Research Article

Exposure-Based Validation List for Developmental Toxicity Screening Assays

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Validation of alternative assays requires comparison of the responses to toxicants in the alternative assay with in vivo responses. Chemicals have been classified as "positive" or "negative" in vivo, despite the fact that developmental toxicity is conditional on magnitude of exposure. We developed a list of positive and negative developmental exposures, with exposure defined by toxicokinetic data, specifically maternal plasma C_{max}. We selected a series of 20 chemicals that caused developmental toxicity and for which there were appropriate toxicokinetic data. Where possible, we used the same chemical for both positive and negative exposures, the positive being the C_{max} at a dose level that produced significant teratogenicity or embryolethality, the negative being the C_{max} at a dose level not causing developmental toxicity. It was not possible to find toxicokinetic data at the no-effect level for all positive compounds, and the negative exposure list contains C_{max} values for some compounds that do not have developmental toxicity up to the highest dose level tested. This exposure-based reference list represents a fundamentally different approach to the evaluation of alternative tests and is proposed as a step toward application of alternative tests in quantitative risk assessment. *Birth Defects Res (Part B)* 101:423–428, 2014. © 2014 Wiley Periodicals, Inc.

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INTRODUCTION

There is continuing interest in the development and validation of alternative tests for developmental toxicity to decrease the time, expense, and use of vertebrate animals associated with standard developmental toxicity protocols. Proposals for the use of alternative tests have included screening large groups of compounds to establish priority for in vivo testing, incorporation into risk assessment strategies as a replacement for a second species, and use in adverse outcome pathway based strategies for chemical evaluation.

Early efforts to develop and validate alternative tests were reviewed by Webster et al. (1997) and by us (Daston et al., 2010). Although some of the validation schemes developed by early workers remain in use today, there is little consensus among scientists in the field on which, if any, of the approaches should be considered a gold standard.

A limitation of previous approaches has been the designation of compounds as unambiguously positive or negative. Such a dichotomization of the chemical world as toxic or nontoxic does not correspond to the biological reality in which a chemical is only positive or negative in the context of dose level and other features of the exposure, for example, timing or duration of exposure. To that

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Table 1
Exposures Producing Positive Effects

Compound	Concentration	Comments	References
1. Abacavir, mw 286.34	23 mg/L; 80 μM (expressed as base)	Maternal C_{max} associated with fetal malformations and death	FDA (1998a); GlaxoSmithKline (2012)
2. Acetazolamide, mw 222.2	27 mg/L; 121 μM	Maternal RBC concentration at exposure level causing fetal death and malformation	Wilson et al. (1968)
3. All-*trans*-retinoic acid, mw 300.44	59.3 μg/L; 200 nM	↑Malformations and resorptions with sc dose in rats; conceptus concentration from the same study. Note: Administration of all-*trans*-retinoic acid results in the presence of many other retinoids through metabolism	Tzimas et al. (1997)
4. Artesunate, mw 384.4	7.6 μg/L; 20 nM	C_{max} at a clearly embryocidal dose level; however, probably requires metabolism to dihydroartemisinin for which the corresponding C_{max} is 49.8 μg/L (175 nM)	Clark et al. (2004, 2008); Li et al. (2009)
5. Caffeine, mw 194.1906	63 mg/L; 325 μM	Concentration at clearly teratogenic dose level	Collins et al. (1981)
6. Dabigatran, mw 627.74	4.3 mg/L; 7 μM	C_{max} at dose level causing resorption and a decrease in viable fetuses	FDA (2010a)
7. D,L-3-hydroxy-3-ethyl-3-phenylpropionamide (HEPP), mw 193.2456	50 mg/L; 260 μM	Embryo concentration at embryocidal and fetocidal dose level	Gómez-Martínez (2007)
8. Ethylene glycol, mw 62.07	3528 mg/L; 57 mM	↑Malformations in rats treated by gavage; C_{max} from pregnant rats of similar strain (ethylene glycol requires metabolism to the proximate teratogen, glycolic acid. If the test system is positive, it must have the capability to perform oxidative metabolism of the glycol)	Neeper-Bradley et al. (1995); Pottenger et al. (2001)
9. Fingolimod, mw 307.471	21 μg/L; 67 nM	Fetal concentration at a dose level that was 45 times the lowest teratogenic exposure level—this concentration is the only available fetal measurement. In male rats, C_{max} after a dose equal to the lowest teratogenic dose was 3 μg/L (10 nM).	FDA (2010b)
10. Glycolic acid, mw 76.05	452 mg/L; 5 mM	↑Malformations in rats treated by gavage; C_{max} from pregnant rats of similar strain	Price et al. (1985); Pottenger et al. (2001)
11. Hydroxyurea, mw 76.06	26.5 mg/L; 350 μM	↑Malformations in rats treated ip; dose is based on C_{max} for embryonic concentration, but since hydroxyurea distributes to total body water it is likely that this also approximates the maternal plasma concentration.	Asano et al. (1983); Wilson et al. (1975)
12. MEHP, the toxic metabolite of di(2-ethylhexyl) phthalate (DEHP), MEHP mw 278.15, DEHP mw 390.62	146 μM	↑Malformations in rats treated by gavage; C_{max} for MEHP from pregnant rats of similar strain. It is also possible to test DEHP (same molar concentration) to determine if the test system has the capability to hydrolyze it to MEHP, but a negative for DEHP should not be considered to be a false negative.	Hellwig et al. (1997); Laignelet and Lhuguenot (2000)
13. Methanol, mw 32.04	8650 mg/L; 270 mM	↑Malformations in rats exposed by inhalation for almost 23 h/day; C_{max} from nonpregnant females	Nelson et al. (1985)
14. Methoxyacetic acid, mw 90.08	450 mg/L; 5 mM	Maternal C_{max} for methoxyacetic acid after a teratogenic dose of methoxyethanol (PBPK model)	Sleet et al. (1996)
15. Methylmercury, mw 215.62 for the ion, atomic weight 200.59 for mercury	2 mg/L as mercury; 5 μM	Embryo concentration at clearly embryocidal and teratogenic exposure; requires extrapolation between Wistar and Sprague Dawley and from sc to oral gavage (Lewandowski et al. [2002] present argument that routes are comparable)	Fuyuta et al. (1978); Lewandowski et al. (2002)
16. Nilotinib, mw 529.52	14.6 mg/L; 28 μM	C_{max} at clearly teratogenic exposure level	FDA (1998b)
17. Ramelteon, mw 259.34344	21 mg/L; 81 μM	Maternal plasma concentration associated with malformations; not statistically significant on pairwise comparison with control, but confirmed by response in higher dose group (at which no PK were performed)	FDA (2005)

(*Continued*)

Table 1
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Compound	Concentration	Comments	References
18. Salicylic acid, mw 138.12	459 mg/L; 3 mM	Maternal serum concentration of salicylic acid after administration of a single gavage dose (embryo concentration was three times higher); assumes that 100% of teratogenic maternal aspirin dose is converted to salicylic acid, supported by pharmacokinetics in male rats at dose levels of aspirin that were teratogenic in pregnant rats	Kimmel et al. (1971); Gupta et al. (2003); Kapetanovic et al. (2009); Wientjes and Levy (1988)
19. SB-209770 = 3-(2-(Carboxymethoxy)-4-methoxyphenyl)-1-(3,4-(methylenedioxy)phenyl)-5-(prop-1-yloxy)indan-2-carboxylic acid, CAS 157659–79–5, mw 520.5	251 mg/L; 0.5 mM	Maternal C_{max} + 2 SD at teratogenic exposure level	Treinen et al. (1999)
20. Valproate, mw 166.2 (Na salt)	134.5 mg/L; 0.8 mM	C_{max} associated with teratogenic exposure	Vorhees (1987)

end, we suggested that it would be useful to develop a validation list of positive and negative *exposures* in which a chemical plus an exposure level would together characterize a positive or a negative result (Daston et al., 2010). A chemical might be positive at one exposure level and negative at another, lower, exposure level, corresponding to the real world of developmental toxicity testing. We have compiled such a list of exposures from publicly available literature and present it here.

The crux of our argument is that hazard identification for developmental toxicity is not an exercise that can be separated from dose–response considerations. Past attempts to develop validation lists have characterized chemicals as "positive" based on the appearance in whole animal tests of malformations, embryolethality, growth impairment, and/or functional deficits in the absence of maternal toxicity (Smith et al., 1983) or with the incorporation of adult/developmental (A/D) toxicity ratios (Brown, 2002). Difficulties with these schemes included the imprecise definition and identification of maternal toxicity and the lack of concordance across species and sometimes strains in positivity by these criteria. Moreover, the use of potentially reversible alterations such as growth impairment as criteria for positivity raised the question of whether such validation schemes ask too much of candidate alternative tests that are designed to identify coarser endpoints such as malformation or embryo death.

METHODS

After publication of our proposal for an exposure-based approach to developing a validation list (Daston et al., 2010), a workshop of interested scientists was convened under the auspices of the Developmental and Reproductive Toxicology (DART) Technical Committee of the ILSI Health and Environmental Sciences Institute (HESI). Workshop members were given data sets and charged with determining positive or negative exposures based on existing data. Compounds were sought for which there were adequate toxicity and kinetic data for the identification of relevant positive or negative exposure levels based on maternal blood or fetal concentrations of candidate chemicals at administered dosages in toxicological studies. Data sets were restricted to rat studies, because rats are the most commonly used species in developmental toxicity testing and because many alternative test systems seek to predict the results of rat studies.

We defined a positive response in developmental toxicity testing as an increase in embryo-fetal death or structural malformation. We did not use decreased fetal weight, ossification delay, variations, or functional abnormalities as positive endpoints to avoid questions about the permanence of these effects or their association with maternal toxicity, which might not be evaluable in an alternative test system. We do not mean to diminish the importance of these endpoints as evidence of developmental toxicity; rather, we wish to select clear and unambiguous outcomes to give alternative tests the fairest chance to succeed. By the same rationale, we chose negative exposure levels as those associated with a clear lack of any developmental alteration, including effects on fetal weight, structural variations, and delays in development.

The workshop exercise demonstrated that many data sets from good toxicological studies lack adequate kinetic data. The ideal data set would include kinetic data collected in pregnant animals as part of a developmental toxicity study. Such data sets were found in regulatory submissions to the US Food and Drug Administration (FDA) for pharmaceutical products, and we made use of these data when they were publicly available. Our preference was to use the maximum maternal plasma concentration (C_{max}) of a chemical as the dose metric, because for many alternative systems the test agent is placed in the system at a constant concentration for the duration of the test, making area under the time–concentration curve (AUC) equivalent in concept to C_{max}. Although not ideal, in many cases kinetic data were only available from studies using different animals and, sometimes, different strains of rats and nonpregnant or even male rats, rather than the pregnant females the fetuses of which would be evaluated. In a few cases, human concentration data were used

Table 2
Exposures Producing no Effects

Compound	Exposure level	Comments	References
1. Abacavir, mw 286.34	5.3 mg/L; 18 μM	C_{max} associated with developmental NOAEL. Note that this is only four- to fivefold lower than the positive concentration	FDA (1998a); GlaxoSmithKline (2012)
2. All-*trans*-retinoic acid, mw 300.44	0.5 μg/L; 1.7 nM	Endogenous maternal plasma concentration	Collins et al. 1994
3. Butylparaben, mw 194.227	21 mg/L; 110 μM	C_{max} for a 100 mg/kg dose in female rat	Daston (2004); Frederiksen et al. (2008); Aubert et al. (2012)
4. Caffeine, mw 194.19	1.5 mg/L; 7.7 μM	Plasma concentration in humans after one cup of coffee	HSDB (2011)
5. Dabigatran, mw 627.74	0.6 mg/L; 1 μM	Concentration at developmentally nontoxic dose level	FDA (2010a)
6. Desloratadine, mw 310.82	487 mg/L; 1.5 mM	Clear NOAEL in registration study with satellite PK study	FDA (2001)
7. Ethylene glycol, mw 62.07	89 mg/L; 1.4 mM	C_{max} for EG at a dose level of 150 mg/kg/day	Neeper-Bradley et al. (1995); Pottenger et al. (2001)
8. Glycolic acid, mw 76.05	21 mg/L; 275 μM	C_{max} for GA at a dose level of 150 mg/kg/day	Neeper-Bradley et al. (1995); Pottenger et al. (2001)
9. Methanol, mw 32.04	0.7 mg/L; 22 μM	Minimum background concentration in human blood	Batterman and Franzblau (1997)
10. Mono(2-ethylhexyl) phthalate (MEHP), mw 278.16	278 μg/L; 1 μM	(1) Lowest $BMDL_{10}$[a] for DEHP is 13 mg/kg/d. (2) Closest pharmacokinetic measure is MEHP AUC after DEHP dose of 30 mg/kg (0.077 mmol/kg) = 646 mM-h per mmol DEHP/kg. (3) 646 mM-h per mmol DEHP/kg * 0.077 mmol DEHP/kg = 49.7 mM-h. (4) 49.7 mM-h/24 h ≈ 2 mM. (5) Assuming linearity, this concentration was divided by 2 to approximate the concentration at a DEHP dose of 13 mg/kg/d.	Laignelet and Lhuguenot (2000)
11. Nilotinib, mw 529.52	1.3 mg/L; 2 μM	$BMDL_{05}$ for malformations; concentration extrapolated from (but close to) observed range	FDA (1998b)
12. Oseltamivir, mw 312.40452	3.77 mg/L; 12 μM	C_{max} at clearly negative dose level; however, effects at higher dose levels (↓ ossification with ↓maternal weight gain) probably also negative.	FDA (1999)
13. Propylene glycol, mw 76.095	65,000 mg/L; 850 mM	No developmental toxicity in rats; C_{max} from males of same strain	FDRL (1973); Morshed et al. (1988)
14. Ramelteon, mw 259.34344	5 μg/L; 19 nM	Extrapolated from PK data at higher dose levels to the NOAEL dose	FDA (2005)
15. SB-209770 = 3-(2-(Carboxymethoxy)-4-methoxyphenyl)-1-(3,4-(methylenedioxy)phenyl)-5-(prop-1-yloxy)indan-2-carboxylic acid, CAS 157659–79–5, mw 520.5	2 mg/L; 4 μM	Maternal C_{max} associated with nondevelopmentally toxic exposure level and less than $BMDL_{01}$ for fetal weight, which is the most sensitive end point.	Treinen et al. (1999)
16. Sodium saccharin, mw 206.16	5 mg/L; 24 μM	This C_{max} is all that is available for pregnant rat; NOAEL is at least 12 times higher.	Fritz and Hess (1968); Sweatman and Renwick (1982); FDRL (1972); Sweatman et al. (1981)
17. Tapentadol, mw 257.80	252 mg/L; 1mM	C_{max} – 2 SD for clearly nondevelopmentally toxic exposure level	FDA (2008)
18. Zaleplon, mw 305.34	3.54 mg/L; 12 μM	C_{max} associated with NOAEL in developmental study (10-fold below LOAEL)	FDA (2009)
19. Zidovudine, mw 267.242	61 mg/L; 227 μM	Fetal concentration at a nondevelopmentally toxic exposure level	Greene et al. (1996)

[a]$BMDL_{10}$ is the lower bound of the benchmark dose at a 10% effect level (US EPA, 2012).

to establish negative exposures. For example, the baseline concentration of methanol in human blood was used as a negative exposure under the assumption that an alternative test system should be expected to identify such a background exposure as not being developmentally toxic.

After being armed by the workshop with a better understanding of the need for good toxicokinetic information in our approach, we compiled a list of chemicals we thought might have adequate data. We performed literature searches on these chemicals in National Library of Medicine databases and made use of National Toxicology Program reports and publicly available FDA documents to gather additional information. Data were extracted to tables and used to identify suitable maternal plasma or, in some cases, fetal concentrations of a chemical at clearly positive or clearly negative exposure levels. Results compiled by one author were reviewed by at least two other authors before being accepted.

RESULTS

The proposed positive exposures and associated developmental toxicities are presented in Table 1, and the negative exposures (those that should produce no adverse effects) appear in Table 2. To the extent possible, we chose maternal serum concentration as the exposure concentration. Exceptions are noted in the "Comments" column in the tables. As noted in the methods, our intention in choosing positive values was to provide a concentration at which adverse developmental effects were clearly elicited and for negative values a concentration at which the lack of effect was definitive. Therefore, it is possible to have effects at concentrations lower than the concentrations in the "positives" list and still consider the test result a true positive or higher than the negative concentration and still have a true negative.

DISCUSSION

The development of alternatives to whole animal testing in developmental toxicology might permit the use of fewer animals in testing and would be useful for the identification of the least toxic candidates in early selection of pharmaceuticals or other chemicals. The premise of any alternative test system is that it will predict a property of a chemical that will be useful in evaluating developmental risk. Because developmental risk is always contextual, occurring or not occurring depending on exposure level and other conditions of the exposure, we believe that tests developed to predict those risks should also do so in a manner related to the known or anticipated exposure level for the chemical.

To that end, we propose a validation scheme by which proponents of new alternative tests may use these lists to evaluate the performance of their assay. An assay should find that the positive exposures are toxic in the proposed alternative system (inasmuch as they were shown to be positive in intact rats), and correspondingly, the negative exposures should be negative in the alternative assay. In a few instances, we have chosen as negatives the concentration of a chemical that is unequivocally nontoxic in humans, for example, the background serum concentration of methanol or the concentration of caffeine achieved after a single cup of coffee.

Some alternative test systems will be limited by the absence of metabolic systems or by insensitivity to specific mechanisms of developmental toxicity. We do not present any requirements for an alternative test system. Rather, our validation list may serve as a tool for the identification of test system limitations. We envision that alternative test systems generally will be used for testing compounds with unknown toxicity, and an understanding of the limitations of the system will be useful for interpreting the results obtained with an unknown. We likewise have no requirement that test systems operate at chemical concentrations present in intact pregnant rats, but we believe that it is fair to expect any test system to be able to convert the concentrations at which it operates to concentrations that can be compared to in vivo toxic and nontoxic exposure levels.

Our proposed list is limited to the concentration of a chemical at which an alternative test should give positive or negative results. Other important considerations such as duration or timing of exposure are not addressed and might represent a limitation of our proposal.

Our list is necessarily limited to compounds for which there were adequate data and therefore it might not be generalizable to the universe of chemicals that might be subjected to testing. We do not propose that our list will be static, and we hope that investigators will be motivated to expand the list by performing kinetic and developmental toxicity studies with additional compounds, thereby increasing the range of chemicals on the list. It is also likely that we have overlooked existing data sets that may be suitable for inclusion on this list, and we invite our colleagues to contribute the data sets that we have missed. We propose setting up a link on the HESI DART web site where such a dynamic list can be made available.

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REFERENCES

Asano Y, Ariyuki F, Higaki K. 1983. Behavioral effects of hydroxyurea exposure during organogenetic period of rats. Congen Anom 23:279–289.

Aubert N, Ameller T, Legrand JJ. 2012. Systemic exposure to parabens: pharmacokinetics, tissue distribution, excretion balance and plasma metabolites of [14C]-methyl-, propyl- and butylparaben in rats after oral, topical or subcutaneous administration. Food Chem Toxicol 50:445–454.

Batterman SA, Franzblau A. 1997. Time-resolved cutaneous absorption and permeation rates of methanol in human volunteers. Int Arch Occup Environ Health 70:341–351.

Brown NA. 2002. Selection of test chemicals for the ECVAM international validation study on *in vitro* embryotoxicity tests. ATLA 30:177–198.

Clark RL, White T, Clode S, Gaunt I, Winstanley P, Ward S. 2004. Developmental toxicity of artesunate and an artesunate combination in the rat and rabbit. Birth Defects Res B 71:380–394.

Clark RL, Lerman SA, Cox EM, Gristwood WE, White TE. 2008. Developmental toxicity of artesunate in the rat: comparison to other artemisinins, comparison of embryotoxicity and kinetics by oral and intravenous routes, and relationship to maternal reticulocyte count. Birth Defects Res B Dev Reprod Toxicol 83:397–406.

Collins MD, Tzimas G, Hummler H, Bürgin H, Nau H. 1994. Comparative teratology and transplacental pharmacokinetics of all-trans-retinoic

acid 13-*cis*-retinoic acid, and retinyl palmitate following daily administration in rats. Toxicol Appl Pharmacol 127:132–144.
Collins TFX, Welsh JJ, Black TN, Collins EV. 1981. A study of the teratogenic potential of caffeine given by oral intubation to rats. Regul Toxicol Pharmacol 1:355–378.
Daston GP. 2004. Developmental toxicity evaluation of butylparaben in Sprague-Dawley rats. Birth Defects Res B Dev Reprod Toxicol 71(4):296–302.
Daston GP, Chapin RE, Scialli AR, Piersma AH, Carney EW, Rogers JM, Friedman JM. 2010. A different approach to validating screening assays for developmental toxicity. Birth Defects Res B 89:526–530.
FDA. 1998a. Ziagen pharmacology review. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/nda/98/20-977_Ziagen.cfm. Accessed May 4, 2014.
FDA. 1998b. Sustiva pharmacology review. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/nda/98/20972.cfm. Accessed May 4, 2014.
FDA. 1999. Tamiflu pharmacology review. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/nda/99/21087_Tamiflu.cfm. Accessed May 4, 2014.
FDA. 2001. Clarinex pharmacology review. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2001/21-165_Clarinex.cfm. Accessed May 4, 2014.
FDA. 2005. Rozerem pharmacology review. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2005/021782s000_RozeremTOC.cfm. Accessed May 4, 2014.
FDA. 2008. Tapentadol pharmacology review. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2008/022304s000_TOC.cfm. Accessed May 4, 2014.
FDA. 2009. Sonata pharmacology review available at: http://www.accessdata.fda.gov/drugsatfda_docs/nda/99/20859_Sonata.cfm
FDA. 2010a. Pradaza pharmacology review. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2010/022512Orig1s000TOC.cfm. Accessed May 4, 2014.
FDA. 2010b. Gilenya pharmacology review. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2010/022527Orig1s000TOC.cfm. Accessed May 4, 2014.
FDRL (Food and Drug Research Labs). 1972. Teratologic evaluation of FDA 71-11 (sodium saccharin). NTIS PB221772.
FDRL (Food and Drug Research Labs). 1973. Teratologic evaluation of FDA 71-56 (propylene glycol) in mice, rats, hamsters and rabbits. Waverely, NY: Food and Drug Research Laboratories, Inc. Cited by Center for the Evaluation of Risks to Human Reproduction. 2004. NTP-CERHR expert panel report on the reproductive and developmental toxicity of propylene glycol. Reprod Toxicol 18:533–579.
Frederiksen H, Taxvig C, Hass U, Vinggaard AM, Nellemann C. 2008. Higher levels of ethyl paraben and butyl paraben in rat amniotic fluid than in maternal plasma after subcutaneous administration. Toxicol Sci 106(2):376–383.
Fritz H, Hess R. 1968. Prenatal development in the rat following administration of cyclamate, saccharin and sucrose. Experientia 24:1140–1141.
Fuyuta M, Fujimoto T, Hirata S. 1978. Embryotoxic effects of methylmercuric chloride administered to mice and rats during organogenesis. Teratology 18:353–366.
GlaxoSmithKline. 2012. Ziagen product labling. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/020977s024,020978s028lbl.pdf. Accessed April 24, 2014.
Gómez-Martínez LE. 2007. Gestational age dependency in the prenatal toxicity and in the disposition kinetics of the novel anticonvulsant HEPP (D,L-3-hydroxy-3-ethyl-3-phenylpropionamide) after subcutaneous administration in pregnant rats. Int J Toxicol 26(3):237–246
Greene JA, Ayers KM, Tucker WE Jr, de Miranda P. 1996. Nonclinical toxicology studies with zidovudine: reproductive toxicity studies in rats and rabbits. Fundam Appl Toxicol 32(2):140–147.
Gupta U, Cook JC, Tassinari MS, Hurtt ME. 2003. Comparison of developmental toxicology of aspirin (acetylsalicylic acid) in rats using selected dosing paradigms. Birth Defects Res B Dev Reprod Toxicol 68(1):27–37.
Hellwig J, Freudenberger H, Jäckh R. 1997. Differential prenatal toxicity of branched phthalate esters in rats. Food Chem Toxicol 35:501–512.
HSDB (Hazardous Substances Data Bank). 2011. Caffeine. Available at: http://toxnet.nlm.nih.gov/cgi-bin/sis/search. Accessed May 4, 2014.
Available at: http://www.accessdata.fda.gov/drugsatfda_docs/nda/99/20859_Sonata.cfm. Accessed May 4, 2014.
Kapetanovic IM, Bauer KS, Tessier DM, Lindeblad MO, Zakharov AD, Lubet R, Lyubimov A. 2009. Comparison of pharmacokinetic and pharmacodynamic profiles of aspirin following oral gavage and diet dosing in rats. Chem Biol Interact 179(2–3):233–239.
Kimmel CA, Wilson JG, Schumacher HJ. 1971. Studies on metabolism and identification of the causative agent in aspirin teratogenesis in rats. Teratology 4:15–24.
Laignelet L, Lhuguenot J. 2000. Di-(2-ethylhexyl)phthalate (DEHP). Absorption, excretion, metabolism and pharmacokinetic profile in pregnant Wistar rats. Dijon: Ecole Nationale Supérieure de Biologie Appliquée à la Nutrition et à l'Alimentation (ENSBANA); submitted by the American Chemistry Counsel to OEHHA, California EPA, on May 12, 2004.
Lewandowski TA, Pierce CH, Pingree SD, Hong S, Faustman EM. 2002. Methylmercury distribution in the pregnant rat and embryo during early midbrain organogenesis. Teratology 66(5):235–241.
Li Q, Si Y, Xie L, Zhang J, Weina P. 2009. Severe embryolethality of artesunate related to pharmacokinetics following intravenous and intramuscular doses in pregnant rats. Birth Defects Res B Dev Reprod Toxicol 86(5):385–393.
Morshed KM, Nagpaul JP, Majumdar S, Amma MK. 1988. Kinetics of propylene glycol elimination and metabolism in rat. Biochem Med Metab Biol 39:90–97.
Neeper-Bradley TL, Tyl RW, Fisher LC, Kubena MF, Vrbanic MA, Losco PE. 1995. Determination of a no-observed-effect level for developmental toxicity of ethylene glycol administered by gavage to CD rats and CD-1 mice. Fundam Appl Toxicol 27:121–130.
Nelson BK, Brightwell WS, MacKenzie DR, Khan A, Burg JR, Weigel WW, Goad PT. 1985. Teratological assessment of methanol and ethanol at high inhalation levels in rats. Fundam Appl Toxicol 5:727–736.
Pottenger LH, Carney EW, Bartels MJ. 2001. Dose-dependent nonlinear pharmacokinetics of ethylene glycol metabolites in pregnant (GD 10) and nonpregnant Sprague–Dawley rats following oral administration of ethylene glycol. Toxicol Sci 62:10–19.
Price CJ, Kimmel CA, Tyl RW, Marr MC. 1985. The developmental toxicity of ethylene glycol in rats and mice. Toxicol Appl Pharmacol 81:113–127.
Sleet RB, Welsch F, Myers CB, Marr MC. 1996. Developmental phase specificity and dose-response effects of 2-methoxyethanol in rats. Fundam Appl Toxicol 29(1):131–139.
Smith MK, Kimmel GL, Kochhar DM, Shepard TH, Spielberg SP, Wilson JG. 1983. A selection of candidate compounds for in vitro teratogenesis test validation. Teratogen Carcinog Mutagen 3:461–480.
Sweatman TW, Renwick AG. 1982. Tissue levels of saccharin in the rat during two-generation feeding studies. Toxicol Appl Pharmacol 62(3):465–473.
Sweatman TW, Renwick AG, Burgess CD. 1981. The pharmacokinetics of saccharin in man. Xenobiotica 11(8):531–540.
Treinen KA, Louden C, Dennis MJ, Wier PJ. 1999. Developmental toxicity and toxicokinetics of two endothelin receptor antagonists in rats and rabbits. Teratology 59(1):51–59.
Tzimas G, Thiel R, Chahoud I, Nau H. 1997. The area under the concentration-time curve of all-trans-retinoic acid is the most suitable pharmacokinetic correlate to the embryotoxicity of this retinoid in the rat. Toxicol Appl Pharmacol 143(2):436–444.
US EPA. 2012. Benchmark dose technical guidance. EPA/100/R-12/002. Available at: http://www.epa.gov/raf/publications/pdfs/benchmark_dose_guidance.pdf. Accessed May 30, 2014.
Vorhees CV. 1987. Teratogenicity and developmental toxicity of valproic acid in rats. Teratology 35(2):195–202.
Webster WS, Brown-Woodman PDC, Ritchie HE. 1997. A review of the contribution of whole embryo culture to the determination of hazard and risk in teratogenicity testing. Int J Dev Biol 41:329–335.
Wientjes MG, Levy G. 1988. Nonlinear pharmacokinetics of aspirin in rats. J Pharmacol Exp Ther 245(3):809–815.
Wilson JG, Maren TH, Takano K, Ellison A. 1968. Teratogenic action of carbonic anhydrase inhibitors in the rat. Teratology 1:51–60.
Wilson JG, Scott WJ, Ritter EJ, Fradkin R. 1975. Comparative distribution and embryotoxicity of hydroxyurea in pregnant rats and rhesus monkeys. Teratology 11:169–178.