TOXICOLOGICAL ASSESSMENT

FOR

PHENYTOIN
(5,5-diphenylhydantoin)

CAS #57-41-0

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INTERIM DRAFT

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SYNONYMS

Phenytoin; 5,5-diphenyl-2,4-imidazolidinedione; diphenylhydantoin; Difhydan; Dihycon; Di-Hydan; Di-Lan; Dilabid; Dilantin; Ekko; Hydantol; Lehydan; Phenydan; Zentropil; $C_{15}H_{12}N_2O_2$.

RESOURCES

In the preparation of this Toxicological Assessment, the following sources and databases were consulted to identify relevant physical and chemical data, toxicological studies, and reports to support the development of a toxicity endpoint and risk-based cleanup criteria: Environmental Protection Based-Chemical Criteria Database, which is a Michigan Department of Natural Resources and Environment (DNRE) in-house database, Integrated Risk Information System (IRIS), Pubmed (Medline), Chemical Abstracts Service, Google Scholar, ChemIDPlus (U.S. National Library of Medicine), PhysProp (Syracuse Research Corporation), Hazardous Substances Data Bank (HSDB), CHEMFATE, PBT Profiler, International Agency for Research on Cancer (IARC), National Institute for Occupational Safety and Health Pocket Guide, the U.S. Environmental Protection Agency (U.S. EPA) EPI-suite, the U.S. EPA List of Lists, National Toxicology Program (NTP), The Merck Index, Goodman and Gilman's The Pharmacological Basis of Therapeutics, 8th Edition, and the United States Pharmacopeial Convention, Inc. Specific references are as indicated.

GENERAL INFORMATION

History of use: Phenytoin has been used for more than 70 years as an anticonvulsant to treat epilepsy and seizures. Dosage is individualized because of the great variation of response among patients and the relatively narrow therapeutic serum concentration range. A therapeutic serum concentration of phenytoin is 10 to 20 micrograms (μ)/milliliter, which results from approximately 4 to 8 milligrams/kilogram body weight per day (mg/kg BW-d) (14 to 164 mg/d) for infants to age 6, to 3 to 15 mg/kg BW-d (minimum adult dose is 300 mg/d) for children over age 6 to adults (Alehan *et al.*, 1999; Gilman *et al.*, 1990; Pfizer, 2009; U.S. CDC, 2000).

Carcinogenicity: Malignancies, including neuroblastoma, in children whose mothers were on phenytoin during pregnancy have been reported. IARC classification of carcinogenicity: evidence in humans is inadequate and evidence in animals is sufficient, with an overall summary evaluation of carcinogenic risk to humans classified as Group 2B: the agent is possibly carcinogenic to humans. NTP assessment of carcinogenicity: phenytoin is reasonably anticipated to be a human carcinogen.

Teratogenicity: Phenytoin is classed as a U.S. Food and Drug Administration pregnancy category risk factor D (positive evidence of human fetal risk, but the benefits from use in pregnant women may be acceptable despite the risk) (HSDB). The epileptic pregnant woman taking phenytoin, either alone or in combination with other anticonvulsants, has a two to three times greater risk of delivering a child with congenital defects. It is not known if this increased risk is due to antiepileptic drugs, the disease itself, genetic factors, or a combination of these; although, evidence from animal studies indicates that phenytoin is most likely the causative factor. A recognizable pattern of congenital malformations and developmental effects, known as the "fetal hydantoin syndrome," has been described and includes prenatal growth deficiency, microcephaly, craniofacial abnormalities (e.g., cleft lip and cleft palate), hypoplasia of the fingernails, and mental deficiency.

Nutrient interactions: Nutrient interactions for phenytoin include decreasing blood folate levels (Lewis et al., 1995) and increasing the metabolism of vitamins D and K (Pronsky, 2008), with a net effect of reducing the amount of all three nutrients in the body.

Other toxic effects: Abnormalities in children (other than those included in the "fetal hydantoin syndrome") whose mothers used phenytoin during pregnancy are heart malformations and hemorrhage in the neonate (usually within 24 hours of birth). Additional side effects frequently reported that require medical attention are: central nervous system toxicity (nystagmus, ataxia, confusion, mood or mental changes, muscle weakness, increased frequency of seizures, slurred speech or stuttering, trembling of hands, unusual excitement, nervousness, or irritability); gingival hyperplasia; lupus erythematosus; phenytoin hypersensitivity syndrome; Stevens-Johnson syndrome; and toxic epidermal necrolysis.

Additional side effects less frequently reported that need medical attention are: blood dyscrasias such as agranulocytosis, leucopenia, pancytopenia, and thrombocytopenia; cholestatic jaundice or hepatitis; choreoathetoid movements; cognitive impairment; periarteritis nodosa; Peyronie's disease; pulmonary infiltrates of fibrosis; vitamin D and/or calcium imbalance (frequent bone fractures, bone malformations, and slowed growth); and peripheral polyneuropathy.

Recently, cerebellar atrophy has been associated with long term use ($\geq$2 months) of phenytoin (De Marco et al., 2003).

CHEMICAL STRUCTURE
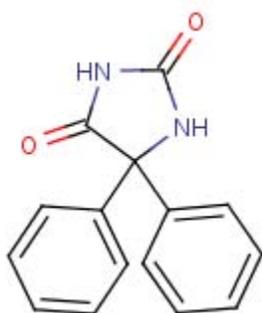
Phenytoin

PHYSICAL AND CHEMICAL PARAMETERS

Available physical and chemical parameters of phenytoin are presented in Table 1.

Table 1. Physical and Chemical Parameters of Phenytoin

Parameter	Value	Source
Molecular weight (grams/mole [mol])	252.2718	ChemID
Physical state at ambient temperature	white powder	HSDB
Melting point (degrees Celsius [°C])	286	ChemID
Boiling point (°C)	511.82	EPI-suite
Water solubility (mg/liter [L] at 22°C)	32	ChemID
Vapor pressure (millimeter mercury at 25°C)	1.2×10^{-10}	ChemID
Henry's Law constant (HLC) (atm-m^3/mol at 25°C)	1.02×10^{-11}	ChemID
log Kow (log P; octanol-water)	2.47	ChemID
Koc (organic carbon; L/kg)	1473	EPI-suite
Permeability Coefficient (Kp) (centimeter/hour)	2.63×10^{-3}	EPI-suite

EXPOSURE

For its use as a human pharmaceutical, exposure to phenytoin occurs most commonly through the oral route and secondarily by administration in parenteral solutions. Occupational exposure to phenytoin may occur through dermal contact with this compound at work places where phenytoin is produced or used. Monitoring data indicate that in some areas of the United States, the general population may be exposed to phenytoin via ingestion of contaminated drinking water (Benotti *et al.*, 1999).

TOXICOKINETICS

Phenytoin is slowly, but almost completely absorbed from the gastro-intestinal tract; the rate of absorption is variable and its bioavailability can differ markedly with different pharmaceutical formulations. Phenytoin is widely distributed throughout the body and is extensively (87 to 93 percent) bound to protein. Plasma binding is almost exclusively to albumin; in individuals with normal plasma albumin concentration and in absence of displacing agents, phenytoin is about 90 percent plasma bound.

Phenytoin is extensively metabolized in the liver to 5-(4-hydroxyphenyl)-5 phenyl-hydantoin, which is pharmacologically inactive. This para-hydroxylation of phenytoin is carried out by cytochrome P450 2C9. The para-hydroxylated phenytoin is, in turn, conjugated to its glucuronide. Phenytoin hydroxylation is capacity limited because of the saturable enzyme systems in the liver. The para-hydroxylated phenytoin can be oxidized to 3,4-dihydroxyphenyl-phenylhydantoin, the catechol metabolite of phenytoin, and further to the 3-O-methylated catechol metabolite of phenytoin. These metabolites of phenytoin are of possible toxicological interest. Phenytoin is more rapidly metabolized in children. The rate of metabolism appears to be subject to genetic polymorphism.

Phenytoin is mainly excreted in the urine as its para-hydroxylated metabolite (23 to 70 percent), either free or in conjugated form (5 percent). About 4 percent is excreted unchanged in the urine and 5 percent in the feces. Small amounts are excreted in the milk. Phenytoin undergoes entero-hepatic recycling.

Phenytoin binds to a specific site on voltage-dependent sodium channels and is thought to exert its anticonvulsant effect by suppressing the sustained repetitive firing of neurons by inhibiting sodium flux through these voltage dependent channels. Phenytoin may also inhibit potassium channels (Danielsson *et al.*, 2003).

Phenytoin stabilizes membranes, protecting the sodium pump in the brain and in the heart. It limits the development of maximal convulsive activity and reduces the spread of convulsive activity from a discharging focus without influencing the focus itself. Although phenytoin has minimal effect on the electrical excitability of the cardiac muscle, it decreases the force of contraction, depresses pacemaker action, and improves atrioventricular conduction. It also prolongs the effective refractory period relative to the action potential duration.

GENOTOXICITY ASSAYS

The results of several *in vitro* and *in vivo* genotoxicity assays are presented in Table 2. These findings are inconclusive. Due to insufficient data, it is not possible at this time to make any conclusions regarding the *in vivo* genotoxicity of phenytoin.

Table 2. Genotoxicity Studies for Phenytoin

Study	*In vitro/vivo*	Finding	Source
Salmonella	vitro	Negative	NTP
Mouse Lymphoma	vitro	Negative	NTP
Chinese Hamster Ovary (CHO) Cell Cytogenetics - Chromosome Aberrations	vitro	Negative	NTP
CHO Cell Cytogenetics - Sister Chromatid Exchange (SCE)	vitro	Positive	NTP
Micronucleus - Peripheral Blood, Bone Marrow	vivo	Negative	NTP
Drosophila	vivo	Negative	NTP
Rodent Bone Marrow Cytogenetics - Chromosome Aberrations	vivo	Negative	NTP
Rodent Bone Marrow Cytogenetics - SCE	vivo	Equivocal	NTP

TOXICITY STUDIES

Cancer

Human: No human cancer studies suitable for development of an oral cancer slope factor were located in the literature.

Nonhuman: Two animal studies reported cancer effects caused by phenytoin. The NTP study (NTP, 1993) included two-year feed experiments for F344/N rats and B6C3F1 mice. The study by Dethloff *et al.* (1996) used identical experimental conditions for Wistar rats and B6C3F1 mice. Both of these were used for the development of the oral cancer slope factor, as detailed below.

Noncancer

Human:

PHENYTOIN EXPOSURE IN UTERO: Scolnik *et al.* (1994) prospectively studied 34 mother-child pairs exposed to phenytoin monotherapy during pregnancy and compared them to mother-child pairs exposed to nonteratogens (e.g., penicillin and acetaminophen). The mean maternal phenytoin dose was 5.9 ± 1.9 standard deviation (SD) mg/kg BW-d (actual range not given). Each mother-child pair exposed to phenytoin was paired with a mother-child pair with similar age (± four years), gravidity (± 1), parity (± 1), and socioeconomic class (± 2 points on the Hollingshead and Redlich scale). All mothers treated with phenytoin had epilepsy, except for one who received the drug for postcraniotomy prophylaxis. Mothers who used phenytoin were similar to their matched controls in rate of cigarette smoking and alcohol consumption: none drank heavily or smoked more than 10 cigarettes per day. Additionally, the mothers had comparable intelligence quotient (IQ) scores (phenytoin group = 90 ± 12.2, control

group = 93.9 ± 11.4). The children exposed to phenytoin *in utero* were compared to the control group children at 18 to 36 months (toddler life stage) and found to have lower (probability value [*p*] = 0.038) global IQ and lower (*p* < 0.05) Reynell language development scores.

PHENYTOIN EXPOSURE DURING OTHER LIFE STAGES: Chung *et al.* (2002), Akaho (1996), and Meador *et al.* (1995), detected decreased/detrimental cognitive effects (alertness, attention, and memory) following one day, seven day, and one month, respectively, of phenytoin exposure. The one month exposure also detected mood effects. Phenytoin dosage was 10 mg/kg BW-d, 200 to 250 mg/d (average 3.3 mg/kg BW-d for 21.7 ± 2.32 year age [U.S. EPA, 1997]), and 200 to 600 mg/d (mean 404 mg/d and average 5.6 mg/kg BW-d for 30 year mean age [U.S. EPA, 1997]), respectively, administered to adolescents, adults, and/or mature adults.

De Marco *et al.* (2003) measured cerebellar volume using magnetic resonance imaging scans of 23 male and 33 female epilepsy patients aged 4 to 65 years (mean 33.6 years) exposed to phenytoin for two or more months. Mean daily dose of phenytoin was 301 mg (range 100 to 650 mg) (mean 4.2 mg/kg BW-d for 33.6 years [U.S. EPA, 1997]). Blood serum levels of phenytoin were available in 18 patients, with toxic levels found in 9 patients. History of alcohol consumption was positive in 12 (21.5 percent) patients. Smoking was not included as a risk factor in this study. Cerebellar volumes were transformed into *Z*-scores and volumes below minus 2 SD from the mean of the control group were considered abnormal. Abnormal (decreased volume) cerebellar atrophy in this group was noted when volumes were compared to a healthy human control group. The atrophy correlated with duration of epilepsy (*p* = 0.01) and duration of treatment with phenytoin (*p* = 0.001) but, not with age, age at seizure onset, maximum dosage used, or mean daily dosage of phenytoin.

MOST SENSITIVE EFFECT(S): Evaluation of the five human studies above revealed doses ranging from 3.3 to 10 mg/kg BW-d, which is within the 3 to 15 mg/kg BW-d range of the human therapeutic dose (Alehan *et al.*, 1999; Gilman *et al.*, 1990; Pfizer, 2009; U.S. CDC, 2000). The adverse effects reported in the human studies (lower IQ, decreased/detrimental cognitive effects, mood effects, and cerebellar atrophy) are consistent with reported phenytoin side effects (mood or mental changes and cognitive impairment). Therefore, the low end of the therapeutic dose range (3 mg/kg BW-d) (Gilman *et al.*, 1990) is identified as the lowest-observed-adverse-effect-level (LOAEL). Use of the low end of the therapeutic dose range for the LOAEL for the development of an oral Reference Dose (RfD) is consistent with the U.S. EPA toxicological evaluation of warfarin, another human pharmaceutical identified in the environment (IRIS, 1988).

Nonhuman:

PHENYTOIN EXPOSURE DURING PREGNANCY (*LOAEL range 50 to 200 mg phenytoin/kg BW-d*): Decreased maternal weight during gestation (*200*) (Schilling *et al.*, 1999) and decreased maternal weight gain (*50*) (McCartney *et al.*, 1999) were detected in rats. Consistent with these observations are studies that found increased resorptions

and decreased litter size in rabbits (*100*) (McClain and Langhoff, 1980) and decreased litter weight in rats (*150*) (Makatsori *et al.*, 2005).

PHENYTOIN EXPOSURE <u>IN</u> <u>UTERO</u> (*LOAEL range 19 to 250 mg phenytoin/kg BW-d*): Decreased fetal weight was observed in mice (*250*) (Paulson *et al.*, 1979) and decreased whole brain (*100*) (Tsutsumi *et al.*, 1998) and hindbrain weights (*150*) (McCartney *et al.*, 1999) were detected in rats. Body weight differences were detected in neonatal mice (*19*) (increased, NTP 1993), pubertal rats (*200*) (decreased, Schilling *et al.*, 1999), and sexually mature rats (*150*) (decreased, Makatsori *et al.*, 2005). Additionally, neonatal survival was decreased in rats (*150*) (Makatsori *et al.*, 2005), (*200*) (Schilling *et al.*, 1999). Consistent with the symptoms of fetal hydantoin syndrome in humans, the teratogenicity (cleft palate) of phenytoin was documented in mice (*45*) (Miller and Becker, 1975), (*125*) (Paulson *et al.*, 1979). A possible correlation with this observation is a detection of altered craniofacial gene expression in embryonic mice (*60*) (Gellineau-Vanwaes *et al.*, 1999).

Phenytoin effected changes in developmental assessments in rats and included accelerated eye opening and olfactory orientation (*50*) (McCartney *et al.*, 1999), delayed reflex function (*50*) (Tsutsumi *et al.*, 1998), and decreased startle response (*50*) (McCartney *et al.*, 1999). Memory and learning in rats was also affected by phenytoin and included decreased radial maze and decreased nonmatching-to-sample abilities (*50*) (Tsutsumi *et al.*, 1998), spatial reference memory-based learning deficit (*200*) (Schilling *et al.*,1999), and complex maze deficit (*100*) (Vorhees *et al.*, 1995). Brain concentrations of neuropeptides were affected by phenytoin in rats and found to be decreased in the mesolimbic cortex for somatostatin and increased in the hippocampus and amygdala for neuropeptide Y (*100*) (Tsutsumi *et al.*, 1998). Phenytoin also caused increased adrenaline and noradrenaline concentrations in response to stress in rats (*150*) (Makatsori *et al.*, 2005) and increased hyperexcitability in monkeys (*20*) (Phillips and Lockard, 1996).

NEONATAL EXPOSURE TO PHENYTOIN (MICE) (*LOAEL range 10 to 35 mg phenytoin/kg BW-d*): Oral exposure by gavage (not by mother's milk) to phenytoin during this life stage caused decreased total brain, brainstem, cerebral, and cerebellar weights (*17.5-35*) (Hatta *et al.*, 1999; Ogura *et al.*, 2002; Ohmori *et al.*, 1997; Ohmori *et al.*, 1999). Decreased early motor function, motor coordination, locomotor activity, and learning were also observed (*10-35*) (Hatta *et al.*, 1999; Ogura *et al.*, 2002; Ohmori *et al.*, 1999).

PHENYTOIN EXPOSURE DURING OTHER LIFE STAGES (*LOAEL range 18-150 mg phenytoin/kg BW-d*): Rats exposed to phenytoin during puberty exhibited decreased body weight gains (*18*) (NTP, 1993) and decreased learning (*45-150*) (Churchill *et al.*, 2003; Hudzik and Palmer, 1995). Adult exposure to phenytoin also caused decreased body weight gains in mice (*21*) (NTP, 1993) and decreased learning (*50*) (Banks *et al.*, 1999) in rats.

MOST SENSITIVE EFFECT(S): Evaluation of the preceding phenytoin doses and toxic effects reveals that effects due to neonatal exposure are the most sensitive (they were seen at the lowest doses). Toxic effects documented during neonatal exposure include decreases in the following: total brain/brainstem/cerebral/cerebellar weights, early motor function, motor coordination, locomotor activity, and learning. These effects may be interrelated, since motor function/coordination/activity is controlled by the brainstem, cerebrum, and cerebellum, while learning is centered in the cerebrum (Guyton and Hall, 1996).

Similar toxicological endpoints have been identified in human studies. A corresponding human study for the brain weight reductions is De Marco *et al.* (2003), who detected cerebellar atrophy (decreased volume) in males and females exposed to phenytoin. Since some neonatal events in rodents occur *in utero* in humans (U.S. EPA, 2002), a corresponding human study for the neonatal-exposure decreased-learning endpoint is Scolnik *et al.* (1994), who reported that children exposed to phenytoin *in utero* had significantly lower global IQ and language scores than a control group. Since it is not known if *in utero* or neonatal exposure is the more sensitive life stage exposure for humans, corresponding human studies for the decreased motor function/coordination/activity endpoints were not located; however, corresponding phenytoin side effects listed above are nystagmus, ataxia, trembling of hands, and choreoathetoid movements.

Of the four neonatal mouse phenytoin exposure studies Ogura *et al.* (2002) and Ohmori *et al.* (1999) used 35 mg phenytoin/kg BW-d, while Ohmori *et al.* (1997) and Hatta *et al.* (1999) used 35, 25, 17.5, and 10 mg phenytoin/kg BW-d and detected effects at the lower phenytoin doses. Ohmori *et al.* (1997) and Hatta *et al.* (1999) share the following identical characteristics: experimental design, Jcl:ICR mice; oral gavage delivery of 35, 25, 17.5, and 10 mg phenytoin/kg BW-d once a day during postnatal days 2 to 4. Ohmori *et al.* (1997) reported total brain and cerebellar weight effects only, while Hatta *et al.* (1999) included brain weight changes over time and early motor function test results. The lowest dose producing an adverse effect (delayed early motor development) was reported in the Hatta *et al.* (1999) mouse study and is 10 mg phenytoin/kg BW-d.

Noncancer Toxicity Value:

In the case of phenytoin, the human studies reported adverse effects using doses within the human therapeutic dose range of 3 to 15 mg phenytoin/kg BW-d, so the low end of the therapeutic dose range (3 mg/kg BW-d) (Gilman *et al.*, 1990) is chosen as the LOAEL. A no-observed-adverse-effect-level (NOAEL) could not be identified. The lowest dose producing an adverse effect in animals is 10 mg phenytoin/kg BW-d, which is also within the human therapeutic dose range.

The biological activity of the human therapeutic dose range of 3 to 15 mg phenytoin/kg BW-d is well established and has been associated with various adverse effects. These effects may be manageable and acceptable under medical supervision; however, in an

exposed general population these effects would be unacceptable. Therefore, the lowest therapeutic human dose of 3 mg phenytoin/kg BW-d is considered to be the LOAEL and is used for development of the oral RfD. This is consistent with development of an oral RfD for another human pharmaceutical, warfarin (IRIS, 1988).

DEVELOPMENT OF THE TOXICITY ENDPOINTS

Cancer Slope Factor

A cancer slope factor is a plausible upper-bound estimate of the probability of a response per unit dose of a hazardous substance over a lifetime. It is used to estimate an upper bound probability of an individual developing cancer as a result of a lifetime exposure to a particular level of a potential carcinogen (Part 201, Environmental Remediation, of the Natural Resources and Environmental Protection Act, 1994 PA 451, as amended [Act 451], administrative rule R 299.5701(d)). A cancer slope factor must be developed if the weight of evidence for carcinogenicity is sufficient (R 299.5738(2)), then considered for use in developing Part 201 cleanup criteria (Section 324.20120a(4)).

Two well-conducted lifetime (chronic) oral exposure cancer studies in rats and mice are available: NTP (1993) and Dethloff *et al*. (1996). The NTP study included two-year feed (oral diet) experiments for F344/N rats and B6C3F1 mice. This study found that chronic feeding of phenytoin did not increase tumor incidences in female F344 rats or male B6C3F1 mice. The male rats that were fed a diet containing 2,400 parts per million (ppm) (122 mg/kg) were found to have marginally increased incidence ($p = 0.054$) of liver neoplasms when compared to controls. There was clear evidence of carcinogenicity in female mice, with increased incidence ($p < 0.001$) of hepatocellular neoplasms. The adult female mice (F1 generation) that were fed diets containing 0, 200, or 600 ppm (0, 50, or 160 mg/kg) provide this evidence. The combined liver neoplasms (hepatocellular adenoma, hepatocellular carcinomas, and hepatoblastomas) incidences were 5, 14, and 30, respectively, for each dose. The adjusted combined liver neoplasms incidence rates were reported as 13.3, 34.8, and 66.4 percent, respectively, for each dose. The calculated combined liver neoplasms incidences were 5/38, 14/40, and 30/45 for the three dose levels (5/0.133=38, 14/0.348=40, 30/0.664=45).

The Dethloff *et al*. (1996) study is a second chronic oral exposure cancer study of phenytoin in rats (Wistar) and mice (B6C3F1). This study found that feeding of phenytoin did not increase tumor incidences in the female rats. The male rats were found to have an increased incidence ($p < 0.01$) of skin pilomatricoma at the highest phenytoin dose (100 mg/kg). No other statistically significant increases in any tumor type, including all liver tumors singly or combined, were found for the rats. Phenytoin was administered in the diet at doses of 0, 10, 25, and 45 mg/kg for mice. Increased incidences ($p < 0.01$) of hepatocellular adenomas were noted in male mice; however, the control group had many of these tumors, which reduces the effectiveness of Benchmark Dose runs for developing a cancer slope factor. The female mice had

increased incidences ($p < 0.01$) of hepatocellular ademonas (5, 4, 7, 24) and combined liver tumors that included hemangioma, hemangiosarcoma, hepatocellular adenoma, hepatocellular carcinoma, and Kupffer cell sarcoma (8, 5, 10, 25). This study did not give details in order to determine time to first tumor or how many animals were at risk. Therefore, it is necessary to assume all 50 animals/group at the start of the study were at risk.

A slope factor of 0.051 (mg/kg-d)$^{-1}$ was developed based on Benchmark Dose evaluation of the NTP study cancer data for the combined liver tumors in female mice as it provided the best model fit. Additional details are available in Appendix A.

Noncancer Reference Dose

As discussed above, the lowest therapeutic dose of 3 mg phenytoin/kg BW-d is chosen for development of the oral RfD. Since this is the lowest dose that is associated with adverse effects (neurological and developmental), it is considered to be the LOAEL.

An uncertainty factor (UF) is applied to the LOAEL to approximate a NOAEL. A value of 10 is used to account for the adverse effects that were seen at doses close to the lowest therapeutic dose: decreased IQ and language development in offspring in humans (Scolnik *et al.*, 1994), decreased cognition (Akaho, 1996), decreased cognition and mood effects (Meador *et al.*, 1995), and cerebellar atrophy (De Marco *et al.*, 2003).

A UF of 10 is applied to account for intraspecies variation. Human studies using *in utero* exposure (Scolnik *et al.*, 1994) and adult exposure (Akaho, 1996; Meador *et al.*, 1995) found detrimental cognitive effects from doses within the therapeutic range. Additionally, cerebellar atrophy was found in humans ranging from age 4 to 65 (De Marco *et al.*, 2003), also within the therapeutic range. The neonatal mice (which corresponds to the third trimester in humans) (U.S. EPA, 2002) studies (Hatta *et al.*, 1999; Ogura *et al.*, 2002; Ohmori *et al.*, 1997; and Ohmori *et al.*, 1999) found brain atrophy and corresponding decreased early motor function and learning with phenytoin intake. Since there are no human studies comparing these effects using *in utero* versus neonatal/later exposure, it is not known which life stage exposure would be more sensitive to humans. Also, from the history of use of phenytoin as a human pharmaceutical, it has been observed that there is a great variation of response among patients. Therefore, a UF of 10 is applied for intraspecies variation to account for sensitive subpopulations.

Application of a UF of 10 for LOAEL to NOAEL approximation and a UF of 10 for intraspecies variation yields a total UF of 100. Applying a total UF of 100 to the LOAEL is consistent with development of an oral RfD for two other human pharmaceuticals, warfarin (IRIS, 1988) and chloral hydrate (IRIS, 2000).

The phenytoin oral RfD is then calculated as,

$$RfD = \frac{LOAEL}{UF} \qquad (1)$$

$$RfD = \frac{3 \text{ mg/kg BW-d}}{100} \qquad (2)$$

$$RfD = 0.03 \text{ mg/kg BW-d} \qquad (3)$$

An oral RfD of 0.03 mg/kg BW-d is then used in the calculation of environmental cleanup criteria for phenytoin.

TOXICITY TO TERRESTRIAL FAUNA AND FLORA

It is expected that terrestrial fauna will respond similarly to laboratory animals following exposure to phenytoin. No information was located regarding phenytoin toxicity to plants.

ENVIRONMENTAL FATE AND TRANSPORT

Air

If released to air, an estimated vapor pressure of 1.2×10^{-10} millimeter mercury at 25°C indicates that phenytoin will exist solely in the particulate phase in the atmosphere. Particulate-phase phenytoin will be removed from the atmosphere by wet or dry deposition. Phenytoin does not absorb at wavelengths >290 nanometers and, therefore, is not expected to be susceptible to direct photolysis by sunlight.

Soil

If released to soil, phenytoin's half-life in soil is estimated at 75 days, which exceeds the U.S. EPA criteria for persistence. Therefore, phenytoin is estimated to be persistent in the environment. Volatilization from moist soil surfaces is not expected to be an important fate process based upon an estimated HLC of 1.02×10^{-11} atmosphere-cubic meter/mole. Phenytoin is expected to have moderate mobility based upon an estimated Koc (soil organic carbon partition coefficient) of 1473 L/kg.

Water

If released to water, phenytoin is expected to absorb to suspended solids and sediment based upon the estimated Koc. Volatilization from water surfaces is not expected to be an important fate process based upon this compound's estimated HLC. Hydrolysis is not expected to be an important environmental fate process since this compound lacks functional groups that hydrolyze readily under environmental conditions. Wastewater treatment appears to be less effective in removing phenytoin than several other

pharmaceuticals (Yu *et al.*, 2006); therefore, phenytoin may be resistant to biodegradation. Monitoring data indicate that in some areas of the United States the general population may be exposed to phenytoin via ingestion of contaminated drinking water (Benotti *et al.*, 2009).

ENVIRONMENTAL ANALYSIS

Groundwater and soil analysis for phenytoin is performed using the U.S. EPA Method 8270. Preliminary method detection limits have been set at 13 µ/L for water and 208 µ/kg for soil.

MICHIGAN REGULATORY STANDARDS

Phenytoin is regulated as a toxic hazardous waste under Part 111, Hazardous Waste Management, of Act 451. The DNRE, Water Bureau, Surface Water Assessment Section, has developed Rule 57 water quality values for phenytoin, and the current values can be obtained from their Web site. The DNRE, Air Quality Division, has developed Initial Risk Screening Level and Secondary Risk Screening Level values for phenytoin (as Dilantin), and the current values can be obtained from their Web site.

OTHER REGULATORY STANDARDS

Phenytoin is a U.S. EPA Hazardous Air Pollutant (as polycyclic organic matter). Phenytoin is subject to reporting under Sections 313 and 6607 of the Pollution Prevention Act, Emergency Planning and Community Right-to-know Act (Title 40 of the Code of Federal Regulations, Part 372), and Section 112(r) of the Clean Air Act (Section 313).

HAZARDOUS WASTE CLASSIFICATION

Phenytoin is regulated as toxic hazardous waste with Michigan Hazardous Waste Numbers 116U (phenytoin) and 117U (phenytoin sodium).

HAZARDOUS SUBSTANCE DESIGNATION

Based on its regulation as a toxic hazardous waste and its toxic effects detailed above, phenytoin is designated as a Part 201 hazardous substance per Part 201, Section 20101(1)(t).

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APPENDIX A
Development of the Cancer Slope Factor
March 1, 2010

If the mode of action (MOA) for a carcinogenic substance is anticipated to be mutagenic, a linear (nonthreshold) approach is appropriate for risk assessment. Other MOAs may be modeled with either linear or nonlinear (threshold) approaches (U.S. EPA, 2005a).

To assess phenytoin for a mutagenic MOA, both *in vitro* and *in vivo* genetic toxicity tests have been conducted (Table 2). The following three *in vitro* tests were negative: Salmonella, mouse lymphoma, and chromosome aberrations in CHO cells. One *in vitro* test was positive: SCE in CHO. The following three *in vivo* tests were negative: bone marrow micronucleus, Drosophila, and chromosome aberrations in bone marrow. One *in vivo* test was equivocal: SCE in bone marrow.

Some evidence suggests that phenytoin may increase tumors through a promotion rather than an initiation mechanism. Specifically, an increased number of male mice exhibited hepatocarcinogenesis when phenytoin was administered orally, in addition to an intraperitoneal (ip) administration of diethylnitrosamine (DEN - a known carcinogen with a mutagenic MOA [U.S. EPA, 2005b]), compared to (1) male mice receiving ip DEN alone, and (2) male mice receiving oral phenytoin alone (Diwan *et al.*, 1993). Inspection of the data reveals the possibility of a synergistic effect rather than an additive one when both phenytoin and DEN are administered; however, the paper did not include this type of data analysis. Phenytoin is structurally similar to phenobarbital (PB) (Diwan *et al.*, 1993), and PB exhibits a dose-response with cytochrome P450 (P450) induction, cell proliferation, and tumor promotion (Whysner *et al.*, 1996). Since phenytoin increases hepatic P450 activity (Diwan *et al.*, 1993), this similarity with PB suggests that phenytoin may also be a tumor promoter; however, a mechanism of hepatocarcinogenesis by enzyme inducing agents remains unknown (Dethloff *et al.*, 1996).

Since the eight genotoxicity tests resulted in six negatives, one positive, and one equivocal, a mutagenic MOA for phenytoin can neither be ruled out nor accepted. Elevation of hepatic P450 activity caused by phenytoin is promising for support of a threshold phenytoin MOA, but not definitive. Therefore, the MOA for phenytoin has not been established and the default linear (nonthreshold) extrapolation (U.S. EPA, 2005a) is used for the cancer evaluation.

As described earlier in this Toxicological Assessment, two chronic feed experiments in F344/N or Wistar rats and B6C3F1 mice identified combined liver tumors in female mice as the critical effect for cancer following phenytoin administration. The liver tumor data is summarized, as follows, in Table A1.

Table A1. Liver tumors in B6C3F1 female mice.

Dataset #1 (NTP, 1993)	Dose mg phenytoin/ kg BW-d	# of female mice	Incidence of combined liver tumors	Incidence percentage
	0	38	5	13.158
	50	40	14	35.000
	160	45	30	66.667
Dataset #2 (Dethloff *et al*., 1996)	Dose mg phenytoin/ kg BW-d	# of female mice	Incidence of combined liver tumors	Incidence percentage
	0	50	8	16.000
	10	50	5	10.000
	25	50	10	20.000
	45	50	25	50.000
Dataset #3 (Dethloff *et al*., 1996)	Dose mg phenytoin/ kg BW-d	# of female mice	Incidence of liver adenoma	Incidence percentage
	0	50	5	10.000
	10	50	4	8.000
	25	50	7	14.000
	45	50	24	48.000

Datasets #1, #2, and #3 were analyzed individually by the U.S. EPA BenchMark Dose Software (BMDS), Version 2.0.0.33, Multistage Cancer Version 1.7 (May 16, 2008). Also, Datasets #1 and #2 were combined, as appropriate (U.S. EPA, 2005a), for additional analysis. Combining Datasets #1 and #2, and combining them with elimination of the highest dose, as detailed in Table A2, are possible since one experiment from each study included identical characteristics: species (mouse), strain (B6C3F1), sex (female), feed (Purina Certified Rodent Chow 5002), dose initiation (age 7 to 8 weeks), dose duration (104 to 107 weeks), and endpoint (combined liver tumors). Elimination of the highest dose in the combined data set is justified because when using BMDS the highest dose group(s) may be dropped as long as there are enough data left to adequately define the low dose region (U.S. EPA, 2009).

Results are, as follows, in Table A2:

Table A2. Cancer Slope Factors from Benchmark Dose Modeling.

Dataset(s)	Endpoint selection rationale	Dose-response model chosen	Rationale for model choice	Mouse Cancer Slope Factor (mg/kg-day)-1	Human Cancer Slope Factor[a] (mg/kg-day)-1
#1 (NTP, 1993)	trend test $p<0.001$	Multistage Cancer Version 1.7 (5/16/08), effect 0.10, degree 1, extra risk	Lower AIC (142.679) $p = 0.946$ Scaled Residual = 0.056	0.007770	0.05084
#2 (Dethloff et al., 1996)	trend test $p<0.01$	Same, with degree 3	Lowest AIC (200.79) $p = 0.6195$ Scaled Residual = 0.706	0.006008	0.03931
#3 (Dethloff et al., 1996)	trend test $p<0.01$	Same, with degree 3	Lowest AIC (174.602) $p = 0.7839$ Scaled Residual = 0.463	0.005493	0.03594
Combined #1 AND #2		Same, with degree (1,2,3,4,5)	(FAILED THE CURVE FIT TESTS)		
Combined #1 AND #2 (minus highest dose)		Same, with degree 2	Lowest AIC (286.366) $p = 0.1742$ Scaled Residual = 1.485	0.007045	0.04610
[a]SF adjustment: (70 human kg/.03820 mouse kg)0.25 power = 6.543 adjustment.					

Given the above results, there are four slope factors from which to choose. Dataset #1 appears to best fit the model overall; this dataset produced the lowest Akaike Information Criterion (AIC), highest Chi squared p value, and smallest (maximum) scaled residual when compared to those from the other datasets. Therefore, the slope factor calculated from Dataset #1 (0.050842) is the best choice based on the best model fit and is used as 0.051 (mg/kg-d)$^{-1}$ for the calculation of environmental cleanup criteria for phenytoin.