenytoin-treated group (mean ± SE : 4.5 ± 0.6, 4.7 ± 0.6 and 2.9 ± 0.6 ($p < 0.025$, Student's $t$ test) in the three groups, respectively) (Levo, 1974).

Groups of 25 male D2B6F1 mice, five weeks of age, were given 0 or 90 mg/kg bw *N*-nitrosodiethylamine (NDEA; 99% pure) in tricaprylin as a single intraperitoneal injection. Two weeks later, the mice were given 0, 125, 250 or 500 mg/kg diet (ppm) phenytoin [purity not specified] in the diet. Ten mice from each group were killed at 30 weeks of age, at which time the incidence of hepatocellular lesions (foci) in NDEA-

treated mice was 2 (lesions)/10, 4 (eosinophilic foci)/10, 0/10 and 10 (lesions)/10 ($p < 0.01$, Mann-Whitney U test) in the groups treated with 0 (control), 125, 250 and 500 ppm phenytoin, respectively. Three NDEA-treated mice receiving 500 ppm phenytoin developed hepatocellular adenomas. Control mice and mice given only phenytoin displayed no hepatocellular lesions at 30 weeks. All surviving mice were killed at 60 weeks of age. Selected portions of liver lobe (two sections per lobe), all liver lesions and lesions in other organs were examined histologically. Survival was not affected by phenytoin in NDEA-treated or vehicle-treated mice. Phenytoin enhanced the hepatocarcinogenesis initiated by NDEA at week 60 (see Table 5). No significant difference in either incidence or multiplicity of lung adenomas was observed between the groups (Diwan *et al.*, 1993).

**Table 5. Effect of phenytoin on hepatocellular carcinogenesis initiated by *N*-nitrosodiethylamine (NDEA) in mice**

| Treatment groups | No. of mice with tumours/total no. at risk (%)[a] | No. of tumours per tumour-bearing mouse (mean ± SD) | | |
|---|---|---|---|---|
| | | Adenomas | Carcinomas | Hepato-blastomas |
| NDEA | 9/15 (60) | 1.8 ± 0.8 | 0 | 0 |
| NDEA/125 ppm phenytoin | 10/14 (71) | 2.6 ± 1.3 | 0 | 0 |
| NDEA/250 ppm phenytoin | 10/13 (77) | 4.4 ± 3.3[b] | 0 | 0 |
| NDEA/500 ppm phenytoin | 15/15 (100)[b] | 11.6 ± 5.6[c] | 2.3 ± 2.2 | 1.4 ± 0.5 |
| Vehicle/250 ppm phenytoin | 1/15 (7) | 1 ± 0 | 0 | 0 |
| Vehicle/500 ppm phenytoin | 1/15 (7) | 1 ± 0 | 0 | 0 |
| Vehicle | 0/15 | 0 | 0 | 0 |

From Diwan *et al.* (1993)
[a] Animals with severe post-mortem changes were not included
[b] Statistically significant compared with NDEA group ($p < 0.05$, Fisher's exact test)
[c] Statistically significant compared with NDEA group ($p < 0.001$, Fisher's exact test)

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

## 4.1 Absorption, distribution, metabolism and excretion

### 4.1.1 *Humans*

Phenytoin is well absorbed following oral dosing with well formulated pharmaceutical preparations (capsules, tablets, suspensions); absorption occurs predominantly in the duodenum, from which the absorption rate is limited by its dissolution in intestinal fluids (solubility approximately 100 µg/mL) and plasma (75 µg/mL). Peak plasma concentrations are usually reached within 4–8 h. The drug is widely distributed in the body

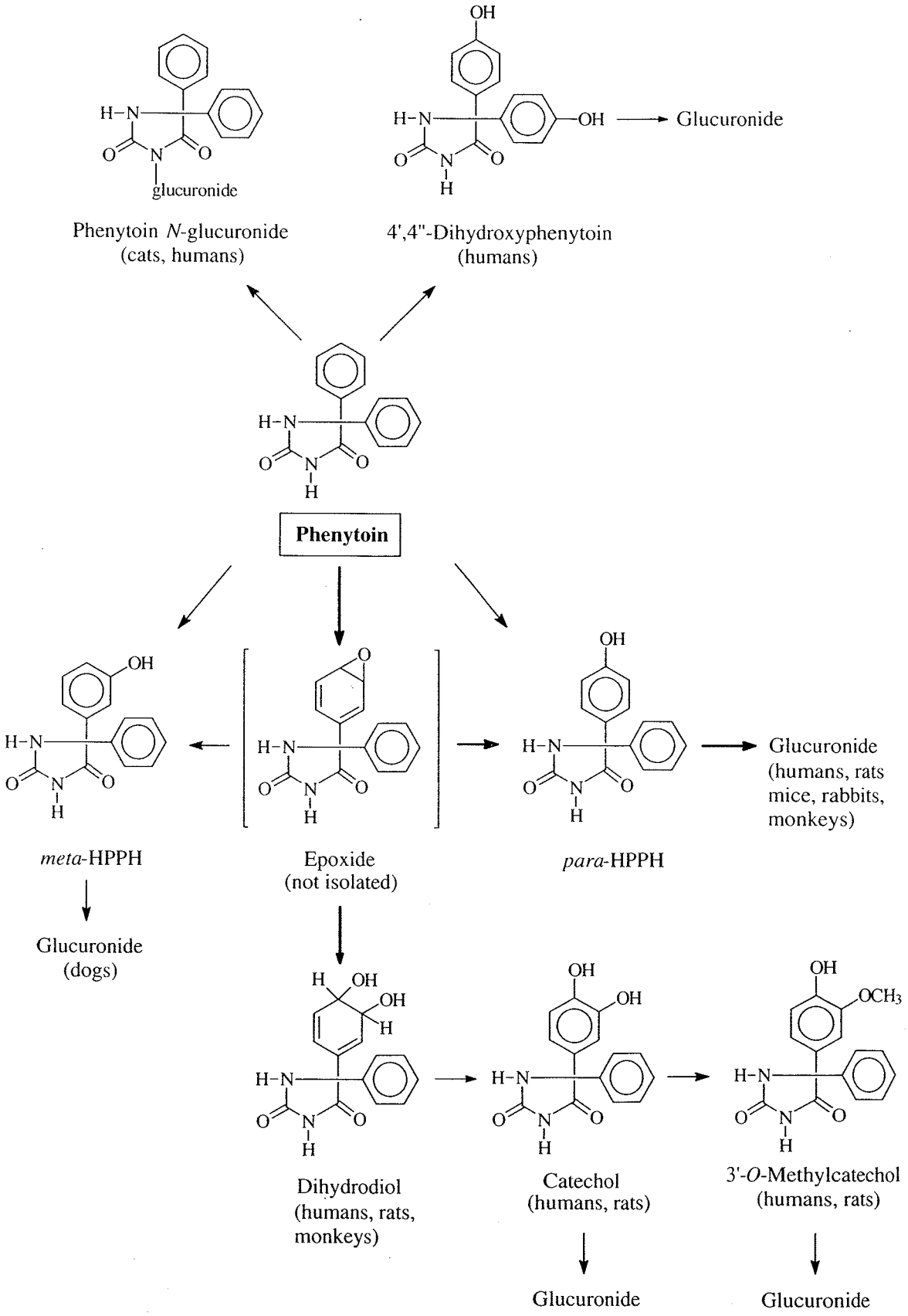
(volume of distribution averages about 0.8 L/kg), readily crosses the blood–brain barrier and is bound extensively (~90%) to plasma protein, predominantly albumin (Treiman & Woodbury, 1995). Phenytoin is metabolized extensively (Figure 1), principally to 5-(4′-hydroxyphenyl)-5-phenylhydantoin (*para*-HPPH), which is excreted as the glucuronide conjugate in the urine, typically accounting for 67–88% of the dose. This is formed via the well known epoxide-diol pathway, like the 5-(3′,4′-dihydrodiol), which is also found in urine, representing some 7–11% of the dose (Browne & LeDuc, 1995). The arene oxide, which is the primary metabolite on this pathway, has never been isolated but its occurrence is inferred. This class of metabolic intermediates has attracted considerable attention because of their possible role in the mechanisms of toxicity, mutagenicity and teratogenicity (see Section 4.3.3) (Van Dyke *et al.*, 1991; Finnell *et al.*, 1992). In addition to the two major excretion products mentioned, a number of minor metabolites including the 3′,4′-catechol, the corresponding 3′-*O*-methylcatechol, a bis-(4-hydroxyphenyl) derivative and the *N*-glucuronide of phenytoin have been identified. These never account for more than trace quantities of a phenytoin dose in humans. The *meta*-isomer of HPPH (namely the 5-(3′-hydroxyphenyl) derivative) has been reported (Browne & LeDuc, 1995), but this may be an analytical artefact. Early workers also reported the identification of two ring-opened products, diphenylhydantoic acid and α-aminodiphenylacetic acid, as minor metabolites. Since phenytoin is prochiral, any metabolic change in one of the phenyl substituents will give rise to chiral derivatives. In humans, the *para*-HPPH excreted in urine is predominantly the *S*-enantiomer (the $S:R$ ratio is typically about 3 : 1) (Poupaert *et al.*, 1975; Maguire *et al.*, 1980; Browne & LeDuc, 1995).

There has recently been considerable study of the cytochrome P450 isoforms responsible for the hydroxylation of phenytoin. Doecke *et al.* (1990) suggested that isoforms of the CYP2C subfamily were responsible for phenytoin metabolism in rabbits and humans, and recent work by Veronese *et al.* (1993) strongly suggests that CYP2C9/10 isoforms are responsible for most of the *para*-HPPH formation in humans.

Phenytoin displays non-linear elimination pharmacokinetics which are adequately described by a model based on the Michaelis–Menten equation (Browne & LeDuc, 1995). One consequence of this non-linearity is that the apparent elimination half-life varies with plasma concentration. Strictly speaking, it is inappropriate to refer to a 'half-life', but in practical terms a mean value of around 22 h is a useful guide. It seems clear that the non-linearity in the elimination of phenytoin results from saturation of the enzyme(s) responsible for the formation of the arene oxide metabolite.

Phenytoin crosses the placenta (Mirkin, 1971). Similar concentrations have been measured in maternal and umbilical cord plasma at delivery, indicating an equilibrium state after long-term administration (Nau *et al.*, 1982). Neonatal elimination of placentally transferred phenytoin was very slow on post-partum days 1 and 2, increased markedly on post-partum day 3 and was complete by post-partum day 5. Phenytoin levels are low in breast milk obtained from epileptic mothers (Mirkin, 1971). The maternal serum level falls during the second and third trimesters of pregnancy (Eadie *et al.*, 1977; Nau *et al.*, 1982). Free and total plasma levels of phenytoin were determined

**Figure 1. Postulated metabolic pathways of phenytoin**




From Browne & Leduc (1995)

in a prospective study of 29 pregnant women receiving phenytoin monotherapy. Total clearance of plasma phenytoin increased from the first trimester and a less pronounced, but significant, increase was observed for clearance of free phenytoin during the third trimester (Tomson *et al.*, 1994). In a study of 10 epileptic women, the proportion of the phenytoin dose excreted as *para*-HPPH tended to rise during pregnancy and overall the increased excretion of *para*-HPPH appeared sufficient to account for the elimination of the entire increase in the daily dose of phenytoin required during pregnancy. Excretion of unmetabolized phenytoin and certain of its minor metabolites appeared essentially unaltered. Thus pregnancy does not enhance uniformly the various pathways of phenytoin metabolism (Eadie *et al.*, 1992). The plasma level of phenytoin returns to normal values during the post-partum period (Lander *et al.*, 1977).

#### 4.1.2 *Experimental systems*

*para*-HPPH glucuronide appears to be the major metabolite in all species studied (e.g., rats, mice, rabbits, monkeys) except dogs, in which the major excretion product is the glucuronide of *meta*-HPPH (Atkinson *et al.*, 1970), and cats, in which the *N*-glucuronide of phenytoin predominates (Hassell *et al.*, 1984). All of the major metabolites found in humans have been identified in most of the animal species studied, although there are some interspecies differences in the relative quantities and in the extent of conjugation (Browne & LeDuc, 1995).

A single intragastric dose of phenytoin to pregnant Sprague-Dawley rats was transferred to the fetuses and was concentrated more in the kidney than in the liver, in contrast to the situation in adult rats (Gabler & Falace, 1970). Transplacental transport has been documented in mice (Waddell & Mirkin, 1972; Stevens & Harbison, 1974), rats (Mirkin, 1971; Stevens & Harbison, 1974), Syrian hamsters (Stevens & Harbison, 1974), goats (Shoeman *et al.*, 1972) and rhesus monkeys (Gabler & Hubbard, 1972).

Studies with CYP2C3 purified from rabbit liver have shown that this enzyme plays a major role in the 4′-hydroxylation of phenytoin, and further suggested than an orthologue to this enzyme was in part responsible for this metabolic reaction in humans (Doecke *et al.*, 1990). The hydroperoxidase component of prostaglandin synthetase, as well as thyroid peroxidase and other peroxidases, can bioactivate phenytoin to a reactive free radical intermediate, which may have toxicological relevance (Kubow & Wells, 1989). Whether this free radical or the arene oxide intermediate is the principal mediator of toxic effects remains unclear.

### 4.2 Toxic effects

#### 4.2.1 *Humans*

##### (*a*) *Acute toxicity*

Death from acute phenytoin overdosage in humans is very uncommon (reviewed by Dollery *et al.*, 1991). Intravenous administration of high doses of phenytoin (e.g., in the emergency treatment of cardiac arrhythmias or status epilepticus) may induce severe cardiac arrhythmias and hypotension as well as central and peripheral nervous system

toxicity. Nystagmus, ataxia, diplopia, dysarthria, vertigo and other cerebellar-vestibular effects are common symptoms of phenytoin intoxication; acute oral overdosage has been reported to cause irreversible cerebellar atrophy (Earnest *et al.*, 1983; Masur *et al.*, 1989, 1990; Reynolds, 1993). Since phenytoin has a narrow therapeutic range and patients respond with considerable interindividual variability, intoxications are frequently iatrogenic or due to inappropriate self-adjustment of doses (Manon-Espaillat *et al.*, 1991).

Phenytoin is a potent inducer of certain hepatic cytochrome P450 activities, increasing the clearance of antipyrine, dicoumarol, primidone, carbamazepine, prednisolone, dexamethasone and other glucocorticoids (Nation *et al.*, 1990a,b; Dollery *et al.*, 1991).

### (*b*) *Chronic toxicity*

#### (i) *Effects on the nervous system*

Chronic phenytoin intake may induce a variety of adverse effects on the nervous system, including drowsiness, nystagmus, ataxia, blurred vision and diplopia; in the past when patients were treated with very high doses for many years, intellectual dulling and depression of mood were frequently reported (reviewed by Dollery *et al.*, 1991).

Although central and peripheral nervous system toxicity is the most consistent effect of acute phenytoin overdosage, the chronic effects of phenytoin on the nervous system are a subject of debate, since it remains unclear if they can be attributed solely to the drug or if and to what extent they result from recurrent seizures. The most severe histopathological feature associated with chronic phenytoin administration is development of cerebellar atrophy. However, literature on neuropathology from before the introduction of phenytoin documents that convulsions are associated with cerebellar atrophy. The molecular mechanism of phenytoin damage to the cerebellum has not been elucidated and the causal role of phenytoin in the induction of cerebellar atrophy cannot be considered as proven (Ghatak *et al.*, 1976; Rapport & Shaw, 1977; McLain, *et al.*, 1980; Hammond & Wilder, 1983; Ney *et al.*, 1994).

A rare, albeit well documented complication of phenytoin administration is the induction of dyskinesias consisting mainly of generalized choreoathetosis with or without orofacial symptoms. Phenytoin has been shown to interfere with the dopaminergic system; however, the precise mechanism remains to be elucidated (Harrison *et al.*, 1993).

#### (ii) *Gingival overgrowth*

Phenytoin-induced gingival overgrowth was first reported in 1939 (Kimball, 1939); later studies revealed extremely high incidences of up to 50% of gingival hyperplasia in patients medicated with phenytoin (Hassell, 1981). However, these high incidences were based mainly on investigations carried out in hospital patients usually taking several anticonvulsant drugs because they experienced complications with the control of their epilepsy (Angelopoulos, 1975). A recent community-based study of patients in general medical practice revealed a 13% incidence of phenytoin-induced, clinically significant gingival overgrowth (Thomason *et al.*, 1992).

Gingival overgrowth can commence within three months after initiation of phenytoin therapy and is most rapid in the first year of medication. Early signs often arise as diffuse swelling of the interdental papillae; in severe cases, the overgrowth may induce impairment of speech and eating (Thomason *et al.*, 1992; Seymour, 1993). There is no strict dose–effect relationship. Polypharmacy significantly increases the prevalence of gingival overgrowth, especially co-medication with sodium valproate, phenobarbital and carbamazepine, which can result in alterations in phenytoin kinetics (Maguire *et al.*, 1986). The overgrowth of tissue appears to involve altered collagen metabolism, which in rare cases and after many years of treatment may result in facial coarsening (Ohta *et al.*, 1995), mainly manifested as broadening of the lips and nose. Rodents and other small mammals do not appear to be susceptible to phenytoin-induced gingival overgrowth, but this side-effect can be reproduced in the monkey *Macaca arctoides* (Staple *et al.*, 1977) and cats (Hassell *et al.*, 1982; Thomason *et al.*, 1992).

Studies on the mechanisms underlying phenytoin-induced gingival hyperplasia show that fibroblasts derived from patients with phenytoin-associated gingival hyperplasia synthesize a higher amount of total protein and collagen and have higher proliferation rates compared with control cells or with cells from patients with idiopathic gingival fibromatosis (Hou, 1993). Phenytoin inhibits calcium transport and, since gingival hyperplasia is also induced by nifedipine and other calcium-channel blockers, it has been suggested that interference with calcium transport may be involved in the gingival overgrowth (Fujii *et al.*, 1994).

(iii) *Folic acid deficiency*

Patients taking phenytoin may exhibit folic acid deficiency (Mallek & Nakamoto, 1981; Reynolds, 1993). In addition to increasing the risk for developing megaloblastic anaemia, folic acid deficiency has been implicated in the development of gingival hyperplasia by impairing maturation of the gingival epithelium, thus rendering the underlying connective tissue more susceptible to inflammation and increased proliferation.

(iv) *Hypersensitivity reactions — effects on the immune system*

Phenytoin has been associated with numerous cutaneous hypersensitivity reactions. Morbilliform rash arises in 2–5% of patients and in rare cases more severe reactions may be observed, such as toxic epidermal necrolysis (Stevens–Johnson syndrome) or lupus erythematosus. The skin reactions may be accompanied by lymphadenopathy, eosinophilia, fever, diverse haematological disorders (leukocytosis, leukopenia, thrombocytopenia) and systemic organ manifestations such as hepatitis or nephritis (Reynolds, 1993). This hypersensitivity syndrome complex, referred to as the phenytoin hypersensitivity syndrome, usually appears early (e.g., two to four weeks) after initiation of treatment, and does not exhibit a dose–response relationship (Delattre *et al.*, 1988; Braddock *et al.*, 1992).

In some patients exhibiting the triad of skin reactions, fever and lymphadenopathy, biopsy specimens of enlarged lymph nodes display a broad spectrum of histopathological changes, ranging from benign hyperplasia to malignant-appearing cells (Saltzstein & Ackerman, 1959; Hyman & Sommers, 1966; Anthony, 1970). The possible effects of

phenytoin on the lymphatic tissue can be categorized into four groups, although some symptoms may overlap: benign lymphoid hyperplasia, pseudolymphoma, pseudo-pseudolymphoma and malignant lymphoma (see Section 2.1.1) (Harris *et al.*, 1992).

Lymphoid hyperplasia and pseudolymphoma are clinically indistinguishable; however, histological examination reveals normal lymph node structure in the hyperplasia group, while loss of normal lymph node architecture, focal necrosis and infiltration with eosinophils are seen in the pseudolymphoma group. Development of pseudolymphoma has been reported in a few hundred patients receiving normal doses of phenytoin (100 mg daily) and, in view of the many hundreds of thousands of treated patients, can be considered as a rare, albeit severe, side-effect (see Section 2.1.1). The clinical symptoms develop within an average of three weeks, five days to three months after initiation of therapy. In addition to high spiking fever and intensely pruritic maculopapular rash (sometimes with pustulation progressing to exfoliative erythroderma), marked localized or generalized enlargement of lymph nodes is observed. Myalgia and arthralgia as well as hepatitis and in rare cases splenomegaly and histological skin features suggestive of mycosis fungoides have also been reported in some patients. The symptoms resolve in most cases after withdrawal of the drug and recurrence is usually observed upon readministration (Gams *et al.*, 1968; Rosenthal *et al.*, 1982; Cooke *et al.*, 1988; Rijlaarsdam *et al.*, 1991; Singer *et al.*, 1993).

(v) *Endocrine effects*

A variety of endocrine effects have been associated with chronic phenytoin administration. Osteomalacia with hypocalcaemia (Dollery *et al.*, 1991) and elevated alkaline phosphatase activity may arise from altered vitamin D metabolism, inhibition of intestinal absorption of calcium and reduced concentrations of vitamin K, which is important for normal calcium metabolism in the bone (Keith *et al.*, 1983; Kumar *et al.*, 1993). Hyperglycaemia and glucosuria appear to be due to inhibition of insulin secretion (Dollery *et al.*, 1991).

Long-term administration of phenytoin affects blood thyroid hormone levels in a specific manner. Phenytoin reduces the plasma concentration of total thyroxine ($T_4$) as well as that of free $T_4$, while there are reports of variable effects upon the concentrations of other thyroid and related hormones. Deda *et al.* (1992) reported that, in addition to $T_4$, there were reductions in total triiodothyronine ($T_3$), free $T_3$ and reverse $T_3$ levels in phenytoin-treated epileptic children. Similar effects on $T_3$ and reverse $T_3$ were found in adults in one study (Yeo *et al.*, 1978), while in another study on adults, $T_3$ and thyrotropin were within normal ranges (Larkin *et al.*, 1989). In a number of studies, the $T_4$ and $T_3$ reductions were accompanied by unaltered thyroid stimulating hormone (TSH) levels (see Schröder-Van der Elst & Van der Heide, 1990). Patients appear to be clinically euthyroid, but experimental studies do not indicate whether phenytoin acts as a thyroid hormone agonist or antagonist. Although much emphasis has been placed on the role of occupancy by phenytoin of binding sites on the circulating thyroxine-binding globulin, the experimental evidence does not support impairment of hormone-binding capacity in the observed side-effects (Yeo *et al.*, 1978; Larkin *et al.*, 1989; Deda *et al.*, 1992).

4.2.2 *Experimental systems*

(*a*) *Acute toxicity*

The nervous system is the major target of acute phenytoin toxicity in experimental animals. Seizures may be induced after single intraperitoneal administration of 75 mg/kg bw phenytoin in seven-day-old rats, while doses of about 200 mg/kg bw are required in 18-day-old rats. Lethal doses after single intraperitoneal administration are in the range of 1000 mg/kg bw phenytoin (Mareš *et al.*, 1987).

Treatment of mice with phenytoin (150 mg/kg intraperitoneally for three days) induced a 33–43% increase in hepatic cytochrome P450 content compared with controls (Kim *et al.*, 1993). Analysis of the specific enzymes by immunoblot quantification and by metabolic activities revealed a several-fold increase in CYP2B and hexobarbital hydroxylase activities. In contrast, phenytoin treatment did not raise CYP1A activity in mice.

(*b*) *Subacute and chronic toxicity*

Groups of 10 male and 10 female B6C3F1 mice 7–9 weeks old were given phenytoin in the diet at concentrations between 0 and 1200 mg/kg diet (ppm) for 13 weeks. Nine males and all females exposed to 1200 ppm died before the end of the study and all treated animals showed depressed body-weight gain, although food consumption was not significantly lower than that of the controls. Dose-dependent centrilobular hypertrophy was observed in animals exposed to doses of 300 ppm or more (United States National Toxicology Program, 1993). In another 13-week study, B6C3F1 mice were given 60–4000 ppm phenytoin in the diet. Most mice receiving 1000 ppm or more phenytoin died before the end of the study. Weight gain impairment and focal necrosis of liver cells were observed in the groups receiving 500 and 250 ppm, while no sign of toxicity was observed in animals given 120 ppm (Maeda *et al.*, 1988).

Groups of 10 male and 10 female Fischer 344/N rats, 7–9 weeks old, were given phenytoin in the diet at concentrations between 0 and 4800 mg/kg diet (ppm) for 13 weeks. Mean body-weight gains were significantly lower in the 2400-ppm and 4800-ppm males and females. There was no clinical sign of toxicity and no gross lesion of any organ related to chemical exposure. Chemical-related microscopic lesions were limited to mild centrilobular hypertrophy in the liver of the 4800-ppm group, which was slightly more prominent in males than in females (United States National Toxicology Program, 1993). Similar impairment in body weight-gain and histopathological changes in the liver were reported in another 13-week study in Fischer 344/N rats given phenytoin at concentrations of 1000 and 2000 ppm in the diet, while no change was induced with concentrations of 120–500 ppm. In addition, the relative weights of the liver and spleen in females and of the liver and kidney in males were increased by 1000 and 2000 ppm phenytoin. Increases were measured in alkaline phosphatase activity and in levels of triglyceride and total protein in males and in total cholesterol, total protein and blood urea nitrogen in females (Jang *et al.*, 1987).

(i) *Effects on the liver*

In male D2B6F1 mice given diets containing 250 or 500 mg/kg diet (ppm) phenytoin, hepatic CYP2B-mediated benzyloxyresorufin *O*-dealkylase activity was induced 30-fold and 43-fold after 30 weeks and 41-fold and 57-fold after 60 weeks, respectively. Male B6C3F1 mice given 125, 250, 500 or 1000 ppm phenytoin for 14 days showed significant increases in liver weights at all but the lowest dose and in hepatic CYP2B-mediated benzyloxyresorufin *O*-dealkylase activity at all doses relative to controls (Diwan *et al.*, 1993).

(ii) *Effects on the nervous system*

The chronic effects of phenytoin on the peripheral nervous system were investigated in female Sprague-Dawley rats given 300 mg/kg bw phenytoin per day orally for 180 days. A time-dependent slowing of sensory and motor conduction velocity was observed, and animals with impaired motor conduction velocity also had histopathological changes in the myelinated fibres of the sciatic nerve (Moglia *et al.*, 1981). In male Wister rats, a single intraperitoneal dose of 150 mg/kg bw phenytoin induced a complete blockage of muscle action potential in the dorsal segmental muscles of the tail evoked by electric stimulation of the caudal nerve and a 40% decrease in the $Na^+,K^+$-ATPase activity of the sciatic nerve compared with control values (Raya *et al.*, 1992).

Exposure of immortalized mouse hippocampal neurons to 1–100 μM phenytoin (concentrations spanning the human therapeutic serum levels and the concentrations in the human brain) impaired the formation of normal neuronal processes by preventing the assembly of several cytoskeletal constituents such as actin, tubulin, neurofilament, tau protein and MAP 5; these constituents accumulated within membrane blebs or cytoplasmic condensations instead of forming normally organized processes. However, at these concentrations, no reduction in the overall cell protein synthesis was seen, an effect observed with concentrations above 200 μM (Bahn *et al.*, 1993).

In addition to its established anticonvulsive activity, phenytoin has been reported to have beneficial effects on the nervous system in other situations. Six-day-old Wistar rats were given 50 mg/kg bw phenytoin (a dose known to give anticonvulsive activity in rats) intraperitoneally, 1 h before induction of brain hypoxia by ligation of the left carotid artery. This dose markedly reduced hypoxic-ischaemic infarction in the cerebral cortex and in the striatum assessed histopathologically 72 h later. This protective effect was not observed when phenytoin was administered immediately after the hypoxia. The authors proposed attenuation of hypoxia-induced release of glutamate (an excitatory amino acid) as a mechanism for this neuroprotective action of phenytoin (Hayakawa *et al.*, 1994). In albino rats of the Wistar-derived Sabra strain, systemically (1–10 mg into the left carotid artery) or topically (0.1–1 mM) applied phenytoin on the neuroma surface suppressed spontaneous ectopic discharge in sciatic nerve neuromas without blocking impulse conductance. Since phenytoin is known to provide effective relief in some kinds of human neuralgia, the authors suggested that the clinical analgesic action of phenytoin may involve direct suppression of ectopic impulses generated in the region of nerve damage (Yaari & Devor, 1985).

(iii) *Effects on the immune system*

Oral administration of phenytoin (25, 50 or 100 mg/kg bw per day for seven days) to BALB/c mice significantly depressed both humoral and cellular immune responses compared with control animals, as assessed by the enumeration of direct and indirect splenic plaque-forming cells and the delayed-type hypersensitivity reaction against sheep red blood cells. Furthermore, spleen cells and lymphocytes obtained from mice treated with 100 mg/kg bw phenytoin suppressed the physiological responses of normal cells in these systems. These immunosuppressing effects were observed in spite of the fact that phenytoin induced a rise in spleen cellularity in the treated mice (Andrade-Mena *et al.*, 1994). Similar results concerning the effects of phenytoin on the murine immune system were also obtained in previous studies in mice (de Souza Queiroz & Mullen, 1980; Margaretten & Warren, 1987).

Lorand *et al.* (1976) assessed morphological changes in lymphoid organs induced by administration of phenytoin (10 or 50 mg/kg bw) to female Wistar rats for two to four months. The morphological changes indicated blockage of cellular differentiation in the thymus; in the lymph nodes, depletion of T lymphocytes together with an increase in plasma cells implied a functional disturbance of the lymphoreticular system. Atypical cells or other morphological features potentially indicative of lymphoma production were not found in this study. Experimental results relating to lymphoma induction are described in Section 3.1.1.

(iv) *Endocrine effects*

Dietary administration of phenytoin to male Wistar rats (50 mg/kg bw for 8 or 20 days) resulted in 20–30% reductions in the serum concentrations of thyroxine ($T_4$) and triiodothyronine ($T_3$) but no change in TSH (Schröder-Van der Elst & Van der Heide, 1990; Cageao *et al.*, 1992). At the same doses, the pituitary deiodination of $T_4$ to $T_3$ was significantly increased and this increase in the conversion rate is known to inhibit the feedback response of TSH. The observations in rats are consistent with the clinical findings (see Section 4.2.1).

(v) *Bone effects*

Long-term treatment of epileptic patients with phenytoin has been associated with increased thickness mainly of craniofacial bones. This side-effect has been observed in Sprague-Dawley rats given 5 mg/kg bw per day phenytoin for 36 days by intraperitoneal injection, in which increased histomorphometric (osteoblast number, bone mineral apposition rate) and biochemical parameters (skeletal alkaline phosphatase activity, osteocalcin concentration in the serum) of bone formation were measured. Simultaneous administration of sodium fluoride (50 mg/L (ppm)) in drinking water for 36 days acted in collaboration to stimulate bone formation and to increase bone volume (Ohta *et al.*, 1995).

(vi) *Effects on cell proliferation*

Phenytoin is commonly used in brain tumour patients with epileptic seizures. Although phenytoin cannot be considered as a cytostatic drug, it has been reported to

inhibit microtubule polymerization (MacKinney *et al.*, 1978), and it enhances the cytotoxicity of vincristine, a prototype microtubule inhibitor, in multidrug-resistant human glioma cell lines (Ganapathi *et al.*, 1993).

## 4.3 Reproductive and prenatal effects

### 4.3.1 *Humans*

Four kinds of effects and consequences of phenytoin treatment can be differentiated.

(*a*) Reproductive effects concerning germ cells, sexual hormones and sexual activity;

(*b*) Structural abnormalities, i.e. classical teratogenic effects, when used mainly in the first trimester of gestation;

(*c*) Short-term functional alterations, manifested postnatally and related mainly to treatment in the perinatal period;

(*d*) Long-term developmental (including behavioural) effects, in general connected with use in the second and third trimesters of gestation.

#### (*a*) *Reproductive effects*

Swanson *et al.* (1978) found phenytoin in the semen at a concentration of 0.2 relative to plasma. A diminution of sperm production in epileptic males is due to a decrease in free testosterone level (Cramer & Jones, 1991). This phenomenon results from an increased prolactin level (Aminoff *et al.*, 1984; Laxer *et al.*, 1985) caused by seizures which can inhibit gonadotrophin-releasing hormone secretion from the hypothalamus that in turn inhibits follicle-stimulating hormone (FSH) and luteinizing hormone (LH) production. Taneja *et al.* (1994) compared the effect of phenytoin on semen with data from untreated epileptic and healthy males. A smaller volume of seminal fluid, and lower spermatozoal concentration and total sperm count were seen in untreated epileptics and phenytoin-treated patients compared with the healthy males, but no difference was evident between the two patient groups. The number of morphologically abnormal sperm was greater in untreated patients than in phenytoin-treated or control subjects.

Male patients treated with phenytoin commonly complain of diminished libido and impotence (Toone *et al.*, 1983). Hyposexuality may be the result of altered levels of pituitary hormones, sex hormone-binding globulin and free testosterone (Mattson & Cramer, 1985; Macphee *et al.*, 1988). Phenytoin decreases free testosterone levels by induction of aromatase and sex hormone-binding globulin synthetase, enhancing conversion of free testosterone to oestradiol. Oestradiol exerts a potent inhibitory influence on LH secretion and it may have a major role in negative feedback in men as well as women. Suppression of LH secretion results in hypogonadotropic hypogonadism, which accounts for reproductive and sexual dysfunction among men with epilepsy (Herzog *et al.*, 1991). The higher level of oestrogen during pregnancy can lower the seizure threshold (Mattson & Cramer, 1985).

*(b) Congenital abnormalities*

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

In Europe, about 0.4% of pregnant women have epilepsy (Czeizel *et al.*, 1992). During epileptic convulsive attacks there may be breathing disorders up to apnoea and alterations in cardiac output that, in pregnancy, may effect fetal well-being, especially in the case of frequent attacks (Yerby, 1991). About 40% of women have increased frequency of seizures, mainly due to the decreased serum level of phenytoin, particularly in the first trimester, in parallel with hyperemesis gravidarum, during labour (Schmidt *et al.*, 1983) or if the fetus is male (Dansky & Finnel, 1991). Pregnant epileptic women therefore need higher doses of anticonvulsant treatment. The risk of stillbirth, microcephaly and mental retardation is doubled in the offspring of women with seizures during pregnancy (Nelson & Ellenberg, 1982).

Meadow (1968) reported five cases of congenital malformation in the offspring of epileptic women treated with phenytoin. Subsequently, a total of 32 children born to mothers who took anticonvulsants, including phenytoin, throughout pregnancy were found to have a similar constellation of abnormalities including cleft lip and palate, and cardiovascular and skeletal (limb, skull, face) abnormalities (Meadow, 1970). Subsequent case reports of epileptic women treated during pregnancy with phenytoin and phenobarbital have described affected infants with similar abnormalities (IARC, 1977).

In the early 1970s, nearly all analytical epidemiological studies indicated a 2–3-fold greater risk for congenital abnormalities after phenytoin treatment in the first trimester of gestation (IARC, 1977). However, the risk is five- to six-fold higher for the fetal hydantoin syndrome (Hanson & Smith, 1975; Hanson, 1976). The most characteristic symptom of this syndrome is hypoplasia of the nails and distal phalanges, which occurs in up to 18% of the exposed fetuses (Barr *et al.*, 1974; Hill *et al.*, 1974). Distal digital and nail hypoplasia has been documented through radiographic hand analyses in about 30% of exposed children (Kelly, 1984). Other abnormalities include cleft lip and palate or unusual face (hypoplasia of the mid-face, broad depressed nasal bridge, short upturned nose, mild hypertelorism, inner epicanthal folds, ptosis of the eyelid), microcephaly and congenital cardiovascular malformations. Mild to moderate mental retardation and prenatal-onset growth retardation may also occur. Hanson *et al.* (1976) concluded that as many as 11% of exposed fetuses develop the fetal hydantoin syndrome. However, there is no obvious borderline between lesser manifestations of this syndrome and normal babies. Thus, at present there is evidence for the teratogenicity of phenytoin ingested at 100–800 mg/day during the first trimester of gestation (Adams *et al.*, 1990).

Gaily *et al.* (1988) reported that several minor anomalies previously regarded as symptoms of fetal hydantoin syndrome were genetically linked to epilepsy. Only hypertelorism and digital hypoplasia were specifically associated with phenytoin exposure.

Most studies in which epileptic mothers taking phenytoin alone (namely those receiving monotherapy) were identified indicated a significantly higher rate of fetal hydantoin syndrome compared with untreated non-epileptic women (Lowe, 1973; Fedrick, 1973; Bertollini *et al.*, 1987; Kaneko *et al.*, 1992). It is difficult to calculate the

specific teratogenic risk of phenytoin as the drug is frequently given with other anticonvulsants (polytherapy) and because maternal epilepsy itself is teratogenic. The teratogenic risk of polytherapy has consistently been found to be higher than that of monotherapy. For example, Kaneko *et al.* (1988, 1992) found that the overall rate of congenital abnormalities was 6.2–6.5% in children born to epileptic women with monotherapy, while the rate was 13.5–15.6% after polytherapy.

The teratogenicity of phenytoin afflicts only some exposed fetuses, possibly due to genetic variation in susceptibility. Strickler *et al.* (1985) demonstrated a genetic defect in the detoxification of arene oxide metabolites of phenytoin, which may increase the risk for fetal hydantoin syndrome. Buehler *et al.* (1990) assessed epoxide hydrolase levels in cultures of both fetal fibroblasts and amniocytes and found a deficiency of the enzyme in fetuses at high risk for fetal hydantoin syndrome. In a prospective study of 19 pregnancies monitored by amniocentesis, an adverse outcome was predicted for four fetuses on the basis of low epoxide hydrolase activity (< 30% of the standard). In all four cases, the mother was receiving phenytoin monotherapy and, after birth, the infants had clinical findings compatible with the fetal hydantoin syndrome. The fetuses with enzyme activity above 30% of the standard after use of the same doses of phenytoin were not considered to be at risk and all 15 neonates lacked any characteristic features of the fetal hydantoin syndrome.

Data on congenital abnormality rates among babies born to women with epilepsy but without anticonvulsant therapy are contradictory (Speidel & Meadow, 1972; Monson *et al.*, 1973; Lowe, 1973; Shapiro *et al.*, 1976; Czeizel *et al.*, 1992). A summary of 12 prospective studies (Kaneko & Kondó, 1995) showed that the overall rate of congenital abnormalities was 11.1% (122/1099), 5.7% (6/105) and 4.8% (4081/85 361) in children born to epileptic women treated with anticonvulsants during pregnancy, to epileptic women without anticonvulsant therapy and to non-epileptic control women, respectively.

### (*c*) *Short-term functional alterations*

Clinical manifestations of withdrawal syndrome including hyperirritability, tremor, seizures, vomiting, poor suckling and sleep disturbances may occur shortly after birth in infants exposed to phenytoin during pregnancy (Watson & Spellacy, 1971; Koch *et al.*, 1985). These symptoms do not affect psychomotor development (Hirano *et al.*, 1984).

In newborns exposed to phenytoin during pregnancy, a tendency to bleeding may develop during the first day of life due to decreased levels of vitamin K-dependent clotting factors, despite normal levels in the mother (Bleyer & Skinner, 1976). Haemorrhage secondary to thrombocytopenia may also be caused occasionally by phenytoin (Page *et al.*, 1982).

### (*d*) *Long-term developmental effects*

Developmental and behavioural neurotoxicity in humans has been studied (Hirano *et al.*, 1984; Adams *et al.*, 1990). The prospective study of Scolnik *et al.* (1994) indicated a mean global IQ in children of mothers treated with phenytoin during pregnancy 10 points lower (95% CI, 4.9–15.8) than that of matched controls, and specific weakness in performance of non-verbal tasks. The deficits were unrelated to seizure activity, but

correlated with serum levels of phenytoin. However, behavioural dysfunction was generally limited to global measures of intellectual function. [The Working Group noted that the studies were limited by the short study period, which in general did not extend past infancy.]

### 4.3.2 *Experimental systems*

In rabbits (as in humans), Swanson *et al.* (1978) found phenytoin in the semen at a concentration of 0.2 relative to plasma. A single intragastric dose of phenytoin to pregnant Sprague-Dawley rats was transferred to the fetuses and was concentrated more in the kidney than in the liver, in contrast to the situation in adult rats (Gabler & Falace, 1970). Transplacental transport has been documented in mice, rats, hamsters, goats and monkeys (see Section 4.1.2).

A/Jax mice were treated with 12.5, 25 or 50 mg/kg bw phenytoin sodium by subcutaneous injection at various times from days 9 to 15 of pregnancy; the highest dose produced cleft palates in 26–43% of the offspring (Massey, 1966). Similar treatment with 50 mg/kg bw phenytoin sodium on days 7–9 or 11–13 of gestation induced significant increases in the incidence of cleft palates in the offspring of Swiss-Webster (15%) and A/J mice (31%) (Gibson & Becker, 1968). The teratogenicity of phenytoin has been confirmed in other experiments in mice (Elshove, 1969; Harbison & Becker, 1969; Marsh & Fraser 1973; Sullivan & McElhatton, 1975), rats (Harbison & Becker, 1972; Mercier-Parot & Tuchman-Duplessis, 1974) and rabbits (McClain & Langhoff, 1980). In a mouse model, it has been shown that phenytoin, and not epileptic disease state nor seizure frequency, was associated with congenital abnormalities (Finnel, 1981; Finnel *et al.*, 1989). The major metabolite of phenytoin, *para*-HPPH, and diphenylhydantoic acid and α-aminodiphenylacetic acid were less teratogenic *in vivo* in mice than phenytoin itself (Harbison & Becker, 1974).

Phenytoin (100 mg/kg bw) administered by gastric instillation on gestational days 9, 11 and 13 to Sprague-Dawley rats interfered with sexual dimorphism in cranofacial pattern (Zengel *et al.*, 1989).

Groups of 10 female Fischer 344/N rats and C57Bl/6N mice were given phenytoin in the diet for two weeks before breeding and throughout gestation and lactation (United States National Toxicology Program, 1993). The concentrations were 0, 80, 240, 800 and 2400 mg/kg diet (ppm) for the rats and 0, 20, 60, 200 and 600 mg/kg diet (ppm) for the mice. The females were mated with unexposed males. Four pregnant rats only from each group were killed on gestational day 18 and their uterine contents assessed. The remaining rats and all of the mice were permitted to litter and rear their pups until lactational day 28, after which the pups were weaned and continued to be exposed to the experimental diets for another four weeks. None of the sperm-positive rats given the 2400-ppm diet and none of the mice given 600 ppm delivered any litters. In offspring of the group of rats given 800 ppm phenytoin, there was increased postnatal death. No gross external malformation was observed among rat fetuses or rat or mouse pups surviving to term in any dose group. No gross or histological lesion was found in the rats exposed to 800 ppm or in any of the groups of mice during the four weeks following weaning.

No obvious teratogenic effects (major defects) were seen in monkeys (rhesus macaque, *Macaca mulata*, Wilson, 1974; Hendrie *et al.*, 1990; *Macaca fascicularis*, Phillips & Lockard, 1985, 1989, 1990, 1993), but there was an increased risk for hyperexcitability.

Phenytoin produces multiple behavioural dysfunctions in rat offspring at subteratogenic and non-growth-retarding doses. Elmazar and Sullivan (1981) found an impairment of motor function, primarily of gross motor coordination. Mullenix *et al.* (1983) reported an increase in the activity level of males but not of females. Exposure to phenytoin *in utero* caused a permanent alteration in the hypothalamic-pituitary-thyroid axis in rats (Theodoropoulos *et al.*, 1990). Significant learning and memory deficits, delayed auditory startle, increased locomotor activity and an extremely abnormal spontaneous circling behaviour have been reported (Vorhees, 1983, 1987a,b; Minck *et al.*, 1989; Vorhees & Minck, 1989; Vorhees *et al.*, 1989; Adams *et al.*, 1990). These dysfunctions were dose-dependent and exposure period-dependent in offspring of treated rats. Pizzi and Jersey (1992) found lower birth weight, later lower body weight and a significant increase in locomotor activity.

### 4.3.3 *Possible mechanisms of prenatal effects of phenytoin*

Various mechanisms have been suggested for the origin of phenytoin-induced cleft palate and other congenital abnormalities.

#### *Genetic susceptibility*

The genetic basis of susceptibility to the fetal hydantoin syndrome became clear from the difference in the incidence and pattern of defects in three inbred mouse strains (Finnel & Chernoff, 1984a,b). It was possible to characterize 'fast' and 'slow' metabolizers among several inbred strains of mice, but there was no correlation between the rate of metabolism and the sensitivity to the teratogenic effect of phenytoin (Atlas *et al.*, 1980). The available human data (Strickler *et al.*, 1985; Buehler *et al.*, 1990) were discussed in Section 4.3.1.

#### *Reactive intermediates*

Phenytoin is a potent inducer of liver microsomal oxidation in humans (Conney, 1969). The human fetus has hepatic and extrahepatic monooxygenase systems which may catalyse the formation of epoxide (Pelkonen & Kärki, 1975) and has hepatic and extrahepatic enzymes (e.g. glutathione *S*-transferase, epoxide hydrolase) that metabolize epoxides (Pacifici *et al.*, 1981; Pacifici & Rane, 1983). In human fetal liver, sulfhydryl groups and glutathione are also present which react with metabolites of phenytoin (Rollins *et al.*, 1981). Martz *et al.* (1977) demonstrated the binding of an oxidized metabolite of phenytoin to cellular macromolecules (trichloroacetic acid-precipitable material) in mouse fetuses and showed that this binding was associated with an increased teratogenic potential of phenytoin. These findings suggest that an intermediary metabolite such as an arene oxide may be the causative agent in the teratogenic effect. Phenytoin enhances epoxide metabolism in cultured fetal hepatocytes (Rane & Peng,

1985) and may also induce the metabolism of its own epoxide. Thus, the balance between the enzyme activities that catalyse the formation and the elimination of epoxides could be a determinant of the risk for developmental abnormalities.

Human evidence to support a role for an epoxide intermediate in phenytoin-induced teratogenesis was presented by Strickler *et al.* (1985), who found that increased toxicity of murine microsome-generated phenytoin metabolites to the lymphocytes of 24 children exposed to phenytoin throughout gestation was highly correlated with major birth defects. Each of the 14 children with a 'positive' assay had a parent whose lymphocytes also scored 'positive'. However, no association was found with the presence of minor anomalies, which seem to be independent, mainly familial traits, a conclusion also reached by Gaily *et al.* (1988), who found that only hypertelorism and digital hypoplasia were associated with phenytoin exposure. Thus, a genetic defect of detoxification of arene oxide metabolites of phenytoin may increase the risk for or can select fetuses having abnormalities. This hypothesis was supported by the study of Buehler *et al.* (1990) described in Section 4.3.1, who found that epoxide hydrolase activity in amniocytes of fetuses with fetal hydantoin syndrome was significantly lower than that in fetuses without this syndrome.

The arene oxide of phenytoin can be formed through cytochrome P450-mediated oxidation and yields the dihydrodiol metabolite, 5-(3,4-dihydroxy-1,5-cyclohexadien-1-yl-5-phenylhydantoin), in a reaction catalysed by the enzyme epoxide hydrolase (Chang *et al.*, 1970) and possibly direct or indirect interactions with glutathione (Harbison, 1978; Pantarotto *et al.*, 1982; Wong *et al.*, 1989). Substitution of hydrogen by deuterium at the *para* position of one of the phenyl rings, which favours arene oxide formation, causes an increase in the teratogenic effect of phenytoin (Lambotte-Vandepaer *et al.*, 1989). Embryonic peroxidase-catalysed bioactivation of phenytoin and glutathione-dependent detoxifying and cytoprotective pathways are critical determinants of phenytoin teratogenicity (Miranda *et al.*, 1994). When the rate of bioactivation exceeds the detoxifying capacity of the organism, the electrophilic centre of the arene oxide is capable of binding covalently to nucleophilic sites found in fetal macromolecules, such as nucleic acid (Jerina & Daly, 1974), and such irreversible binding at the critical periods of development may explain the teratogenic effect of phenytoin. Finnel *et al.* (1993, 1994) reduced the teratogenic effect of phenytoin in mice by co-administration of stiripentol, a potent inhibitor of cytochrome P450 enzymes. This suggests that cytochrome P450 oxidation products may be the primary teratogenic agent(s) in phenytoin-induced teratogenesis.

Besides an arene oxide, free radicals may also be reactive intermediates generated by cytochromes P450 and/or prostaglandin synthetase (Kubow & Wells, 1986; Wells *et al.*, 1989). Increased oxygen consumption and free radical intermediates were detected during bioactivation of phenytoin by prostaglandin synthetase *in vitro* (Kubow & Wells, 1989). Phenytoin embryopathy was enhanced by concurrent treatment with 12-*O*-tetradecanoylphorbol 13-acetate, which activates phospholipase $A_2$, leading to release of membrane-bound arachidonic acid and enhanced prostaglandin biosynthesis (Wells & Vo, 1989). Furthermore, administration to CD-1 mice of phenytoin together with acetylsalicylic acid (an irreversible inhibitor of prostaglandin synthetase), caffeic acid (an

antioxidant) or α-phenyl-*N*-*tert*-butylnitrone (a free radical spin-trapping agent) led to 50%, 71% and 82% reductions in the rate of phenytoin-caused cleft palate, respectively (Wells *et al.*, 1989). These results indicated that prostaglandin synthetase contributes to the enzymatic bioactivation of phenytoin to a teratogenic free radical intermediate. Phenytoin teratogenicity in mice was also reduced by the antioxidant vitamin E (Sanyal & Wells, 1993) and by dietary *n*–3 fatty acids (Kubow, 1992) via inhibition of embryonic prostaglandin synthetase. Liu and Wells (1994) showed that peroxidase-catalysed bioactivation of phenytoin may initiate oxidative damage to lipids and proteins in embryonic tissues.

It has been suggested that phenytoin and glucocorticoids disrupt normal palatal development by the same or a very similar mechanism (Fritz, 1976; McDevitt *et al.*, 1981). Phenytoin treatment of A/J mice increased endogenous maternal corticosterone concentrations for approximately 48 h after dosing (Hansen *et al.*, 1988). Thus, Hansen *et al.* (1992) hypothesized that phenytoin causes clefting through the glucocorticoid-mediated effect; however, it appeared that phenytoin is capable of producing clefts in the absence of endogenous maternal corticosterone. Involvement of glucocorticoids or of interaction between phenytoin and the glucocorticoid receptor (Sonawane & Goldman, 1981; Katsumata *et al.*, 1982; Hansen & Hodes, 1983; Gupta *et al.*, 1985; Katsumata *et al.*, 1985; Hansen, 1991) has not been ruled out. Binding of phenytoin to the glucocorticoid receptor could stimulate the release of a protein that can inhibit the phospholipase $A_2$-mediated release from membrane phospholipids of arachidonic acid, a precursor in the synthesis of leukotrienes, thromboxanes and prostaglandins (Kay *et al.*, 1988). In experimental systems, addition of arachidonic acid decreased the incidence of phenytoin-induced abnormalities, suggesting that phenytoin somehow caused a deficiency of arachidonic acid (Kay *et al.*, 1990). However, a significant amount of work remains to be done to examine the involvement of the arachidonic acid cascade in phenytoin-induced teratogenicity *in vivo*.

In conclusion, the bulk of experimental data and some human evidence suggests some role for reactive intermediates in phenytoin-induced teratogenicity. These observations may also contribute to the understanding of the effect of phenytoin on the central nervous system (Kempermann *et al.*, 1994).

*Effect on folate metabolism*

Phenytoin alters the metabolism of folate (Malpas *et al.*, 1966; Reynolds, 1973). This water-soluble B vitamin is required for DNA synthesis (it provides one-carbon units for the de-novo synthesis of guanine, adenine and thymine) and plays an important role in the methylation cycle in supplying the methyl group (Czeizel, 1995). Folate deficiency is seen in epileptics, mainly in pregnant epileptic women (Hiilesmaa *et al.*, 1983; Dansky *et al.*, 1987), although there is an increased demand for folate during pregnancy.

In experimental systems, conflicting results have been obtained when phenytoin and folate were given concurrently. Folic acid had no effect on the rate of abnormalities produced by phenytoin treatment in mice (Marsh & Fraser, 1973; Mercier-Parot & Tuchmann-Duplessis, 1974). Although folic acid was initially said to decrease the rate of

cleft palate (Marsh & Fraser, 1973), this was not confirmed in subsequent reports (Schardein *et al.*, 1973; Sullivan & McElhatton, 1975). The embryonic concentration of folate decreased after intraperitoneal administration of phenytoin (Netzloff *et al.*, 1979), but not after dietary supplementation (Hansen & Billings, 1985), and further intervention studies (e.g., Chatot *et al.*, 1984; Zhu & Zhou, 1989) have also given contradictory results. Nevertheless, phenytoin and/or its arene oxide metabolite can decrease the activity of hepatic 5,10-methylenetetrahydrofolate reductase (Billings, 1984) and, consequently, may affect *S*-adenosylmethionine synthesis, which is central to methyl group transfer reactions. Neither folate absorption (Nelson *et al.*, 1983) nor folate catabolism (Guest *et al.*, 1983) is adversely affected by phenytoin. Data from human randomized double-blind intervention studies suggest that a high dose (4 mg) of folic acid alone can reduce the recurrence (MRC Vitamin Study Research Group, 1991) and a low dose (0.8 mg) of folic acid-containing multivitamins can prevent the first occurrence of neural-tube defects (Czeizel & Dudás, 1992) and some other major congenital abnormalities (Czeizel, 1993), including oral clefts. This is supported by a case–control study (Shaw *et al.*, 1995).

*Fetal hypoxia*

The experimental studies of Danielson *et al.* (1992) and Danielsson *et al.* (1992) indicated that phenytoin exerts its teratogenic effects by inducing fetal hypoxia, leading to vascular disruption and necrosis of existing and developing structures.

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

### 4.4.1 *Humans*

Bone-marrow cells from epileptic patients treated with phenytoin were analysed in two studies. Alving *et al.* (1977) found no evidence of either chromosomal damage or induction of hyperploidy in 10 adult patients (four women and six men), aged 19–49 years (mean, 33 years), treated for 4–20 years; in none of the patients did the serum level of phenytoin exceed 20 mg/L during the last year of treatment. The controls in this study were bone-marrow aspirates and peripheral blood samples from 10 patients without haematological disorders who had not received treatment with ionizing radiation or cytostatic drugs. The bone-marrow smears from five of the patients were also studied for the presence of micronuclei: a doubling of the number of erythroblasts with micronuclei was observed, but the difference was not statistically significant. Knuutila *et al.* (1977) reported on a series of 22 patients with epilepsy (12 women and 10 men), aged 4–47 years (mean, 21 years); the effective serum concentrations of phenytoin varied between 4 and 113 μmol/L [1 and 29 mg/L]. The results of the study were compared with those from a simultaneously conducted study of 20 healthy persons (11 women and nine men), aged 23–37 years (mean, 27 years): none had taken medicines and none of the women contraceptive pills for at least half a year before the study (Knuutila *et al.*, 1976). The mean frequency of all chromosomal structural changes among the patients was 0.5%

## Table 6. Genetic and related effects of phenytoin or its sodium salt

| Test system | Result[a] | | Dose[b] (LED/HID) | Reference |
|---|---|---|---|---|
| | Without exogenous metabolic system | With exogenous metabolic system | | |
| PRB, SOS repair test (chromo-test) | – | NT | 6300 | Brams *et al.* (1987) |
| SA0, *Salmonella typhimurium* TA100, reverse mutation | – | – | 385 | Sezzano *et al.* (1982) |
| SA0, *Salmonella typhimurium* TA100, reverse mutation | – | – | 323 | Haworth *et al.* (1983) |
| SA0, *Salmonella typhimurium* TA100, reverse mutation | – | – | 185 | Léonard *et al.* (1984) |
| SA0, *Salmonella typhimurium* TA100, reverse mutation | – | – | 370 | Brams *et al.* (1987) |
| SA3, *Salmonella typhimurium* TA1530, reverse mutation | – | – | 185 | Léonard *et al.* (1984) |
| SA5, *Salmonella typhimurium* TA1535, reverse mutation | – | – | 385 | Sezzano *et al.* (1982) |
| SA5, *Salmonella typhimurium* TA1535, reverse mutation | – | – | 323 | Haworth *et al.* (1983) |
| SA7, *Salmonella typhimurium* TA1537, reverse mutation | – | – | 385 | Sezzano *et al.* (1982) |
| SA7, *Salmonella typhimurium* TA1537, reverse mutation | – | – | 323 | Haworth *et al.* (1983) |
| SA7, *Salmonella typhimurium* TA1537, reverse mutation | – | – | 185 | Léonard *et al.* (1984) |
| SA8, *Salmonella typhimurium* TA1538, reverse mutation | – | (+) | 100 | Sezzano *et al.* (1982) |
| SA8, *Salmonella typhimurium* TA1538, reverse mutation | – | – | 370 | Léonard *et al.* (1984) |
| SA9, *Salmonella typhimurium* TA98, reverse mutation | – | – | 385 | Sezzano *et al.* (1982) |
| SA9, *Salmonella typhimurium* TA98, reverse mutation | – | – | 323 | Haworth *et al.* (1983) |
| SA9, *Salmonella typhimurium* TA98, reverse mutation | – | – | 370 | Léonard *et al.* (1984) |
| SA9, *Salmonella typhimurium* TA98, reverse mutation | – | – | 370 | Brams *et al.* (1987) |
| SAS, *Salmonella typhimurium* TA97, reverse mutation | – | – | 185 | Léonard *et al.* (1984) |
| SAS, *Salmonella typhimurium* TA97, reverse mutation | – | – | 370 | Brams *et al.* (1987) |
| DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation | – | | 100 inj. | Woodruff *et al.* (1985) |
| DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation | – | | 5000 feed | Woodruff *et al.* (1985) |
| GCO, Gene mutation, Chinese hamster ovary cells, *hprt* locus, *in vitro* | ? | ? | 800 | Oberly *et al.* (1993) |
| G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus, *in vitro* | – | – | 400 | Oberly *et al.* (1993) |
| SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro* | – | – | 300 | Riedel & Obe (1984) |
| SIA, Sister chromatid exchange, Wg 3h cells *in vitro* | 0 | + | 75 | Tan *et al.* (1985) (abstract) |

**Table 6 (contd)**

| Test system | Result[a] | | Dose[b] (LED/HID) | Reference |
|---|---|---|---|---|
| | Without exogenous metabolic system | With exogenous metabolic system | | |
| CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro* | – | – | 300 | Riedel & Obe (1984) |
| CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro* | – | – | 300 | Kindig *et al.* (1992) |
| CIM, Chromosomal aberrations, mouse primary embryonic fibroblasts *in vitro* | – | NT | 200 | de Oliveira & Machado-Santelli (1987) |
| AIA, Aneuploidy, mouse primary embryonic fibroblasts *in vitro* (hyperdiploidy) | + | NT | 200 | de Oliveira & Machado-Santelli (1987) |
| T7S, Cell transformation, SA7/Syrian hamster embryo cells *in vitro* | + | NT | 50 | Hatch *et al.* (1986) |
| SHL, Sister chromatid exchange, human lymphocytes *in vitro* | + | NT | 15 | Maurya & Goyle (1985) |
| CHL, Chromosomal aberrations, human lymphocytes *in vitro* | – | NT | 70 | Bishun *et al.* (1975) |
| CHL, Chromosomal aberrations, human lymphocytes *in vitro* | – | NT | 100 | Alving *et al.* (1976) |
| CHL, Chromosomal aberrations, human lymphocytes *in vitro* | – | NT | 500 | Léonard *et al.* (1984) |
| CHL, Chromosomal aberrations, human lymphocytes *in vitro* | + | NT | 54 | Ramadevi *et al.* (1984) |
| CHL, Chromosomal aberrations, human lymphocytes *in vitro* | + | NT | 10 | García Sagredo (1988) |
| CIH, Chromosomal aberrations, human amnion cells *in vitro* | – | NT | 200 | de Oliveira & Machado-Santelli (1987) |
| AIH, Aneuploidy, human lymphocytes *in vitro* (hyperdiploidy and polyploidy) | – | NT | 70 | Bishun *et al.* (1975) |
| AIH, Aneuploidy, human lymphocytes *in vitro* (hyperdiploidy) | – | NT | 100 | Alving *et al.* (1976) |
| AIH, Aneuploidy, human amnion cells *in vitro* (hyperdiploidy) | + | NT | 200 | de Oliveira & Machado-Santelli (1987) |
| TVI, Transformation of mouse peritoneal macrophages treated *in vivo*, scored *in vitro* | + | | 50 ip × 1 | Massa *et al.* (1990) |
| TVI, Cell transformation, human fibroblasts treated *in vivo*, scored *in vitro* | + | | NG | Dhanwada *et al.* (1992) |
| MVM, Micronucleus test, mouse (Swiss) bone marrow *in vivo* | + | | 100 po × 2 | Das *et al.* (1983) |

**Table 6 (contd)**

| Test system | Result[a] | | Dose[b] (LED/HID) | Reference |
|---|---|---|---|---|
| | Without exogenous metabolic system | With exogenous metabolic system | | |
| MVM, Micronucleus test, mouse (BALB/c) bone marrow *in vivo* | + | | 0.5 iv × 1 | Montes de Oca-Luna *et al.* (1984) |
| MVM, Micronucleus test, mouse (Swiss CD-1) liver *in utero* | (+)[c] | | 100 ip × 1 | Barcellona *et al.* (1987) |
| MVM, Micronucleus test, mouse (Swiss CD-1) bone marrow *in vivo* | – | | 100 ip × 1 | Barcellona *et al.* (1987) |
| MVM, Micronucleus test, mouse (CD-1) bone marrow *in vivo* | – | | 40 ip × 2 | Kindig *et al.* (1992) |
| MVM, Micronucleus test, mouse (BALB/c) bone marrow *in vivo* | – | | 20 iv × 1 | McFee *et al.* (1992) |
| MVM, Micronucleus test, mouse (B6C3F1) bone marrow *in vivo* | – | | 70 ip × 3 | McFee *et al.* (1992) |
| MVM, Micronucleus test, mouse (B6C3F1) peripheral blood erythrocytes *in vivo* | – | | 70 ip × 3 | McFee *et al.* (1992) |
| SVA, Sister chromatid exchange, mouse (ICR) bone marrow *in vivo* | – | | 80 ip × 1 | Sharma *et al.* (1985) |
| SVA, Sister chromatid exchange, mouse (ICR) liver *in utero* | (+) | | 80 ip × 1 | Sharma *et al.* (1985) |
| SVA, Sister chromatid exchange, mouse (B6C3F1) bone marrow *in vivo* | – | | 250 ip × 1 | McFee *et al.* (1992) |
| SVA, Sister chromatid exchange, female mouse (CD-1) bone marrow *in vivo* | – | | 40 ip × 1 | Kindig *et al.* (1992) |
| SVA, Sister chromatid exchange, mouse (ICR) liver *in utero* | – | | 40 ip × 2 | Kindig *et al.* (1992) |
| CBA, Chromosomal aberrations, rat (Lister) bone-marrow cells *in vivo* | – | | 50 × 3 | Alving *et al.* (1976) |
| CBA, Chromosomal aberrations, mouse (BALB/c) bone marrow *in vivo* | – | | 0.48 po 3d/wk 2 m | de Oliveira *et al.* (1987) |
| CBA, Chromosomal aberrations, mouse (BALB/c) bone marrow *in vivo* | – | | 100 ip × 3 | de Oliveira *et al.* (1987) |
| CBA, Chromosomal aberrations, mouse (B6C3F1) bone marrow *in vivo* | – | | 500 ip × 1 | McFee *et al.* (1992) |
| DLM, Dominant lethal test, mouse *in vivo* | + | | 145 ip × 1 | Epstein *et al.* (1972) |
| AVA, Aneuploidy, rat (Lister) bone-marrow *in vivo* (hyperdiploidy) | – | | 50 × 3 | Alving *et al.* (1976) |
| AVA, Aneuploidy, mouse (BALB/c) bone marrow *in vivo* | – | | 0.48 po 3d/wk 2 m | de Oliveira *et al.* (1987) |

**Table 6 (contd)**

| Test system | Result[a] | | Dose[b] (LED/HID) | Reference |
|---|---|---|---|---|
| | Without exogenous metabolic system | With exogenous metabolic system | | |
| AVA, Aneuploidy, mouse (BALB/c) bone marrow *in vivo* | + | | 50 ip × 3 | de Oliveira *et al.* (1987) |
| SLH, Sister chromatid exchange, human lymphocytes *in vivo* | + | | NG | Habedank *et al.* (1982) |
| SLH, Sister chromatid exchange, human lymphocytes *in vivo* | – | | NG | Sinués *et al.* (1982) |
| SLH, Sister chromatid exchange, human lymphocytes *in vivo* | + | | NG | Schaumann *et al.* (1985) |
| SLH, Sister chromatid exchange, human lymphocytes *in vivo* | – | | NG | Tan *et al.* (1985) (abstract) |
| SLH, Sister chromatid exchange, human lymphocytes *in vivo* | – | | NG | Schaumann *et al.* (1989) |
| SLH, Sister chromatid exchange, human lymphocytes *in vivo* | – | | 270[d] daily > 1 yr | Flejter *et al.* (1989) |
| SLH, Sister chromatid exchange, human lymphocytes *in vivo* | + | | NG | Taneja *et al.* (1992) |
| MVH, Micronucleus test, human lymphocytes *in vivo* | – | | 6 daily > 6 mo | Hashem & Shawki (1976) |
| MVH, Micronucleus test, human bone-marrow cells *in vivo* | – | | NG | Alving *et al.* (1977) |
| CBH, Chromosomal aberrations, human bone-marrow cells *in vivo* | – | | NG | Alving *et al.* (1977) |
| CBH, Chromosomal aberrations, human bone-marrow cells *in vivo* | – | | NG | Knuutila *et al.* (1977) |
| CLH, Chromosomal aberrations, human lymphocytes *in vivo* | + | | 6 daily > 6 mo | Hashem & Shawki (1976) |
| CLH, Chromosomal aberrations, human lymphocytes *in vivo* | – | | NG | Alving *et al.* (1977) |
| CLH, Chromosomal aberrations, human lymphocytes *in vivo* | – | | 6 daily > 8 mo | Esser *et al.* (1981) |
| CLH, Chromosomal aberrations, human lymphocytes *in vivo* | – | | NG | Tan *et al.* (1985) (abstract) |
| CLH, Chromosomal aberrations, human lymphocytes *in vivo* | – | | 270[d] daily > 1 yr | Flejter *et al.* 1989) |

**Table 6 (contd)**

| Test system | Result[a] | | Dose[b] (LED/HID) | Reference |
|---|---|---|---|---|
| | Without exogenous metabolic system | With exogenous metabolic system | | |
| AVH, Polyploidy, human lymphocytes *in vivo* | + | | 6 daily > 6 mo | Hashem & Shawki (1976) |
| AVH, Aneuploidy, human bone-marrow cells *in vivo* | – | | NG | Alving *et al.* (1977) |
| AVH, Aneuploidy, human lymphocytes *in vivo* | – | | NG | Alving *et al.* (1977) |
| AVH, Aneuploidy, human bone-marrow cells *in vivo* | – | | NG | Knuutila *et al.* (1976) |
| *, Inhibition of tubulin polymerization *in vitro* | – | 0 | 2520 | Léonard *et al.* (1984) |
| ICR, Inhibition of gap-junctional intercellular communication, Chinese hamster (V79) cells *in vitro* | + | 0 | 23 | Welsch & Stedman (1984) |
| ICR, Inhibition of gap-junctional intercellular communication, Chinese hamster (V79) cells *in vitro* | +[e] | 0 | 60 | Jone *et al.* (1985) |
| SPM, Sperm morphology, mouse (Swiss) *in vivo* | + | | 10 po × 5 | Ramaniah *et al.* (1980) |
| *, Increase in c-*sis* expression | + | | 10 | Dill *et al.* (1993) |

* Not shown on profile

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

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

[c] Pretreatment with epoxide hydrolase inhibitor 1,2-epoxy-3,3,3-trichloropropane (100 mg/kg bw sc) increased genotoxicity

[d] Average daily dose from 10 patients

[e] Combination treatment with phenobarbital enhanced the effect of phenytoin

(range, 0–5.4%), compared with 0.4% (0–2%) in controls. No increase in polyploidy or hyperdiploidy (except one cell in two patients) was found.

Many studies have been conducted on possible cytogenetic effects induced by phenytoin in lymphocytes (and two in bone marrow) of children and adult patients affected by grand mal or petit mal seizure. Almost all of the adequately conducted studies had negative findings for micronucleus frequencies (2/2 studies), chromosomal aberrations (5/6 studies) and aneuploidy (2/2 studies). The only positive finding was that of Hashem and Showki (1976) for chromosomal aberrations and polyploidy. Their study subjects were 21 epileptic patients aged 3–12 years (11 boys and 10 girls) treated for grand mal epilepsy with the sodium salt of phenytoin at a dose of 3–6 mg/kg bw per day for durations ranging between six months and five years; serum levels were not reported. The control group comprised 25 subjects (3–15 years); 10 were epileptics and 15 were normal subjects aged 3–14 years (nine boys and six girls). There was no difference between epileptics not receiving any treatment and normal controls, but a significant increase was seen in gaps, chromatid breaks, chromosome pulverization, polynuclei and polyploidy in treated epileptics relative to untreated epileptics or normal controls. In normal controls, the percentages of cells with single chromatid breaks and polyploidy, were 2.5 ± 0.25 and 0.2, respectively; neither fragmentation/pulverization nor gaps were observed. In untreated epileptic patients, the percentages of cells with single chromatid breaks and polyploidy were 3.0 ± 0.2 and 0.3, respectively. In treated patients, the corresponding frequencies were 6.8 ± 0.87 and 2.8 ± 0.35; induction of gaps (5.2 ± 0.31%) and isochromatid breaks (5.6 ± 0.21%), as well as fragmentation/pulverization (1.8 ± 0.67%), was observed.

Results on sister chromatid exchange (SCE) in lymphocytes are contradictory. Habedank *et al.* (1982) were the first to describe increased SCE in lymphocyte cultures of nine children (6–16 years of age) suffering from epilepsy and therefore treated with phenytoin monotherapy for 6–60 months; serum levels of phenytoin were rather uniform (40–80 μM) [10–20 mg/L]. Their average SCE rate (10.03 ± 1.31 per metaphase) clearly exceeded that of a control group of healthy children (6.49 ± 0.46 per metaphase). Schaumann *et al.* (1985) also found significantly increased frequencies of SCE in lymphocyte cultures of 12 adult male epileptic patients (37.25 ± 2.26 years of age; 12.28 ± 0.69 SCE/metaphase) on long-term monotherapy (1.3–14 years) compared with 12 controls matched for age and smoking (37.08 ± 2.21 years of age; 7.81 ± 0.44 SCE/metaphase); the serum levels of the patients were consistently within the therapeutic range of 10–20 mg/L (with exception of two patients with subtherapeutic levels). More recently, an extensive study (Taneja *et al.*, 1992) compared SCE frequencies in 29 phenytoin-treated epileptics (8.29 ± 2.67 SCE/metaphase in 22 men; 7.52 ± 2.92 SCE/metaphase in seven women), 32 untreated epileptics (8.44 ± 2.40 SCE/metaphase in 16 men; 9.76 ± 2.73 SCE/metaphase in 16 women) and 32 normal healthy controls (5.0 ± 1.13 SCE/metaphase in 16 men; 4.95 ± 1.08 SCE/metaphase in 16 women). Age ranged from 16 to 41 years (20.9 ± 7.8 years) in the patients and from 16 to 38 (24.5 ± 5.5 years) in the controls, but no information was available on smoking habits. The authors concluded that SCE frequencies were similar in untreated patients and in patients receiving phenytoin monotherapy, but that both groups had significantly increased frequencies compared

with controls. No correlation of SCE frequency with sex or duration of therapy was observed. None of the subjects in this study were heavy smokers or on any drugs. The disease itself might therefore be responsible for inducing genetic damage. [The Working Group noted that the distribution of smokers in the groups was not presented.]

In contrast with these results, Schaumann *et al.* (1989) undertook a larger, better controlled study than that published in 1985 and observed no increase in SCE frequency in lymphocytes of treated patients. Sixteen adult male patients with epilepsy receiving long-term phenytoin monotherapy (serum level, 3–20 mg/L; age, 38.8 ± 2.4 years) were matched with 16 healthy controls (age, 38.9 ± 2.4 years) for sex, age and smoking habits. Statistical analysis did not reveal any significant difference between rates of SCE in phenytoin-treated persons (5.52 ± 0.25 per metaphase) and controls (5.78 ± 0.29 per metaphase). These data agree with those from three other studies, one published as an abstract (Tan *et al.*, 1985), on 12 adult epileptics and two more extensive studies. Sinués *et al.* (1982) analysed the lymphocytes of 64 epileptic adults (6.84 ± 1.18 per metaphase) and 30 controls taking no medication (6.66 ± 1.15 per metaphase); no information was reported on age, smoking habits or serum levels of phenytoin. Flejter *et al.* (1989) compared a mixed population of five epileptic children and four adults [6.10 ± 1.93 per metaphase] with 10 control adults aged 23–52 years and taking no medication (6.83 ± 1.65 per metaphase). The blood levels of phenytoin ranged from 2.7 to 22.0 mg/L.

### 4.4.2 *Experimental systems*

In bacteria, all gene mutation tests performed without exogenous metabolic systems were negative, while one of these studies was positive in the presence of an exogenous metabolic activation system.

No sex-linked recessive lethal mutations were observed in *Drosophila melanogaster* after phenytoin was injected or fed to the adults.

No mutation was induced at the *tk* locus in mouse lymphoma L5178Y cells; the results concerning the *hprt* locus in Chinese hamster ovary cells are unclear.

There was no evidence for the induction of SCE in one study on Chinese hamster cells *in vitro*, but one abstract reported positive effects in Wg 3h cells in the presence of an exogenous metabolic system. Chromosomal aberrations were not found in Chinese hamster ovary cells or in mouse primary embryonic fibroblasts. Aneuploidy induction, mitotic delay and abnormal chromosome/spindle segregation were observed in one study of primary embryonic fibroblasts *in vitro* (de Oliveira & Machado-Santelli, 1987).

Cell transformation was induced in one study with Syrian hamster embryo cells. Gap-junctional intercellular communication in Chinese hamster lung V79 cells was inhibited by phenytoin treatment.

In cultured human lymphocytes, SCE was observed in a single study and chromosomal aberrations were increased in two of five studies. In one of these, there was an increase in hyperdiploidy, but without a dose–response relationship, while the results of two others were negative for both aneuploidy and polyploidy. Mitotic delay and abnormal chromosome/spindle segregation have also been reported in human lymphocyte or amnion cell cultures (Maurya & Goyle, 1985; de Oliveira & Machado-Santelli, 1987).

The frequency of cell transformation was enhanced *in vitro* in hyperplastic gingival tissue derived from one patient treated with phenytoin and in murine peritoneal macrophages in a host-mediated assay.

Micronuclei were induced in two of six studies performed *in vivo* in mouse bone marrow but not in single studies in mouse liver and circulating erythrocytes. Inhibition of epoxide hydrolase by subcutaneous injection of mice on day 13 of gestation with 1,2-epoxy-3,3,3-trichloropropane (TCPO) (100 mg/kg) 1 h before intraperitoneal injection of phenytoin increased the frequency of micronuclei in liver cells of the fetal mice (TCPO control, 0.23 ± 0.120 versus TCPO + phenytoin, 1.05 ± 0.658) over that observed with phenytoin, but without TCPO treatment (control, 0.23 ± 0.116 versus phenytoin, 0.43 ± 0.206) (Barcellona *et al.* 1987). There is no evidence for the induction of either SCE or chromosomal aberrations in adult mouse in bone marrow *in vivo* or in mouse liver *in utero*. Increased frequencies of aneuploidy were observed in mouse bone marrow after three intraperitoneal injections of 50 mg/kg bw phenytoin, but not after two months oral dosing with 0.48 mg/mouse/day three days per week; no increased hyperploidy was reported with the former protocol in rat bone marrow. Phenytoin administered to male mice induced dominant lethal effects and sperm-head morphological abnormalities.

*5-(4′-Hydroxyphenyl)-5-phenylhydantoin (para-HPPH)*

This metabolite of diphenylhydantoin was reported to slightly increase mutations in *S. typhimurium* in the presence of S9 in one study (Sezzano *et al.*, 1982), but not in another in which higher dose levels were used (Léonard *et al.*, 1984).

Following intraperitoneal treatment of pregnant mice, micronuclei were not induced in the bone-marrow cells of the adults or in the liver cells of the fetuses (Barcellona *et al.*, 1987).

# 5. Summary of Data Reported and Evaluation

## 5.1 Exposure data

Phenytoin, often administered as its sodium salt, has been widely used since the 1930s as an anticonvulsant in the treatment of epilepsy and, to a lesser extent and more recently, in the treatment of certain cardiac arrhythmias.

## 5.2 Human carcinogenicity data

Many case reports have suggested that there may be a relationship between lymphomas and anticonvulsants, especially phenytoin. In a cohort study in Denmark of epileptic patients exposed to anticonvulsants, including phenytoin, there was an increase in overall cancer risk, attributable to an excess of brain and lung cancer. Nevertheless, brain tumours probably caused the seizure disorder; an evaluation of brain tumour risk over time showed that these tumours were unlikely to be drug-related.

Nested case–control studies based on the Danish cohort investigated in detail the influence of several treatments with anticonvulsants on the risk of cancers of the lung, bladder and liver and non-Hodgkin lymphoma. Anticonvulsant treatment with phenytoin was not associated with lung, bladder or liver cancer. There was an elevated risk for non-Hodgkin lymphoma associated with phenytoin use, but this was not significant.

Two case–control studies investigated the relationship between multiple myeloma and the use of phenytoin, among many other factors. One found no association between phenytoin use and at risk for multiple myeloma risk. The other study found a nonsignificantly elevated risk associated with the use of phenytoin. The power of both studies to assess an effect of phenytoin was low.

## 5.3 Animal carcinogenicity data

Phenytoin was tested for carcinogenicity by oral administration in three experiments in mice and in two experiments in rats. It was also tested by perinatal/adult exposure in one study in mice and rats and by intraperitoneal administration in one study in mice.

In one experiment in three strains of female mice, oral administration of the sodium salt of phenytoin was reported to increase the incidence of lymphomas. Oral administration to female mice in another study decreased the incidence of mammary gland adenocarcinomas, leukaemias and polyps of the endometrium; in a further study, the incidence of hepatocellular tumours was reduced in males. Oral administration to rats did not increase the incidence of tumours in two studies.

In the experiment using combinations of adult and perinatal exposure, adult exposure resulted in a dose-dependent increase in the incidence of hepatocellular tumours in female mice. Perinatal treatment followed by adult exposure increased the incidence of hepatocellular tumours in both male and female mice and slightly in male rats.

Following intraperitoneal injection of phenytoin into mice, leukaemias and lymphomas were observed.

In one experiment in mice, phenytoin increased the incidence of hepatocellular tumours induced by *N*-nitrosodiethylamine. In a mouse lung adenoma assay, phenytoin decreased the multiplicity of lung adenomas induced by urethane.

## 5.4 Other relevant data

Phenytoin is well absorbed in humans. It is eliminated mainly as the glucuronide of the major metabolite, 5-(4′-hydroxyphenyl)-5-phenylhydantoin, which typically accounts for 67–88% of the dose in urine. Several other metabolites are known. The elimination kinetics are non-linear, but an apparent mean half-life of 22 h is a useful guide.

5-(4′-Hydroxyphenyl)-5-phenylhydantoin is the main metabolite in all animal species except dogs (5-(3′-hydroxyphenyl)-5-phenylhydantoin) and cats (the *N*-glucuronide).

Acute phenytoin intoxication in humans presents usually with cerebellar-vestibular effects such as nystagmus, ataxia, diplopia, vertigo and dysarthria. Chronic administration of phenytoin at therapeutic doses may rarely induce various adverse health effects such as symptoms associated with impairment of the nervous system described above.

Gingival overgrowth, sometimes together with increased thickness of the craniofacial bones as well as folic acid deficiency and development of megaloblastic anaemia, are well established adverse effects of the drug. Phenytoin has also been associated with various forms of cutaneous hypersensitivity reactions, sometimes accompanied by lymphadenopathy and benign lymphoid hyperplasia. In rare cases, the histological architecture of the lymph nodes is lost (pseudolymphoma). Phenytoin may also induce a variety of endocrine effects such as reduction of thyroxine concentrations, hypocalcaemia, osteomalacia and hyperglycaemia.

The nervous system appears to be the major target of acute and chronic phenytoin toxicity in experimental animals. In addition, repeated administration of phenytoin induces increased liver and kidney weights, centrilobular hepatic hypertrophy and diverse immunosuppressive effects. Phenytoin may reduce thyroxine concentrations and increase bone thickness in rodents, but gingival hyperplasia has been observed only in cats and monkeys and not in rodents.

Phenytoin is an inducer of certain hepatic cytochrome P450 activities in humans and mice.

There is evidence for the teratogenicity of phenytoin in humans ingesting 100–800 mg per day during the first trimester of gestation. Phenytoin is teratogenic in mice and rats. Animal and a few human studies suggest that neurobehavioural deficits occur at doses which produce no dysmorphic effect.

Phenytoin induced mutations in *Salmonella typhimurium* in the presence of a metabolic activation system in one study.

No mutagenic effect was observed in *Drosophila* or in mammalian cells *in vitro* in the absence of an exogenous metabolic system. Aneuploidy was induced in one study in primary mouse embryonic fibroblasts *in vitro*. Cell transformation was induced in Syrian hamster embryo. A single study showed increased clone sizes of murine macrophages in a host-mediated assay. Phenytoin inhibited gap-junctional intercellular communication.

In human lymphocytes *in vitro*, sister chromatid exchanges were induced in one study and chromosomal aberrations were induced in two of five studies. Aneuploidy was observed in human amnion cells but not in lymphocytes.

Phenytoin induced micronuclei in three of five studies in rodents *in vivo*. Aneuploidy, in one of two studies, aberrant sperm morphology and dominant lethal mutations were induced, but not sister chromatid exchange or chromosomal aberrations.

In general, studies of human lymphocytes *in vivo* showed no induction of micronuclei, chromosomal aberrations or aneuploidy but an increase of polyploidy was found in one study and of sister chromatid exchange frequencies in three of seven studies. Neither chromosomal aberrations nor aneuploidy were induced in human bone marrow.

The metabolite 5-(4′-hydroxyphenyl)-5-phenylhydantoin was mutagenic in *Salmonella typhimurium* in the presence of a metabolic activation system; it did not induce micronuclei in mouse bone marrow *in vivo*.

*Mechanistic considerations*

Evidence is available to support the conclusion that phenytoin induces liver tumours in mice by a promoting mechanism. The increase in liver weight, centrilobular hypertrophy and pattern of cytochrome P450 induction are similar to those observed with other non-genotoxic mouse liver tumour promoters such as phenobarbital. In addition, the inhibition of cell–cell communication by phenytoin *in vitro* supports the role of promotion in mouse carcinogenesis.

The metabolic activation of phenytoin to a reactive intermediate has been proposed to account for the teratogenicity and possible genotoxicity of phenytoin. One possible intermediate is an arene oxide, that is hypothesized to result in binding to cellular macromolecules. However, this possibility has not been evaluated definitively, and studies of potential DNA damage in mouse liver or hepatocytes have not been reported.

The mechanism of induction of aneuploidy by phenytoin *in vitro* is unclear, as is its relationship to carcinogenicity in mouse liver.

### 5.5 Evaluation[1]

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

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

### Overall evaluation

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

## 6. References

Adams, J., Vorhees, C.V. & Middaugh, L.D. (1990) Developmental neurotoxicity of anticonvulsants: human and animal evidence on phenytoin. *Neurotoxicol. Teratol.*, **12**, 203–214

Allen, R.W., Jr, Ogden, B., Bentley, F.L. & Jung, A.L. (1980) Fetal hydantoin syndrome, neuroblastoma and hemorrhagic disease in a neonate. *J. Am. med. Assoc.*, **244**, 1464–1465

Al-Shammri, S., Guberman, A. & Hsu, E. (1992) Neuroblastoma and fetal exposure to phenytoin in a child without dysmorphic features. *Can. J. neurol. Sci.*, **19**, 243–245

Alving, J., Jensen, M.K. & Meyer, H. (1976) Diphenylhydantoin and chromosome morphology in man and rat: a negative report. *Mutat. Res.*, **40**, 173–176

Alving, J., Jensen, M.K. & Meyer, H. (1977) Chromosome studies of bone marrow cells and peripheral blood lymphocytes from diphenylhydantoin-treated patients. *Mutat. Res.*, **48**, 361–366

American Hospital Formulary Service (1995) *AHFS Drug Information® 95*, Bethesda, MD, American Society of Health-System Pharmacists, pp. 1441–1444

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

Aminoff, M.J., Simon, R.P. & Wiedemann, E. (1984) The hormonal responses to generalized tonic-clonic seizures. *Brain*, **107**, 569–578

Anderson, R.C. (1976) Cardiac defects in children of mothers receiving anticonvulsant therapy during pregnancy. *J. Pediatr.*, **89**, 318–319

Andrade-Mena, C.E., Sardo-Olmedo, J.A.J. & Ramirez-Lizardo, E.J. (1994) Effects of phenytoin administration on murine immune function. *J. Neuroimmunol.*, **50**, 3–7

Angelopoulos, A.P. (1975) A clinicopathological review: diphenylhydantoin gingival hyperplasia: 2. Aetiology, pathogenesis, differential diagnosis and treatment. *J. Can. dent. Assoc.*, **5**, 275–277

Anisimov, V.N. (1980) Effect of buformin and diphenylhydantoin on the life span, oestrous function and spontaneous tumours incidence in rats. *Vopr. Onkol.*, **26**, 42–48 (in Russian)

Anthony, J.J. (1970) Malignant lymphoma associated with hydantoin drugs. *Arch. Neurol.*, **22**, 450–454

Atkinson, A.J, Jr, MacGee, J., Strong, J., Garteiz, D. & Gaffney, T.E. (1970) Identification of 5-*meta*-hydroxyphenyl-5-phenylhydantoin as a metabolite of diphenylhydantoin. *Biochem. Pharmacol.*, **19**, 2483–2491

Atlas, S.A., Zweier, J.L. & Nebert, D.W. (1980) Genetic differences in phenytoin pharmacokinetics. In vivo clearance and in vitro metabolism among inbred strains of mice. *Dev. Pharmacol. Ther.*, **1**, 281–304

Bahn, S., Ganter, U., Bauer, J., Otten, U. & Volk, B. (1993) Influence of phenytoin on cytoskeletal organization and cell viability of immortalized mouse hippocampal neurons. *Brain Res.*, **615**, 160–169

Barcellona, P.S., Barale, R., Campana, A., Zucconi, D., Rossi, V. & Caranti, S. (1987) Correlations between embryotoxic and genotoxic effects of phenytoin in mice. *Teratog. Carcinog. Mutag.*, **7**, 159–168

Barr, M., Jr, Poznanski, A.K. & Schmickel, R.D. (1974) Digital hypoplasia and anticonvulsants during gestation: a teratogenic syndrome? *J. Pediatr.*, **84**, 254–256

Bertollini, R., Kallen, B., Mastroiacova, P. & Robert, A. (1987) Anticonvulsant drugs in monotherapy. Effect on the fetus. *Eur. J. Epidemiol.*, **3**, 164–171

Billings, R.E. (1984) Decreased hepatic 5,10-methylenetetrahydrofolate reductase activity in mice after chronic phenytoin treatment. *Mol. Pharmacol.*, **25**, 459–466

Bishun, N.P., Smith, N.S. & Williams, D.C. (1975) Chromosomes and anticonvulsant drugs. *Mutat. Res.*, **28**, 141–143

Blattner, W.A., Henson, D.E., Young, R.C. & Fraumeni, J.F., Jr (1977) Malignant mesenchymoma and birth defects. Prenatal exposure to phenytoin. *J. Am. med. Assoc.*, **238**, 334–335

Bleyer, W.A. & Skinner, A.L. (1976) Fatal neonatal hemorrhage after maternal anticonvulsant therapy. *J. Am. med. Assoc.*, **235**, 626–627

Bostrom, B. & Nesbit, M.E., Jr (1983) Hodgkin disease in a child with fetal alcohol-hydantoin syndrome. *J. Pediatr.*, **103**, 760–762

Braddock, S.W., Harrington, D. & Vose, J. (1992) Generalized nodular cutaneous pseudolymphoma associated with phenytoin therapy. Use of T-cell receptor gene rearrangement in diagnosis and clinical review of cutaneous reactions to phenytoin. *J. Am. Acad. Dermatol.*, **27**, 337–340

Brams, A., Buchet, J.P., Crutzen-Fayt, M.C., de Meester, C., Lauwerys, R. & Léonard, A. (1987) Comparative study, with 40 chemicals, of the efficiency of the *Salmonella* assay and the SOS chromotest (kit procedure). *Toxicol. Lett.*, **38**, 123–133

British Medical Association/Royal Pharmaceutical Society of Great Britain (1994) *British National Formulary Number 27 (March 1994)*, London, pp. 189–190, 193–194

British Pharmacopoeial Commission (1993) *British Pharmacopoeia* 1993, Vols. I & II, London, Her Majesty's Stationery Office, pp. 511–512, 1056–1058

Browne, T.R. & LeDuc, B. (1995) Phenytoin: chemistry and biotransformation. In: Levy, R.H., Mattson, R.H. & Meldrum, B.S., eds, *Antiepileptic Drugs*, 4th Ed., New York, Raven Press, pp. 283–300

Budavari, S., ed. (1995) *The Merck Index*, 12th Ed., Rahway, NJ, Merck & Co.

Buehler, B.A., Delimont, D., van Waes, M. & Finnell, R.H. (1990) Prenatal prediction of risk of the fetal hydantoin syndrome. *New Engl. J. Med.*, **322**, 1567–1572

Burke, J.T. & Thénot, J.P. (1985) Determination of antiepileptic drugs. *J. Chromatogr.*, **340**, 199–241

Cageao, L.F., Ceppi, J.A., Boado, R.J. & Zaninovich, A.A. (1992) Diphenylhydantoin stimulates the intrapituitary conversion of thyroxine to triiodothyronine in the rat. *Neuroendocrinology*, **56**, 453–458

Chang, T., Savory, A. & Glazko, A.J. (1970) A new metabolite of 5,5-diphenylhydantoin (dilantin). *Biochem. biophys. Res. Comm.*, **38**, 444–449

Chatot, C.L., Klein, N.W., Clapper, M.L., Resor, S.R., Singer, W.D., Russman, B.S., Holmes, G.L., Mattson, R.H. & Cramer, J.A. (1984) Human serum teratogenicity studied by rat embryo culture: epilepsy, anticonvulsant drugs, and nutrition. *Epilepsia*, **25**, 205–216

Chhabra, R.S., Bucher, J.R., Haseman, J.K., Elwell, M.R., Kurtz, P.J. & Carlton, B.D. (1993) Comparative carcinogenicity of 5,5-diphenylhydantoin with or without perinatal exposure in rats and mice. *Fundam. appl. Toxicol.*, **21**, 174–186

Conney, A.H. (1969) Microsomal enzyme induction by drugs. *Pharmacol. Phys.*, **3**, 1–6

Cooke, L.E., Hardin, T.C. & Hendrickson, D.J. (1988) Phenytoin-induced pseudolymphoma with mycosis fungoides manifestations. *Clin Pharmacol.*, **7**, 153–157

Cramer, J.A. & Jones, E.E. (1991) Reproductive function in epilepsy. *Epilepsia*, **32** (Suppl. 6), S19–S26

Czeizel, A.E. (1993) Prevention of congenital abnormalities by periconceptional multivitamin supplementation. *Br. med. J.*, **306**, 1645–1648

Czeizel, A.E. (1995) Folic acid in the prevention of neural tube defect. *J. Pediatr. Gastroenterol. Nutr.*, **20**, 4–16

Czeizel, A.E. & Dudás, I. (1992) Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *New Engl. J. Med.*, **327**, 1832–1835

Czeizel, A.E., Bod, M. & Halász, P. (1992) Evaluation of anticonvulsant drugs during pregnancy in a population-based Hungarian study. *Eur. J. Epidemiol.*, **8**, 122–127

Dabee, V., Hart, A.G. & Hurley, R.M. (1975) Teratogenic effects of diphenylhydantoin. *Can. med. Assoc. J.*, **112**, 75–76

Danielson, M.K., Danielsson, B.R.G., Marchner, H., Lundin, M., Rundqvist, E. & Reiland, S. (1992) Histopathological and hemodynamic studies supporting hypoxia and vascular disruption as explanation to phenytoin teratogenicity. *Teratology*, **46**, 485–497

Danielsson, B.R.G., Danielson, M.K., Rundqvist, E. & Reiland, S. (1992) Identical phalangeal defects induced by phenytoin and nifedipine suggest fetal hypoxia and vascular disruption behind phenytoin teratogenicity. *Teratology*, **45**, 247–258

Dansky, L.V. & Finnel, R.H. (1991) Parental epilepsy, anticonvulsant drugs, and reproductive outcome: epidemiologic and experimental findings spanning three decades. II. Human studies. *Reprod. Toxicol.*, **5**, 301–335

Dansky, L.V., Andermann, E., Rosenblatt, D., Sherwin, A.L. & Andermann, F. (1987) Anticonvulsants, folate levels, and pregnancy outcome: a prospective study. *Ann. Neurol.*, **21**, 176–182

Das, U.N., Devi, G.R., Rao, K.P., Nandan, S.D. & Rao, M.S. (1983) Prevention and/or reversibility of genetic damage induced by phenytoin in the bone marrow cells of mice by colchicine: possible relevance to prostaglandin involvement. *IRCS med. Sci.*, **11**, 122–123

Deda, G., Akinci, A., Teziç, T. & Karagöl, U. (1992) Effects of anticonvulsant drugs on thyroid hormones in epileptic children. *Turk. J. Pediatr.*, **34**, 239–244

DeLattre, J.-Y., Safai, B. & Posner, J.B. (1988) Erythema multiforme and Stevens-Johnson syndrome in patients receiving cranial irradiation and phenytoin. *Neurology*, **38**, 194–198

Dhanwada, K.R., Veerisetty, V., Zhu, F., Razzaque, A., Thompson, K.D. & Jones, C. (1992) Characterization of primary human fibroblasts transformed by human papilloma virus type 16 and herpes simplex virus type 2 DNA sequences. *J. gen. Virol.*, **73**, 791–799

Dill, R.E., Miller, E.K., Weil, T., Lesley, S., Farmer, G.R. & Iacopino, A.M. (1993) Phenytoin increases gene expression for platelet-derived growth factor B chain in macrophages and monocytes. *J. Periodontol.*, **64**, 169–173

Dilman, V.M. & Anisimov, V.N. (1980) Effect of treatment with phenformin, diphenylhydantoin or *L*-dopa on life span and tumour incidence in C3H/Sn mice. *Gerontology*, **26**, 241–246

Diwan, B.A., Hennemen, J.R., Nims, R.W. & Rice, J.M. (1993) Tumor promotion by an anticonvulsant agent, phenytoin, in mouse liver: correlation with CYP2B induction. *Carcinogenesis*, **14**, 2227–2231

Doecke, C.J., Sansom, L.N. & McManus, M.E. (1990) Phenytoin 4-hydroxylation by rabbit liver P450IIC3 and identification of orthologs in human liver microsomes. *Biochem. biophys. Res. Commun.*, **166**, 860–866

Dollery, C., Boobis, A.R., Burley, D., Davies, D.M., Davies, D.S., Harrison, P.I., Orme, M.E., Park, B.K. & Goldberg, L.I. (1991) *Therapeutic Drugs: Phenytoin (sodium)*, Edinburgh, Churchill Livingstone, pp. P93–P98

Eadie, M.J., Lander, C.M. & Tyrer, J.H. (1977) Plasma drug level monitoring in pregnancy. *Clin. Pharmacokinet.*, **2**, 427–436

Eadie, M.J., McKinnon, G.E., Dickinson, R.G., Hooper, W.D. & Lander, C.M. (1992) Phenytoin metabolism during pregnancy. *Eur. J. clin. Pharmacol.*, **43**, 389–392

Earnest, M.P., Marx, J.A. & Drury, L.R. (1983) Complications of intravenous phenytoin for acute treatment of seizures. Recommendations for usage. *J. Am. med. Assoc.*, **249**, 762–765

Ehrenbard, L.T. & Chaganti, R.S.K. (1981) Cancer in the fetal hydantoin syndrome (Letter to the Editor). *Lancet*, **ii**, 97

Elmazar, M.M.A. & Sullivan, F.M. (1981) Effect of prenatal phenytoin administration on postnatal development of the rat: a behavioral teratology study. *Teratology*, **24**, 115–124

Elshove, J. (1969) Cleft palate in the offspring of female mice treated with phenytoin (Letter to the Editor). *Lancet*, **ii**, 1074

Epstein, S.S., 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**, 288–325

Esser, K.J., Kotlarek, F., Habedank, M., Mühler, U. & Mühler, E. (1981) Chromosomal investigations in epileptic children during long-term therapy with phenytoin or primidone. *Hum. Genet.*, **56**, 345–348

Fedrick, J. (1973) Epilepsy and pregnancy: a report from the Oxford Record Linkage Study. *Br. med. J.*, **ii**, 442–448

Finnel, R.H. (1981) Phenytoin-induced teratogenesis: a mouse model. *Science*, **211**, 483–484

Finnel, R.H. & Chernoff, G.F. (1984a) Editorial comment: Genetic background: the elusive component in the fetal hydantoin syndrome. *Am. J. med. Genet.*, **19**, 459–462

Finnel, R.H. & Chernoff, G.F. (1984b) Variable pattern of malformation in the mouse fetal hydantoin syndrome. *Am. J. med. Genet.*, **19**, 463–471

Finnel, R.H., Abbott, L.C. & Taylor, S.M. (1989) The fetal hydantoin syndrome: answers from a mouse model. *Reprod. Toxicol.*, **3**, 127–133

Finnell, R.H., Buehler, B.A., Kerr, B.M., Ager, P.L. & Levy, R.H. (1992) Clinical and experimental studies linking oxidative metabolism to phenytoin-induced teratogenesis. *Neurology*, **42** (Suppl. 5), 25–31

Finnel, R.H., Van Waes, M., Musselman, A., Kerr, B.M. & Levy, R.H. (1993) Differences in the patterns of phenytoin-induced malformations following stiripentol coadministration in three inbred mouse strains. *Reprod. Toxicol.*, **7**, 439–448

Finnel, R.H., Kerr, B.M., Van Waes, M., Stewart, R.L. & Levy, R.H. (1994) Protection from phenytoin-induced congenital malformations by coadministration of the antiepileptic drug striripentol in a mouse model. *Epilepsia*, **35**, 141–148

Flejter, W.L., Astemborski, J.A., Hassel, T.M. & Cohen, M.M. (1989) Cytogenetic effects of phenytoin and/or carbamazepine on human peripheral leukocytes. *Epilepsia*, **30**, 374–379

Friedman, G.D. (1986) Multiple myeloma: relation to propoxyphene and other drugs, radiation and occupation. *Int. J. Epidemiol.*, **15**, 424–426

Fritz, H. (1976) The effect of cortisone on the teratogenic action of acetylsalicylic acid and diphenylhydantoin in the mouse. *Experientia*, **32**, 721–722

Fujii, A., Matsumoto, H., Nakao, S., Teshigawara, H. & Akimoto, Y. (1994) Effect of calcium-channel blockers on cell proliferation, DNA synthesis and collagen synthesis of cultured gingival fibroblasts derived from human nifedipine responders and non-responders. *Arch. oral Biol.*, **39**, 99–104

Gabler, W.L. & Falace, D. (1970) The distribution and metabolism of dilantin in non-pregnant, pregnant and fetal rats. *Arch. int. Pharmacodyn.*, **184**, 45–58

Gabler, W.L. & Hubbard, G.L. (1972) The distribution of 5,5-diphenylhydantoin (DPH) and its metabolites in maternal and fetal rhesus monkey tissues. *Arch. int. Pharmacodyn.*, **200**, 222–230

Gabrys, K., Medras, E., Kowalski, P. & Gola, A. (1983) Malignant lymphoma in the course of antiepileptic therapy. *Polsk. Tyg. Lek.*, **38**, 505–507 (in Polish)

Gaily, E., Granström, M.-L., Hiilesmaa, V. & Bardy, M. (1988) Minor anomalies in the offspring of epileptic mothers. *J. Pediatr.*, **112**, 520–529

Gams, R.A., Neal, J.A. & Conrad, F.G. (1968) Hydantoin-induced pseudo-pseudolymphoma. *Ann. intern. Med.*, **69**, 557–568

Ganapathi, R., Hercbergs, A., Grabowski, D. & Ford, J. (1993) Selective enhancement of vincristine cytotoxicity in multidrug-resistant tumor cells by dilantin (phenytoin). *Cancer Res.*, **53**, 3262–3265

García Sagredo, J.M. (1988) Effect of anticonvulsants on human chromosomes. 2. In vitro studies. *Mutat. Res.*, **204**, 623–626

Gennaro, A.R., ed. (1995) *Remington: The Science and Practice of Pharmacy*, 19th Ed., Vol. II, Easton, PA, Mack Publishing Co., pp. 1177–1178

Ghatak, N.R., Santoso, R.A. & McKinney, W.M. (1976) Cerebellar degeneration following long-term phenytoin therapy. *Neurology*, **26**, 818–820

Gibson, J.E. & Becker, B.A. (1968) Teratogenic effects of diphenylhydantoin in Swiss-Webster and A/J mice. *Proc. Soc. exp. Biol. Med.*, **128**, 905–909

Glazko, A.J. (1982) Phenytoin: chemistry and methods of determination. In: Woodbury, D.M., Penry, J.K. & Pippenger, C.E., eds, *Antiepileptic Drugs*, New York, Raven Press, pp. 177–189

Goodman Gilman, A., Rall, T.W., Nils, A.S. & Taylor, P., eds (1990) *Goodman and Gilman's. The Pharmacological Basis of Therapeutics*, 8th Ed., New York, Pergamon, pp. 439–443; 857–861

Griswold, D.P., Jr, Casey, A.E., Weisburger, E.K., Weisburger, J.H. & Schabel, F.M., Jr (1966) On the carcinogenicity of a single intragastric dose of hydrocarbons, nitrosamines, aromatic amines, dyes, coumarins and miscellaneous chemicals in female Sprague-Dawley rats. *Cancer Res.*, **26**, 619–625

Griswold, D.P., Jr, Casey, A.E., Weisburger, E.K. & Weisburger, J.H. (1968) The carcinogenicity of multiple intragastric doses of aromatic and heterocyclic nitro or amino derivatives in young female Sprague-Dawley rats. *Cancer Res.*, **28**, 924–933

Guest, A.E., Saleh, A.M., Pheasant, A.E. & Blair, J.A. (1983) Effects of phenobarbitone and phenytoin on folate catabolism in the rat. *Biochem. Pharmacol.*, **32**, 3179–3182

Gupta, C., Katsumata, M. & Goldman, A.S. (1985) H-2 histocompatibility region influences the inhibition of arachidonic acid cascade by dexamethasone and phenytoin in mouse embryonic palates. *J. craniofac. Genet. dev. Biol.*, **9**, 277–285

Habedank, M., Esser, K.-J., Brüll, D., Kotlarek, F. & Stumpf, C. (1982) Increased sister chromatid exchanges in epileptic children during long-term therapy with phenytoin. *Hum. Genet.*, **61**, 71–72

Halery, S. & Feuerman, E.J. (1977) Pseudolymphoma syndrome. *Dermatologica*, **155**, 321–327

Hammond, E.J. & Wilder, B.J. (1983) Immunofluorescent evidence for a specific binding site for phenytoin in the cerebellum. *Epilepsia*, **24**, 269–274

Hansen, D.K. (1991) The embryotoxicity of phenytoin: an update on possible mechanisms. *Proc. Soc. exp. Biol. Med.*, **197**, 361–368

Hansen, D.K. & Billings, R.E. (1985) Phenytoin teratogenicity and effects on embryonic and maternal folate metabolism. *Teratology*, **31**, 363–371

Hansen, D.K. & Hodes, M.E. (1983) Comparative teratogenicity of phenytoin among several inbred strains of mice. *Teratology*, **28**, 175–179

Hansen, D.K., Holson, R.R., Sullivan, P.A. & Grafton, T.F. (1988) Alterations in maternal plasma corticosterone levels following treatment with phenytoin. *Toxicol. appl. Pharmacol.*, **96**, 24–32

Hansen, D.K., Branham, W.S., Sheehan, D.M. & Holson, R.R. (1992) Embryotoxicity of phenytoin in adrenalectomized CD-1 mice. *Proc. Soc. exp. Biol. Med.*, **199**, 501–508

Hanson, J.W. (1976) Fetal hydantoin syndrome. *Teratology*, **13**, 185–187

Hanson, J.W. & Smith, D.W. (1975) The fetal hydantoin syndrome. *J. Pediatr.*, **87**, 285–290

Hanson, J.W., Myrianthopoulos, N.C., Sedgwick Harvey, M.A. & Smith, D.W. (1976) Risks to the offspring of women treated with hydantoin anticonvulsants, with emphasis on the fetal hydantoin syndrome. *J. Pediatr.*, **89**, 662–668

Harbison, R.D. (1978) Chemical-biological reactions common to teratogenesis and mutagenesis. *Environ. Health Perspectives*, **29**, 87–100

Harbison, R.D. & Becker, B.A. (1969) Relation of dosage and time of administration of diphenylhydantoin to its teratogenic effect in mice. *Teratology*, **2**, 305–311

Harbison, R.D. & Becker, B.A. (1972) Diphenylhydantoin teratogenicity in rats. *Toxicol. appl. Pharmacol.*, **22**, 193–200

Harbison, R.D. & Becker, B.A. (1974) Comparative embryotoxicity of diphenylhydantoin and some of its metabolites in mice. *Teratology*, **10**, 237–242

Harris, D.W.S., Ostlere, L., Buckley, C., Whittaker, S., Sweny, P. & Rustin, M.H.A. (1992) Phenytoin-induced pseudolymphoma. A report of a case and review of the literature. *Br. J. Dermatol.*, **127**, 403–406

Harrison, M.B., Lyons, G.R. & Landow, E.R. (1993) Phenytoin and dyskinesias: a report of two cases and review of the literature. *Movement Disorders*, **8**, 19–27

Hashem, N. & Shawki, R. (1976) Cultured peripheral lymphocytes: one biologic indicator of potential drug hazard. *Afr. J. Med. med. Sci.*, **5**, 155–163

Hassell, T.M. (1981) *Epilepsy and the Oral Manifestation of Phenytoin Therapy*, Basel, Karger, pp. 116–202

Hassell, T.M., Roebuck, S., Page, R.C. & Wray, S.H. (1982) Quantitative histopathologic assessment of developing phenytoin-induced gingival overgrowth in the cat. *J. clin. Periodontol.*, **9**, 365–372

Hassell, T.M., Maguire, J.H., Cooper, C.G. & Johnson, P.T. (1984) Phenytoin metabolism in the cat after long-term oral administration. *Epilepsia*, **25**, 556–563

Hatch, G.G., Anderson, T.M., Lubet, R.A., Kouri, R.E., Putman, D.L., Cameron, J.W., Nims, R.W., Most, B., Spalding, J.W., Tennant, R.W. & Schechtman, L.M. (1986) Chemical enhancement of SA7 virus transformation of hamster embryo cells: evaluation of interlaboratory testing of diverse chemicals. *Environ. Mutag.*, **8**, 515–513

Haworth, S., Lawlor, T., Mortelmans, K., Speck, W. & Zeiger, E. (1983) *Salmonella* mutagenicity test results for 250 chemicals. *Environ. mol. Mutagen.*, **5** (Suppl. 1), 3–142

Hayakawa, T., Hamada, Y., Maihara, T., Hattori, H. & Mikawa, H. (1994) Phenytoin reduces neonatal hypoxic-ischemic brain damage in rats. *Life Sci.*, **54**, 387–392

Hendrie, T.A., Rowland, J.R., Binkerd, P.E. & Hendricks, A.G. (1990) Developmental toxicity and pharmacokinetics of phenytoin in the rhesus macaque: an interspecies comparison. *Reprod. Toxicol.*, **4**, 257–266

Herzog, A.G., Levesque, L.A., Drislane, F.W., Ronthal, M. & Schomer, D.L. (1991) Phenytoin-induced elevation of serum estradiol and reproductive dysfunction in men with epilepsy. *Epilepsia*, **32**, 550–553

Hiilesmaa, V.K., Teramo, K., Granström, M.-L. & Bardy, A.H. (1983) Serum folate concentrations during pregnancy in women with epilepsy: relation to antiepileptic drug concentrations, number of seizures, and fetal outcome. *Br. med. J.*, **287**, 577–579

Hill, R.M., Verniaud, W.M., Horning, M.G., McCulley, L.B. & Morgan, N.F. (1974) Infants exposed *in utero* to antiepileptic drugs: a prospective study. *Am. J. Dis. Child.*, **127**, 645–653

Hirano, T., Kaneko, S. & Fujioka, K. (1984) Prospective study on psychomotor development in the offspring of epileptic mother: risk factors for developmental retardation. *Ann. Rep. pharmacopsychiat. Res. Found.*, **15**, 282–291

Hou, L.-T. (1993) Synthesis of collagen and fibronectin in fibroblasts derived from healthy and hyperplastic gingivae. *J. Formos. med. Assoc.*, **92**, 367–372

Hyman, G.A. & Sommers, S.C. (1966) The development of Hodgkin's disease and lymphoma during anticonvulsant therapy. *Blood*, **28**, 416–427

IARC (1977) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, Vol. 13, *Some Miscellaneous Pharmaceutical Substances*, Lyon, pp. 201–225

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Update of* IARC Monographs *Volumes 1 to 42*, Lyon, pp. 319–321

Ichiba, M., Kanazawa, H., Kobo, M., Takenobu, S., Taguchi, S., Senga, K. & Tamura, Z. (1984) Analysis of drugs in fallen dust in a dispensary using thin-layer chromatography and chemical ionization mass spectrometry. *Yakuzaigaku*, **44**, 231–238 (in Japanese)

Ichiba, M., Kanazawa, H., Shimizu, N., Kobo, M., Senga, K. & Tamura, Z. (1986) Identification of drugs with optical, fluorescence and polarizing microscopes: drugs in fallen and floating dust in a dispensary. *Bunseki Kagaku*, **35**, 157–160 (in Japanese)

Isobe, T., Horimatsu, T., Fujita, T., Miyazaki, K. & Sugiyama, T. (1980) Adult T-cell lymphoma following diphenylhydantoin therapy. *Acta haematol. jpn.*, **43**, 711–714

Jang, J.J., Takahashi, M., Furukawa, F., Toyoda, K., Hasegawa, R., Sato, H. & Hayashi, Y. (1987) Long-term in vivo carcinogenicity study of phenytoin (5,5-diphenylhydantoin) in F344 rats. *Food chem. Toxicol.*, **25**, 697–702

Jerina, D.M. & Daly, J.W. (1974) Arene oxides: a new aspect of drug metabolism. *Science*, **185**, 573–582

Jimenez, J.F., Brown, R.E., Seibert, R.W., Seibert, J.J. & Char, F. (1981) Melanotic neuroectodermal tumor of infancy and fetal hydantoin syndrome. *Am. J. pediatr. Hemat./Oncol.*, **3**, 9–15

Jone, C.M., Erickson, L.M., Trosko, J.E., Netzloff, M.L. & Cheng, C.-C. (1985) Inhibition of metabolic cooperation by the anticonvulsants, diphenylhydantoin and phenobarbital. *Teratog. Carcinog. Mutag.*, **5**, 379–391

Jones, G.L. & Wimbish, G.H. (1985) Hydantoins. In: Frey, H.-H. & Janz, D., eds, *Handbook of Experimental Pharmacology*, Vol. 74, *Anti-epileptic Drugs*, Berlin, Springer-Verlag, pp. 351–419

Juhász, J., Baló, J. & Szende, B. (1968) Experimental tumours developing upon the effect of diphenylhydantoin treatment. *Magyar Onkol.*, **12**, 39–44 (in Hungarian)

Kaneko, S. & Kondo, T. (1995) Antiepileptic agents and birth defects. Incidence, mechanisms and prevention. *CNS Drugs*, **3**, 41–55

Kaneko, S., Otani, K., Fukushima, Y., Ogawa, Y., Nomura, Y., Ono, T., Nakane, Y., Teranishi, T. & Goto, M. (1988) Teratogenicity of antiepileptic drugs: analysis of possible risk factors. *Epilepsia*, **29**, 459–467

Kaneko, S., Otani, K., Kondo, T., Fukushima, Y., Nakamura, Y., Ogawa, Y., Kan, R., Takeda, A., Nakane, Y. & Teranishi, T. (1992) Malformation in infants of mothers with epilepsy receiving antiepileptic drugs. *Neurology*, **42** (Suppl. 5), 68–74

Katsumata, M., Gupta, C., Baker, M.K., Sussdorf, C.E. & Goldman, A.S. (1982) Diphenylhydantoin: an alternative ligand of a glucocorticoid receptor affecting prostaglandin generation in A/J mice. *Science*, **218**, 1313–1315

Katsumata, M., Gupta, C. & Goldman, A.S. (1985) Glucocorticoid receptor IB: mediator of anti-inflammatory and teratogenic functions of both glucocorticoids and phenytoin. *Arch. Biochem. Biophys.*, **243**, 385–395

Kay, E.D., Goldman, A.S. & Daniel, J.C. (1988) Arachidonic acid reversal of phenytoin-induced neural tube and craniofacial defects *in vitro* in mice. *J. craniofac. Genet. dev. Biol.*, **8**, 179–186

Kay, E.D., Goldman, A.S. & Daniel, J.C. (1990) Common biochemical pathway of dysmorphogenesis in murine embryos: use of the glucocorticoid pathway by phenytoin. *Teratog. Carcinog. Mutag.*, **10**, 31–39

Keith, D.A., Gundberg, C.M., Japour, A., Aronoff, J., Alvarez, N. & Gallop, P.M. (1983) Vitamin K-dependent proteins and anticonvulsant medication. *Clin. Pharmacol. Ther.*, **34**, 529–532

Kelly, T.E. (1984) Teratogenicity of anticonvulsant drugs. III. Radiographic hand analysis of children exposed *in utero* to diphenylhydantoin. *Am. J. med. Genet.*, **19**, 445–450

Kempermann, G., Knoth, R., Gebicke-Haerter, P.J., Stolz, B.-J. & Volk, B. (1994) Cytochrome P450 in rat astrocytes *in vivo* and *in vitro*: intracellular localization and induction by phenytoin. *J. Neurosci. Res.*, **39**, 576–588

Kim, N.D., Yoo, J.K., Won, S.M., Park, S.S. & Gelboin, H.V. (1993) Phenytoin induction of cytochrome P4502B in mice: effects on hexobarbital hydroxylase activity. *Xenobiotica*, **23**, 217–225

Kimball, O.P. (1939) The treatment of epilepsy with sodium diphenylhydantoinate. *J. Am. med. Assoc.*, **112**, 1244–1246

Kindig, D., Garriott, M.L., Parton, J.W., Brunny, J.D. & Beyers, J.E. (1992) Diphenylhydantoin is not genotoxic in a battery of short-term cytogenetic assays. *Teratog. Carcinog. Mutag.*, **12**, 43–50

Kirchhoff, L.V., Evans, A.S., McClelland, K.E., Carvalho, R.P.S. & Pannuti, C.S. (1980) A case–control study of Hodgkin's disease in Brazil. I. Epidemiological aspects. *Am. J. Epidemiol.*, **112**, 595–608

Knuutila, S., Simell, O., Lipponen, P. & Saarinen, I. (1976) Bone-marrow chromosomes in healthy subjects. *Hereditas*, **82**, 29–35

Knuutila, S., Siimes, M., Simell, O., Tammisto, P. & Weber, T. (1977) Long-term use of phenytoin: effects on bone-marrow chromosomes in man. *Mutat. Res.*, **43**, 309–312

Koch, S., Göpfert-Geyer, E., Häuser, I., Hartmann, A., Jakob, S., Jäger-Roman, E., Nau, H., Rating, D. & Helge, H. (1985) Neonatal behaviour disturbance in infants of epileptic women treated during pregnancy. *Prof. clin. biol. Res.*, **163B**, 453–461

Koren, G., Demitrakoudis, D., Weksberg, R., Rieder, M., Shear, N.H., Sonely, M., Shandling, B. & Spielberg, S.P. (1989) Neuroblastoma after prenatal exposure to phenytoin: cause and effect? *Teratology*, **40**, 157–162

Kramer, S., Ward, E., Meadows, A.T. & Malone, K.E. (1987) Medical and drug risk factors associated with neuroblastoma: a case–control study. *J. natl Cancer Inst.*, **78**, 797–804

Krüger, G., Harris, D. & Sussman, E. (1972) Effect of dilantin in mice. II. Lymphoreticular tissue atypia and neoplasia after chronic exposure. *Z. Krebsforsch.*, **78**, 290–302

Kubow, S. (1992) Inhibition of phenytoin bioactivation and teratogenicity by dietary n-3 fatty acids in mice. *Lipids*, **27**, 721–728

Kubow, S. & Wells, P.G. (1986) In vitro evidence for prostaglandin synthetase-catalysed bioactivation of phenytoin to a free radical intermediate (Abstract no. 23). *Pharmacologist*, **28**, 195

Kubow, S. & Wells, P.G. (1989) In vitro bioactivation of phenytoin to a reactive free radical intermediate by prostaglandin synthetase, horseradish peroxidase, and thyroid peroxidase. *Mol. Pharmacol.*, **35**, 504–511

Kumar, N., Khwaja, G.A., Gupta, M. & Sharma, S. (1993) Antiepileptic drug induced osteomalacic myopathy with hyperparathyroidism and nephrolithiasis. *J. Assoc. Phys. India*, **41**, 748–749

Lambotte-Vandepaer, M., Brams, A., Crutzen-Fayt, M.C., Duverger-Van Bogaert, M., Dumont, P. & Léonard, A. (1989) Comparison of teratogenic properties in mice, strain BALB-c by diphenylhydantoin and $^{3}$H-diphenylhydantoin. *C.R. Soc. Biol.*, **183**, 358–361 (in French)

Lander, C.M., Edwards, V.E., Eadie, M.J. & Tyrer, J.H. (1977) Plasma anticonvulsant concentrations during pregnancy. *Neurology*, **27**, 128–131

Larkin, J.G., Macphee, G.J.A., Beastall, G.H. & Brodie, M.J. (1989) Thyroid hormone concentrations in epileptic patients. *Eur. J. clin. Pharmacol.*, **36**, 213–216

Laxer, K.D., Mullooly, J.P. & Howell, B. (1985) Prolactin changes after seizures classified by EEG monitoring. *Neurology*, **35**, 31–35

Léonard, A., de Meester, C., Fabry, L., de Saint-Georges, L. & Dumont, P. (1984) Lack of mutagenicity of diphenylhydantoin in in vitro short-term tests. *Mutat. Res.*, **137**, 79–88

Levo, Y. (1974) The protective effect of hydantoin treatment on carcinogenesis. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **285**, 29–30

Li, F.P, Willard, D.R., Goodman, R. & Vawter, G. (1975) Malignant lymphoma after diphenylhydantoin (dilantin) therapy. *Cancer*, **36**, 1359–1362

Linet, M.S., Harlow, S.D. & McLaughlin, J.K. (1987) A case–control study of multiple myeloma in whites: chronic antigenic stimulation, occupation, and drug use. *Cancer Res.*, **47**, 2978–2981

Liu, L. & Wells, P.G. (1994) In vivo phenytoin-initiated oxidative damage to proteins and lipids in murine maternal hepatic and embryonic tissue organelles: potential molecular targets of chemical teratogenesis. *Toxicol. appl. Pharmacol.*, **125**, 247–255

Lorand, I.G.H., Hadler, W.A. & Prigenzi, L.S. (1976) Morphological changes in the lymphoid organs induced by diphenylhydantoin sodium (DPH). *Virchows Arch. A. Pathol. Anat. Histol.*, **372**, 81–88

Lowe, C.R. (1973) Congenital malformations among infants born to epileptic women. *Lancet*, **i**, 9–10

MacKinney, A.A., Vyas, R.S. & Walker, D. (1978) Hydantoin drugs inhibit polymerization of pure microtubular protein. *J. Pharmacol. exp. Ther.*, **204**, 189–202

Macphee, G.J.A., Larkin, J.G., Butler, E., Beastall, G.H. & Brodie, M.J. (1988) Circulating hormones and pituitary responsiveness in young epileptic men receiving long-term anti-epileptic medication. *Epilepsia*, **29**, 468–475

Maeda, T., Sano, N., Togei, K., Shibata, M., Izumi, K. & Otsuka, H. (1988) Lack of carcinogenicity of phenytoin in (C57Bl/6 × C3H)$F_1$ mice. *J. Toxicol. environ. Health*, **24**, 111–119

Maguire, J.H., Butler, T.C. & Dudley, K.H. (1980) Absolute configurations of the dihydrodiol metabolites of 5,5-diphenylhydantoin (phenytoin) from rat, dog, and human urine. *Drug Metab. Dispos.*, **8**, 325–331

Maguire, J.H., Greenwood, R.S., Lewis, D.V. & Hassell, T.M. (1986) Phenytoin-induced gingival overgrowth incidence is dependent upon co-medication. *J. dent. Res.*, **65**, 249

Mallek, H.M. & Nakamoto, T. (1981) Dilantin and folic acid status. Clinical implications for the periodontist. *J. Periodontol.*, **14**, 255–259

Malpas, J.S., Spray, G.H. & Witts, L.J. (1966) Serum folic-acid and vitamin-$B_{12}$ levels in anticonvulsant therapy. *Br. med. J.*, **i**, 955–957

Manon-Espaillat, R., Burnstine, T.H., Remler, B., Reed, R.C. & Osorio, I. (1991) Antiepileptic drug intoxication: factors and their significance. *Epilepsy*, **32**, 96–100

Mareš, P., Lišková-Bernášková, K. & Mudrochová, M. (1987) Convulsant action of diphenylhydantoin overdose in young rats. *Act. Nerv. Sup. (Praha)*, **29**, 30–35

Margaretten, N.C. & Warren, R.T. (1987) Effect of phenytoin on antibody production: use of a murine model. *Epilepsia*, **28**, 77–80

Marsh, L. & Fraser, F.C. (1973) Studies on dilantin-induced cleft palate in mice (Abstract). *Teratology*, **7**, A-23

Martz, F., Failinger, C., III & Bake, D.A. (1977) Phenytoin teratogenesis: correlation between embryopathic effect and covalent binding of putative arene oxide metabolite in gestational tissue. *Pharmacol. exp. Ther.*, **203**, 231–239

Massa, T., Gerber, T., Pfaffenholz, V., Chandra, A., Schlatterer, A. & Chandra, P. (1990) A host-mediated in vivo/in vitro assay with peritoneal murine macrophages for the detection of carcinogenic chemicals. *J. Cancer Res. clin. Oncol.*, **116**, 357–364

Massey, K.M. (1966) Teratogenic effects of dyphenylhydantoin sodium. *J. oral Ther. Pharmacol.*, **2**, 380–385

Masur, H., Elger, C.E., Ludolph, A.C. & Galanski, M. (1989) Cerebellar atrophy following acute intoxication with phenytoin. *Neurology*, **39**, 432–433

Masur, H., Fahrendorf, G., Oberwittler, C. & Reuther, G. (1990) Cerebellar atrophy following acute intoxication with phenytoin. *Neurology*, **40**, 1800

Mattson, R.H. & Cramer, J.A. (1985) Epilepsy, sex hormones and antiepileptic drugs. *Epilepsia*, **26** (Suppl.), S40–S51

Maurya, A.K. & Goyle, S. (1985) Mutagenic potential of anticonvulsant diphenylhydantoin (DPH) on human lymphocytes *in vitro*. *Meth. Find exp. clin. Pharmacol.*, **7**, 109–112

Maya, M.T., Farinha, A.R., Lucas, A.M. & Morais, J.A. (1992) Sensitive method for the determination of phenytoin in plasma, and phenytoin and 5-(4-hydroxyphenyl)-5-phenylhydantoin in urine by high-performance liquid chromatography. *J. pharm. biomed. Analysis*, **10**, 1001–1006

McClain, R.M. & Langhoff, L. (1980) Teratogenicity of diphenylhydantoin in the New Zealand white rabbit. *Teratology*, **21**, 371–379

McDevitt, J.M., Gautieri, R.F. & Mann, D.E., Jr (1981) Comparative teratogenicity of cortisone and phenytoin in mice. *J. pharm. Sci.*, **70**, 631–634

McDonald, D.F. (1969) Lack of effect of diphenylhydantoin ingestion on vesical transitional epithelium in the rat. *J. surg. Oncol.*, **1**, 77–79

McFee, A.F., Tice, R.R. & Shelby, M.D. (1992) In vivo cytogenetic activity of diphenylhydantoin in mice. *Mutat. Res.*, **278**, 61–68

McLain, L.W., Jr, Martin, J.T. & Allen, J.H. (1980) Cerebellar degeneration due to chronic phenytoin therapy. *Ann. Neurol.*, **7**, 18–23

Meadow, S.R. (1968) Anticonvulsant drugs and congenital abnormalities (Letter to the Editor). *Lancet*, **ii**, 1296

Meadow, S.R. (1970) Congenital abnormalities and anticonvulsant drugs. *Proc. roy. Soc. Med.*, **63**, 48–49

Medical Economics (1996) *PDR®: Physicians' Desk Reference*, 50th Ed., Montvale, NJ, Medical Economics Data Production Co., pp. 1906–1913

Mercier-Parot, L. & Tuchmann-Duplessis, H. (1974) The dysmorphogenic potential of phenytoin: experimental observations. *Drugs*, **8**, 340–353

Minck, D.R., Erway, L.C. & Vorhees, C.V. (1989) Preliminary findings of a reduction of otoconia in the inner ear of adult rats prenatally exposed to phenytoin. *Neurotoxicol. Teratol.*, **11**, 307–311

Miranda, A.F., Wiley, M.J. & Wells, P.G. (1994) Evidence for embryonic peroxidase-catalyzed bioactivation and glutathione-dependent cytoprotection in phenytoin teratogenicity. Modulation by eicosatetraynoic acid and buthionine sulfoximine in murine embryo culture. *Toxicol. appl. Pharmacol.*, **124**, 230–241

Mirkin, B.L. (1971) Diphenylhydantoin: placental transport, fetal localisation, neonatal metabolism, and possible teratogenic effects. *J. Pediatr.*, **78**, 329–337

Moglia, A., Tartara, A., Arrigo, A., Poggi, P., Scelsi, M. & Scelsi, R. (1981) Chronic treatment with phenytoin in rats: effects on peripheral nervous system. *Farm. Sci.*, **36**, 419–424

Monson, R.R., Rosenberg, L., Hartz, S.C., Shapiro, S., Heinonen, O.P. & Slone, D. (1973) Diphenylhydantoin and selected congenital malformations. *New Engl. J. Med.*, **289**, 1049–1052

Montes de Oca-Luna, R., Leal-Garza, C.H., Baca-Sevilla, S. & Garza-Chapa, R. (1984) The effect of diphenylhydantoin on the frequency of micronuclei in bone-marrow polychromatic erythrocytes of mice. *Mutat. Res.*, **141**, 183–187

Morris, J.E., Price, J.M., Lalich, J.J. & Stein, R.J. (1969) The carcinogenic activity of some 5-nitrofuran derivatives in the rat. *Cancer Res.*, **29**, 2145–2156

MRC Vitamin Study Research Group (1991) Prevention of neural tube defects. Results of the Medical Research Council Vitamin Study. *Lancet*, **338**, 131–137

Mullenix, P., Tassinari, M.S. & Keith, D.A. (1983) Behavioral outcome after prenatal exposure to phenytoin in rats. *Teratology*, **27**, 149–157

Nation, R.L., Evans, A.M. & Milne, R.W. (1990a) Pharmacokinetic drug interactions with phenytoin (Part I). *Clin. Pharmacokinet.*, **18**, 37–60

Nation, R.L., Evans, A.M. & Milne, R.W. (1990b) Pharmacokinetic drug interactions with phenytoin (Part II). *Clin. Pharmacokinet.*, **18**, 131–150

Nau, H., Kuhnz, W., Egger, H.-J., Rating, D. & Helge, H. (1982) Anticonvulsants during pregnancy and lactation. Transplacental, maternal and neonatal pharmacokinetics. *Clin. Pharmacokinet.*, **7**, 508–543

Nelson, K.B. & Ellenberg, J.H. (1982) Maternal seizure disorder, outcome of pregnancy, and neurological abnormalities in the children. *Neurology*, **32**, 1247–1254

Nelson, E.W., Crick, W.F., Cerda, J.J., Wilder, B.J. & Streiff, R.R. (1983) The effect of diphenylhydantoin (phenytoin) on the sequential stages of intestinal folate absorption. *Drug. Nutr. Interact.*, **2**, 47–56

Netzloff, M.L., Streiff, R.R., Frias, J.L. & Rennert, O.M. (1979) Folate antagonism following teratogenic exposure to diphenylhydantoin. *Teratology*, **19**, 45–50

Ney, G.C., Lantos, G., Barr, W.B. & Schaul, N. (1994) Cerebellar atrophy in patients with long-term phenytoin exposure and epilepsy. *Arch. Neurol.*, **51**, 767–771

Oberly, T.J., Michaelis, K.C., Rexroat, M.A., Bewsey, B.J. & Garriott, M.L. (1993) A comparison of the CHO/HGPRT$^{+}$ and the L5178Y/TK$^{+/-}$ mutation assays using suspension treatment and soft agar cloning: results for 10 chemicals. *Cell Biol. Toxicol.*, **9**, 243–257

Ohta, T., Wergedal, J.E., Matsuyama, T., Baylink, D.J. & Lau, K.-H.W. (1995) Phenytoin and fluoride act in concert to stimulate bone formation and to increase bone volume in adult male rats. *Calcif. Tissue int.*, **56**, 390–397

de Oliveira, A.R. & Machado-Santelli, G.M. (1987) Diphenylhydantoin and mitotic spindle abnormalities in cultured mouse and human cells. *Mutat. Res.*, **187**, 91–97

de Oliveira, A.R., Mori, L. & Machado-Santelli, G.M. (1987) Diphenylhydantoin effects in Balb/c mouse bone marrow cells: cytogenetic aspects (Short communication). *Rev. Brasil. Genet.*, **10**, 127–134

Olsen, J.H., Boice, J.D., Jr, Jensen, J.P.A. & Fraumeni, J.F., Jr (1989) Cancer among epileptic patients exposed to anticonvulsant drugs. *J. natl Cancer Inst.*, **81**, 803–808

Olsen, J.H., Wallin, H., Boice, J.D., Jr, Rask, K., Schulgen, G. & Fraumeni, J.F., Jr (1993) Phenobarbital, drug metabolism, and human cancer. *Cancer Epidemiol. Biomarkers Prev.*, **2**, 449–452

Olsen, J.H., Schulgen, G., Boice, J.D., Jr., Whysner, J., Travis, L.B., Williams, G.M., Johnson, F.B. & McGee, J.O'D. (1995) Antiepileptic treatment and risk for hepatobiliary cancer and malignant lymphoma. *Cancer Res.*, **55**, 294–297

Pacifici, G.M. & Rane, A. (1983) Epoxide hydrolase in human fetal liver. *Pharmacology*, **26**, 241–248

Pacifici, G.M., Norlin, A. & Rane, A. (1981) Glutathione S-transferase in human fetal liver. *Biochem. Pharmacol.*, **30**, 3367–3371

Page, T.E., Hoyme, H.E., Markarian, M. & Jones, K.L. (1982) Neonatal haemorrhage secondary to thrombocytopenia: an occasional effect of prenatal hydantoin exposure. *Birth Defect. orig. Art. Ser.*, **18**, 47–50

Pantarotto, C., Arboix, M., Sezzano, P. & Abbruzi, R. (1982) Studies of 5,5-diphenylhydantoin irreversible blinding to rat liver microsomal proteins. *Biochem. Pharmacol.*, **31**, 1501–1507

Parke-Davis (1995) *Phenytoin*, Morris Plains, NJ, USA

Pelkonen, O. & Kärki, N.T. (1975) Epoxidation of xenobiotics in the human fetus and placenta: a possible mechanism of transplacental drug-induced injuries. *Biochem. Pharmacol.*, **24**, 1445–1448

Pendergrass, T.W. & Hanson, J.W. (1976) Fetal hydantoin syndrome and neuroblastoma (Letter to the Editor). *Lancet*, **ii**, 150

Peraino, C., Fry, R.J.M., Staffeldt, E. & Christopher, J.P. (1975) Comparative enhancing effects of phenobarbital, amobarbital, diphenylhydantoin and dichlorodiphenyltrichloroethane on 2-acetylaminofluorene-induced hepatic tumorigenesis in the rat. *Cancer Res.*, **35**, 2884–2890

Philip, J., Holcomb, I.J. & Fusari, S.A. (1984) Phenytoin. In: Florey, K., ed., *Analytical Profiles of Drug Substances*, Vol. 13, New York, Academic Press, pp. 417–445

Phillips, N.K. & Lockard, J.S. (1985) A gestational monkey model: effects of phenytoin versus seizures on neonatal outcome. *Epilepsia*, **26**, 697–703

Phillips, N.K. & Lockard, J.S. (1989) Stiripentol, phenytoin, valproate and pregnancy in monkey (Abstract). *Epilepsia*, **30**, 666

Phillips, N.K. & Lockard, J.S. (1990) Object permanence development in monkey infants prenatally exposed to phenytoin or stiripentol (Abstract). *Epilepsia*, **31**, 600

Phillips, N.K. & Lockard, J.S. (1993) Phenytoin and/or stiripentol in pregnancy: infant monkey hyperexcitability. *Epilepsia*, **34**, 1117–1122

Pizzi, W.J. & Jersey, R.M. (1992) Effects of prenatal diphenylhydantoin treatment on reproductive outcome, development, and behavior in rats. *Neurotoxicol. Teratol.*, **14**, 111–117

Poupaert, J.H., Cavalier, R., Claesen, M.H. & Dumont, P.A. (1975) Absolute configuration of the major metabolite of 5,5-diphenylhydantoin, 5-(4′-hydroxyphenyl)-5-phenylhydantoin. *J. med. Chem.*, **18**, 1268–1271

Ramadevi, G., Das, U.N., Rao, K.P. & Rao, M.S. (1984) Prostaglandins and mutagenesis: modification of phenytoin induced genetic damage by prostaglandins in lymphocyte cultures. *Prostaglandins Leukotrienes Med.*, **15**, 109–113

Ramaniah, T.V., Nandan, S.D., Rao, K.P. & Rao, M.S. (1980) Mutagenicity of phenytoin in the male germ cells of Swiss mice. *IRCS med. Sci.*, **8**, 853

Ramilo, J. & Harris, V.J. (1979) Neuroblastoma in a child with the hydantoin and fetal alcohol syndrome. The radiographic features. *Br. J. Radiol.*, **52**, 993–995

Rane, A. & Peng, D. (1985) Phenytoin enhances epoxide metabolism in human fetal liver culture. *Drug Metab. Dispos.*, **13**, 382–385

Rapport, R.L., II. & Shaw, C.-M. (1977) Phenytoin-related cerebellar degeneration without seizures. *Ann. Neurol.*, **2**, 437–439

Raya, A., Gallego, J., Hermenegildo, C., Puertas, F.J., Romero, F.J., Felipo, V., Miñana, M.D., Grisolía, S. & Romá, J. (1992) Prevention of the acute neurotoxic effects of phenytoin on rat peripheral nerve by H7, an inhibitor of protein kinase C. *Toxicology*, **75**, 249–256

Reynolds, E.H. (1973) Anticonvulsants, folic acid, and epilepsy. *Lancet*, **i**, 1376–1378

Reynolds, J.E.F., ed. (1993) *Martindale: The Extra Pharmacopoeia*, 30th Ed., London, The Pharmaceutical Press, pp. 304–309

Riedel, L. & Obe, G. (1984) Mutagenicity of antiepileptic drugs. II. Phenytoin, primidone and phenobarbital. *Mutat. Res.*, **138**, 71–74

Rijlaarsdam, U., Scheffer, E., Meijer, C.J.L.M., Kruyswijk, M.R.J. & Willemze, R. (1991) Mycosis fungoides-like lesions associated with phenytoin and carbamazepine therapy. *J. Am. Acad. Dermatol.*, **24**, 216–220

Rikihisa, T., Takeda, Y., Nakata, K.-I. & Kanakubo, Y. (1984) Composition of fallen dust in a dispensing room. *Yakuzaigaku*, **44**, 169–174 (in Japanese)

Rollins, D., Larsson, A., Steen, B., Krishnaswamy, K., Hagenfeldt, L., Moldéns, P. & Rane, A. (1981) Glutathione and gamma-glutamyl cycle enzymes in fetal liver. *J. Pharmacol. exp. Ther.*, **217**, 697–700

Rosenthal, C.J., Noguera, C.A., Coppola, A. & Kapelner, S.N. (1982) Pseudolymphoma with mycosis fungoides manifestations, hyperresponsiveness to diphenylhydantoin, and lymphocyte disregulation. *Cancer*, **49**, 2305–2314

Rubinstein, I., Langevitz, P. & Shibi, G. (1985) Isolated malignant lymphoma of the jejunum and long-term diphenylhydantoin therapy. *Oncology*, **42**, 104–106

Saltzstein, S.L. & Ackerman, L.V. (1959) Lymphadenopathy induced by anticonvulsant drugs and mimicking clinically and pathologically malignant lymphomas. *Cancer*, **12**, 164–182

Sanders, B.M. & Draper, G.J. (1979) Childhood cancer and drugs in pregnancy. *Br. med. J.*, **i**, 717–719

Sanyal, S. & Wells, P.G. (1993) Reduction in phenytoin teratogenicity by pretreatment with the antioxidant D-α-tocopherol acetate (vitamin E) in CD-1 mice (Abstract no. 950). *Toxicologist*, **13**, 252

Schardein, J.L., Dresner, A.J., Hentz, D.L., Petrere, J.A., Fitzgerald, J.E. & Kurtz, S.M. (1973) The modifying effect of folinic acid on diphenylhydantoin-induced teratogenicity in mice. *Toxicol. appl. Pharmacol.*, **24**, 150–158

Schaumann, B.A., Johnson, S.B., Wang, N. & Van Brunt, S. (1985) Sister chromatid exchanges in adult epileptic patients on phenytoin therapy. *Environ. Mutag.*, **7**, 711–714

Schaumann, B.A., Winge, V.B. & Garry, V.F. (1989) Lack of sister chromatid exchange induction in phenytoin-treated patients with epilepsy. *Epilepsia*, **30**, 240–245

Schmidt, D., Canger, A., Avanzini, G., Battino, D., Cusi, C., Beck-Mannagetta, G., Koch, S., Rating, D. & Janz, D. (1983) Changes in seizure frequency in pregnant epileptic women. *J. Neurol. Neurosurg. Psychiat.*, **46**, 751–755

Schöder-Van der Elst, J.P. & Van der Heide, D. (1990) Effects of 5,5′-diphenylhydantoin on thyroxine and 3,5,3′-triiodothyronine concentrations in several tissues of the rat. *Endocrinology*, **126**, 186–191

Schreiber, M.M. & McGregor, J.G. (1968) Pseudolymphoma syndrome, a sensitivity to anticonvulsant drugs. *Arch. Dermatol.*, **97**, 297–300

Scolnik, D., Mulman, D., Rovet, J., Gladstone, D., Czuchta, D., Gardner, H.A., Gladstone, R., Ashby, P., Weksberg, R., Einarson, T. & Koren, G. (1994) Neurodevelopment of children exposed *in utero* to phenytoin and carbamazepine monotherapy. *J. Am. med. Assoc.*, **271**, 767–770

Scoville, B. & White, B.G. (1981) Carcinogenicity of hydantoins: history, data, hypotheses, and public policy. In: Dam, M., Gram, L. & Penry, J.K., eds, *Advances in Epileptology: XIIth Epilepsy International Symposium*, New York, Raven Press, pp. 589–596

Selby, J.V., Friedman, G.D. & Fireman, B.H. (1989) Screening prescription drugs for possible carcinogenicity: eleven to fifteen years of follow-up. *Cancer Res.*, **49**, 5736–5747

Seymour, R.A. (1993) Drug-induced gingival overgrowth. *Adverse Drug React. Toxicol. Rev.*, **12**, 215–232

Sezzano, P., Raimondi, A., Arboix, M. & Pantarotto, C. (1982) Mutagenicity of diphenylhydantoin and some of its metabolites towards *Salmonella typhimurium* strains. *Mutat. Res.*, **103**, 219–228

Shapiro, S., Hartz, S.C., Siskind, V., Mitchell, A.A., Slone, D., Rosenberg, L., Monson, R.R., Heinonen, O.P., Idänpään-Heikkilä, J., Härö, S. & Saxén, L. (1976) Anticonvulsants and parental epilepsy in the development of birth defects. *Lancet*, **i**, 272–275

Sharma, R.K., Jacobson-Kram, D., Lemmon, M., Bakke, J,. Galperin, I. & Blazak, W.F. (1985) Sister-chromatic exchange and cell replication kinetics in fetal and maternal cells after treatment with chemical teratogens. *Mutat. Res.*, **158**, 217–231

Shaw, G.M., Lammer, E.J., Wasserman, G.R., O'Malley, C.D. & Tolarevo, M.M. (1995) Risks of orofacial clefts in children born to women using multivitamins containing folic acid periconceptionally. *Lancet*, **345**, 393–396

Sherman, S. & Roizen, N. (1976) Fetal hydantoin syndrome and neuroblastoma. *Lancet*, **ii**, 517

Shoeman, D.W., Kauffman, R.E., Azarnoff, D.L. & Boulos, B.M. (1972) Placental transfer of diphenylhydantoin in the goat. *Biochem. Pharmacol.*, **21**, 1237–1243

Singer, J., Schmid, C., Souhami, R. & Isaacson, P.G. (1993) Bone marrow involvement in phenytoin induced 'pseudolymphoma'. *Clin. Oncol.*, **5**, 397–398

Sinués, B,. Martínez, P., Tamparillas, M. & Bartolomé, M. (1982) Cytogenetic study of the treatment with phenytoin using SCE. *Arch. Farmacol. Toxicol.*, **8**, 165–170 (in Spanish)

Society of Japanese Pharmacopoeia (1992) *The Pharmacopoeia of Japan JP XII*, 12th Ed., Tokyo, pp. 451–453

Sonawane, B.R. & Goldman, A.S. (1981) Susceptibility of mice to phenytoin-induced cleft palate correlated with inhibition of fetal palatal RNA and protein synthesis. *Proc. Soc. exp. biol. Med.*, **168**, 175–179

de Souza Queiroz, M.L. & Mullen, P.W. (1980) The effects of phenytoin, 5-(*para*-hydroxyphenyl)-5-phenylhydantoin and valproic acid on humoral immunity in mice (Abstract no. 31). *Int. J. Immunopharmacol.*, **2**, 224–225

Speidel, B.D. & Meadow, S.R. (1972) Maternal epilepsy and abnormalities of the fetus and newborn. *Lancet*, **ii**, 839–843

Staple, P.H., Reed, M.J. & Mashimo, P.A. (1977) Diphenylhydantoin gingival hyperplasia in *Macaca arctoides*: a new human model. *J. Periodontol.*, **48**, 325–336

Stevens, M.W. & Harbison, R.D. (1974) Placental transfer of diphenylhydantoin: effects of species, gestational age and route of administration. *Teratology*, **9**, 317–326

Strickler, S.M., Dansky, L.V., Miller, M.A., Seni, M.-H., Andermann, E. & Spielberg, S.P. (1985) Genetic predisposition to phenytoin-induced birth defects. *Lancet*, **ii**, 746–749

Sullivan, F.M. & McElhatton, P.R. (1975) Teratogenic activity of the antiepileptic drugs phenobarbital, phenytoin and primidone in mice. *Toxicol. appl. Pharmacol.*, **34**, 271–282

Swanson, B.N., Leger, R.M., Gordon, W.P., Lynn, R.K. & Gerber, N. (1978) Excretion of phenytoin into semen of rabbits and man. Comparison with plasma levels. *Drug. Metab. Dispos.*, **6**, 70–74

Tan, Y.-D., Qiu, X.-F. & Xie, J.-R. (1985) Diphenylhydantoin: clinical investigation and cytogenetic research on mammalian cells *in vitro* (Abstract). *Environ. Mutag.*, **7**,35

Taneja, N., Jain, S., Maheswari, M.C., Tandon, J.K. & Kucheria, K. (1992) Induction of sister chromatid exchanges in phenytoin treated and untreated patients with epilepsy. *Indian J. med. Res.*, **96**, 302–305

Taneja, N., Kucheria, K., Jain, S. & Maheshwari, M.C. (1994) Effect of phenytoin on semen. *Epilepsia*, **35**, 136–140

Taylor, W.F., Myers, M. & Taylor, W.R. (1980) Extrarenal Wilm's tumour in an infant exposed to intrauterine phenytoin (Letter to the Editor). *Lancet*, **ii**, 481–482

Theodoropoulos, T.J., Pappolla, M.A., Goussis, O.S., Zolman, J.C. & Benson, D.M. (1990) Permanent alterations in the hypothalamic-pituitary-thyroid axis in the rat following phenytoin exposure in utero. *Horm. Metab. Res.*, **22**, 521–523

Thomas, J., ed. (1991) *Prescription Products Guide 1991*, 20th Ed., Victoria, Australian Pharmaceutical Publishing Co., pp. 652–653

Thomason, J.M., Seymour, R.A. & Rawlins, M.D. (1992) Incidence and severity of phenytoin-induced gingival overgrowth in epileptic patients in general medical practice. *Community Dent. oral Epidemiol.*, **20**, 288–291

Tomson, T., Lindbom, U., Ekqvist, B. & Sundqvist, A. (1994) Disposition of carbamazepine and phenytoin in pregnancy. *Epilepsia*, **35**, 131–135

Toone, B.K., Wheeler, M., Nanjee, M., Fenwick, P. & Grant, P. (1983) Sex hormones, sexual activity and plasma anticonvulsant levels in male epileptics. *J. Neurol. Neurosurg. Psychiat.*, **46**, 824–826

Treiman, D.M. & Woodbury, D.M. (1995) Phenytoin: absorption, distribution, and excretion. In; Levy, R.H., Mattson, R.H. & Meldrum, B.S., eds, *Antiepileptic Drugs*, 4th Ed., New York, Raven Press, pp. 301–314

United States National Library of Medicine (1996) *RTECS Database*, Bethesda, MD

United States National Toxicology Program (1993) *Toxicology and Carcinogenesis Studies of 5,5-Diphenylhydantoin (Phenytoin) (CAS No. 57-41-0) in F344/N Rats and B6C3F1 Mice (Feed Studies)* (Technical Report Series 404; NIH Publication no. 94-2859), Research Triangle Park, NC

United States Pharmacopeial Convention (1994) *The 1995 US Pharmacopeia*, 23rd Rev./*The National Formulary*, 18th Rev., Rockville, MD, pp. 1216–1221

United States Tariff Commission (1939) *Synthetic Organic Chemicals, US Production and Sales, 1938*, Report No. 136, Second Series, Washington DC, US Government Printing Office, p. 103

United States Tariff Commission (1948) *Synthetic Organic Chemicals, US Production and Sales, 1946*, Report No. 159, Second Series, Washington DC, US Government Printing Office, p. 103

Van Dyke, D.C., Berg, M.J. & Olson, C.H. (1991) Differences in phenytoin biotransformation and susceptibility to congenital malformations: a review. *Drug Intell. clin. Pharm.*, **25**, 987–992

Veronese, M.E., Doecke, C.J., Mackenzie, P.I., McManus, M.E., Miners, J.O., Rees, D.L.P., Gasser, R., Meyer, U.A. & Birkett, D.J. (1993) Site-directed mutation studies of human liver cytochrome *P*-450 isoenzymes in the CYP2C subfamily. *Biochem. J.*, **289**, 533–538

Vidal (1995) *Dictionnaire Vidal*, 71st Ed., Paris, Editions du Vidal, pp. 449–450, 1247

Vorhees, C.V. (1983) Fetal anticonvulsant syndrome in rats: dose- and period-response relationships of prenatal diphenylhydantoin, trimethadione and phenobarbital exposure on the structural and functional development of the offspring. *J. Pharmacol. exp. Ther.*, **227**, 274–287

Vorhees, C.V. (1987a) Fetal hydantoin syndrome in rats: dose-effect relationship of prenatal phenytoin on postnatal development and behavior. *Teratology*, **35**, 287–303

Vorhees, C.V. (1987b) Maze learning in rats: a comparison of performance in two water mazes in progeny prenatally exposed to different doses of phenytoin. *Neurotoxicol. Teratol.* , **9**, 235–241

Vorhees, C.V. & Minck, D.R. (1989) Long-term effects of prenatal phenytoin exposure on offspring behavior in rats. *Neurotoxicol. Teratol.*, **11**, 295–305

Vorhees, C.V., Weisenburger, W.P. & Minck, D.R. (1989) An analysis of factors influencing water maze learning in rats: effects of escape assistance, task complexity, and path order using prenatal exposure to phenytoin as a positive control (Abstract no. BTS41). *Teratology*, **39**, 513

Waddell, W.J. & Mirkin, B.L. (1972) Distribution and metabolism of diphenylhydantoin-$^{14}$C in fetal and maternal tissues of the pregnant mouse. *Biochem. Pharmacol.*, **21**, 547–552

Watson, J.D. & Spellacy, W.N. (1971) Neonatal effects of maternal treatment with the anticonvulsant drug diphenylhydantoin. *Obstet. Gynec.*, **37**, 881–885

Wells, P.G. & Vo, H.P.N. (1989) Effect of the tumor promoter 12-*O*-tetradecanoylphorbol-13 acetate on phenytoin-induced embryopathy in mice. *Toxicol. appl. Pharmacol.*, **97**, 398–405

Wells, P.G., Zubovits, J.T., Wong, S.T., Molinari, L.M. & Ali, S. (1989) Modulation of phenytoin teratogenicity and embryonic covalent binding by acetylsalicylic acid, caffeic acid, and α-phenyl-*N*-t-butylnitrone: implications for bioactivation by prostaglandin synthetase. *Toxicol. appl. Pharmacol.*, **97**, 192–202

Welsch, F. & Stedman, D.D. (1984) Inhibition of metabolic cooperation between Chinese hamster V79 cells by structurally diverse teratogens. *Teratog. Carcinog. Mutag.*, **4**, 285–301

Willington, S.E., Zajac, W., McGregor, D.B. & Combes, R.D. (1989) Methods and criteria for assessing the V79 metabolic cooperation assay (Abstract no. 36). *Mutat. Res.*, **216**, 283

Wilson, J.G. (1974) Teratologic causation in man and its evaluation in non-human primates. In: Motulsky, A.G., Lenz, W. & Ebling, F.J.G., eds, *Birth Defects*, Amsterdam, Excerpta Medica, pp. 191–203

Wong, M., Helston, L.M.J. & Wells, P.G. (1989) Enhancement of murine phenytoin teratogenicity by the gamma-glutamylcysteine synthetase inhibitor L-buthionine-(S,R)-sulfoximine and by the glutathione depletor diethyl maleate. *Teratology*, **40**, 127–141

Woodruff, R.C., Mason, J.M., Valencia, R. & Zimmering, S. (1985) Chemical mutagenesis testing in *Drosophila*. V. Results of 53 coded compounds tested for the National Toxicology Program. *Environ. Mutag.*, **7**, 677–702

Yaari, Y. & Devor, M. (1985) Phenytoin suppresses spontaneous ectopic discharge in rat sciatic nerve neuromas. *Neurosci. Lett.*, **58**, 117–122

Yeo, P.P.B., Bates, D., Howe, J.G., Ratcliffe, W.A., Schardt, C.W., Heath, A. & Evered, D.C. (1978) Anticonvulsants and thyroid function. *Br. med. J.*, **i**, 1581–1583

Yerby, M.S. (1991) Pregnancy and epilepsy. *Epilepsia*, **32** (Suppl. 6), S51–S59

Zengel, A.E., Keith, D.A. & Tassinari, M.S. (1989) Prenatal exposure to phenytoin and its effect on postnatal growth and craniofacial proportion in the rat. *J. craniofac. Genet. dev. Biol.*, **9**, 147–160

Zhu, M.-X. & Zhou, S.-S. (1989) Reduction of the teratogenic effects of phenytoin by folic acid and a mixture of folic acid, vitamins, and amino acids: a preliminary trial. *Epilepsia*, **30**, 246–251