Phenytoin

Evaluation of the effects on reproduction, recommendation for classification

To: the State Secretary of Social Affairs en Employment
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Health Council of the Netherlands

contents

samenvatting

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid (SZW) heeft de Gezondheidsraad het effect beoordeeld dat het geneesmiddel fenytoïne heeft op de voortplanting.
Dit advies is opgesteld door de commissie Classificatie reproductietoxische stoffen – hierna aangeduid als de commissie – een subcommissie van de vaste commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS).
De Gezondheidsraad heeft een vaste rol bij de bescherming van werknemers tegen mogelijke schadelijke effecten van stoffen waar zij tijdens hun werk mee in aanraking kunnen komen. Meer informatie over die rol staat op www.gezondheidsraad.nl.

Gebruik van fenytoïne

Fenytoïne is een synthetisch geneesmiddel. Het wordt voornamelijk gebruikt tegen epileptische aanvallen, maar soms ook bij hartritmestoornissen. Mensen die werkzaam zijn in de farmaceutische industrie, in apotheken of in ziekenhuizen kunnen tijdens hun werk in aanraking komen met fenytoïne.

Classificeren naar bewijskracht voor schadelijk effect

Bij de beoordeling van effecten op de voortplanting kijkt de commissie zowel naar de effecten op de fertiliteit (vruchtbaarheid) van mannen en vrouwen als naar de effecten op de ontwikkeling van het nageslacht. Daarnaast worden de effecten op de lactatie (hoeveelheid en kwaliteit van moedermelk) beoordeeld en de effecten via de moedermelk op de zuigeling.
De commissie beoordeelt of er aanwijzingen zijn dat de stof een schadelijk effect kan hebben. Als dergelijke aanwijzingen bestaan stelt ze voor om de stof in te delen in een bepaalde gevarencategorie, die aangeeft hoe sterk de bewijskracht is voor het schadelijke effect van de stof. Op basis van dat voorstel kan de minister van SZW besluiten om de stof al dan niet als reproductietoxische stof aan te merken.
De indeling in gevarencategorieën is gebaseerd op EU-verordening (EG) 1272/2008.

Advies aan de staatssecretaris

Op grond van de beschikbare wetenschappelijke gegevens stelt de commissie voor om fenytoïne alleen voor effecten op de ontwikkeling in te delen in een gevarencategorie. Over de effecten

op de vruchtbaarheid en op of via lactatie zijn onvoldoende geschikte gegevens beschikbaar.

Classificatievoorstel commissie voor fenytoïne

- voor effecten op de fertiliteit adviseert de commissie fenytoïne niet in te delen in een gevarencategorie wegens onvoldoende geschikte gegevens.
- voor effecten op de ontwikkeling adviseert de commissie fenytoïne in te delen in categorie 1B (*stoffen waarvan verondersteld wordt dat zij toxisch zijn voor de menselijke voortplanting*) en te etiketteren met H360D (*kan het ongeboren kind schaden*).
- voor effecten op of via lactatie adviseert de commissie om fenytoïne niet te etiketteren wegens onvoldoende geschikte gegevens.

executive summary

At the request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluated the effects of phenytoin on reproduction. This advisory report has been drafted by the Subcommittee on the Classification of Reproduction Toxic Substances of the Dutch Expert Committee on Occupational Safety (DECOS) of the Health Council, hereafter called the Committee. The Health Council has a permanent task in assessing the hazard of substances to which man can be occupationally exposed. More information about this task can be found at www.gezondheidsraad.nl.

Use of phenytoin

Phenytoin is a synthetic anticonvulsant, which can be used in the treatment of most types of seizure disorders and status epilepticus. Occasionally, phenytoin is used as an antiarrhythmic drug. Workers can be occupationally exposed to phenytoin in the pharmaceutical industry, in pharmacies or in hospitals.

Classification according to strength of evidence for toxic effect

For assessing the effects on reproduction, the Committee evaluates the effects on male and female fertility and on the development of the offspring. Moreover, the Committee considers the effects of a substance on lactation and on the offspring via lactation.
If there are data indicating hazardous properties, the Committee recommends classification in a category based on the strength of the evidence. Based on that proposal, the Minister of Social Affairs and Employment can decide whether to classify the substance as toxic to reproduction. The classification is performed according to EU-regulation (EC) 1272/2008.

Recommendations to the State Secretary

Based on the available scientific data, the Committee recommends to classify phenytoin only for effects on offspring development. There are insufficient data for classification with regard to effects on paternal and maternal fertility and effects on or via lactation.

The Committee's classification proposal for phenytoin:

- For fertility, the Committee recommends not classifying phenytoin due to a lack of appropriate data.
- For developmental toxicity, the Committee recommends to classify phenytoin in category 1B (*presumed human reproductive toxicant) and to label it H360D (may damage the unborn child*).
- For effects on or via lactation, the Committee recommends not labelling phenytoin due to a lack of appropriate data.

01 scope

1.1 Background

As a result of the Dutch regulation on registration of compounds toxic to reproduction that came into force on 1 April 1995, the Minister of Social Affairs and Employment requested the Health Council of the Netherlands to classify compounds toxic to reproduction. This classification is performed by the Health Council's Subcommittee on the Classification of Reproduction Toxic Substances of the Dutch Expert Committee on Occupational Safety (DECOS). The classification is performed according to European Union Regulation (EC) 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. The CLP regulation is based on the Globally Harmonised System of Classification and Labelling of Chemicals (GHS). The Subcommittee's advice on the classification will be applied by the Ministry of Social Affairs and Employment to extend the existing list of compounds classified as reproductive toxicant (category 1A and 1B and 2) or compound with effects on or via lactation.

1.2 Committee and procedure

This document contains the recommendations for classification of phenytoin by the Health Council's Subcommittee on the Classification of Reproduction Toxic Substances, hereafter called the Committee. The members of the Committee are listed on the last page of this report. The classification is based on the evaluation of published human and animal studies concerning adverse effects with respect to fertility and offspring development as well as adverse effects on or via lactation.

Classification concerning fertility (F/f) and offspring development (D/d)[a]:	
Category 1	Known or presumed human reproductive toxicant (H360(F/D)).
Category 1A	Known human reproductive toxicant .
Category 1B	Presumed human reproductive toxicant.
Category 2	Suspected human reproductive toxicant (H361(f/d)).
No classification for effects on fertility or development.	
Classification for lactation	
	Effects on or via lactation (H362).
	No labelling for lactation.
Hazard statement codes:	
H360F	May damage fertility.
H360D	May damage the unborn child.
H361f	Suspected of damaging fertility.
H361d	Suspected of damaging the unborn child.
H360FD	May damage fertility. May damage the unborn child.
H361fd	Suspected of damaging fertility. Suspected of damaging the unborn child.
H360Fd	May damage fertility. Suspected of damaging the unborn child.
H360Df	May damage the unborn child. Suspected of damaging fertility.
H362	May cause harm to breast-fed children.

[a] Substances in category 1 are assigned F or D, substances in category 2 f or d.

The classification and labelling of substances is performed according to the guidelines of the European Union (Regulation (EC)1272/2008). The classification of compounds is the result of an integrated assessment of the nature of all parental and developmental effects observed, their specificity and adversity, and the dosages at which the various effects occur. The guideline necessarily leaves room for interpretation, dependent on the specific data set under consideration. In the process of using the regulation, the Committee has agreed upon a number of additional considerations.

Additional considerations to Regulation (EC) 1272/2008

If there is sufficient evidence to establish a causal relationship between human exposure to the substance and impaired fertility or subsequent developmental toxic effects in the offspring, the compound will be classified in category 1A, irrespective of the general toxic effects (see Regulation (EC) 1272/2008, 3.7.2.2.1.).
Adverse effects in a reproductive study, reported without information on the parental or maternal toxicity, may lead to a classification other than category 1B, when the effects occur at dose levels which cause severe toxicity in general toxicity studies.
Clear adverse reproductive effects will not be disregarded on the basis of reversibility per se.
The Committee does not only use guideline studies (studies performed according to OECD[a] standard protocols) for the classification of compounds, but non-guideline studies are taken into consideration as well.

[a] Organisation for Economic Cooperation and Development

Regarding fertility, the Committee takes into account data on parameters related to fertility, such as seminal fluid volume and spermatozoa concentration, that are related to male fertility. The Committee excludes publications containing only data on sex hormone levels from the assessment, because the relationship between these hormone levels and functional fertility (ability to conceive children) is too uncertain.
In 2017, the President of the Health Council released a draft of the report for public review. The Committee has taken the comments received into account in deciding on the final version of the report. These comments, and the reply by the Committee, can be found on the website of the Health Council.

1.3 Labelling for lactation

The recommendation for classifying substances for effects on or via lactation is also based on Regulation (EC) 1272/2008. The criteria define that substances which are absorbed by women and have been shown to interfere with lactation or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled. Unlike the classification of substances for fertility and developmental effects, which is based on hazard identification only (largely independent of dosage), the labeling for effects on or via lactation is based on risk characterization and therefore, it also includes consideration of the level of exposure of the breastfed child.
Consequently, a substance should be labelled for effects on or via lactation when it is likely that the substance would be present in breast milk at potentially toxic levels. The Committee considers a concentration of a compound as potentially toxic to the breastfed child when this concentration leads to exceeding the exposure limit for children, or, if that level is unknown, the exposure limit for the general population, e.g. the acceptable daily intake (ADI).

1.4 Data

Literature searches were conducted in the on-line databases Current Contents and Medline, from 1966 up to May 2011 and by searches on internet; updates were performed in TOXNET, the last search was

performed in July 2017. Literature was selected primarily on the basis of the text of the abstracts. Publications cited in the selected articles, but not selected during the primary search, were reviewed if considered appropriate. In addition, handbooks and a collection of most recent reviews were consulted as well as several websites regarding (publications on) toxicology and health. References are divided in literature cited and literature consulted, but not cited.
The Committee describes both human and animal studies in the text. The animal data are described in more detail in Annex A as well. Of each study, the quality of the study design (performed according to internationally acknowledged guidelines) and the quality of documentation is considered.
In the assessment of the potential adverse effects of phenytoin on reproduction, the Committee also used data on adverse effects related to its application as a therapeutic agent.

1.5 Presentation of conclusions

The classification is given with key effects, species and references specified. In case a substance is not classified as toxic to reproduction, one of two reasons is given:

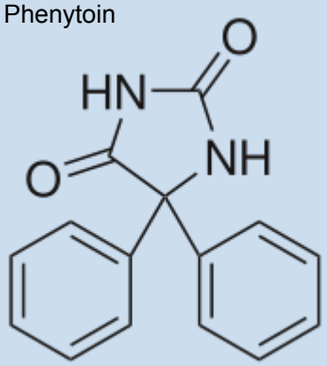
- Lack of appropriate data precludes assessment of the compound for reproductive toxicity.
- Sufficient data show that no classification for toxicity to reproduction is indicated.

1.6 Final remark

The classification of compounds is based on hazard evaluation (Niesink et al., 1995)[1] only, which is one of a series of elements guiding the risk evaluation process. The Committee emphasizes that for derivation of health based occupational exposure limits these classifications should be placed in a wider context. For a comprehensive risk evaluation, hazard evaluation should be combined with dose-response assessment, human risk characterization, human exposure assessment and recommendations of other organizations.

02
phenytoin

2.1 Properties

Name	:	Phenytoin
CAS-no	:	57-41-0
Synonyms	:	Diphenylhydantoin; Fenitoina; Phenantoinum; Phenytoinum; 5,5-Diphenylhydantoin; 5,5-Diphenylimidazoline-2,4-dione
Trade names	:	Dantoin, Dilantin, Diphenlyn, Phenyltoin, Divulsan, Novo-diphenyl, Phentoin sodium, Denyl sodium, Dilantin sodium, Diphentoin, Diphenylan sodium, Kessodanten, Elsanutin, Phentoin, Di-Hydan, Phenhydan
Structural formula	:	Phenytoin 
Use	:	Phenytoin is a commonly prescribed synthetic anti-epileptic, which can be used in the treatment of most types of seizure disorders and status epilepticus. Occasionally, phenytoin is used as an antiarrhythmic drug. It is incidentally prescribed off-label for treatment of skin disorders. Phenytoin may be administered orally or intravenously at doses of 300 – 600 mg/day. The therapeutic plasma level is 10-20 µg/ml.
Occupational exposure	:	Can occur in the pharmaceutical industry, in pharmacies or in hospitals.
Mol weight	:	252.268 g/mol
Chemical formula	:	$C_{15}H_{12}N_2O_2$
General toxicity	:	Phenytoin can cause a wide range of adverse idiosyncratic effects such as cosmetic changes, blood dyscrasias, Stevens-Johnson syndrome and hepatotoxicity. The most common signs of toxicity of phenytoin are referable to the central nervous system and are usually dose-related. At plasma drug concentrations higher than 20 µg/ml drowsiness, dysarthria, tremor, ataxia and cognitive difficulties were reported (Brodie, 1996).[2]
Mechanism	:	The motor cortex appears to be the primary site of action where spread of seizure activity is inhibited. Phenytoin tends to stabilize the threshold against hyperexcitability possibly by promoting sodium efflux from neurons. This includes the reduction of posttetanic potentiation at synapses. Loss of posttetanic potentiation prevents cortical seizure foci from detonating adjacent cortical areas. Phenytoin can reduce the maximal activity of brain stem centres responsible for the tonic phase of tonic-clonic (grand mal) seizures.
Kinetics	:	Oral bioavailability of phenytoin is high (90%). Phenytoin is widely distributed throughout the body and more than 90% of the circulating drug is protein-bound, mainly to albumin. The half-life is dose-dependent and has an average of 20 to 30 hours for a therapeutic dose. Phenytoin is metabolized in the liver by cytochrome P450-2C9 and -2C19. Most of the drug is excreted in the bile as inactive metabolites which are then reabsorbed from the intestinal tract and excreted in the urine. Urinary excretion of phenytoin and its metabolites occurs partly with glomerular filtration but more importantly by tubular secretion. Phenytoin accumulates in fat tissue.

Data from HSDB[3], unless otherwise noted

2.2 Context

Epilepsy is the most common neurological disorder in women of reproductive age. Women with epilepsy are at risk of reproductive dysfunction, through ovulatory failure for instance (Morrell et al., 2002; Bauer et al., 2008).[4,5] Approximately eight in 1,000 pregnancies occur among women with epilepsy part of whom are taking antiepileptic drugs (Fairgrieve et al., 2000).[6] Phenytoin is a classic antiepileptic drug used therapeutically as early as 1938.

In many human studies, the duration of exposure to phenytoin and the dose levels used are not mentioned. Furthermore, (pregnant) epileptic women are very often treated with a combination of antiepileptic drugs, making an evaluation of the effect of phenytoin on reproductive outcomes

more difficult. The assessment in this report is restricted to phenytoin exposure given as monotherapy.

2.3 Human studies

2.3.1 Fertility studies

Male fertility

Herzog et al. (2006) compared serum levels of neuroactive steroids among men with epilepsy who took various antiepileptic drugs, untreated men with epilepsy and non-epileptic controls. Twenty-five men with epilepsy who had been taking phenytoin only, ten men who had untreated epilepsy and 25 non-epileptic controls were included in the study. In addition to serum analysis, sexual interest and function were assessed with a questionnaire. The phenytoin group had lower sexual interest and function levels ($p<0.001$), lower serum dehydroepiandrosterone sulfate ($p<0.001$), bioactive testosterone ($p<0.001$) and bioactive androstanediol ($p<0.05$) levels and a lower ratio of bioactive androstanediol to bioactive estradiol ($p<0.05$) compared to non-epileptic controls. The bioactive estradiol concentration was not different from the control group. The untreated men with epilepsy had lower dehydroepiandrosterone sulfate and bioactive androstanediol levels (both $p<0.01$) and a lower ratio of bioactive androstanediol to bioactive estradiol ($p<0.05$) compared to non-epileptic controls.[7]

Taneja et al. (1994) evaluated the effect of seizures and phenytoin monotherapy on the male reproductive system by studying seminal fluid. Three groups of men were included in this study: 42 epileptic men treated with phenytoin, 13 untreated epileptic men and 28 control men (non-epileptic and untreated). After collection of semen, a minimum number of 500-1,000 mature sperm cells was scored for morphologic abnormalities of head, neck, and tail. Serum samples of treated and untreated patients were analyzed for testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), hormones influencing testis function. The treated and untreated epileptic patients showed no difference in total seminal fluid volume, spermatozoa concentration, total sperm count, or number of morphologically abnormal sperm. Both treated and untreated patients showed lower seminal fluid volumes ($p<0.05$) and spermatozoa concentrations ($p<0.001$) than the control group. In addition, the percentages of abnormal sperm were increased in untreated patients (15.39 ± 6.87; $p<0.005$) and in phenytoin-treated patients (12.77 ± 7.17, not statistically significant) as compared to controls (9.47 ± 4.04). Testosterone levels were lower than normal (<13 nM) in 4/9 untreated patients and 5/12 phenytoin-treated patients. LH levels were increased (>9.8 IU/L) in 1/3 untreated patients and 2/6 phenytoin-treated patients. According to the investigators, the results of this study are indicative for an indirect effect of epilepsy on the male reproductive function rather than a direct effect of phenytoin.[8]

Chen et al. (1992) investigated the effects of phenytoin on sperm motility. Six epileptic patients treated with phenytoin monotherapy for at least five years (serum levels varied from 7.2 to 13.4 µg/ml) were observed for effects on sperm motility immediately after semen had liquefied or after a 2-h pre-incubation at 37ºC (to detect effects on the duration of activity). Forty-five healthy volunteers served as control group. Sperm motility was decreased in all treated patients when motility was measured immediately after semen liquefication (22.9±11.7) or after a 2-h pre-incubation at 37ºC (13.5±8.7) compared to healthy donors (42.6±12.5 and 35.2±7.0 respectively). In addition, the duration of sperm activity of epileptic patients was shorter than that of the controls ($p<0.05$). The effect on sperm motility showed no correlation with duration of therapy, serum phenytoin levels or seizure types.[9]

The in vitro experiment published by Chen et al. in 1992[9] but originally reported by Shen and Chen (1990) showed reduced sperm motility upon 2-h pre-incubation with phenytoin concentrations 1.6, 4.2 and 5.0 mM, compared to incubation medium (all $p<0.01$). The inhibitory effect on sperm motility appeared to be dependent on both duration of pre-incubation and the concentration of the drug.[10]

Hong et al. (1982) also measured the effects of phenytoin on human sperm motility in vitro. Using concentrations as high as 2mM, phenytoin did not have an effect on sperm motility.[11]

Female fertility

Morrell et al. (2005) investigated sexual dysfunction, sex steroid hormone abnormalities, and depression in women with epilepsy treated with antiepileptic drug monotherapy. Subjects were women with epilepsy receiving an antiepileptic drug in monotherapy for at least six months prior to study participation, as well as women without epilepsy not receiving antiepileptic drugs who served as controls. Thirteen epileptic women receiving phenytoin were included in the study, as well as thirteen nonepileptic controls. All subjects were between the ages of eighteen and 40, cycling, and at least four years postmenarche. Women with epilepsy were recruited from the patient populations of two U.S. Epilepsy Centres. Nonepileptic controls were recruited from friends and family of the participants. Subjects provided medication history and completed a reproductive history form and a seizure history form. Sexual function was determined by the subject's response to sexual inventories examining sexual experience and attitude. The subjects also completed an internally developed questionnaire examining sexual desire and arousal to determine whether a sexual dysfunction was present as defined by the Diagnostic and Statistical Manual of Diseases Classification (4th Edition). Sexual dysfunction and sexual anxiety were higher and sexual arousal was lower than in controls ($p<0.05$).[12]

2.3.2 Developmental toxicity studies

Treatment with phenytoin during pregnancy has been associated with a distinct set of malformations called the foetal hydantoin syndrome (Hanson and Smith, 1975; Smith's Recognizable Patterns of Human Malformations, 2014).[13,14] Among these developmental disturbances are abnormal facial features, anomalies of the distal phalanges, pre- and postnatal growth deficiency, as well as mental performance deficiency. Other defects reported less frequently include cleft lip and/or palate, cardiovascular anomalies, renal defects, positional limb deformities and diaphragmatic hernias. Clinicians generally consider phenytoin as a teratogen. A drawback of most of the epidemiological studies this conclusion is based upon, is that they concern pregnant women receiving combinations of phenytoin and other antiepileptic drugs (polytherapy). The Committee assesses the strength of the epidemiological evidence for the purpose of classification focused on data regarding monotherapy.

Structural defects

Surveillance systems

Puhó et al. (2007) used the Hungarian Case-Control Surveillance of Congenital Abnormalities to investigate the developmental toxicity of all drugs used in at least 0.5% of pregnant women who later delivered children with orofacial clefts. A total of 1,975 cases with orofacial clefts, 38,151 population-based controls (without birth defects) and 20,868 malformed controls with other defects were included. There were 1,374 cases of isolated cleft lip with or without cleft palate. Maternal phenytoin treatment was recorded by gestational month. Phenytoin use during the second and third months of pregnancy was evaluated. Phenytoin use in gestational months before and after the above period was not evaluated, However, if pregnant women took phenytoin in the first gestational month and continued to use it in the second, third and/or fourth months, these treatments were evaluated as well. The odds ratios (ORs) with 95% confidence intervals (CIs) were adjusted for maternal age and employment status, parity and acute maternal disease in the second and/or third months of pregnancy. Polytherapy was not mentioned as exclusion criterion. An increased risk of isolated cleft lip with or without cleft palate was shown for phenytoin (OR=3.0; 95% CI 1.5-5.8, compared to population controls, and OR=4.4; 95% CI 2.1-9.1, compared to malformed controls). The ORs for the comparison with population controls differed between mothers who used folic acid during the critical period (OR=2.3; 95% CI 0.7-7.7) and mothers who did not (OR=3.6; 95% CI 1.6-8.0).[15]

Arpino et al.. (2000) used an international database on congenital malformations and drug exposure (MADRE) to assess the teratogenic effects of five antiepileptic drugs, one of them being phenytoin. Among 8,005 cases of congenital malformations, 24 infants had been exposed to phenytoin monotherapy during the first trimester of pregnancy. Cases were defined as infants presenting with a specific malformation, and

controls as infants presenting with any other birth defect. Infants exposed to drugs other than antiepileptic drugs were classified as nonexposed. ORs with 95% CIs were estimated for each of the nine contributing registries separately and combined. An association between phenytoin and a congenital malformation, i.e. spina bifida, was observed in only one registry (two exposed cases; OR=62.33; 95% CI 4.9-785.9).[16]

Prospective studies

Vajda et al. (2012) analysed prospectively collected data from the Australian Pregnancy Register to provide information on the relative teratogenicity of antiepileptic drugs. The database contained pregnancy outcomes from 1,317 women with epilepsy. Information on the duration of the prenatal exposure of the children was not provided. Whether the exclusion criteria were those of EURAP, was not mentioned. Excluded were pregnancies that ended in spontaneous abortions or chromosomal or genetic abnormalities, those in which the women had treatment changes in the first trimester, and those involving other diseases or treatment that could affect foetal outcome. The prevalence of malformations associated with phenytoin monotherapy was 1/35 (2.9%). Their prevalence in the untreated population was 6/106 (5.2%).[17]

Hernández-Diaz et al. (2012) examined the offspring of women who enrolled in the North American AED Pregnancy Registry between 1997 and 2011. The risk of major malformations was calculated among infants exposed to specific antiepileptic drugs in monotherapy during the first trimester of pregnancy and compared to an unexposed group. Women were eligible for analysis if they had a liveborn infant, a stillborn infant, or a pregnancy terminated because of a foetal abnormality. They were ineligible if they had a spontaneous abortion, withdrew from the registry, or were lost to follow-up. Internal controls were pregnant women not taking an antiepileptic drug and without epilepsy who had been recruited among the friends and relatives of antiepileptic drug-exposed participants since 2003. The phenytoin-exposed group consisted of 416, the control group of 442 subjects. In addition, an external control group of 206,224 infants was captured by a surveillance system at Brigham and Women's Hospital in Boston using the same inclusion and exclusion criteria for outcome definition. Twelve phenytoin-exposed infants had major malformations (2.9%; 95% CI 1.5-5.0) and five unexposed internal controls (1.1%; 95% CI 0.4-2.6), which led to a relative risk (RR) of 2.6 (95% CI 0.9-7.4). Comparison with the external controls gave a similar result.[18]

Thomas et al. (2008) examined the prevalence of cardiac malformations and its association with antiepileptic drugs in a prospective study evaluating several drugs as mono- or polytherapy, based on the Indian Kerala Registry of Epilepsy and Pregnancy. The evaluation concerned 740 pregnant women with epilepsy enrolled between April 1998 and December 2004. Thirty-one infants were born from mothers treated with phenytoin monotherapy during the first trimester of pregnancy. At three

months of age, a cardiologist carried out a clinical examination and echocardiography on all live-born babies. None of the babies showed cardiac malformations.[19]

Thomas et al. (2017) estimated the RR of major congenital malformations in the offspring of 2,454 pregnant women with epilepsy enrolled in the Kerala registry from 1 April 1998 until 31 December 2013. They evaluated the effects of prenatal exposure to various antiepileptic drugs as mono- or polytherapy. One hundred and six children were born to mothers who received phenytoin monotherapy. Two control groups were included: women with epilepsy not taking any antiepileptic drugs (n=252) and women without epilepsy in the first trimester of pregnancy and not using antiepileptic drugs, from an antenatal clinic (n=319). Their socioeconomic backgrounds were similar. The RR of major congenital malformations in the children exposed to phenytoin prenatally was not increased, compared to the offspring of women with epilepsy not using antiepileptic drugs (RR=1.0; 95% CI 0.0-2.5), or the offspring of unexposed healthy women (RR=1.6; 95% CI 0.6-4.3).[20]

Meador et al. (2006) investigated the foetal outcome after phenytoin intake during pregnancy in a prospective study across 25 epilepsy centres, aimed at comparison of four commonly used antiepileptic drugs. In total, 354 mother/child pairs were included in the study. Most mothers (81%) were seizure-free during pregnancy; only 3% had more than five convulsions during their pregnancy. Fifty-six children were exposed to phenytoin monotherapy during the first trimester of pregnancy. In 3.6% (2/56) foetal death was observed and in 7.1% (4/56) congenital malformations were seen. These congenital malformations were observed in the brain (agenesis of corpus callosum), heart (ventricular septal defect) and genital region (hydronephrosis with extrarenal pelvis and undescended testicle). Controls were not included in this study.[21]

Morrow et al. (2006) studied the risk of major congenital malformation from *in utero* exposure to antiepileptic drugs. Prospective data collected by the UK Epilepsy and Pregnancy Register during the period 1996-2005 were analysed. Included were 82 children born to women taking phenytoin as monotherapy and 239 children born to women with epilepsy but not taking any antiepileptic drug, who served as controls. The investigators adjusted the data for the age at delivery, parity of the mother, family history of major congenital malformations, periconceptional folic acid exposure, and sex of the infant. The phenytoin-exposed children did not show a higher percentage of malformations than the controls. [22]

Holmes et al. (2001) investigated the presence of major malformations, signs of hypoplasia of the midface and fingers, microcephaly and small body size in children born to women with epilepsy taking antiepileptic drugs at five American maternity hospitals from 1986 to 1993. Women were excluded if they did not speak English, had a multiple-gestation

pregnancy, or had another potentially teratogenic factor, such as type 1 diabetes mellitus. They completed questionnaires to provide demographic and medical data. Included were infants exposed to antiepileptic drugs (n=316), as well as infants not exposed to antiepileptic drugs whose mother reported having had epilepsy (n=98). For each of these children, a control was recruited from the ten infants born closest in time to him or her (n=508). In 87 cases the exposed infants were born to mothers on phenytoin monotherapy. The infants of mothers with a history of epilepsy who had not taken antiepileptic drugs did not show more abnormalities than the control infants, neither in term of individual outcomes, nor overall. Compared to the controls, the phenytoin-exposed infants had a higher frequency of at least one of the following abnormalities: major malformations, microcephaly, growth retardation, midface hypoplasia and hypoplasia of the fingers (OR=2.8; 95% CI 1.1-8.8).[23]

Wide et al. (2000) assessed minor anomalies in infants exposed to antiepileptic drugs *in utero* in a population-based follow-up study of children born to women with epilepsy, treated during pregnancy, and enrolled from 1985 to 1995. Twenty-two women received phenytoin monotherapy at a mean dose of 274 mg/day (range 75-450). They took the drug throughout pregnancy. Twenty-one exposed children were examined for minor facial, digital, genital, skin and other anomalies. The control group consisted of 87 unexposed children, born in the same hospital within two days of the birth of a phenytoin-exposed child and matched for gestational age, gender and mode of delivery. Four controls were lost to follow-up. At the age of nine months, children exposed to phenytoin *in utero* and unexposed children showed facial anomalies in 2/21 vs 6/83 cases, digital anomalies in 2/21 vs 8/83, genital anomalies in 0/21 vs 0/83, skin anomalies in 4/21 vs 9/83, and other anomalies in 0/21 vs 2/83 cases, respectively).[24]

Kaneko et al. (1999) identified the major risk factors for the occurrence of congenital malformations, the relative teratogenic risks of several antiepileptic drugs and advisable ranges of antiepileptic drug doses to reduce the risk of congenital malformations in offspring in a prospective study. A total of 423 children were exposed to phenytoin *in utero* during at least the first trimester at daily doses of 50 mg/day (3.0 µg/ml drug level). A group of 98 children born to epileptic women not taking any antiepileptic drugs during gestation served as control. The children were examined for congenital malformations at birth, at five days and at one month of age. Three controls had malformations (3.1%). The prevalence of malformations in offspring exposed to phenytoin monotherapy (n=132) was 9.1% (OR=3.2; 95% CI 0.9-11.5 (CI calculated by the Committee)). The types of malformation were not reported.[25]

Canger et al. (1999) analysed the prevalence of malformations among infants of mothers with epilepsy treated with antiepileptic drugs during pregnancy. The first pregnancies of 517 women were studied

prospectively from December 1977 to 1996. Fifty-eight pregnancies that had ended in early spontaneous (n=38) or early elected (n=20) abortions were excluded from the analyses. Seven of the remaining pregnancies were lost to follow-up. Another eight ended in a therapeutic abortion due to a malformation. Thirty-one of the remaining 444 pregnancies were in women receiving phenytoin monotherapy. No information on duration of treatment was given and no control group was included. The children born to three of the women taking phenytoin as monotherapy, at 200 or 375 mg/day, had malformations: one child had a club foot, one a hip dislocation, and one an umbilical hernia.[26]

Samrén et al. (1997) pooled and reanalyzed data from five prospective studies in Europe to quantify the risk of congenital malformations following intrauterine antiepileptic drug exposure in mono- and polytherapy. Data were available for 1,221 children of which 141 were exposed to phenytoin *in utero*. A control group of 158 children of unexposed pregnancies among non-epileptic women from Germany was included. The results showed an overall increased risk of major congenital abnormalities in children of mothers with epilepsy treated with anti-epileptic drugs during pregnancy as compared with children of healthy controls. The number of malformed children after phenytoin monotherapy was 9/141 (6%), compared to 12/158 (8%) among the nonexposed controls. When the analyses were restricted to the exposed and unexposed pregnancies from Germany, matched for maternal age, parity, social class, smoking habits and previous abortions, an OR of 2.2 (95% CI 0.7-6.7) was observed, with 5 congenital malformations occurring among 33 phenytoin-exposed pregnancies. The malformations observed among phenytoin-exposed infants were inguinal hernia, heart defects, cleft lip with or without cleft palate, pre- or postaxial polydactyly, hypospadias, microcephaly, and megacolon.[27]

Koch et al. (1992) reviewed data on congenital malformations obtained in a collaborative study on epilepsy, antiepileptic drugs and pregnancy outcome. The aim of the study was to clarify the role of antiepileptic drugs versus genetic predisposition and other confounding factors in the major and minor anomalies observed at birth and at one and four years of age. Pregnant women with epilepsy treated with phenytoin monotherapy (n=24), women with epilepsy without treatment (n=25) and women whose partners had epilepsy (n=22) were included in the study and matched with healthy controls on socioeconomic status and maternal age, previous abortions, parity, and smoking habits during the third trimester of pregnancy. The duration of the prenatal exposure to phenytoin of the offspring was not reported. Two major malformations, a heart defect and a megacolon occurred in offspring of mothers with phenytoin monotherapy. Specific minor anomalies more frequent in children exposed to phenytoin than in controls at one year of age were hypoplasia of the nails (n=6, $p<0.001$) and hypoplasia of the phalanges (n=7, $p<0.001$). These

anomalies were less frequent at four years of age and in children of untreated mothers with epilepsy and fathers with epilepsy.[28]

Oguni et al. (1992) investigated the prevalence of abnormal pregnancy outcomes in the offspring of 103 epileptic women who had 115 pregnancies and were followed during pregnancy between 1982 and 1989. Ninety pregnancies had sufficient follow-up of the pregnancy outcomes. Twelve women were included with more than one pregnancy each. Therapeutic abortions were excluded except when the reason for abortion was a foetal abnormality. Five epileptic women not receiving any anticonvulsants served as controls. Twenty epileptic women received phenytoin monotherapy during the first trimester of pregnancy. Their pregnancies led to spontaneous abortion in two cases, but not to any babies with major malformations. There were no spontaneous abortions in the control group, nor babies with malformations.[29]

Gaily et al. (1988) reported the outcome of a prospective study in which 121 children of epileptic mothers were examined at 5.5 years of age. A control group of healthy children was included (n=105). Selection criteria for inclusion in the control group were absence of maternal epilepsy or other chronic disorder in the mother, absence of intrauterine drug exposure, gestational period at least 37 weeks, and no major perinatal illness or complication. Eighty-two of the 121 children of epileptic mothers had been exposed to phenytoin *in utero* (46 to monotherapy and 36 to polytherapy). The presence of nine minor craniofacial and digital features was examined clinically. The effects of phenytoin mono- and polytherapy were not analysed separately. The *in utero* exposure duration was not reported. Maternal phenytoin levels were measured. Moderate to high (>40 µmol/L) phenytoin levels in the first 20 weeks of pregnancy were associated with hypertelorism (RR=6.3; 95% CI 1.5-26.8), nail hypoplasia (RR=6.2; 95% CI 1.9-20.0), three or more dermal arches (RR=3.9; 95% CI 1.0-15.7), distal phalangeal hypoplasia (RR=11.9; 95% CI 3.7-38.8) and digital hypoplasia combined[a] (RR=10.6; 95% CI 1.8-40.2).[30]

Gaily (1990) studied the phalangeal and metacarpal bone length in children from epileptic mothers (n=111) and in control children of nonepileptic mothers (n=96) as a follow-up. Gaily used radiological measurement of the degree of distal digital hypoplasia, a more discrete and objective method than clinical examination. The mean age of both groups at the time of the examination was 5.5 years (range 5.2-5.8 years). Seventy-six of the children born to epileptic mothers had been exposed *in utero* to phenytoin, 21 to other anti-epileptic drugs and 14 had not been exposed to any anti-epileptic drug. Maternal phenytoin levels were measured at least once during the first 20 weeks of pregnancy. Phenytoin exposure during this period was associated with an elevated prevalence of radiologically defined distal phalangeal hypoplasia of mainly the second

[a] Nail/distal phalangeal hypoplasia and ≥ 3 dermal arches combined

and fifth digits (11% versus 1% among controls and not phenytoin-exposed children, p<0.01). The eight children exposed to the highest phenytoin levels (>40 µmol/L) showed the most prominent effects. Only 44 of the 76 women treated with phenytoin received no other antiepileptic drugs. Separate results for this monotherapy group were not reported.[31]

D'Souza et al. (1990) studied the outcome among 22 children born to mothers treated with phenytoin as monotherapy during pregnancy in a prospective study including 61 epileptic women. Maternal phenytoin levels were 10-25 mg/L. A control group, matched for maternal age, parity and social class, without any medical complications and not taking any drugs regularly (n=62), was included. Twelve of the 22 children exposed to phenytoin *in utero* throughout pregnancy had congenital anomalies versus none of the controls. The congenital anomalies observed were: epicanthic folds, extra digits, heart disease, dislocation of hip, hypoplastic nails, distal phalangeal hypoplasia, hypospadias, ptosis, craniosynostosis and Down's syndrome. One child that had been exposed to phenytoin during the first trimester of pregnancy only showed no anomalies.[32]

Kelly et al. (1984) investigated the prevalence of abnormalities in the offspring until two years of age in a prospective study among mothers from low socioeconomic status with high frequency of tonic-clonic generalized seizures and the use of anticonvulsants. The duration of the *in utero* exposure of the children was not reported. Of the 41 children exposed to phenytoin monotherapy *in utero*, 15 were diagnosed with distal digital hypoplasia (in most cases in hands and feet) and five with minor craniofacial abnormalities. One case of microcephaly and one with major abnormality were also observed. A control group was not described.[33]
As part of the study, hand radiographs were obtained on 51 children born to epileptic mothers and phalanges and metacarpals were measured. The results of these analyses were reported separately (Kelly, 1984).[34] In 20 pregnancies phenytoin was taken during pregnancy at doses of 200-600 mg/day. Seven of the 20 children (35%) had distal digital hypoplasia.

Follow-up studies of pharmacovigilance centre data
In a follow-up study of women reported to the Motherisk Program described by Gladstone et al. (1992), children exposed to phenytoin *in utero* were investigated for major and minor anomalies. The study included 16 women with epilepsy who had taken phenytoin during the first trimester of pregnancy. Fifteen of them had taken phenytoin as monotherapy and one had taken a second anti-epileptic drug. The study included a matched control group (n=16) of women exposed to other drugs without medical complications. Abnormalities observed among phenytoin-exposed offspring were minor anomalies (3/16; flattened nasal bridge, nail hypoplasia, or absence of the distal phalanx in the right index finger), adrenal haemorrhage (1/16) and developmental delay (1/16). One elective abortion and one neonatal death, but no malformations occurred in the control group. Statistics were not reported.[35]

In another study among women reported to the Motherisk Program, Scolnik et al. (1994) investigated children exposed *in utero* to phenytoin monotherapy for the presence of major malformations. Thirty-four women exposed to phenytoin were matched with controls exposed to other drugs. These women came in for counselling after exposure to other drugs. Other matching criteria were age, gravidity, parity, and socioeconomic class. Two phenytoin-exposed infants showed major malformations, one of them having cleft palate and hypospadias, the other demonstrating meningomyelocele and hydrocephalus. None of the control children showed major malformations.[36] It is unclear whether this study includes the women reported about by Gladstone et al.[35]

In a third follow-up study of women reported to the Motherisk Program, Nulman et al. (1997), investigated the effects of phenytoin monotherapy in the treatment of maternal epilepsy and separated them from those of epilepsy itself. The study comprised a group of pregnant women with epilepsy treated with phenytoin (n=29), a group of pregnant epileptic women not treated with phenytoin (n=9) and a group of pregnant women treated with phenytoin for conditions other than epilepsy (n=5), all matched to women who came in for counselling after exposure to other drugs (n=34). Other matching criteria were age, gravidity, parity, and socioeconomic class. The phenytoin-using women took the medicine throughout pregnancy. Three out of 34 children exposed to phenytoin *in utero* (born from epileptic and non-epileptic mothers) and two out of 34 controls had congenital malformations. The malformations in the exposed children were clubfoot, hypospadias and missing distal phalanx of the right index finger. Minor anomalies, in particular high forehead, frontal bossing, malar hypoplasia, toe-finger-nail dysplasia and epicanthal folds, were observed more frequently among exposed children compared to controls (overall RR= 2.1, $p<0.01$). [37] It is unclear whether this study includes the women reported about by Gladstone et al.[35]

Retrospective cohort studies

Adab et al. (2004) performed a retrospective cohort study in 249 children aged six months to sixteen years and exposed to antiepileptic drugs *in utero*. An unexposed control group consisted of 101 children, 83 of them aged six years or older (10.5±3.2 years) and eighteen up to five years of age (3.3±0.9 years). A clinician conducted semi-structured interviews of the epileptic mothers to ascertain information about their epilepsy and relevant pregnancy. Clinical records were used to confirm this information. The interviews were also used to collect data for each child on early development, behavioural problems, schooling, additional educational needs, and the need for additonal therapy including speech therapy. The children were examined for dysmorphic features, defined as cosmetic variations without disability, and major malformations, defined as structural abnormalities requiring medical or surgical intervention to prevent disability, using the EUROCAT guidelines. Twenty-one children aged six years or older (10.7±3.5 years) and five children up to five years of age

(2.6±1.4 years) were exposed to phenytoin monotherapy *in utero*. The duration of the prenatal exposure was not reported. The frequencies of dysmorphic features and major malformations were either incompletely or not reported. The only results reported were that phenytoin-exposed children aged six years or older did not have more dysmorphic features than the controls (no statistical details mentioned). The other data from the study are reported in the section *Functional and cognitive effects*. [38]

Orup et al. (2003) identified skeletal deviations in children after prenatal exposure to the anticonvulsant phenytoin. The study comprised 28 children of which seven children were exposed to phenytoin as monotherapy. The exposure lasted the entire nine months of pregnancy. The children exposed to phenytoin monotherapy were examined at five to 14 years of age. Craniofacial structures, hand-wrist structures and dental maturity were evaluated. Normative data from several sources served as reference populations for comparative purposes. Deviations in drug-exposed children as compared to controls were decreased lengths of the maxilla and mandible, several dental changes, decreased face height and decreased nasal bone length and bone angle ($p<0.01$ to $p<0.0001$). Deviations, especially in the maxilla, persisted with age (unclear whether this observation applies to phenytoin monotherapy) [39]

Dean et al. (2002) examined the neonatal and later childhood morbidity in children exposed to antiepileptic drugs prenatally. They investigated the frequencies of neonatal withdrawal, congenital malformations, childhood medical problems, developmental delay and behavioural disorders in the offspring of 149 women who took antiepileptic drugs during pregnancy and delivered between 1976 and 2000. They were identified through review of hospital records, or recruited from the antenatal clinic and postnatal wards. A structured interview including pregnancy histories and standardised assessment of all available offspring was carried out by a trained research nurse. Thirty-eight siblings of exposed cases were not exposed to antiepileptic drugs *in utero*. They served as controls. In 16 cases the mother had epilepsy but took no treatment and in 22 cases the child was born before epilepsy developed. Twenty-four children that had been exposed to phenytoin as single antiepileptic drug were identified. They had a mean age of 11 years at the time of study. The mean age of the controls was 15 years. The phenytoin-exposed children demonstrated increased frequencies of minor congenital malformations and either major congenital malformation or developmental delay, as compared to the controls ($p<0.05$). The neurodevelopment and behaviour findings are reported in the section *Functional and cognitive effects*.[40]

Lu et al. (2000) investigated whether digit anomalies, associated with *in utero* exposure to antiepileptic drugs, could be better identified using radiographs and dermatoglyphics rather than relying only on visual inspection. Forty-six antiepileptic-exposed individuals were examined of which 13 children were exposed to phenytoin monotherapy. An unexposed

control group (n=75) was included. This group consisted of (1) children recruited from the same sources as the phenytoin group, (2) children of families enrolled in a large health maintenance organization and (3) children whose previous radiographs were used for a subjective analysis of the qualitative changes in comparison to those in antiepileptic drug-exposed children and who were not growth retarded, and did not have either a serious medical disorder or exposure *in utero* to anti-epileptic drugs. The age range was only reported for monotherapy-exposed and polytherapy-exposed children combined. The evaluation included: physical examination of the fingers, nail size, dermal ridge patterns, measurements of phalanges and metacarpals, qualitative assessment of radiographs and interrelationships. Information as to the duration of the prenatal exposure to phenytoin was not provided. Nail size was not decreased in phenytoin-exposed children compared to controls (60 out of the 75, selection criteria not specified). Three or more arched fingerprint patterns in ten fingers occurred at a frequency of 14.4% in children exposed to phenytoin monotherapy as compared to 1.3% in controls, $p<0.0005$). Data on other digit changes were not reported for the group exposed to phenytoin monotherapy.[41]

Samrén et al. (1999) performed a large retrospective cohort study to assess the risk of major congenital abnormalities associated with antiepileptic drugs. The study comprised offspring of women with epilepsy, with or without antiepileptic drug use during pregnancy. Nine hundred twenty-one women were using one or multiple antiepileptic drugs during at least the first trimester of pregnancy (1,411 children were exposed *in utero*). One thousand nine hundred fifty-five nonexposed control women (2000 children) were included in this study. Matching criteria were age and parity of the mother, and sex, birth year and hospital of delivery of the child. One-hundred fifty-one women were treated with phenytoin as monotherapy. One child (1%) had major congenital abnormalities (type of defect was not mentioned). Statistical analysis showed no excess risk (RR 0.5; 95% CI 0.1-3.4).[42]

Meta-analyses

Weston et al. (2016) carried out a meta-analysis to assess the effects of prenatal exposure to antiepileptic drugs on the prevalence of congenital malformations in the child. They included prospective cohort studies, cohort studies set within pregnancy registries and randomised controlled trials. Participants were women with epilepsy taking antiepileptic drugs; the two control groups were women without epilepsy and women with epilepsy who were not taking antiepileptic drugs during pregnancy. The primary outcome was the presence of a major congenital malformation. Secondary outcomes included specific types of major congenital malformations. Thirty-one studies, on a variety of antiepileptic drugs, contributed to the meta-analysis. Children born to epileptic women receiving phenytoin monotherapy were at an increased risk of congenital malformation compared with children born to women without epilepsy (five

studies; n=477 vs 987; RR 2.38; 95% CI 1.12-5.03) and to women with untreated epilepsy (15 studies; n=640 vs 1,256; RR=2.40; 95% CI 1.42-4.08). The malformations noted were neural tube, cardiac, oro-facial/craniofacial and skeletal/limb defects.[43]

Veroniki et al. (2017) conducted a systematic review and network meta-analysis of congenital malformations and prenatal outcomes of pregnancies of women receiving antiepileptic drugs for any indication. Included were monotherapy and polytherapy. The comparators were placebo, no antiepileptic treatment (women not exposed to any antiepileptic drug, but with the same indications for use), or other antiepileptic drugs alone or in combination. The primary outcomes were the prevalence of overall and specific types of major congenital malformation. When studies also reported on major congenital malformation cases that were diagnosed prenatally and resulted in elective terminations, these were included in the congenital malformation analysis. For specific types of congenital malformation, the six most frequently occurring in the literature were selected, namely cardiac defect, cleft lip/palate, club foot, hypospadias, inguinal hernia and undescended testes (boys only). The secondary outcomes of interest were foetal loss, prenatal growth retardation, preterm birth, and minor congenital malformations. The investigators included 92 cohort studies, three case-control studies, and one randomised clinical trial. Phenytoin monotherapy was associated with a higher risk of developing major congenital malformations than control (OR=1.67; 95% CI 1.30-2.17). It was also associated with two types of specific malformation: cleft lip/palate (OR=3.11; 95% CI 1.31-7.72), and club foot (OR=2.73; 95% CI 1.13-6.18). Phenytoin monotherapy was not associated with an increased risk of cardiac malformation, hypospadias, inguinal hernia, or undescended testes, nor with an increased risk of foetal loss, prenatal growth retardation, preterm birth, or any minor congenital malformations.[44]

Growth

Prospective studies

Arulmozhi et al. (2006) evaluated the physical growth and psychomotor development of infants exposed to antiepileptic drugs *in utero* in a prospective study of 30 pregnant women. Eighteen women exposed to phenytoin monotherapy (200-300 mg/day) throughout pregnancy were included in this study. A control group (n=30) was included, matched for socioeconomic status, education of the parents and nutritional status of the mothers. The mothers were interviewed regarding the feeding pattern of the child – whether breastfed or started on supplementary feeds and intercurrent illness if any was noted. The physical growth of the babies was examined at birth and at the 1st, 6th, and 12th month of age. It was assessed by measuring the body weight, head circumference, length and anterior fontanelle. The body weight, head circumference and length of the case and control babies did not differ at birth and at one month of age.

At six and 12 months of age, the case babies had a lower body weight and a smaller head circumference, and they were shorter than the controls ($p<0.01$ or $p<0.001$). Phenytoin monotherapy was associated with a larger area of the anterior fontanelle at birth and at 12 months of age ($p<0.001$). The data on psychomotor development of the babies are reported in the section *Functional and cognitive effects*.[45]

Gaily and Granström (1989) investigated the delay of early postnatal physical growth and intelligence in drug-exposed children of epileptic mothers. Forty-eight women on phenytoin monotherapy during pregnancy (duration not reported) and 103 control children were included in this study. The control children were born to non-epileptic mothers at the same hospital during the study period. The sampling criteria for the control children were gestation of at least 37 weeks, no major pre- or perinatal complication, and no drug exposure. Crown-heel length and weight was routinely measured up to 18 months of age. There was no evidence of intrauterine phenytoin exposure causing growth retardation, indicated by length or weight increments in the first postnatal month. The intelligence findings are reported in the section *Functional and cognitive effects*.[46]

Hiilesmaa et al. (1981) studied foetal growth retardation in babies born to 133 epileptic mothers on antiepileptic medication. After delivery, a control pair (mother and child) matched for maternal age, parity, social class, and foetal sex was selected for each epileptic woman. Fifty-five women received phenytoin monotherapy, but its duration was not mentioned. Phenytoin exposure did not cause any change in offspring body weight, body length, or head circumference compared to unexposed controls.[47]

Retrospective cohort studies

Artama et al. (2013) examined the effect of antiepileptic drug use during pregnancy on perinatal health in offspring in a nationwide, retrospective cohort study in Finland. This register-based study was based on all pregnancies ending in birth in Finland between 1996 and 2008. The outcomes included preterm birth, low birth weight, weight for gestational age, low Apgar score, need for respiratory treatment, perinatal death and infant death. The data concern 751,139 singleton births. In cases with conflicting or missing information, the register data were confirmed and supplemented with information from the maternity hospital records. Maternal age at delivery, parity, maternal residence district, socioeconomic status and offspring major congenital anomalies were included in the analyses as potential confounding factors. Twenty-six infants were born to epileptic women receiving phenytoin monotherapy one month prior to and/or during pregnancy. The controls were 721,948 women without epilepsy or antiepileptic drug use and their singleton offspring. All 26 children were born alive, 21 of them full term. The OR for preterm birth) was 1.85 (95% CI 0.43-7.88), for low birth weight 1.33 (0.18-9.92), for large-for-gestational-age 3.23 (0.96-10.83). The outcome small-for-gestational-age was not assessed. None of the 21 infants in the phenytoin-exposed group

that were born full term had a low Apgar score or neonatal respiratory problems needing treatment.[48]

Functional and cognitive effects

Prospective studies

Thomas et al. (2008) investigated the motor and mental development of infants exposed to antiepileptic drugs *in utero*. They evaluated the offspring of 395 women enrolled in the Indian Kerala Registry of Epilepsy and Pregnancy between 1998 and 2004. Twenty-nine infants from mothers receiving phenytoin monotherapy at any time during pregnancy were included in the study. Thirty-two infants born to epileptic mothers not prenatally exposed to any antiepileptic drug served as controls. The Mental Developmental Quotient and Motor Developmental Quotient of the infants were measured at 12 months of age. The Mental Development Quotient of the phenytoin-exposed infants was 90.3 (95% CI 77.3-103.3) and that of the controls 92.3 (95% CI 81.4-103.2). The Motor Developmental Quotients of these groups were 100 (95% CI 91.6-108.4) and 94.7 (95% CI 84.9-104.5), respectively.[49]

Gopinath et al. (2015) further followed up this cohort when the children were between ten and 12 years of age. They studied IQ, attention, memory and other neuropsychological functions in the 190 children completing the study and whose mothers enrolled between April 1998 and December 2001. The investigators selected 149 age- and sex-matched controls from among the children attending two nearby schools in order to avoid any socioeconomic bias. Eleven children exposed to phenytoin prenatally were included in the study. Only the full scale IQ (measured with the Wechsler Intelligence Scale for Children, fourth edition) was reported for this group separately. The full scale IQ of the phenytoin-exposed children was 82.6±13.5 (mean±SD) and that of the controls 80.7±13.7.[50]

Meador et al. (2011) examined dose-related effects of foetal antiepileptic drug exposure on verbal and non-verbal cognitive measures at the age of three years as part of a prospective multi-centre study. A control group was not included in the study and the duration of the foetal exposure to the drugs was not mentioned. Forty-one children were exposed to phenytoin monotherapy during pregnancy at an average daily dose of 401 mg (range 67-750). Their cognitive performance was measured using the Differential Ability Scales, Preschool Language Scale (fourth edition), Peabody Picture Vocabulary Test (fourth edition) and Developmental Test of Visual-Motor Integration (fifth edition). Testing was conducted at 36-45 months of age; standardized scores were calculated. The verbal index was 95.9 (95% CI 91.0-100.8), the non-verbal index 102.0 (95% CI 96.9-107.2). Partial Pearson correlation for verbal and non-verbal indices to pregnancy average standardized dose, controlling for maternal IQ, were 0.09 and -0.20, respectively. No dose-related effects were seen.[51]

Arulmozhi et al. (2006) evaluated the physical growth and psychomotor development of infants exposed to antiepileptic drugs *in utero* in a prospective study of 30 pregnant women. Eighteen women exposed to phenytoin monotherapy (200-300 mg/day) throughout pregnancy were included in this study. A control group (n=30) was included, matched for socioeconomic status, education of the parents and nutritional status of the mothers. The mothers were interviewed regarding the feeding pattern of the child – whether breastfed or started on supplementary feeds and intercurrent illness if any was noted. The data on the physical growth of the babies are reported in the section *Growth*. The psychomotor development of the babies was examined at the 2nd, 6th, and 12th month of age. It was assessed using the Griffiths scales and babies were examined for locomotor score, reaching behaviour and personal-social development. Phenytoin monotherapy was associated with a delayed locomotor development at the 2nd, 6th and 12th month, indicated by a negative impact on sitting progression (compared to controls, $p<0.001$). The reaching behaviour and the personal-social scores of the phenytoin-exposed babies did not differ from those of the controls.[45]

Wide et al. (2000) assessed the psychomotor development in infants exposed to antiepileptic drugs *in utero* in a population-based follow-up study of children born to women with epilepsy, treated during pregnancy, and enrolled from 1985 to 1995. Twenty-two women received phenytoin monotherapy at a mean dose of 274 mg/day (range 75-450). They took the drug throughout pregnancy. Twenty-one exposed children were tested with the Griffiths test for psychomotor development, which consisted of the subsets: gross motor function, personal and social behaviour, hearing and speech, eye and hand coordination, and performance. The control group consisted of 87 unexposed children, born in the same hospital within two days of the birth of a phenytoin-exposed child and matched for gestational age, gender and mode of delivery. At the age of nine months children exposed to phenytoin *in utero* showed a mean total score in the Griffiths test that was similar to that of unexposed children (346 versus 344; 95% CI for difference of the mean: -7.1 to 11.3).[24]

Wide et al. (2002) also assessed the psychomotor development of the children at the preschool age of 4.5-5 years. Follow-up at this age was started in 1992. At that time, the children born in 1985-1986 were too old to be included. Sixteen women were exposed to phenytoin monotherapy at a mean dose of 253 mg/day (range 75-450), throughout pregnancy. Fifteen exposed children were tested with the Griffiths test for psychomotor development, which consisted of the subsets: locomotor function, personal and social behaviour, hearing and speech, eye and hand coordination, performance, and practical reasoning. The control group consisted of 66 unexposed children, matched for gestational age, gender and mode of delivery, recruited within ±2 days of the birth of a phenytoin-exposed child. At the age of 4.5-5 years children exposed to phenytoin *in utero* showed a reduction in mean scores for locomotor

development compared with unexposed children (98 versus 106; 95% CI for difference of the mean: -14.0 to -0.4). The performance in the other subsets of the test was not affected.[52]

Gaily and Granström (1989) investigated delay of early postnatal physical growth and intelligence in drug-exposed children of epileptic mothers. Forty-eight women on phenytoin monotherapy during pregnancy (duration not reported) and 103 control children were included in this study. The control children were born to non-epileptic mothers at the same hospital during the study period. The sampling criteria for the control children were gestation of at least 37 weeks, no major pre- or perinatal complication, and no drug exposure. The data on physical growth of the children are reported in the section *Growth*. Intelligence was assessed at 5.5 years by a verbal (Wechsler Preschool and Primary Scale of Intelligence) and a non-verbal (Leiter International Performance Scale) method. There was no evidence of intrauterine phenytoin exposure affecting intelligence.[46]

Gaily et al. (1988) investigated the intellectual performance of 148 children of epileptic mothers enrolled in a prospective study during pregnancy, and of 105 control children. Control selection criteria were absence of maternal epilepsy or other chronic disorder in the mother, absence of intrauterine drug exposure, gestational period at least 37 weeks, and no major perinatal illness or complication. One hundred twenty-nine out of the 148 children born to epileptic mothers had been exposed to antiepileptic drugs during the first 20 weeks of pregnancy. One hundred twenty-one of the 148 children were examined together with the control group at the age of 5.5 years. The intelligence of 117 exposed and 104 control children was assessed by both verbal (Wechsler Preschool and Primary Scale of Intelligence) and nonverbal (Leiter International Performance Scale) methods. Of the exposed children, 103 had been exposed *in utero* to phenytoin, 54 of which to this drug as monotherapy. The results of children exposed *in utero* to mono- or polytherapy were not analysed separately. The intelligence scores of the treated and control groups did not differ.[53]

Follow-up study of pharmacovigilance centre data

Scolnik et al. (1994) investigated the neurodevelopment of children exposed *in utero* to phenytoin monotherapy in a study among women reported to the Motherisk Program. Thirty-four women exposed to phenytoin were matched with controls. These women came in for counselling after exposure to other drugs. Other matching criteria were age, gravidity, parity, and socioeconomic class. All 34 women were treated with phenytoin during the first trimester; 29 women took phenytoin throughout pregnancy. Complete physical and neurologic examination of the children was performed, followed by neurobehavioral testing of both mothers and offspring. Children between 18 and 30 months of age were tested by means of the Bayley scales of infant development (1969) and children beyond that age with the McCarthy scales (1972). All children were also tested with the Reynell developmental language scales (1977).

Children exposed to phenytoin had lower (mean±SD) scores of global IQ (103±25.2) than their controls (113.4±13.1) ($p<0.05$). Additionally, the children exposed to phenytoin scored lower on the Reynell developmental language scales for verbal comprehension (0.2±1.6 versus control value of 1.1±0.95) and expressive language (-0.47±1.2 versus control value of 0.2±0.96) ($p<0.05$).[36]

Retrospective cohort studies

Forsberg et al. (2011) studied children's school grades at age sixteen in order to evaluate long-term effects on neurodevelopment in children born to women with epilepsy during pregnancy. The Patient Register, the Medical Birth Register, and a local study at a Stockholm hospital were used to identify women with epilepsy in Sweden who had given birth between 1973 and 1986. Exposed children were compared to all other children born in Sweden between 1973 and 1986. The analysis was adjusted for child's year of birth, maternal age, parity and maternal education level. Among the 1,070 children in this study, 429 had been exposed to anticonvulsants in polytherapy and 316 to phenytoin monotherapy. The duration of the prenatal exposure was not reported. The ORs with 95% CIs for 'not passed' and 'passed in excellence' in sports, mathematics, English and Swedish were measured. The ORs for 'not passed' were 1.00 (95% CI 0.68-1.47); 1.13 (0.81-1.54); 1.16 (0.81-1.66) and 1.17 (0.81-1.69) for sports, mathematics, English and Swedish, respectively, for the children exposed to phenytoin monotherapy *in utero*. The ORs for 'passed with excellence' were 0.76 (0.59-1.07); 0.82 (0.62-1.07); 0.92 (0.70-1.20) and 0.95 (0.73-1.24) for the four subjects. There was no increased risk of leaving compulsory school without a final grade (OR=1.19 (0.79-1.80).[54]

Adab et al. (2004) reported similar findings in a retrospective cohort study performed in 249 children aged six months to sixteen years and exposed to antiepileptic drugs *in utero*. An unexposed control group consisted of 101 children, 83 of them aged six years or older (10.5±3.2 years) and eighteen up to five years of age (3.3±0.9 years). A clinician conducted semi-structured interviews of the epileptic mothers to ascertain information about their epilepsy and relevant pregnancy. Clinical records were used to confirm this information. Twenty-one children aged six years or older (10.7±3.5 years) and five children up to five years of age (2.6±1.4 years) were exposed to phenytoin monotherapy *in utero*. The duration of the prenatal exposure was not reported. The 21 phenytoin-exposed children aged six or older were tested for verbal IQ, performance IQ and fullscale IQ using the Wechsler IntelligenceTest for Children III. Eighty of the 83 unexposed children over 6 years of age served as controls. Verbal IQ in the phenytoin group (mean 98.5; 95% CI 90.6-106.4) was not different from that in unexposed control children from epileptic mothers (90.9; 95% CI 87.2-94.6). Neither were performance IQ (mean 97.1; 95% CI 91.7-102.6 vs 90.2; 95% CI 86.1-93.0) and full scale IQ (mean 97.6; 95% CI 90.3-105.0 vs 89.5; 95% CI 85.5-93.4). Educational problems (indicated

by the presence of additional educational needs, that were not specified any further) were assessed in children aged four and above. These problems occurred in one (4.5%) of the phenytoin-exposed children and in seven (8%) of the unexposed children. The OR for additional educational needs was 0.98 (95% CI 0.36-2.68).[38]

Vinten et al. (2009) analysed the behaviour of children aged between six and sixteen from the above cohort who had undergone a full neuropsychological assessment and whose mothers had completed the Vineland Adaptive Behavior Scales (VABS). Women and their children were excluded from the study if they had a progressive neurological deficit, a major learning difficulty, or symptomatic generalized epilepsy. A total of 150 mothers completed the VABS regarding 242 children. The mothers were also asked to complete the Parenting Stress Index (PSI). The VABS measures communication, daily living skills and socialization skills, while the PSI assesses the behavioural and temperament qualities of the child. Twenty children were exposed to phenytoin monotherapy *in utero*, whereas 80 children were not exposed to any antiepileptic drugs *in utero*, although their mothers had a diagnosis of epilepsy. The data were adjusted for maternal and child IQ. The exposed children showed no statistically significant effects in the VABS compared to the controls. PSI data were available from 13 phenytoin cases and 52 controls. The percentages of exposed children versus controls falling within the highest stress ranges for the domains of the PSI were: for distractibility 7.7% vs 19.2%, for adaptability 7.7% vs 13.5%, for reinforces-parent 30.8% vs 48.1%, for being demanding 7.7% vs 26.9%, for mood 46.2% vs 42.3%, for acceptability 0% vs 25.0%, and for total domains 15.4% vs 25.0%.[55]

Dean et al. (2002) examined the neonatal and later childhood morbidity in children exposed to antiepileptic drugs prenatally. They investigated the frequencies of neonatal withdrawal, congenital malformations, childhood medical problems, developmental delay and behaviour disorders in the offspring of 149 women who took antiepileptic drugs during pregnancy and delivered between 1976 and 2000. They were identified through review of hospital records, or recruited from the antenatal clinic and postnatal wards. A structured interview including pregnancy histories and standardised assessment of all available offspring was carried out by a trained research nurse. Thirty-eight siblings of exposed cases were not exposed to antiepileptic drugs *in utero*. They served as controls. In sixteen cases, the mother had epilepsy but took no treatment and in 22 cases the child was born before epilepsy developed. Twenty-four children that had been exposed to phenytoin as single antiepileptic drug were identified. They had a mean age of 11 years at the time of the study. The mean age of the controls was 15 years. The phenytoin-exposed children did not have a higher frequency of speech delay, motor delay, or behaviour disorders. The structural defect findings are reported in the section *Structural defects*.[40]

Meta-analysis

Veroniki et al. (2017) conducted a systematic review and network meta-analysis to compare the safety of antiepileptic drugs for neurodevelopment of infants exposed *in utero* and/or during breast feeding. Randomized clinical trials, quasi-randomised clinical trials and observational studies were eligible. Included studies assessed infants or children ≤ 12 years of age whose mothers used antiepileptic drugs during pregnancy and/or while breast feeding. Both monotherapy and polytherapy antiepileptic drugs were eligible. Placebo, no antiepileptic drug treatment, or other antiepileptic drugs alone or in combination were considered as comparators. The primary neurological outcomes were cognitive developmental delay and autism/dyspraxia, and the secondary outcomes included attention-deficit hyperactivity disorder, language delay, neonatal seizures, psychomotor developmental delay and social impairment. The investigators included 29 cohort studies (5,100 patients). Phenytoin monotherapy during pregnancy and/or breast feeding was not associated with cognitive developmental delay, autism/dyspraxia, neonatal seizure, psychomotor developmental delay, or attention-deficit hyperactivity disorder in the child. Adequate data on its effects on language delay and social impairment were not available.[56]

2.3.3 Lactation

Effects via lactation

Meador et al. (2010) investigated the effect of breastfeeding during phenytoin monotherapy on subsequent cognitive abilities in children in a prospective multicentre observational study. They used the Differential Ability Scales method to measure the children's cognitive abilities. A nonexposed control group was not included. Mothers with an IQ below 70 were excluded to avoid floor effects and because maternal IQ was considered the major predictor of child IQ in population studies. Other exclusion criteria included positive syphilis or HIV serology, progressive cerebral disease, other major diseases (e.g., diabetes), exposure to teratogenic agents other than antiepileptic drugs, poor antiepileptic drug compliance, drug abuse in the prior year, or drug abuse sequelae. At the age of three years the IQ of children breastfed (n=17) and non-breastfed (n=23) was measured. The mean IQ for breastfed children, 91 (95% CI 84-98), was reported not to differ from the mean IQ for non-breastfed children, 99 (95% CI 93-105).[57]

Meador et al. (2014) also investigated the effect of breastfeeding during phenytoin monotherapy on subsequent cognitive abilities in children after a period of three more years. All mothers in the study continued taking the drug after delivery. At the age of six the IQ of children breastfed (n=17) and non-breastfed (n=20) was measured using the same method. The

former had a mean IQ of 104 (95% CI 99-110), the latter a mean IQ of 108 (95% CI 103-113), values that were reported not to differ.[58]

Transfer via lactation

Shimoyama et al. (1998) determined the concentrations of phenytoin in breast milk and plasma samples from five lactating women. These women had been treated with 100 (n=1), or 300 mg (n=5) phenytoin/kg bw/d. The investigators reported an average milk to maternal plasma ratio of 0.289, with ratios ranging from 0.13 to 0.52 (13-52%).

The concentrations in breast milk ranged between 0.41 and 1.30 µg/ml. The authors calculated that a 4-kg infant drinking 1L of milk daily would ingest approximately 0.1-0.33 mg phenytoin/kg bw/d when the standard dose was administered.[59]

Fleishaker et al. (1987) analysed the phenytoin concentration in breast milk and plasma samples from four lactating women. They reported a milk to plasma concentration ratio of 0.34±0.09 (34±9%).[60]

Steen et al. (1982) examined the milk to plasma concentration ratios of six women receiving phenytoin at a daily dose of 200, 300 or 400 mg. They also analysed the plasma concentrations of their infants. The study was carried out between one and three months after delivery. The mean plasma phenytoin concentrations of the six mothers ranged from 3.2 to 19.8 µg/ml. The milk to plasma concentration ratios ranged from 6 to 18%. The mean plasma concentrations in two of the infants were 0.18 and 0.12 µg/ml. They were below the detection level in the remaining four. Assuming a total daily milk intake of 160 ml/kg bw, the investigators calculated that the daily dose to the infant would vary between 0.03 to 0.47 mg/kg bw.[61]

Kaneko et al. (1979) analysed the concentrations of phenytoin in serum and breast milk of nine phenytoin-treated women with epilepsy at postnatal days 3-32. The dose of phenytoin they received was not reported, but the concentration in their serum was 4.5±1.4 µg/ml and the concentration in their milk 0.8±0.3 µg/ml. The milk to serum ratio was 18±6%.[62]

Rane et al. (1974) analysed the transfer of phenytoin from mother to child in seven pairs of mothers taking phenytoin therapy and their newborn children. The mothers took 200-500 mg/day, with or without additional medicines. Their plasma concentrations ranged from 0.7 to 15 µg/ml phenytoin at 0.03-3.6 hours before delivery. The first observed plasma concentrations in the newborn infants were between 0.43 and 13 µg/ml (blood samples taken 0 to 48 hours after birth). The amount of breast milk given to the infants was reported. The concentration of phenytoin in the milk was measured and reported in only one case. The breast milk of that mother contained 0.26-0.38 µg/ml, and the corresponding milk to maternal plasma ratio was 45%. The plasma levels of the infants were measured

daily during the first week after birth. The data show that the plasma levels of the infants decreased during nursing. The half-life of phenytoin in plasma was determined in five cases and varied between 6.6 and 34.0 hours.[63]

Mirkin (1971) investigated the milk to plasma ratio in two women treated chronically with phenytoin at 300 mg/day. Three measurements were done during the period of one to 33 days after birth. The plasma concentrations of one subject were 2.5 to 3.2 µg/ml, of the other 5.4 to 8.4 µg/ml. The average concentrations in their milk were 1.5 and 1.7 µg/ml, respectively. The women had average milk to plasma ratios of 51% (range 43-59) and 27% (range 15-42), respectively (ranges calculated by the Committee, no consistent increasing or decreasing trend).[64]

2.4 Animal studies

In Tables 1 and 2 (Annex A), fertility studies and developmental studies performed in animals are summarized.

2.4.1 Fertility studies

Male fertility

Rats

Shetty (2007) treated male Wistar rats (9-13 weeks old; n=5 per group) intraperitoneally with 3.5, 5.5 or 7.0 mg phenytoin/day (i.e. approximately 14-28 mg/kg/day) on five consecutive days. Concurrent negative controls (n=5) were treated intraperitoneally with 0.5 ml water/day. Information on general toxicity was not provided. Epididymal sperm morphology, evaluated on days 14 and 35 after the last treatment, showed no differences in the numbers of normal and abnormal spermatozoa between rats treated with phenytoin and controls.[65]

Cohn et al. (1982) administered 20 mg phenytoin/kg bw/day by subcutaneous injection to male Wistar rats (n=11) during three months, starting at weaning, postnatal day (PND) 21. The control group (n=10) received saline subcutaneously (volume not reported). Necropsy was performed two days after the last injection. There were no signs of general toxicity (growth was the only endpoint reported). Fertility rate was determined at the end of treatment by caging each male with two untreated females for five days. The males were considered fertile if at least one of the females became pregnant. Fertility was not affected by phenytoin (phenytoin: 5/10 fertile; control: 7/11). Relative reproductive

organ weights (testis, seminal vesicle, prostate, coagulating gland, epididymis) and epididymal sperm content and motility were not affected either.[66]

Cohn et al. (1978) treated male albino rats (age and strain not reported; 5 or 7 rats per group) subcutaneously with 5, 10 or 20 mg phenytoin/day (corresponding to about 20, 40 and 80 mg/kg bw/day, respectively) on 70 consecutive days. The study included a control group of five rats (treatment not reported). Mean phenytoin concentrations measured in tissues from four male rats given 20 mg phenytoin/day subcutaneously for 12 days were 10.9 µg/ml (serum), 1.9 µg/g (testis), 2.15 µg/g (epididymis), 3.1 µg/g (coagulating gland) and 2.34 µg/g (prostate). Phenytoin concentration in post-coital uterine fluid was below the limit of detection (uterine fluid was collected from seven proestrous female rats mated with males treated with 20 mg phenytoin/day subcutaneously for seven days). Phenytoin treated rats showed extensive cutaneous necrosis at the injection sites (no other information on general toxicity was given). Fertility rate, determined at the end of treatment by caging each male with two untreated females for five days, was reduced at the highest dose level (high dose phenytoin 2/5; control 7/7; $p<0.05$). Relative reproductive organ weights (testis, seminal vesicle, prostate, coagulating gland, epididymis), epididymal sperm content and motility, Leydig cell count and blood testosterone concentration were not affected by exposure to phenytoin.[67]

Female fertility

Mice

Roberts et al. (1991) treated two strains of female mice (C57BL/6 and A/J; n=10-13 per group) with phenytoin sodium salt by gastric intubation (0, 40, 65 or 105 mg/kg bw/day) every 48 hours, starting at least one week prior to breeding and continuing throughout pregnancy. Controls (n=10 for C57BL/6, n=12 for A/J) received 5 ml/kg bw/day of the vehicle (water adjusted with NaOH to pH 11). On gestation day (GD) 10-12 (vaginal plug = GD0), blood was obtained by tail-tip bleeding for determination of dam phenytoin pharmacokinetics. On GD18, dams were sacrificed. Maximal phenytoin serum concentrations were reached 4-8 hr after dosing and approximated 23 (low-dose), 30 (mid-dose) and 33 (high-dose) µg/ml in C57 mice and 15 (low-dose) and 25 (mid-dose) µg/ml in A/J mice. Serum phenytoin remained above the human therapeutic concentration (5 µg/ml) for most of the interdose interval. Mortality occurred in nonpregnant C57 females at 65 and 105 mg/kg bw/day (1/11 and 5/12, respectively ($p<0.05$)). It was preceded by weight loss and tremors. Mortality did not occur in nonpregnant A/J dams. The proportion of mated females that became pregnant was decreased at the highest dose in C57 mice (phenytoin 2/12; control 8/10, $p<0.01$). It was decreased dose-dependently in A/J mice (phenytoin 9/12, 5/11 and 0/13 at 40, 60 and 105 mg/kg bw/day, respectively; control 10/12), but the decrease was only statistically significant at the highest dose ($p<0.01$). The proportion of

females that mated, total number of implantations per litter and number of viable implantations per litter were not affected by exposure to phenytoin.[68]

2.4.2 Developmental toxicity studies

Structural defects

Gavage

Rats

Rowland et al. (1990) daily administered phenytoin (2,2-diphenylhydantoin sodium salt) to pregnant Sprague-Dawley rats (n=4-9 per group) by gavage on GD8 to GD17. Doses of 0, 150, 375, 750, 1125, and 1500 mg/kg bw/day phenytoin were administered. Reproductive outcomes were determined at GD20.

After the oral dosing, a dose-dependent increase of maternal toxicity was observed, which was characterized by impaired motor function, decreased maternal weight gain ($p<0.05$), hepatic necrosis and gastritis. Maternal deaths occurred between GD10 and GD17 in the groups treated with 1,125 mg/kg/day (2/8 dams dead) and 1,500 mg/kg/day (3/4 dams dead). The reproductive outcomes of the remaining dams in these dose groups were affected. An increase in embryonic loss occurred at 1125 mg/kg/day: all foetuses were resorbed in two out of six litters; 11/14 foetuses were dead in one out of six litters. Additionally, all foetuses of the one dam examined after 1500 mg/kg phenytoin per day were resorbed. A dose-dependent intrauterine growth retardation was observed, which was characterized by decreases in foetal weight, crown-rump length and ossification of the appendicular and axial skeleton (compared to controls, all $p<0.05$). An increased percentage of malformed foetuses per litter was observed at 750 and 1,125 mg/kg/day doses (compared to controls, $p<0.05$). Visceral malformations included cardiovascular malformations (mainly absent aortic arches), urogenital anomalies (mainly hydronephrosis and ectopic kidneys) and craniofacial malformations (mainly highly arched palates often associated with a thin, tapered snout). Skeletal malformations included hemivertebrae and fused vertebrae in the cervical and thoracic regions.[69]

Zengel et al. (1989) evaluated the effect of prenatal phenytoin on the postnatal growth and craniofacial morphology in mature rats. Pregnant females were treated with 1,000 mg/kg bw phenytoin (suspended in 1% solution of carboxymethylcellulose) by gavage on days 9, 11 and 13 of gestation. Controls received an equivalent volume of the vehicle on the same days. Group size and maternal toxicity were not described. After delivery, pup weight was recorded frequently till postnatal day (PND) 135, when pups were sacrificed and skeletons were prepared for analysis. Average litter size after delivery was comparable, but a higher pup mortality was observed in phenytoin-treated animals (94% compared to

1% in controls). At the time of weaning on PND25 all viable pups from the phenytoin-treated dams which were left, were used for the examinations. Non-viable pups were not further examined. Six male and six female pups of the control group were randomly selected from five control litters. After weaning, body weight accumulation was reduced in the offspring of treated females (compared to controls, $p<0.01$, 0.02 or 0.05, at several time points from PND 36 on). Male pups were more affected than female pups. All pups from treated females showed less protrusion of the eyes, absent or rudimentary lacrimal bones and presence of nasolacrimal canals. Examination of the skeletons revealed reduced body length (compared to controls, $p<0.05$) and changes in craniofacial morphology in both male and female pups from phenytoin-treated females. The craniofacial changes included reduced cranial length ($p<0.005$), bizygomatic width ($p<0.005$), facial height ($p<0.005$) and maxtransfrontal width ($p<0.01$).[70]

Lorente et al. (1981) treated pregnant CD rats with phenytoin by gavage at doses of 700, 800 or 1,000 mg/kg bw/d, either on single or multiple days of gestation in the periods GD9-11, GD9-13, GD10-12, GD10-14, GD11-13, GD12-14 or GD13-15. With these high-dosage regimens, the authors aimed to produce the greatest number of anomalies with the lowest level of maternal mortality. The multiple-dose regimen of 1,000 mg/kg bw/d on GD9-13 was chosen, because it produced a relatively low maternal mortality, but the longest exposure of the foetus to phenytoin. However, the maternal mortality or toxicity was not specified. Furthermore, dam group size was not reported.

Rats exposed *in utero* to 1,000 mg/kg bw/d on GD9-13 exhibited foetal onset growth retardation, abnormalities of the craniofacial region and axial skeleton. In addition, the exposed offspring had lower body weights than controls at all foetal ages examined (GD14.5-18) and at birth (all $p<0.05$). They also had a shortened snout and high-arched, irregular palate, and delays in skeletal maturation, demonstrated by reduced bone length and ossified bone length at birth (both $p<0.05$). No consistent information was provided on other dosage regimens, but retarded palatal growth was also observed at a 15% incidence at single day administration of 700-1,000 mg/kg bw.[71]

Kim et al. (2012) treated pregnant Sprague-Dawley rats with phenytoin by gavage at doses of 0, 50, 150, or 300 mg/kg bw/day (11 animals per dose group) from GD6 through GD15. They investigated the dose-response effects of phenytoin on pregnant dams and embryo-foetal development as well as the relationship between maternal and developmental toxicity. At 300 mg/kg bw/day, various signs of maternal toxicity were observed: increased clinical changes such as ataxia and seizure, suppressed body weight and body weight gain ($p<0.01$), decreased food intake ($p<0.01$), decreased absolute weights of lung, spleen, heart, and brain and increased relative weights of adrenal glands, kidneys, brain, ovary and liver as compared to the control group ($p<0.01$ or $p<0.05$, depending on

the organ). At 150 mg/kg bw/day, maternal toxicity signs similar to those at 300 mg/kg were present (p<0.01), though less strongly: suppressed body weight and body weight gain, decreased food intake, a decreased absolute weight of the heart and increased relative weights of adrenal glands and brain. No treatment-related maternal effects were observed at 50 mg/kg bw/day.
At 300 mg/kg bw/day, developmental toxicity, including decreased foetal and placental weights (p<0.01), an increased incidence of morphological alterations (p<0.01) and a delay in foetal ossification occurred (p<0.01 or p<0.05, depending on the parameter), as compared to the controls. Developmental toxicity was less severe at 150 mg/kg bw/day than at 300 mg/kg bw/day. It was restricted to a decreased placental weight (p<0.01) and an increased incidence of visceral and skeletal alterations (p<0.01). No treatment-related developmental effects were observed at 50 mg/kg bw/day.[72]

Mice
Eluma et al. (1984) administered phenytoin to pregnant CD 1 mice at levels of 0, 50, 75 and 125 mg/kg bw/d by gavage during GD8-10, GD11-13, GD14-16 or GD8-16 (2 mice/group/time point; six mice per control group). Information on maternal toxicity was not presented. Foetal weight showed a window-dependent and dose-related decrease in all treatment groups (p-values not mentioned). In treated offspring, a high incidence of cleft palate was noted, and a lower incidence of foetal death, cleft lips, haematomas, hydroencephaly and exencephaly (compared to controls, p-values ranged from <0.001 to <0.0001). These lesions were however, not specified per time point and dose level.[73]

Fritz et al. (1976) treated mated (Tif/MAG) mice (n=30/group) with 0, 15, 50, 100 or 170 mg phenytoin/kg bw/d during GD6-15 by gavage. Offspring was subjected to visceral (one third) and skeletal examination (two thirds). Ten dams in the high-dose group died. In the lower dose groups, there was no maternal mortality. At the next higher dose of 100 mg/kg food intake was diminished. The reactions of the dams in the lower dose groups (15 and 50 mg/kg) did not differ from those of the controls. No further information on maternal toxicity was given. Early embryonic death occurred at 100 and 170 mg/kg bw/d, and mean foetal weight was reduced in these groups, as compared to the controls (p<0.01). There was a dose-related increase in cleft palate at 15, 50, 100 and 170 mg/kg bw/d (0.3%, 2%, 5.2% and 9.3%, respectively, versus 0.13% in historical control data from 500 mice), which was statistically significant from 50 mg/kg bw/d onwards (compared to controls, p<0.01). Skeletal examination showed incomplete ossification of the fore-limbs at 170 mg/kg bw/d (p<0.01), but no malformations.[74]

Roberts et al. (1991) treated two strains of female mice (C57BL/6 and A/J; n=10-13/group) with phenytoin sodium salt by gastric intubation (0, 40, 65 or 105 mg/kg bw/day) every 48 hours, starting at least 1 week prior to

breeding and continuing throughout pregnancy. Controls (n=10 for C57, n=12 for A/J) received 5 ml/kg bw/day of the vehicle (water adjusted with NaOH to pH 11). On GD10-12 (vaginal plug = GD0), blood was obtained by tail-tip bleeding for determination of dam phenytoin pharmacokinetics. On GD18, dams were sacrificed. Gestational weight gain, gravid uterus weight and foetal weight were determined. Foetuses were examined for visceral and skeletal abnormalities. These endpoints could not be examined in A/J mice at 105 mg/kg because there were no pregnant females at this dose. Maximal phenytoin serum concentrations were reached 4-8 hr after dosing and approximated 23 (low-dose), 30 (mid-dose) and 33 (high-dose) µg/ml in C57 mice and 15 (low-dose) and 25 (mid-dose) µg/ml in A/J mice. Serum phenytoin remained above the human therapeutic concentration (5 µg/ml) for most of the interdose interval. Mortality occurred in nonpregnant C57 females at 65 and 105 mg/kg bw/day (1/11 and 5/12, respectively) and was preceded by weight loss and tremors. Gestational weight gain and gravid uterus weight were dose-dependently decreased in C57 mice (compared to controls, statistically significant by a linear trend analysis, $p<0.05$). Postimplantation loss was decreased in A/J mice given 65 mg/kg bw/day ($p<0.05$). Foetal weight was only lower at 65 mg/kg in C57 ($p<0.05$). There was decreased ossification of the sternebrae (in C57 at 65 mg/kg, $p<0.05$) and of the thoracic centra, metacarpals and metatarsals (in A/J at 65 mg/kg, $p<0.05$). The frequencies of hydroencephaly (at 65 mg/kg) and open eyelid (at 40 and 65 mg/kg) were increased in A/J mice, and the frequency of cardiac calcium deposit (inside the apex of the left ventricle) was increased (at 40 mg/kg) in C57 mice (all $p<0.05$).[68]

Cats

Khera (1979) treated pregnant cats (n=13-14 per group) with 0, 1 or 2 mg phenytoin sodium salt/kg bw/d by gavage during GD10-22, and necropsied them on GD 43. Foetuses were examined for external, visceral and skeletal malformations.

Maternal body weights were decreased at 2 mg/kg bw/d on GD20 and GD30 (compared to controls, $p<0.05$), but normal at GD40 and GD43. Maternal body weights were normal at 1 mg/kg bw/d at all time points. The incidences of abortion, non-pregnancy, live or dead foetuses and mean foetal weights were within control range. The number of resorptions was higher in the 2 mg/kg bw/d group than in controls ($p<0.05$). There were no treatment-related anomalies in live foetuses. A high incidence of malformations however, was noted in test foetuses from four aborting cats (on GD31-37): one control cat, two cats given 1 mg/kg bw/d and one cat given 2 mg/kg bw/d. None of the seven foetuses from the control cat were anomalous, whereas all eleven foetuses from the two cats given 1 mg/kg bw/d had cleft palate and open eyelids and four out of the ten foetuses from the cat given 2 mg/kg bw/d had umbilical hernia and one had open eyelids.[75]

Water intake

Mice

Finnell et al. (1989) exposed three strains of virgin female mice (SWV, LM/Bc and C57BL/6J) to phenytoin (0, 10, 20, 40, or 60 mg/kg bw) orally via the drinking water for a 15-day period (before mating) and throughout gestation until GD18. Phenytoin was administered before mating in order to reach a steady state concentration of phenytoin, which was within the desired therapeutic range for a mouse (within 2.5 and 12.5 µg/ml plasma). No information on maternal toxicity was reported. A dose-dependent decrease in foetal weight was observed in all strains (compared to controls, $p<0.05$). Additionally, the incidence of offspring with one or more congenital abnormality was enhanced with increasing doses of phenytoin in all mice strains ($p<0.05$). These included both skeletal defects (mainly a pattern of multiple malformations and ossification delays in supraoccipital bones, sternebrae, distal phalanges and midfacial bones) and soft tissue defects (mainly dilated or immaturely developed cerebral ventricles and renal defects, digital, cardiac and ocular anomalies). A correlation was observed between the risk of offspring with abnormalities and plasma phenytoin ($p<0.05$).[76]

Feed

Mice

Hansen and Billings (1986) treated pregnant A/J mice with phenytoin via the diet prior to and throughout gestation (daily dose of 0, 60 or 75 mg/kg) to investigate the effects of chronic exposure to phenytoin. On GD18 or -19, mice were sacrificed and foetuses were examined. Blood samples were collected from phenytoin-treated dams and their phenytoin concentrations were determined. These concentrations were 9.2±0.9 µg/ml of plasma at 60 mg/kg body weight and 13.0±0.9 µg/ml at 75 mg/kg. Maternal toxicity, indicated by food consumption, weight gain rate, sedation and ataxia was not observed after phenytoin administration in the diet (data not shown). A decrease in foetal weight was observed at both doses (compared to controls, $p<0.05$) and a decrease in the number of implantation sites in the highest dose group ($p<0.05$). No increase in the incidence of clefts was found.[77]

Intraperitoneal injection

Rats

Soysal (2011) treated female Wistar albino rats (n=7) on GD8-10 with 25 mg/kg phenytoin diluted with serum physiologic intraperitoneally. A control group (n=6) was included. On GD20, foetuses were isolated by Caesarean section. Maternal toxicity was not reported upon. Forty

foetuses of the control group and 42 foetuses of the phenytoin-treated group were examined for bone and cartilage defects. Mean length (2.75± 0.29 cm) and weight (3.04±0.42 g) of foetuses from drug-treated animals were different from the control group (length 3.21±0.27 cm and weight 3.51±0.35 g; p<0.001). Ossification of the skull bones in drug-treated foetuses was deteriorated (increased costal separation in 10/42 foetuses of drug-treated mothers) and shape malformations in the ribs were observed.[78]

Shapiro et al. (1987) treated female Sprague-Dawley rats with 10, 50 or 100 mg phenytoin sodium salt/kg body weight/day intraperitoneally from GD17 through day 7 post partum (PP). Vehicle controls received 0.93 ml water (pH 11.6)/kg bw/day. On the basis of previous studies 100 mg/kg bw was assumed to be within the human therapeutic range. Information on general toxicity in the dams was not provided. At 100 mg/kg about half of the dams lost all their foetuses, the other half delivered two days later than the other groups and had normal litter sizes. Offspring of dams treated with 50 or 100 mg/kg had decreased body weights from birth throughout their life (compared to controls, p<0.05). Male offspring of the 10 and 50 mg/kg groups showed decreased relative testis weights at day 8 PP (p<0.002 and p<0.02, respectively), but not at days 28 or 120 PP (no data for the 100 mg/kg group). At day 120 PP, relative seminal weight was decreased in the 50 and 100 mg/kg groups (p<0.02 and p<0.001, respectively). Serum levels of the hormones testosterone and dihydrotestosterone in male offspring were not affected at days 8, 28 or 120 PP. Levels of the hormone androstenedione in male offspring exposed *in utero* to 50 mg/kg were increased at day 8 PP (p<0.02), and decreased at day 28 PP (p<0.02). Estrous cyclicity (vaginal smears from about 13-week old offspring) and weights of the uterus and ovaries (at about 16 weeks PP) were examined in female offspring. These endpoints were not affected by prenatal exposure to phenytoin.[79]

Mice

Hansen and Hodes (1983) administered phenytoin to groups of 25 pregnant ICR mice at doses of 0, 50, 75 or 100 mg/kg bw/d by intraperitoneal injection on GD10-12. The highest dose was lethal to the dams and omitted from the study. Maternal toxicity (measures not specified) was not observed at doses of 50 and 75 mg/kg bw/d. Mean foetal weight and crown rump length showed a dose-related decrease in both test groups (compared to controls, p<0.01 or p<0.05), while transumbilical distance was decreased in the 75 mg/kg bw/d group only (p<0.01). The incidence of malformed foetuses was increased at 75 mg/kg bw/d (p<0.05), with orofacial anomalies most frequently observed (p<0.01). Other malformations included ectopic kidneys, cryptorchydism and cardiac defects, but no data were presented. Skeletal defects were not found in any group. A dose of 75 mg/kg bw/d was also administered to groups of 19 or 20 pregnant A/J, C57BL/6J, B6AF$_1$, AB6F$_1$, (B6A)F$_2$ and C3H/He mice on GD10-12. Fifteen or 20 pregnant animals of the same

strain received vehicle and served as controls. A higher number of resorptions was observed in two strains, a higher number of malformed foetuses in three strains, a higher number of orofacial anomalies in two strains and a lower foetal weight in three strains treated with phenytoin as compared to control animals ($p<0.01$ or $p<0.05$).[80]

Hansen and Billings (1986) treated pregnant A/J mice with phenytoin by intraperitoneal injection on GD10 (0, 60 or 75 mg/kg) to investigate the effects of acute exposure to phenytoin. The plasma phenytoin levels of the phenytoin-treated dams were determined on GD12. These levels were 5.8±1.2 µg/ml of plasma at 60 mg/kg body weight and 17.0±1.0 µg/ml at 75 mg/kg. On day 18 or 19 of gestation, mice were sacrificed and foetuses were examined. Phenytoin administered by intraperitoneal injection resulted in sedation and ataxia for approximately 24 hours in the high-dose dams. A dose-related increase in resorptions was observed (compared to controls, $p<0.05$) and nearly all of the surviving foetuses had a morphological abnormality, mainly cleft lip and palate ($p<0.05$). No effect was observed on foetal weight.[77]

Intravenous injection

Rats

Rowland et al. (1990) administered phenytoin (Dilantin, phenytoin sodium injection) to pregnant Sprague-Dawley rats (n= 4-9 per group) by intravenous injection on GD8 to GD17. Doses of 0, 25, 50, 75, and 100 mg/kg bw/day were administered. Reproductive outcomes were determined at GD20. After the intravenous dosing, a dose-dependent increase in maternal toxicity was observed, which was characterized by mild to severe imbalance and ataxia in the 50 and 75 mg/kg groups. Due to excessive ataxia in the 100 mg/kg group, this dose was discontinued. No decreases in maternal weight gain were evident after intravenous dosing. Decreases in foetal weights and crown-rump lengths and increases in foetal malformations (mainly craniofacial malformations, i.e. thin, tapered snouts and arched palates) occurred at 75 mg/kg phenytoin (compared to controls, $p<0.05$).
Pharmacokinetic sampling on GD8-9 and GD16-17 revealed an increase in plasma phenytoin exposure between the beginning and the end of the treatment period.[69]

Functional and cognitive effects

Gavage

Rats

Mowery et al. (2008) dosed female Sprague-Dawley rats twice daily with 0 or 50 mg phenytoin/kg bw/d by gavage from ten days before mating, throughout pregnancy and the three week preweaning period. Maternal plasma levels on the last day of dosing approximated the low level of

human therapeutic concentrations (10-20 µg/ml). Information on maternal toxicity was not provided. Behavioural testing was conducted with 80-90 days old female offspring (test group: n=31 from 11 litters; controls: n=22 from 10 litters). Female rats developmentally exposed to phenytoin showed increased performance in simple associative learning tasks (appetitive-to-aversive transfer paradigm; compared to controls, $p<0.05$), but displayed impaired performance in a higher-order learning and memory task (avoidance conditioning, $p<0.05$).[81]

Schilling et al. (1999) dosed pregnant Sprague-Dawley rats (10 controls, 15 treated dams) with 0 or 200 mg phenytoin/kg bw/d by gavage during GD7-18. They tested the effects on learning and memory, as well as on circling behaviour in water. Maternal body weight was decreased in the treatment group during GD14-18 (compared to controls, $p<0.05$). Offspring mortality was increased in the treatment group during the first week($p<0.01$). Per litter, two male offspring were tested, one exhibiting circling in a straight water channel and one in a circling tank. Circling in a water channel occurred in 53% of the exposed offspring. Circling was, however, never seen in the control group. Litter characteristics showed no differences between the groups. Preweaning body weights were not affected. Postweaning body weights were decreased in the treatment group ($p<0.05$ or $p<0.01$).
Noncircling phenytoin-exposed offspring demonstrated impaired reference memory-based spatial learning in the Morris water maze ($p<0.05$). Circling offspring demonstrated impaired reference memory based spatial learning, cued (visible) platform learning, spatial discrimination and working memory-based learning (all $p<0.01$).[82]

McCartney et al. (1999) dosed pregnant Sprague-Dawley rats (≥20 dams per group) with 0, 50, 100 or 150 mg phenytoin sodium salt/kg bw/d by gavage during GD7-18. Examinations were conducted in all pups (preweaning) or in 1 pup/sex/litter (postweaning). Various developmental and behavioural indices were evaluated. Maternal weight gain showed a dose related reduction in all treatment groups (compared to controls, $p<0.05$), and other signs of maternal toxicity, i.e. increased incidences of chromodacryorrhea, lacrimation and circling, were noted in the 100 and 150 mg/kg bw groups (data not reported). Accelerations in developmental landmarks and preweaning behaviour (eye opening, incisor eruption, negative geotaxis and olfactory orientation) and delays in air righting were noted among all offspring treatment groups ($p<0.05$). Locomotor activity was increased in high-dose pups (at PND21) and adults (at PND62) ($p<0.05$). Dose-related performance deficits were noted in a water maze assay (learning/memory impairments, $p<0.01$) and in auditory startle responses ($p<0.05$ in at least one dose group). For a number of these endpoints, male and female rats showed different sensitivity.
Offspring brain weights also showed sex differences in sensitivity, that depended on age. Hind brain weights were reduced in male pups at 150

mg/kg bw/d ($p<0.05$). Forebrain and whole brain weights were reduced in adult females at 100 and 150 mg/kg bw/d ($p<0.05$).[83]

Tsutsumi et al. (1998) dosed pregnant Sprague-Dawley rats (7-10 dams per group) by gavage with 0, 50 or 100 mg phenytoin/kg bw/d by gavage during GD7-18, and subjected male offspring (generally 10-15 males/ group) to a reflex test and several learning/memory tests. No information on maternal toxicity was provided. The completion of the negative geotaxis (reflex) test was delayed in both treatment groups (compared to controls, $p<0.001$). No effects of phenytoin were detected in a figure-eight-maze, a Biel water maze, or a Morris maze test. In a radial maze test, the total number of choices was higher ($p<0.05$), whereas the number of correct choices was lower in the high-dose group ($p<0.05$). The percentage of correct choices in the high-dose group was also lower in a delayed nonmatching-to-sample test (T-shaped maze, $p<0.05$). Offspring whole brain weights were decreased in the high dose group in week 6 ($p<0.05$), whereas their body weights were unaffected. The brain weights had returned to normal at 16 weeks of age.[84]

Vorhees et al. (1995) dosed pregnant Sprague-Dawley rats (5-6 dams per group) with 0 or 100 mg phenytoin sodium salt/kg bw/d in corn oil by gavage during GD7-18. Maternal weight gain, gestation length, pups per litter and sex ratio within litters were not affected. Phenytoin offspring (number/group not indicated) were subdivided according to circling behaviour and tested at approximately 50 days of age. Phenytoin offspring committed more errors and had longer latencies to find the goal than controls in the Cincinnati (water) Maze (compared to controls; circling animals: $p<0.01$; noncircling animals: $p<0.05$). The effects in circling animals were larger than those in noncircling animals.[85]

Pizzi et al. (1992) dosed pregnant Sprague-Dawley rats (10-11 dams per group) with 0, 100 or 200 mg phenytoin sodium salt/kg bw/d by gavage during GD9-18.
Phenytoin administration resulted in a dose-related decrease in maternal weight gain (compared to controls, both doses $p<0.001$). Maternal plasma levels in the low- and high-dose groups were 11.5 and 26.2 mg[a] phenytoin/ml. Offspring had lower birth weights and body weights at PND30 (high dose group males and females: $p<0.001$; low dose group males $p<0.05$). This was accompanied with increased mortality (41% and 61% in the low- and high-dose group, respectively). Phenytoin-exposed offspring frequently showed chromodacryorrhea. Pups exposed *in utero* to 100 mg/kg bw/d showed an increase in pivoting locomotor activity on PND7 and 9 ($p<0.005$). Due to mortality, the 200 mg/kg bw/d group was not examined for this activity. Phenytoin-exposed offspring developed an abnormal spontaneous circling behaviour (12 and 33% in the low- and high-dose group, respectively, compared to 0% of the controls). As adults,

[a] The Committee assumes that µg rather than mg was meant.

the animals exposed to 200 mg/kg bw/d showed increases in locomotor activity measures (at PND45: $p<0.05$; at PND66: $p<0.05$).[86]

Weisenburger et al. (1990) dosed pregnant Sprague-Dawley rats (10-13 litters/group remained) by gavage with 0, 100 or 200 mg phenytoin/kg bw/d by gavage during GD7-18. Where possible, six pups/sex/litter were retained for testing. Maternal serum concentrations on GD18 were 15.1 and 20.9 µg/ml for low- and high-dose, respectively. Maternal weight gain was reduced in both treatment groups (compared to controls, $p<0.05$). Pup mortality was increased at 200 mg/kg bw/d at birth ($p<0.01$), PND7 ($p<0.01$) and PND28 ($p<0.05$). Preweaning and postweaning offspring body weights were reduced at 200 mg/kg bw/d ($p<0.05$). Exposed offspring showed dose-related increases in circling behaviour (both doses: $p<0.01$), preweaning locomotor activity (both doses: $p<0.05$), errors in a complex water maze ($p<0.01$) and impaired performance in a radial-arm maze (both circlers and noncirclers at each dose $p<0.01$).[87]

Vorhees (1987) dosed pregnant Sprague-Dawley rats (13-20 litters/group) by gavage with 0, 100, 150 or 200 mg phenytoin/kg bw/d by gavage during GD7-18. These doses resulted in maternal plasma levels on GD18 (4h after dosing) of about 10, 20 and 24 µg/ml, in the low-, mid- and high-dose group, respectively (with little decline up to 24h). The adult/embryonic plasma ratio in five individual rats of the mid- and high-dose group at 24h post-dosing on GD18 was between 1.3 and 2.4. Maternal weights were decreased in the high-dose group during gestation (compared to controls; GD18 ($p<0.01$) and GD20 ($p<0.05$)). Litter numbers were not affected by prenatal exposure to phenytoin . Offspring mortality was increased in the high-dose group during PND1-21 ($p<0.01$). Offspring of both sexes (number/group not indicated) was tested prior to weaning (for activity only) and afterwards. The highest dose of phenytoin produced increased activity (in various tests of activity, $p<0.01$), delayed dynamic righting development ($p<0.01$), impaired Biel multiple-T water maze learning ($p<0.01$), Y-maze avoidance learning ($p<0.05$), and inhibited tactile startle responses ($p<0.05$). The two lower doses of phenytoin generally showed a dose-effect relationship on most measures. Phenytoin-exposed offspring showed a dose-related increase in circling behaviour ($p<0.05$ or $p<0.01$). Offspring showed no effects on postnatal growth, total brain weight, or brain protein content as adults (at 79-84 days of age).[88]

Ruiz et al. (1987) dosed Sprague-Dawley rats with 0 or 50 mg phenytoin/kg bw/d by gavage from 7 days before fertilization, throughout pregnancy. Pups were also given the drug by gavage, but the dosing regimen was not reported. Information on maternal toxicity was not given either. Neuronal structure in the somatosensory cortex at 30 days of postnatal development was investigated in six offspring/group. The cortical layers III and IV in phenytoin-treated rats and controls showed no major differences. However, the total cell density in layer V was higher in treated

animals (compared to controls, p<0.0001). Numbers and lengths of apical and basilar dendrites were decreased (p<0.05 to p<0.0001). The degree of neuronal maturation at the level of collateral apical dendrites of pyramidal cells was diminished in the surface layers of the cortex, while poor development of the basilar dendrites was also seen in the deeper layers of the sensory cortex of the treated animals. The pattern of phosphorylation of cytoskeletal proteins was changed in the treated animals.[89]

Elmazar and Sullivan (1981) dosed pregnant Wistar rats (10 (first study)-20 (repeat study) litters/group) with 0 or 100 mg phenytoin/kg bw/d by gastric intubation during GD7-19. Previously, 100 mg/kg had been shown to produce a steady-state situation by GD19, with a blood level of 6.7±1.8 µg/ml, 24 hours after dosing, being within the human therapeutic range. The maternal body weight decreased up to day 10 and then increased to about 300 g at day 19. Due to paired feeding, the body weights of the control dams followed the same pattern. After parturition, the offspring was culled to six-eight/litter and reared by fostering or cross fostering. Survival and body weights of the pups from treated mothers were lower than in control pups in two experiments at two days of age (p<0.05), in one of the experiments at 21 days of age (p<0.01) and at 90 days of age (p<0.05), as compared to controls. Both of these effects could be reduced by cross fostering. A number of neurological effects were seen in the phenytoin group; there was a delay of up to fifteen days in the development of the dynamic righting reflex (no statistics reported), a decreased ability of offspring to stay on a rotating rod (p<0.05), and a decrease ability to walk along elevated parallel rods (p<0.01). There were no changes in development of physical landmarks, or in the development of crawling and walking activities at 9-21 days of age, and no changes were seen in a head-dipping test or in a conditioned avoidance test (shuttle box) at 26-34 days of age. There was a decrease in absolute brain weight of the treated group at age three days (p<0.05 or <0.001), which in males remained lower than the controls even at 90 days (p<0.001). There was no difference in cerebellar DNA content. [90]

Vorhees (1983) administered phenytoin or vehicle to pregnant Sprague-Dawley rats by gavage and investigated the effects of different dose regimens in three experiments. Structural effects and postnatal functioning were investigated on GD20 or postnatally. The first experiment served to determine the highest dose for assessing postnatal functional teratogenesis in experiments 2 and 3. In this dose range-finding study with five or six rats per group 200 mg/kg bw/day was chosen as the highest dose to be used in the other experiments. This choice was based on maternal toxicity (reduced maternal weight; compared to controls, p<0.01) and the fraction of resorbed or dead foetuses (not statistically different from controls).
In the second experiment, doses of 0, 5, 50 or 200 mg/kg bw/day were administered on GD7-18 (group size not reported). Maternal weight was

decreased at 200 mg/kg bw/day on GD14 and GD18 ($p<0.05$), and during lactation ($p<0.01$). Length of gestation, external malformations, number of offspring delivered and sex ratio within litters were not affected. Offspring mortality was increased at 50 and 200 mg/kg bw/day on PND0 (both $p<0.0001$) and at 200 mg/kg bw/day on PND 21 ($p<0.001$). The mortality had returned to normal on PND22-70. Before weaning (PND1-21), no reduction in offspring body weight occurred in any dose group compared to controls (data not shown). After weaning (PND22-70), offspring body weight was reduced: the 200 mg/kg group was 10.8% lighter than the controls on PND42 ($p<0.001$) and 7.2% lighter on PND70 ($p<0.05$). No treatment effects were found on lower incisor eruption, eye opening or vaginal patency development. In the preweaning period no treatment effects were found on negative geotaxis, olfactory orientation, figure-8 activity or neonatal T-maze behaviour. Treatment effects were found in tests of righting (data not shown), pivoting and startle (200 mg/kg; compared to controls, both $p<0.001$), as well as swimming (200 mg/kg, at various ages, $p<0.05$). No treatment effects were observed on postweaning M-maze behaviour, passive avoidance or spontaneous alternation. Phenytoin affected postweaning figure-8 activity and Biel water maze learning (200 mg/kg bw/day, $p<0.05$ to $p<0001$).

In the third experiment, doses of 0 or 200 mg/kg bw/ day were given on GD7-10, 11-14 or 15-18 (group size not reported). Maternal weight was not affected. Length of gestation, external malformations, number of offspring delivered and sex ratio within litters were not affected either. Offspring mortality was increased during the preweaning period after treatment at GD7-10 ($p<0.001$) and GD15-18 ($p<0.01$). It was also increased postweaning after treatment at GD11-14 ($p<0.01$). No treatment effects were found on incisor eruption, eye opening or vaginal patency development. In the preweaning period no treatment effects were found on righting, negative geotaxis, olfactory orientation, figure-8 activity or neonatal T-maze behaviour. Phenytoin increased preweaning pivoting locomotion and delayed swimming development in the groups exposed at GD11-14 or GD15-18 ($p<0.05$ or <001), but it did not affect startle. No treatment effects were observed on postweaning M-maze behaviour or spontaneous alternation. Phenytoin also increased postweaning figure 8 ambulation ($p<0.01$) and water maze errors ($p<0.001$) and impaired passive avoidance retention in the GD11-14 exposure group ($p<0.05$).[91]

Mice, newborn

The neonatal period in mice corresponds to the period of development of the central nervous system in the third trimester of human pregnancy. Therefore, the following experiments with neonatal mice are relevant for assessing the effects of phenytoin on the developing central nervous system.

Ogura et al. (2002) dosed newborn C57BL/6 mice (13-15 males/ group) with 0 or 35 mg phenytoin/kg bw/d by gavage during PND5-14. Plasma level of phenytoin was 20 ± 2.8 µg/ml, 3h after the last dose. Brain

concentration of phenytoin was 1.6 times higher (31.9±10.3 µg/ml). Some treated pups showed acute behavioural detoriation, including anorexia, hyperactivity and motor coordination deficits. Mortality (38%) and weight loss was noted in the treated pups, but at 56 days of age the body weights of treated mice had returned to control values. Brainstem weight, cerebral weight, cerebellar weight and total brain weight were reduced in treated 56 days old mice ($p<0.01$). Histopathological examination showed that phenytoin treatment interferes with the development of granule cells in the hippocampus and the cerebellum, and with the dendritic development of Purkinje cells. Treated mice were impaired in the acquisition of a hidden platform task in a water maze test (compared to controls, $p<0.001$), and committed more errors during the learning process in a radial arm maze than controls ($p<0.01$). [92]

Ohmori et al. (1999) administered phenytoin (0 or 35 mg/kg bw/d) to newborn Jcl:ICR mice (13-16/sex/group) by gavage during PND2-4. Plasma level of phenytoin on the third day of administration was 17.7 µg/ml; in the brain, the level was higher (31.4 µg/g). Mortality was >30% in males and females of the treatment group versus none in controls. Weight loss, anorexia, motor hypoactivity and incoordination was noted in treated pups at PND4 (data not shown). Their body weight recovered to normal at PND56. Motor performance of the treated mice in a rotating rod was impaired (compared to controls, $p<0.05$). Their spontaneous locomotor activity, detected by movements in a box, was impaired as well ($p<0.01$). Total brain weight, cerebral weight and cerebellar weight were reduced in treated mice ($p<0.01$). Phenytoin induced neurotoxic damage in the developing cerebellum; it induced cell death of external granule cells and inhibited migration of granule cells in the cerebella, and affected Purkinje cell differentiation. [93]

Hatta et al.(1999) administered phenytoin to newborn Jcl:ICR mice (number per group not indicated) at levels of 0, 10, 17.5, 25 or 35 mg/kg bw/d by gavage on PND2-4. Mortality rate in the high-dose group was 8% in either sex. Pup body weights (on PND5-PND21) did not differ from those in controls. Mice treated with 25 or 35 mg/kg bw/d showed decreased locomotor abilities and righting reflex on PND5 (compared to controls, $p<0.05$ or <0.01). Total brain weight, cerebral weight and cerebellar weight were decreased in pups treated with 25 or 35 mg/kg bw/d ($p<0.05$ or <0.01).[94]

Monkeys

Phillips and Lockard (1993) dosed four adult female monkeys (Macaca fascicularis) with phenytoin via stomach catheter from one month before mating throughout gestation. The animals were dosed twice daily at levels providing plasma concentrations between 4-12 µg/ml phenytoin (the initial dose was 20 mg/kg bw). The infants were investigated for hyperexcitability. Control groups consisted of monkeys treated with stiripentol (n=5), or phenytoin plus stiripentol (n=4), because stiripentol

has been shown to reduce the incidence of phenytoin-induced congenital malformations in mice. Information on maternal toxicity was not given. After birth, infants were transferred to a nursery for testing. They were tested at an age of about two weeks to 45 days (exact times were corrected for gestational age, i.e., age based on conception date rather than birth date). Infants exposed to phenytoin showed hyperexcitability (jerking, screeching, refusing to attend to stimuli, lack of visual orientation) during cognitive testing when compared to stiripentol ($p<0.05$), not compared to phenytoin plus stiripentol.[95]

Intraperitoneal injection

Rats

Wolansky & Azcurra (2005) studied motor and learning disorders in offspring (n= 28-32/group) of female Sprague Dawley rats treated intraperitoneally with 0 or 30 mg phenytoin sodium salt/kg bw/d during GD13-18. Maternal body weights and behaviour, and gestation time were not affected by the treatment (data not shown). Pup body weight was not affected either (data not shown). Circling velocities in a circular maze and spatial error rates for direction of circling were increased in the phenytoin group at all time points investigated (PND40, PND80 and PND150; compared to controls, $p<0.05$). This study shows that gestational exposure to phenytoin results in long-term functional disorders.[96]

2.4.3 Lactation

No relevant animal studies on effects of phenytoin on or via lactation were available.

2.5 Conclusions

2.5.1 Fertility

No data are available on the effects of phenytoin on functional fertility (ability to have children) of men or women. There are only some data on changes in parameters related to fertility. The data regarding men concern the effects of phenytoin on sperm characteristics and sexual interest and function. The data regarding women are limited to effects on sexual function and arousal.

Sexual interest and function and serum levels of neuroactive hormones in men were investigated in one study.[7] They were shown to be lower in men with epilepsy treated with phenytoin.

Sperm characteristics were investigated in men with epilepsy treated with phenytoin by two research groups.[8,9] These groups demonstrated a variety of changes: a lower seminal fluid volume, a decreased sperm concentration, an increased percentage of abnormal sperm, a decreased sperm motility and a decreased duration of sperm activity. However, an effect of epilepsy cannot be excluded, since similar effects were observed in both untreated and phenytoin-treated epileptic patients. Sperm characteristics were also investigated after in vitro incubation of sperm

with phenytoin.[10,11] These studies produced contradictory findings. Together, the data do not clearly indicate an adverse effect of phenytoin exposure on male fertility.

Sexual dysfunction, anxiety and arousal were examined in women with epilepsy treated with phenytoin.[12] The investigators observed decreased sexual function, increased anxiety and reduced arousal. An effect of epilepsy cannot be excluded, because phenytoin-treated epileptic women were compared with non/epileptic controls.Together, the data do not clearly indicate an adverse effect of phenytoin exposure on female fertility.

Overall, the human data are not sufficient for classifying phenytoin for effects on fertility.

In animal studies the effect of phenytoin on fertility was investigated in male rats and female mice. In studies performed in male rats, no effects were seen on fertility at dose levels up to 30 mg/kg/day.[65,66] At higher dose levels of 80 mg/kg/day, close to the therapeutic range, an affected fertility rate was reported, but no effects on reproductive organs, sperm morphology and testosterone levels.[67]

At dose levels above 60 mg/kg/day pregnancy rate in mice was affected, whereas no effect was seen on mating rate and number of implantations.[68] This could have been a nonspecific effect of these dose levels, because maternal mortality was increased.

Together, the animal evidence is insufficient for classifying phenytoin for effects on fertility.

In conclusion, the human data do not clearly indicate that male or female fertility is affected by phenytoin. The same holds true for the animal data. Taken together, the human and animal studies do not provide sufficient evidence for classification. Therefore, the Committee recommends not classifying phenytoin for effects on fertility due to a lack of appropriate human data and data in experimental animals.

2.5.2 Development

Developmental effects in humans

The Committee has assessed the strength of the epidemiological evidence. It is clear that a large number of epidemiological studies has been performed to examine the developmental effects of phenytoin. Many of these studies have flaws, however. Only a subset concerns phenytoin monotherapy, and is relatively well-designed and well-reported upon. In these studies, various effects belonging to the foetal hydantoin syndrome were found after prenatal exposure to phenytoin. These effects comprise major and minor structural defects, as well as functional and cognitive ones.

Structural defects belonging to the foetal hydantoin syndrome were observed in a surveillance system study by Arpino et al. (2000)[16], in prospective studies by D'Souza et al. (1990)[32], Koch et al. (1992)[28], Holmes et al. (2001)[23] and Hernández-Diaz et al. (2012)[18], in retrospective studies by Orup et al. (2003)[39] and Lu et al. (2000)[41], and Dean et al.

(2002)[40], as well as in follow-up studies of pharmacovigilance centre data (Gladstone et al. (1992)[35], Scolnik et al. (1994)[36], Nulman et al. (1997)[37]). However, overlap of datasets in the last three studies cannot be ruled out.[35-37] Among the major malformations observed are spina bifida, cleft lip/palate, hypospadias, extra digits, heart disease, hip dislocation and megacolon. Minor malformations found include a high forehead, frontal bossing, dental changes and nail and finger anomalies.

Functional and cognitive effects of the foetal hydantoin syndrome were shown in three studies, by Arulmozhi et al. (2006)[45], Scolnik et al.(1994)[36] and Wide et al.(2002)[52]. These effects include delayed locomotor development and a lower IQ. There is supportive evidence from a small IQ study, demonstrating that verbal IQ is lower than non-verbal IQ in children exposed prenatally.[51]

Although the studies mentioned above are the most informative ones, they also have some weaknesses. Examples are the nature of the control groups and the size of the studies. The main or only control groups in the prospective studies of D'Souza et al. (1990)[32], Koch et al. (1992)[28], Nulman et al. (1997)[37], Holmes et al. (2001)[23] and Dean et al. (2002)[40] consisted of children born to epileptic women not taking phenytoin. In the other studies, the control groups consisted of children born to non-epileptic women not taking phenytoin. Therefore, an effect of epilepsy in those studies cannot be excluded. The studies of D'Souza et al. (1990)[32], Koch et al. (1992)[28] and Nulman et al. (1997)[37], on the other hand, are small. Statistical analysis of the data was only possible in the latter.

The results of many other studies suggest that taking phenytoin during pregnancy may lead to structural, functional and cognitive effects of the foetal hydantoin syndrome in the offspring. These studies have major shortcomings, however, including combined exposure with other anti-epileptic drugs, lacking information as to co-exposure to such drugs, absence of a control group, a small number of exposed women and incomplete reporting, of statistics for example. Due to these limitations, the findings cannot be ascribed to prenatal phenytoin exposure with certainty. Available data suggest that exposure to phenytoin can cause developmental effects like distal phalangeal and nail hypoplasia and hypertelorism. Many of the studies on minor congenital anomalies, functional and cognitive effects have an additional flaw. Genetic factors play a role in these outcomes. However, the parents often have not, or only partly been examined for the same traits as the children. This also raises doubt as to prenatal phenytoin exposure being the cause of the effects observed.

Two well-performed, large studies provided no evidence for an excess risk of developmental effects in children exposed to phenytoin *in utero*. These are the retrospective cohort studies by Forsberg et al. (2011)[54] and by Samrén et al. (1999)[42].

In addition to the many primary studies, three meta-analyses of the effects of maternal antiepileptic drug use on offspring development have been

reported: one on congenital malformations, one on congenital malformations and pregnancy outcomes, and one on neurological development.[43,44,56] The results of these meta-analyses are in line with the results of the primary studies, with the advantage of being based on larger numbers. Meta-analyses have their own drawbacks, however. An important one is that they cannot correct for the weaknesses of the original studies included.

Overall, there is a large amount of epidemiological data suggesting that phenytoin is a developmental toxicant and clinicians generally consider phenytoin a teratogen. Indeed, multiple studies show effects in terms of one or more features of the foetal hydantoin syndrome. All these studies have shortcomings however, minor or major ones. The Committee considers the quality of the human data insufficient to base the classification of phenytoin primarily on the human data and classify phenytoin as a known human developmental toxicant.

Developmental effects in animals

Many animal studies with phenytoin have been carried out, mainly involving oral or intraperitoneal administration to pregnant rats or mice. These studies show a variety of prenatal and postnatal effects on development.

The Committee considers the oral studies to be the most relevant ones for assessing the safety of occupational (or other) exposure to phenytoin during gestation for offspring development. Fritz et al. (1976)[74] reported structural defects, i.e. cleft palate and diminished limb ossification, in offspring of mice exposed orally. Hansen & Billings (1986)[77] reported decreased foetal growth. Furthermore, Vorhees et al. (1995)[85], Elmazar and Sullivan (1981)[90] and Vorhees (1983)[91] demonstrated a variety of functional effects in offspring of rats exposed orally. Among these effects are impaired motor coordination and abnormal circling behaviour in water (more errors and longer time needed to find the goal). In all of these studies, maternal toxicity was absent.

Three studies in newborn mice also provide evidence for phenytoin causing functional developmental changes. These studies, by Ogura et al. (2002)[92], Ohmori et al. (1999)[93] and Hatta et al. (1999)[94], contribute such evidence, because the murine neuronal system is still developing shortly after birth and known to be sensitive to developmental toxicity at this stage. The investigations with newborn mice extend the range of functional effects demonstrated to decreased motor coordination, hyperactivity, hypoactivity and impaired locomotor activity. These effects were accompanied by lower weights of brain, cerebrum and cerebellum.

Additional evidence comes from two studies with intraperitoneal administration of phenytoin to pregnant mice.[77,80] The effects reported in the offspring were affected growth and occurrence of structural defects, in the absence of maternal toxicity. The effects observed were decreased foetal weight and crown rump length, decreased transumbilical distance, a higher number of foetal deaths and malformed foetuses, and increased incidence of orofacial anomalies (cleft lip and palate).

The levels of phenytoin in plasma were measured in some of the studies mentioned above.[77,90,92,93] The concentrations found were in the range of human therapeutic levels (10-20 µg/mL).[97,98]
In the remaining animal studies maternal toxicity was observed, or information on its presence or absence was lacking. Therefore, the effects found in those studies, that are similar to those described above, might be due to phenytoin, but cannot be ascribed to it with certainty.
Together, the animal studies provide sufficient evidence that prenatal exposure to phenytoin can cause growth retardation, as well as morphological and behavioural abnormalities. Some effects found are identical to those observed in humans, the main one being cleft lip and palate. Many endpoints that can be investigated in animal tests differ from those examined in humans. Taking this fact into account, the structural and functional developmental effects in animals are similar to those in humans.

Overall conclusion on development

The major effects of prenatal exposure to phenytoin in animals are similar to those in humans, i.e. growth retardation and abnormalities in structural and functional development. Additionally, the serum levels producing these effects in animals were within the commonly accepted therapeutic range for humans. Overall, the Committee is of the opinion that the available human and animal data are sufficient to recommend classification of phenytoin in category 1B (presumed human reproductive toxicant) and to label phenytoin with H360D (may damage the unborn child).

2.5.3 Lactation

There are only human data regarding the effects of phenytoin on or via lactation.

Effects via lactation

The analyses of effects via lactation were limited to IQ in breastfed children of women taking phenytoin. The studies did not show an adverse effect.[57,58]

Transfer via lactation and related risk of developmental toxicity

The majority of the human data concerns the transfer from maternal plasma to breast milk.[59-64] The milk to maternal plasma ratio reported ranges from six to 59 percent. The high upper value of this range is a cause for concern. All transfer data concern women with epilepsy taking phenytoin breastfeeding their infants. The breastfeeding in this clinical situation is often regarded as beneficial for the infant as it reduces withdrawal symptoms in the child. During breast feeding, the infant gradually gains weight and, as a consequence, the dose of phenytoin received (in mg/kg bw) is gradually decreasing. The high upper value of the milk to maternal plasma range suggests that breastfed infants of epileptic women taking phenytoin receive a dose postnatally that might

cause developmental toxicity. There are no data regarding the phenytoin plasma level of women only occupationally exposed to phenytoin. This level will probably be much lower than that of epileptic women taking phenytoin. Consequently, withdrawal symptoms in their children, if any, would presumably be lighter and more seldom.

One research group calculated that breast feeding leads to a daily phenytoin dose to the infant between 0.03 and 0.47 mg/kg bw.[61] Assuming a worst-case scenario, this dose range is still tenfold lower than the therapeutic dose range for children (5-10 mg/kg bw).

As stated above, information on the phenytoin plasma level of women exposed to phenytoin occupationally only, is not available. Therefore, it is not possible to assess the risk of developmental toxicity these women mediate through breastfeeding.

Overall conclusion on lactation

Overall, the Committee is of the opinion that, for the effects on or via lactation, phenytoin should not be labelled due to a lack of appropriate data.

2.5.4 Proposed classification and labelling

Proposed classification for fertility

For fertility, the Committee recommends not classifying phenytoin due to a lack of appropriate data.

Proposed classification for developmental toxicity

For developmental toxicity, the Committee recommends to classify phenytoin in category 1B (presumed human reproductive toxicant) and to label it H360D (may damage the unborn child).

Proposed labelling for effects on or via lactation

For effects on or via lactation, the Committee recommends not labelling phenytoin due to a lack of appropriate data.

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annexes

A animal fertility and developmental toxicity studies

Table 1. In vivo fertility studies

Reference	Species	Experimental period/design	Dose and route	General toxicity	Effects on reproductive organs/ effects on reproduction
Male fertility					
Shetty, 2007	Wistar rat (5/group)	Epididymal sperm morphology was evaluated at 14 and 35 days after a 5-day treatment period.	0, 3.5, 5.5 or 7.0 mg phenytoin/ rat/d, i.p.	No information.	No effects on epididymal sperm morphology.
Cohn et al., 1982	Wistar rat (10 or 11/group)	Fertility rate, reproductive organ weighs and sperm content and motility were evaluated after a 3-month treatment period (first dose at 3 wk PP).	0 or 20 mg phenytoin/kg bw/d, s.c.	Growth not affected.	No effects on fertility rate, relative reproductive organ weights, epididymal sperm content or motility.
Cohn et al., 1978	Albino rat (5 or 7/group)	Fertility rate, reproductive organ weights, epididymal sperm content and motility, Leydig cell count and blood testosterone were evaluated after a 70-day treatment period (age at start not reported).	0, 5, 10 or 20 mg phenytoin/rat/d, s.c. (~ 20, 40 or 80 mg/kg bw/d).	Extensive cutaneous necrosis at injection site.	Fertility rate decreased at 20 mg/rat ($p<0.05$). Other endpoints not affected.
Female fertility					
Roberts et al., 1991	C57BL/6J and A/J mice (10-13/group)	Female mice were dosed every 48 hr from ≥1 week prior to mating until sacrifice on GD18. Fertility endpoints comprised % of females that mated, fecundity index, total number of implantations per litter and number of live implantations per litter.	0, 40, 65 or 105 mg phenytoin sodium salt/kg bw/day, p.o. (gastric intubation).	Mortality in non-pregnant C57 females (1/11 at 65 mg/kg, 5/12 at 105 mg/kg, $p<0.05$) preceded by weight loss and tremors.	Fecundity index (% of mated females that became pregnant) decreased at 105 mg/kg in C57 mice ($p<0.01$), and dose-dependently in A/J mice, although only statistically significant at the highest dose ($p<0.01$). Other fertility endpoints not affected.

Table 2. In vivo developmental studies

Reference	Species	Experimental period/design	Dose and route	General toxicity	Developmental toxicity
Structural defects					
Rowland et al., 1990	Sprague-Dawley rats (n=4-9/group).	Pregnant females were daily exposed orally at GD8-17. reproductive outcomes were determined at GD20.	p.o.: 0, 150, 375, 750, 1,125, or 1,500 mg/kg bw/day.	Maternal death, impaired motor function, decreased maternal weight gain at 1,125 and 1,500 mg/kg.	• Embryolethality at 1,125 and 1,500 mg/kg. • Intrauterine growth retardation, associated with reductions in foetal weight at 375-1,135 mg/kg ($p<0.05$), crown-rump length at 750 and 1,125 mg/kg ($p<0.05$) and appendicular and axial skeleton ossification. • Craniofacial, cardiovascular and urogenital malformations at 750 and 1,125 mg/kg ($p<0.05$).

Zengel et al., 1989	Sprague-Dawley rats (group size not reported).	Pregnant females were exposed by gavage on GD9, -11 and -13.	p.o.: 0 or 1000 mg/kg bw/day of phenytoin suspended in 1% carboxymethyl-cellulose.	• Same average litter size (11). • Maternal toxicity not described.	• Pup mortality was higher in treated females (94% compared to 1% in controls). • Offspring with symptoms of the foetal hydantoin syndrome; general growth reduction ($p<0.01$, 0.02 or 0.05, at several time points) and changes in the craniofacial pattern (small head, hypertelorism, broad upturned nose, reduced cranial dimensions; $p<0.005$, 0.01, 0.02 or 0.05).
Lorente et al., 1981	CD rats (group size not reported).	Pregnant rats (1-5 litters/group) were treated by gavage either on single or multiple days of gestation in the periods GD9-11, GD9-13, GD10-12, GD10-14, GD11-13, GD12-14 or GD13-15.	p.o.: 700, 800 or 1000 mg phenytoin/kg bw/d.	The authors aimed to produce the greatest number of anomalies with the lowest level of maternal mortality. Information on maternal mortality was not presented.	• Foetal onset growth retardation, abnormalities of the craniofacial region and axial skeleton at 1,000 mg/kg bw/d on GD9,11 and 13. • Lower foetal weights ($p<0.05$), a shortened snout and high-arched, irregular palate, and delays in skeletal maturation at 1,000 mg/kg bw/d on GD9, -11 and -13 ($p<0.05$). • A 15% incidence of retarded palatal growth at single day administrations of 700-1,000 mg/kg bw.
Kim et al., (2012)	Sprague/Dawley rats (11/group).	Pregnant females were treated with phenytoin by gavage.	p.o.: 0, 50, 150, or 300 mg/kg bw/day on GD6 through GD15.	At 300 mg/kg, various signs of maternal toxicity were observed: increased clinical changes such as ataxia and seizure, suppressed body weight and body weight gain ($p<0.01$), decreased food intake ($p<0.01$), decreased absolute weights of lung, spleen, heart, and brain and increased relative weights of adrenal glands, kidneys, brain, ovary and liver as compared to the control group ($p<0.01$ or $p<0.05$, depending on the organ). At 150 mg/kg bw/day, maternal toxicity signs similar to those at 300 mg/kg were present ($p<0.01$), though less strong: suppressed body weight and body weight gain, decreased food intake, a decreased absolute weight of the heart and increased relative weights of adrenal glands and brain. No treatment-related maternal effects were observed at 50 mg/kg bw/day.	At 300 mg/kg, developmental toxicity, including decreased foetal and placental weights ($p<0.01$), an increased incidence of morphological alterations ($p<0.01$) and a delay in foetal ossification occurred ($p<0.01$ or $p<0.05$, depending on the parameter). Developmental toxicity was less severe at 150 mg/kg bw/day than at 300 mg/kg bw/day. It was restricted to a decreased placental weight ($p<0.01$), an increased incidence of visceral and skeletal alterations ($p<0.01$), a decreased absolute maternal weight of the heart and increased relative maternal weights of adrenal glands and brain. Maternal toxicity signs similar to those at 300 mg/kg were present ($p<0.01$), though less strong. No treatment-related developmental effects were observed at 50 mg/kg bw/day.

Eluma et al., 1984	CD 1 mice (2 mice/group/ time point; 6 mice per control group).	Pregnant mice were treated by gavage during GD8-10, GD11-13, GD14-16 or GD8-16.	p.o.: 0, 50, 75 and 125 mg phenytoin/kg bw/d.	No information.	• Time-dependent and dose-related decrease in foetal weights in all treatment groups (p-values not mentioned). • High incidence of cleft palate, and a lower incidence of foetal death, cleft lips, haematomas, hydroencephaly and exencephaly (p-values ranged from <0.001 to <0.0001) Lesions were not specified per time point and dose level.
Fritz et al., 1976	(Tif/MAG) mice (n=30/group).	Mated mice were treated by gavage during GD6-15. Offspring was subjected to visceral (one third) and skeletal examination (two thirds).	p.o.: 0, 15, 50, 100 or 170 mg phenytoin/kg bw/d.	• Ten high-dose dams died. • No maternal mortality in the lower dose groups. • Food intake diminished at 100 mg/kg. • No effect on mortality or food intake at 15 or 50 mg/kg.	• Early embryonic death at 100 and 170 mg/kg. • Reduced mean foetal weight at 100 and 170 mg/kg ($p<0.01$). • Dose-related increase in cleft palate at 15, 50, 100 and 170 mg/kg bw/d (0.3%, 2%, 5.2% and 9.3%, respectively, versus 0.13% in historical control data from 500 mice) (statistically significant from 50 mg/kg bw/d onwards, $p<0.01$). • Incomplete ossification of the fore-limbs at 170 mg/kg bw/d ($p<0.01$), but no malformations.
Roberts et al. 1991	C57BL/6J and A/J mice (10-13/group).	Female mice were dosed every 48 hr from ≥1 week prior to mating until sacrifice on GD18. Gestational weight gain, gravid uterus weight, foetal weight and visceral and skeletal observations were recorded.	p.o.: 0, 40, 65 or 105 mg phenytoin sodium salt/kg bw/day, p.o. (gastric intubation).	Mortality in non-pregnant C57 females (1/11 at 65 mg/kg, 5/12 at 105 mg/kg), preceded by weight loss and tremors.	• Decreased post-implantation loss in A/J mice at 65 mg/kg ($p<0.05$). • Dose-dependent decrease (≥40 mg/kg) gestational weight gain and gravid uterus weight in C57 (linear trend analysis $p<0.05$). • Decreased foetal weight in C57 (at 65 mg/kg, $p<0.05$). • Decreased ossification in both strains at 65 mg/kg. • Increased frequency of hydroencephaly (65 mg/kg) and open eyelid (40 and 65 mg/kg) in A/J, cardiac calcium deposit (40 mg/kg) in C57 mice (all $p<0.05$).
Khera, 1979	Cats (n=13-14/group).	Mated cats were treated by gavage during GD10-22, and necropsied on GD 43. Foetuses were examined for external, visceral and skeletal malformations.	p.o.: 0, 1 or 2 mg phenytoin sodium salt/kg bw/d.	Decreased maternal body weights at 2 mg/kg bw/d on GD20 and GD30 ($p<0.05$), normal body weights at 1 mg/kg bw/d.	• No effects on abortion, non-pregnancy, live or dead foetuses or mean foetal weights. • Increased number of resorptions at 2 mg/kg bw/d ($p<0.05$). • No anomalies in live foetuses, but a high incidence of malformations in cats aborting on GD31-37; (all 11 foetuses from two cats given 1 mg/kg bw/d had cleft palate and open eyelids and of the 10 foetuses from one cat given 2 mg/kg bw/d, four had umbilical hernia and one had open eyelids).

Finnell et al., 1989	SWV, LM/Bc, and C57BL/6J mice (10-15 litters/ treatment).	Virgin mice daily exposed orally via drinking water 15-days before mating and throughout gestation (until GD18).	p.o.: 0, 10, 20, 40, or 60 mg phenytoin/kg bw.	Not reported.	• Foetal weight was dose-dependently decreased in all strains ($p<0.05$). • The incidence of offspring with one or more congenital abnormalities was increased dose-dependently in all mice strains ($p<0.05$). • Skeletal defects included mainly a pattern of multiple malformations and ossification delays in supraoccipital bones, sternebrae, distal phalanges and midfacial bones. • Soft tissue defects included mainly dilated or immaturely developed cerebral ventricles and renal defects, digital, cardiac and ocular anomalies. • Correlation between risk of abnormalities and plasma phenytoin ($p<0.05$).
Hansen and Billings, 1986	A/J mice.	Pregnant females were exposed orally via the feed daily, prior to and during gestation.	p.o.: 0, 60 or 75 mg/kg phenytoin.	No maternal toxicity, indicated by food consumption, weight gain rate, sedation and ataxia (data not shown).	• Decreased foetal weight at both doses ($p<0.05$) • Decreased number of implantation sites in the highest dose group ($p<0.05$).
Soysal et al., 2011	Wistar albino rats (test group: n=42 foetuses from 7 litters, control group: n=40 foetuses from 6 litters).	Pregnant females were treated i.p. on GD 8, 9 and 10. Foetuses were isolated on GD 20 and examined for bone and cartilage defects.	i.p. 25 mg/kg phenytoin diluted with serum physiologic.	Not reported.	• Mean length (2.75±0.29 cm) and weight (3.04±0.42 g) of foetuses from drug-treated animals were different from the control group (length 3.21±0.27 cm and weight 3.51±0.35 g; $p<0.001$). • Increased costal separation in 10/42 foetuses from drug treated animals. • Shape malformations in the ribs of foetuses from drug-treated animals.
Shapiro et al., 1987	Sprague-Dawley rat.	Dams were dosed from GD17 through day 7 PP. Weights of testis and seminal vesicle, and serum hormone levels (andro-stenedione, testosterone, dihydrotestosterone) were evaluated in male offspring of 8, 28 or 120 days of age.	0, 10, 50 or 100 mg phenytoin sodium salt/kg bw/day, i.p.	No information.	• Decreased body weights from birth throughout life at 50 or 100 mg/kg ($p<0.05$). • Decreased relative testis weights at day 8 PP in male offspring at 10 and 50 mg/kg ($p<0.002$ and $p<0.02$, respectively), but not at days 28 or 120 PP. No data for the 100 mg/kg group. • Relative seminal weight was decreased at day 120 PP at 50 and 100 mg/kg ($p<0.02$ and $p<0.001$, respectively). • Serum levels testosterone and dihydrotestosterone in male offspring were not affected at days 8, 28 or 120 PP. Levels of androstenedione at 50 mg/kg were increased at day 8 PP ($p<0.02$), and decreased at day 28 PP ($p<0.02$). • Estrous cyclicity (vaginal smears from about 13-week old offspring) and weights of the uterus and ovaries (at about 16 weeks PP) in female offspring not affected.

Hansen & Billings, 1986	A/J mice.	Pregnant females were i.p. injected with phenytoin on GD10.	i.p.: 0, 60 or 75 mg/kg phenytoin.	sedation and ataxia (high-dose group).	• A dose-related increase in resorptions (p<0.05) • Nearly all surviving foetuses had an abnormality, mainly cleft lip and palate (p<0.05).
Hansen & Hodes, 1983	ICR mice (25/group).	Pregnant mice were treated by i.p. injection on GD10-12.	i.p.: 0, 50, 75 or 100 mg phenytoin/kg bw/d.	• The high-dose was lethal to the dams and omitted from the study. • No maternal toxicity at 50 and 75 mg/kg bw/d (measures not specified).	• Dose-related decrease in mean foetal weight and crown rump length in both test groups (p<0.01 or p<0.05). • Increased transumbilical distance at 75 mg/kg bw/d (p<0.01). • Increased incidence of malformed foetuses at 75 mg/kg bw/d (p<0.05), with orofacial anomalies most frequently observed (p<0.01). • Other malformations included ectopic kidneys, cryptorchydism and cardiac defects, but no data were presented.. • No skeletal defects in any group.
	C57BL/6J, B6AF$_1$, AB6F$_1$, (B6A)F$_2$ and C3H/He mice (15, 19 or 20 animals per group).		i.p.: 0 or 75 mg/kg bw/d.		A higher number of resorptions was observed in two strains, a higher number of malformed foetuses in three strains, a higher number of orofacial anomalies in two strains and a lower foetal weight in three strains treated with phenytoin as compared to control animals (p<0.01 or p<0.05).
Rowland et al., 1990	Sprague-Dawley rats (n=4-9/group).	Pregnant females were daily exposed intravenously at GD8-17. Reproductive outcomes were determined at GD20.	i.v.: 0, 25, 50, 75, or 100 mg/kg bw/day.	No decreased weight gain, mild to severe imbalance and ataxia at 50and75 mg/kg, excessive ataxia at 100mg/kg (100 mg/kg therefore discontinued).	• Embryolethality • Intrauterine growth retardation, associated with reductions in foetal weight and crown-rump length (p<0.05). • Craniofacial malformations (p<0.05).
Functional and cognitive defects					
Mowery et al., 2008	Sprague Dawley rats (test group: n=31 from 11 litters; controls: n=22 from 10 litters).	Female rats were dosed by gavage twice daily from 10 days before mating, throughout pregnancy and the three week pre-weaning period. Behavioural testing was conducted with 80-90 days old female offspring.	p.o.: 0 or 50 mg phenytoin/kg bw/d. Maternal plasma levels on the last day of dosing approximated the low level of human therapeutic concentrations (10-20 µg/ml).	• No information on maternal toxicity.	• Increased performance in simple associative learning tasks (appetitive-to-aversive transfer paradigm, p<0.05). • Impaired performance in a higher-order learning and memory task (avoidance conditioning, p<0.05).

Schilling et al., 1999	Sprague Dawley rats (10 (controls) or 15 (treated dams)).	Pregnant Sprague Dawley rats were dosed by gavage during GD7-18. Per litter, 2 male offspring were tested in the Morris water maze, one exhibiting circling in a straight water channel and one in a circling tank.	p.o.: 0 or 200 mg phenytoin/kg bw/d.	• Decreased maternal body weight during GD14-18 (p<0.05). • Increased offspring mortality during the first week (p<0.01). • Preweaning body weights not affected. • Decreased post weaning body weights (p<0.05, or <0.01).	• Circling in 53% of the exposed offspring; no circling in the control group. • Impaired reference memory-based spatial learning in the Morris water maze in noncircling offspring (p<0.01). • Impaired reference memory based spatial learning, impaired cued (visible) platform learning (p<0.01), impaired spatial discrimination (p<0.01) and impaired working memory-based learning (p<0.01) in circling offspring.
McCartney et al.,1999.	Sprague Dawley rats (≥20 dams/group). Examinations were conducted in all pups (pre-weaning) or in 1 pup/ sex/ litter (post-weaning).	Pregnant Sprague Dawley rats (≥20 dams/group) were dosed by gavage during GD 7-18.	p.o.: 0, 50, 100 or 150 mg phenytoin sodium salt/kg bw/d.	• Dose-related reduction in maternal weight gain in all treatment groups (p<0.05). • Increased incidences of chromodacryorrhea, lacrimation and circling at 100 and 150 mg/kg bw/d (data not reported).	• Accelerations in developmental landmarks and preweaning behaviour (eye opening, incisor eruption, negative geotaxis and olfactory orientation) in pups of all treatment groups (p<0.05). • Delays in air righting in pups of all offspring treatment groups (p<0.05). • Increased locomotor activity in high-dose pups (at PND21) and adults (at PND62) (p<0.05). • Dose-related performance deficits in a water maze assay (learning/ memory impairments, p<0.01) and in auditory startle responses (p<0.05 in at least one dose group). • Reduced hind brain weights in male pups at 150 mg/kg bw/d (p<0.05). • Reduced forebrain and whole brain weights in adult females at 100 and 150 mg/kg bw/d (p<0.05).
Tsutsumi et al., 1998	Sprague Dawley rats (7-10 dams/group) Examinations were conducted in (generally) 10-15 offspring males/group.	Pregnant rats were dosed by gavage during GD 7-18, and male offspring was subjected to a reflex test and several learning/memory tests.	p.o.: 0, 50 or 100 mg phenytoin/kg bw/d.	• No information on maternal toxicity.	• Delayed time completion of a negative geotaxis (reflex) test in both treatment groups (p<0.001). • No effects in a figure-eight-maze, a Biel water maze, or a Morris maze test. • High total number of choices (p<0.05).and low number of correct choices (p<0.05).in the high-dose group in radial maze test. • Low number of correct choices in the high-dose group in delayed nonmatching-to-sample test (T-shaped maze, p<0.05.). • Decreased offspring whole brain weights in high dose group in wk 6 (p<0.05); body weigths unaffected. Offspring brain weight had returned to normal at 16 weeks.

Vorhees et al., 1995	Sprague Dawley rats (5-6 dams/group) (Offspring number tested was not indicated).	Pregnant rats were dosed by gavage during GD7-18. Offspring was tested at approximately 50 days of age.	p.o.: 0 or 100 mg phenytoin sodium salt/kg bw/d in corn oil.	• Maternal weight gain, gestation length, pups per litter and sex ratio within litters were not affected.	• More errors and longer latencies to find the goal in the Cincinnati (water) Maze (circling animals p<0.01), noncircling animals p<0.05). • The effect in circling animals was larger than in noncircling animals.
Pizzi et al., 1992	Sprague Dawley rats (10-11 dams/group).	Pregnant rats were dosed by gavage during GD9-18.	p.o.: 0, 100 or 200 mg phenytoin sodium salt/kg bw/d. (On GD18, maternal plasma levels in the low- and high-dose group were 11.5 and 26.2 mg[1] phenytoin/ml). [1] The Committee assumes that µg rather than mg was meant.	• Dose-related decrease in maternal weight gain (both doses p<0.001). • Lower birth weights and body weights at PND30 (high dose group males and females p<0.001; low dose group males p<0.05)., and increased offspring mortality (41% and 61% in the lowand high-dose group, respectively). • Exposed offspring frequently showed chromodacryorrhea.	• Increased pivoting locomotor activity on PND7 and 9 at 100 mg/kg bw/d (due to mortality, the 200 mg/kg bw/d group was not examined). • Abnormal spontaneous circling behaviour (2 and 33% in the low- and high-dose group, respectively). • Increases in locomotor activity measures in adult rats that had been exposed to 200 mg/kg bw/d (at PND45: p<0.05; at PND66: p<0.05).
Weisenburger et al., 1990	Sprague Dawley rats (10-13 litters/group). Where possible, six offspring rats/sex/litter were retained for testing.	Pregnant rats were dosed by gavage during GD7-18.	p.o.: 0, 100 or 200 mg phenytoin/kg bw/d. Maternal serum concentrations on GD18 were 15.1 and 20.9 µg/ml for low- and high-dose, respectively which was stated to be in the range of human therapeutic ranges (10-25 µg/ml).	• Reduced maternal weight gain in both treatment groups (p<0.05). • Increased pup mortality at 200 mg/kg bw/d at birth (p<0.01), PND7 (p<0.01).and PND28 (p<0.05). • Reduced preweaning and postweaning offspring body weights at 200 mg/kg bw/d (p<0.05).	• Dose-related increases in circling behaviour (both doses: p<0.01), preweaning locomotor activity (both doses: p<0.05), errors in a complex water maze and impaired performance in a radial-arm maze (both circlers and noncirclers at each dose: p<0.01).
Vorhees, 1987	Sprague Dawley rats (13-20 litters/group) (Offspring number tested per group was not indicated).	Pregnant rats (13-20 litters/group) were dosed by gavage during GD7-18. Offspring of both sexes was tested prior to weaning (for activity only) and afterwards.	p.o.: 0, 100, 150 or 200 mg phenytoin/kg bw/d • These levels resulted in maternal plasma levels on GD 18 (4h after dosing) of about 10, 20 and 24 µg/ml, in the low-, mid- and high-dose group, respectively (with little decline up to 24h).	• Decreased maternal weights in the high-dose group during gestation (at GD18 p<0.01, at GD20 p<0.05). • 23% of the litters in the high-dose group were lost (versus 7% in controls, not statistically significant). • Increased offspring mortality in the high-dose group during PND1-21 (p<0.01).	• Dose-related increase in circling behaviour (p<0.05 or <0.01). • The highest dose produced: - increased activity (in various tests of activity, p<0.01), - delayed dynamic righting development (p<0.01), - impaired Biels (multiple-T water) maze learning (p<0.01), - Y-maze (avoidance) learning (p<0.05), - inhibited tactile startle responses (p<0.05). • The two lower doses showed a dose-effect relationship on most measures. • No effects on postnatal growth, total brain weight, or brain protein content in adulthood (at 79-84 days of age).

Ruiz et al., 1987	Sprague Dawley rats (6 offspring/group).	Rats were dosed by gavage, from 7 days before fertilization, throughout pregnancy, while pups were also given the drug by gavage. The dosing regimen was not reported. Neuronal structure in the somatosensory cortex at 30 days of postnatal development was investigated.	p.o.: 0 or 50 mg phenytoin/kg bw/d.	• No information on maternal toxicity.	• No major differences in cortical layers III and IV. • Total cell density in layer V higher (p<0.0001). • Numbers and lengths of apical and basal dendrites decreased (p<0.05 to p<0.0001) • Diminished degree of neuronal maturation at the level of collateral apical dendrites of pyamidal cells in the surface layers of the cortex. • Poor development of the basilar dendrites in the deeper layers of the sensory cortex. • Altered pattern of phosphorylation of cytoskeletal proteins.
Elmazar & Sullivan, 1981	Wistar rats (10 (first study) - 20 (repeat study) litters/group).	Pregnant rats were dosed by gastric intubation during GD7-19. After parturition, the offspring was culled to six-eight/litter and reared by fostering or cross fostering.	p.o.: 0 or 100 mg phenytoin/kg bw/d.	• Decreased body weights of the treated dams up to GD10. • Due to paired feeding, the body weights of the control dams followed the same pattern.	• Lower survival and decreased body weights in two experiments at PND2 (p<0.05), in one of them at PND21 (p<0.01).and PND90 (p<0.05). • Delay of up to 15 days in the development of the dynamic righting reflex (no statistics reported). • Decreased ability of offspring to stay on a rotating rod (p<0.05). • Decreased ability to walk along elevated parallel rods (p<0.01). • No changes in development of physical landmarks, or in the development of crawling and walking activities at 9-21 days of age, and no changes in a head-dipping test or in a conditioned avoidance test (shuttle box) at 26-34 days of age. • Decreased absolute brain weight at 3 d (p<0.05 or <0.001), which remained until 90 d (p<0.001). • No differences in cerebellar DNA content.
Vorhees, 1983	Sprague-Dawley rats (number not reported).	Pregnant rats received phenytoin or vehicle by gavage. Structural effects and postnatal functioning were investigated on GD20 or postnatally. The first experiment served to determine the highest dose for assessing postnatal functional teratogenesis in experiments 2 and 3. In this dose range-finding study 200 mg/kg bw/ day was chosen as the highest dose to be used in the other experiments. This choice was based on maternal toxicity (reduced maternal weight, p<0.01) and the fraction of resorbed or dead			

	foetuses (not statistically different from controls).			
		Experiment 2: 0, 5, 50 or 200 mg/kg bw/day p.o. on GD7-18.	• Decreased maternal body weight at 200 mg/kg bw/day on GD14 and -18 ($p<0.05$), and during lactation ($p<0.01$). • Length of gestation, external malformations, number of offspring delivered and sex ratio within litters not affected. • Offspring mortality increased at 50 and 200 mg/kg bw/day on PND0 (both $p<0.0001$) and at 200 mg/kg bw/day on PND 21 ($p<0.001$). Mortality returned to normal on PND22-70. • Before weaning (PND1-21), no reduction in offspring body weight in any dose group (data not shown). • After weaning (PND22-70), offspring body weight was reduced: 200 mg/kg group 10.8% lighter than controls on PND42 ($p<0.001$) and 7.2% lighter on PND70 ($p<0.05$).	• No effects on lower incisor eruption, eye opening or vaginal patency development. • No effects on preweaning negative geotaxis, olfactory orientation, figure-8 activity or neonatal T-maze behaviour. • Effects on preweaning righting (data not shown), pivoting (200 mg/kg, $p<0.001$), startle (200 mg/kg, $p<0.001$), and swimming (200 mg/kg, at various ages, $p<0.05$). • No effects on postweaning M-maze behaviour, passive avoidance or spontaneous alternation. • Postweaning figure-8 activity and Biel water maze learning affected (200 mg/kg bw/day, $p<0.05$ to $p<0001$).
		Experiment 3: 0 or 200 mg/kg bw/ day p.o. on GD7-10, 11-14 or 15-18.	• Maternal weight not affected. • Length of gestation, external malformations, number of offspring delivered and sex ratio within litters not affected. • Offspring mortality increased preweaning after treatment at GD7-10 ($p<0.001$) and GD15-18 ($p<0.01$). • Offspring mortality increased postweaning after treatment at GD11-14 ($p<0.01$).	• No effects on incisor eruption, eye opening or vaginal patency development. • No effects on preweaning righting, negative geotaxis, olfactory orientation, figure-8 activity or neonatal T-maze behaviour. • Increased preweaning pivoting locomotion and delayed swimming development in the groups exposed at GD11-14 or GD15-18 ($p<0.05$ or <001); startle not affected. • No effects on postweaning M-maze behaviour or spontaneous alternation. • Increased postweaning figure 8 ambulation ($p<0.01$) and water maze errors ($p<0.001$) and impaired passive avoidance retention in the GD11-14 exposure group ($p<0.05$).

Ogura et al., 2002	C57BL/6 mice (13-15 males/ group).	Newborn mice were dosed by gavage during PND5-14.	p.o.: 0 or 35 mg phenytoin/kg bw/d - Plasma level of phenytoin was 20±2.8 µg/ml, 3h after the last dose. • Brain concentration of phenytoin was 1.6 times higher (31.9±10.3 µg/ml).	• In some pups: acute behavioural detoriation, including anorexia, hyperactivity and motor coordination deficits. • Mortality (38%). • Weight loss (at 56 days, the body weights of treated mice had returned to control values).	• Reduced brainstem weight, cerebral weight, cerebellar weight and total brain weight in 56 days old mice ($p<0.01$). • Interference with the development of granule cells in the hippocampus and the cerebellum, and with the dendritic development of Purkinje cells. • Impaired acquisition of a hidden platform task in a water maze test ($p<0.001$). • Increased errors during learning in a radial arm maze ($p<0.01$).
Ohmori et al., 1999	Jcl:ICR mice (13-16/sex/ group).	Phenytoin was administered by gavage to newborn Jcl:ICR mice during PND2-4.	p.o.: 0 or 35 mg phenytoin/kg bw/d Plasma level of phenytoin on the third day of administration was 17.7 µg/ml. In the brain the level was higher (31.4 µg/g).	• Mortality >30% in males and females. • Weight loss, anorexia, motor hypoactivity and incoordination at PND4 (data not shown). Body weight recovered to normal at PND56.	• Impaired motor performance in a rotating rod ($p<0.05$). • Impaired spontaneous locomotor activity ($p<0.01$). • Reduced total brain weight, cerebral weight and cerebellar weight ($p<0.01$). • Neurotoxic damage in the developing cerebellum (cell death of external granule cells, inhibited migration of granule cells and impaired Purkinje cell differentiation).
Hatta et al., 1999	Jcl:ICR mice (n not indicated).	Newborn mice were dosed by gavage on PND2-4.	p.o.: 0, 10, 17.5, 25 or 35 mg phenytoin/kg bw/d. Dose levels were stated to correspond to therapeutic plasma levels in humans.	• 8% mortality rate in the high-dose group in either sex. • No effects on pup body weights (PND5-PND21).	• Decreased locomotor abilities and righting reflex at 25 or 35 mg/kg bw/d, on PND5 ($p<0.05$ or <0.01). • Decreased total brain weight, cerebral weight and cerebellar weight at 25 or 35 mg/kg bw/d ($p<0.05$ or <0.01).
Phillips and Lockard, 1993	Monkeys (Macaca fascicularis). (Four adult females were dosed; control groups consisted of monkeys treated with stiripenol (n=5), or phenytoin plus stiripenol (n=4), because stiripenol has been shown to reduce the incidence of phenytoin-induced congenital malformations in mice.)	Adult female monkeys (Macaca fascicularis) were dosed via stomach catheter from 1 month before mating throughout gestation. After birth, the infants were transferred to a nursery for testing. They were tested at an age of about 2 weeks to 45 days (exact times were corrected for gestational age, i.e., age based on conception date rather than birth date).	By stomach catheter at levels providing plasma concentrations between 4-12 µg phenytoin/ml (initial dose was 20 mg/kg bw).	• No information on maternal toxicity.	• Infants showed hyperexcitability (jerking, screeching, refusing to attend to stimuli, lack of visual orientation) during cognitive testing when compared to stiripenol ($p<0.05$), not to phenytoin plus stiripenol.

Wolansky & Azcurra, 2005	Sprague Dawley rats (n= 28-32 offspring/group).	Pregnant females were treated during GD 13-18. Motor and learning disorders of offspring were examined in a circular maze at PND40, PND80 and PND150.	i.p.: 0 or 30 mg phenytoin sodium salt/kg bw/d.	• Maternal body weights and pup body weights not affected (data not shown). • Maternal behaviour not affected (data not shown). • Gestation time not affected.	• Increased circling velocities in a circular maze at all time points ($p<0.05$). • Increased spatial error rates for direction of circling at all time points ($p<0.05$).

B abbreviations

bw body weight

CI confidence interval

d day

GD gestation day

h hours

i.m. intramuscular

i.p. intraperitoneal

i.v. intravenous

n number

NOEL no observed effect level

NOAEL no observed adverse effect level

OR odds ratio of an effect in exposed population versus non exposed population

PND postnatal day

PP post partum day

p.o. oral

RR relative risk

s.c. subcutaneous

the Committee

This advisory report has been prepared by the Subcommittee on the Classification of Substances Toxic to Reproduction, a permanent subcommittee of the Health Council of the Netherlands.
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