Original Article

ILSI/HESI Maternal Toxicity Workshop Summary: Maternal Toxicity and Its Impact on Study Design and Data Interpretation

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Workshops on maternal toxicity were held at the annual Society of Toxicology, Teratology Society, and European Teratology Society meetings in 2009. Speakers presented background information prior to a general discussion on this topic.

The following recommendations/options are based on the outcome of the discussions at the workshops:

1. A comprehensive evaluation of all available data from general toxicity studies, range-finding Developmental and Reproductive Toxicology (DART) studies, class effects, structure–activity relationships, exposure studies, etc. is essential for appropriate dose selection for definitive DART studies. The intent is to avoid marked maternal toxicity leading to mortality or decreased body weight gains of greater than 20% for prolonged periods.

(a) Evaluate alternative endpoints for dose selection and data interpretation (e.g., target tissue effects and pharmacology) for biotherapeutics.

(b) Evaluate additional maternal parameters based on effects and/or target organs observed in short-term (e.g., 2- or 4-week) general toxicity studies.

2. Evaluate all available data to determine a cause–effect relationship for developmental toxicity.

(a) Conduct a pair-feeding/pair-watering study as a follow-up.

(b) Evaluate individual data demonstrating maternal toxicity in the mother with adverse embryo–fetal outcomes in the litter associated with the affected mother.

(c) Conduct single-dose studies at increasing doses as a complement to conventional embryo–fetal toxicity studies for certain classes of compounds that affect the hERG channel.

3. Support statements that embryo–fetal effects are caused by maternal toxicity and/or exaggerated pharmacology, especially for malformations.

(a) Provide mechanistic or other supporting data.

(b) Establish the relevance of the DART findings in animals for human exposures. *Birth Defects Res (Part B)* 92:36–51, 2011. © 2010 Wiley-Liss, Inc.

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INTRODUCTION

Although the demonstration of some degree of maternal toxicity is required in regulatory developmental and reproductive toxicology (DART) studies (US EPA, 1998; US FDA, 2000; OECD, 2001; ICH, 2005), marked maternal toxicity may be a significant confounding factor in study design and data interpretation. To date, there is no clear consensus on what level of maternal toxicity is acceptable versus that which is merely confounding. This is critical in some regulatory settings that explicitly require the identification of developmental toxicity that is not secondary to maternal toxicity. A related issue concerns distinguishing ''true'' maternal toxicity from exaggerated pharmacology. In addition, the interference of maternal toxicity might be confounding in the assessment of interspecies differences in developmental toxicity. Similarly there are conflicting reports in the literature about fetal abnormalities and other effects associated with maternal toxicity. Although recent publications tend to indicate there is no commonly seen causal relationship between maternal toxicity and specific fetal malformations (Hood and Miller, 2006), the link between maternal toxicity and other manifestations of altered fetal development (e.g., growth retardation) appear to be stronger (Chernoff et al., 2008). Therefore, the issue of the impact of maternal toxicity on study design and data interpretation remains a high priority in regulatory toxicology, as it affects both industry scientists and government regulators.

When considering maternal toxicity, especially as it pertains to maternal body weight loss or decreased weight gain, it must be remembered that any effect leading to decreased fetal body weight or decreased litter size will have an effect on the apparent maternal body weight or weight gain. To assess the influence of fetal body weight or litter size on maternal weight change, it is essential to evaluate any correlations among individual maternal body weight (or weight change), gravid uterine weight, litter size, and fetal weights. After such an evaluation, it should be possible to determine whether there was an effect on the maternal carcass weight, with or without an effect on the litter.

If a reduction in maternal body weight/weight gain is the result of late fetal death or reduced litter weight, it will probably occur during the last trimester of gestation, when fetal size influences the overall weight of the maternal animal. However, if a reduction in weight/weight gain occurs near the beginning of the dosing period, it is likely to be the result of an effect on the maternal animal.

Due to the different intended uses of their products, pharmaceutical and chemical industries and their respective international regulatory authorities approach the conduct and interpretation of developmental toxicity studies in a slightly different manner. Pharmaceutical compounds are intended to be given to people to treat specific conditions/diseases. Accordingly, pharmaceutical companies are interested in addressing two key DART questions: (a) do the compounds pose developmental or reproductive risks, which might limit their use in certain populations (e.g., pregnant women), and (b) what is the relationship between effective and toxicologic doses. Chemical companies have much less control over the distribution and use of the chemicals they manufacture, and cannot limit exposures to certain segments of the population.

The basic issue for preclinical pharmaceutical research is to identify potential target organ toxicity and associated exposures prior to clinical trials with the compounds, whereas most chemical companies need to obtain toxicity information for classification and labeling. Pharmaceutical companies generally have a good understanding of the pharmacologic properties of the compounds being developed, which helps in designing preclinical studies for evaluating potential toxicity. However, chemical companies typically do not have this information for novel chemicals and need to conduct the studies to identify toxicologic properties. Similarly, pharmaceutical companies usually have a good understanding of pharmacokinetic and pharmacodynamics properties of their compounds, whereas chemical companies rarely have this information.

This manuscript summarizes the presentations and discussions from workshops on maternal toxicity (Maternal Toxicity and Its Impact on Study Design and Data Interpretation) held at the annual meetings of the Society of Toxicology (Baltimore, MD, March 17, 2009), the Teratology Society (Rio Grande, Puerto Rico, June 30, 2009), and the European Teratology Society (Arles, France, September 9, 2009). The opinions expressed in the presentation summaries are those of the individual presenters.

PRESENTATION SUMMARIES

Overview/Background—R. Hood and J. Rogers

Developmental effects can result from direct effects of a compound on the embryo/fetus, indirect (maternally mediated) effects, or a combination of the two effects. Maternally mediated effects are those that occur secondarily as a result of an effect on the pregnant animal. They differ from direct effects on the conceptus primarily in their immediate source rather than the end result. Therefore, there are two critical concerns in the interpretation of developmental toxicity test results: (1) How commonly does maternal toxicity cause adverse effects on the offspring and (2) Under what circumstances is this likely to occur?

Some degree of maternal toxicity is required under current regulatory testing guidelines (US EPA, 1998; US FDA, 2000; OECD, 2001; ICH, 2005). Toxicity to the pregnant animal must be detectable, quantifiable, and must be distinguishable from exaggerated pharmacological effects when testing pharmaceutical candidates.

However, even pharmacological effects may potentially influence embryo–fetal development and survival.

Khera (1984, 1985) reviewed published studies and proposed that a number of effects on the offspring of rodents and rabbits occurred merely as a consequence of maternally mediated toxicity, such as decreased fetal body weight, certain malformations and developmental variations, and resorptions. Examples of the malformations Khera associated with maternal effects include exencephaly, open eyes, and fused thoracic or lumbar vertebrae in mice, and fused ribs, exencephaly, and eye defects in hamsters, as well as rib, vertebral, and sternebral defects in rats and rabbits. Khera's hypothesis was that such effects were often not dose-related, tended to be species-specific, and were seldom observed at dosages below those that were maternally toxic.

However, it can be argued that it is Khera's interpretation, rather than the developmental toxicity study results themselves, that may be of concern. Khera's literature review indicated a possible association between maternal toxicity and embryo–fetal effects, but it did not establish a causal relationship between these two observations. Additional criticisms of Khera's hypothesis include the fact that his literature review was retrospective, there was a potential selection bias arising from the general tendency not to publish negative data, and the failure to adequately address maternal toxicity endpoints in the published literature of the time. In fact, Khera himself stated that in 40% of the studies he evaluated in support of his hypothesis the maternal toxicity data were ''insufficient or nonexistent'' (Khera, 1987).

Studies conducted to test Khera's hypothesis that maternal toxicity commonly resulted in specific defects did not find a consistent relationship for defects other than supernumerary ribs. Maternal toxicity was not an effective or consistent inducer of most malformations. For example, a mouse study by Kavlock and colleagues (1985) concluded that there was no clear direct relationship between the induction of maternal toxicity, including lethality, and the production of major abnormalities. A follow-up study with rats (Chernoff et al., 1990) reached similar conclusions: overt maternal toxicity, as defined by weight loss or mortality, is not always associated with the same defined syndrome of adverse developmental effects in the rat.

However, there are cases in which adverse effects on the offspring, including malformations, appear to have been maternally mediated. Clark and colleagues (1984) found evidence of maternal toxicity influencing fetal findings in studies with diflunisal in rabbits, in which fetal axial skeletal defects were observed. Diflunisal was found to produce severe maternal hemolytic anemia and greatly decreased erythrocyte ATP levels. The authors were able to demonstrate that the skeletal malformations resulted from maternal hypoxia secondary to anemia, rather than from a direct effect of the drug on the embryo or fetus. In addition, it was demonstrated that diflunisal had no effects on rat erythrocyte ATP levels, and the compound was categorized as ''not teratogenic'' in rats or mice.

Other chemical compounds for which maternal effects have been implicated in developmental toxicity include the following: (1) acetazolamide, where maternal hypercapnia is associated with fetal ectrodactyly in mice, (2) phenytoin, where maternal bradycardia is associated with fetal cleft lip/palate in mice, (3) norfloxacin, where maternal inanition is associated with fetal death and decreased body weight in rabbits, (4) indacrinone, where maternal hypokalemia is associated with fetal skeletal defects in rats, and (5) hydroxyurea, where maternal uterine ischemia is associated with fetal hemorrhage in rabbits.

From a regulatory perspective (US EPA, 1991; US FDA, 2000; OECD, 2007), it is difficult to distinguish between those effects on in utero development that are attributable to direct fetal exposure to the toxicant versus those effects that are due to, or exacerbated by, maternal toxicity. Therefore, adverse effects on the developing organism are considered by most regulators to be toxic manifestations of treatment, regardless of the cause. For that reason, evidence of maternal toxicity does not automatically negate the observation of fetal toxicity at a similar dose level.

Relationships of Maternal/Fetal Weight Changes in Developmental Toxicity Bioassays—N. Chernoff

An analysis of 125 developmental toxicity bioassays in the mouse, rat, and rabbit conducted by the National Toxicology Program (NTP) was reported (Chernoff et al., 2008). Although varying by species, general findings include:

1. Most Lowest-Observed-Adverse-Effect Levels (LOAELs) were determined by reduced maternal gestational weight gain or fetal weight at term. In $\geq$60% of mouse, rat, and rabbit studies, maternal LOAELs were determined solely by reduced weight gain at some point during gestation. In dose levels where weight and another type of toxicity occur together, the percent of LOAELs rises to $\geq$86% of the studies. Reduced weight at term is the sole determinant of fetal LOAELs in the mouse and rat in $\geq$71% of the studies and $\geq$82% when lower weight and any other toxicity are observed at the same dose level. This high incidence of fetal weight alterations does not appear to hold for the rabbit, where a fetal LOAEL involving reduced weight occurred in $\leq$50% of the reviewed studies.
2. Maternal weight reductions are associated with reduced food intake for a variety of chemically unrelated test agents. There is a significant association ($P < 0.01$) between reduced maternal weight gain and reductions in food intake (21/24 studies in the three species where food intake was measured).
3. Lower fetal weights were associated with reduced maternal weight gains late in gestation. Maternal weight reductions in the rat are associated with significant fetal weight reductions in only 40% of the studies. However, analysis of these data based on the period during which the reduced maternal weight gains took place indicated that this factor is a major determinant of fetal weight at term. Significantly reduced fetal weight at term occurred in only 16% (9/55) of dose levels where significant reductions in maternal weight gain occurred only on or before Gestation Day 14 (GD14). In contrast, significantly reduced fetal weight at term occurred in 83% (24/29) of dose levels where significant maternal weight reductions occurred after GD15. The reductions in

food intake and the resultant undernutrition would be more likely to induce fetal growth retardation if these effects occurred late in gestation when fetal growth is greatest. This has also been shown in the rat experiments by Garofano et al. (1998). The relationship between fetal weight at term and gestational period of decreased food intake was also seen in the human population in the Netherlands exposed to famine in WWII during the winter of 1944–1945. Smith (1947) and Stein et al. (1995) examined the birth weights of children born to mothers who experienced the famine at different points during gestation. They concluded that there is a significant effect of gestational period of exposure on birth weight. The average weight of babies born to mothers who experienced the famine during the third trimester was significantly lower as compared to babies of mothers exposed only during the first trimester.

4. The degree of fetal weight reduction is correlated with the extent of the maternal weight loss. In both the mouse and rat, there was a significant ($P < 0.01$) association of the magnitude of maternal and fetal weight reductions.
5. The data do not show an increased sensitivity of the developing embryo/fetus as compared to the maternal animal for the array of test agents in the NTP data sets. Analysis of studies in which either maternal or fetal toxicity occurred indicates that the fetus is more sensitive (that is, adverse effects are induced at lower doses as compared to the mother) to test agents in 12/36 mouse, 4/45 rat, and 1/17 rabbit studies. Of note is the large number of studies in which adverse fetal effects and overt maternal toxicity occur at the same dose level: 17/36 mouse, 13/45 rat, and 6/17 rabbit studies. This high percentage of studies where maternal and developmental LOAELs occurred at the same dose level (37% across the three species) suggests an association between weight changes since this endpoint is the most common one.

The observed inter-species differences in the patterns of maternal and fetal weight reductions may be related to differences in the comparative length of the embryogenesis and fetal growth stages. In the substantial majority of the studies examined, the test agent was administered during the ''period of major organogenesis'', considered to be GD6-15 in the rat and mouse and GD8-19 in the rabbit. The dams were euthanized before term: GD17 in the mouse, GD21 in the rat, and GD29 in the rabbit. The species-specific fetal growth periods and maternal euthanasia times result in differences in the amount of time in which a fetus can ''recover'' from reduced in utero growth during the period of major organogenesis (shortest in the mouse and longest in the rabbit). The mouse, with the shortest interval between the last dose and euthanasia, has the highest association of maternal and fetal weight reductions and the weights of affected fetuses were often below 80% of the concurrent controls. The rabbit, which has the longest interval between dosing and euthanasia, has the weakest association between maternal and fetal effects, and weights of affected fetuses never fell below 80% of controls. The rat has an intermediate interval between dosing and euthanasia; both the concordance of maternal and fetal weight changes and the extent of fetal weight loss as compared to controls lie between the mouse and rabbit.

In a substantial number of the reviewed developmental toxicity studies from the NTP program, reduced fetal weights at term may, therefore, be due to maternal undernutrition and concomitant reduced food intake caused by general toxicity rather than a direct developmental insult. Consequently, such test agents may be erroneously classified as primary developmental toxicants. Experimental approaches to test the hypothesis that maternal undernutrition in standard developmental toxicology bioassays may be responsible for significant term fetal weight decrements were discussed.

Referring to agents that induce fetal weight reductions solely due to maternal undernutrition may not be an accurate representation of their intrinsic potential to adversely affect development.

Exaggerated Pharmacology: Maternal versus Direct Embryonic Effects: Cardiovascular-Active Compounds—B. Danielsson

It is well established that interruption of the oxygen supply to the embryo, resulting in periods of hypoxia/anoxia, is teratogenic. In classical experimental studies (e.g., by temporarily clamping the uterine vessels or with periods of low oxygen concentration in the air), hypoxia produced a wide spectrum of adverse effects. The effects consisted of embryonic death, growth retardation, and stage-specific malformations such as cleft lip/palate, CNS, heart, axial skeletal, and digital defects (e.g., oligo-, brachy-, and syndactyly) (Franklin and Brent, 1964; Grabowski, 1970; Webster and Abela, 2007). However, fetal embryonic hypoxia and subsequent malformations can also be produced by compounds via direct pharmacological action on the embryo or via effects on the maternal side.

Direct action in the embryo. Strong evidence indicates that hypoxia is a common embryotoxic/teratogenic mechanism for a large number of compounds due to their inhibition of cardiac human ether a-go-go channel (hERG) of the embryonic heart. As discussed below, fetal adverse effects for many older pharmaceutical compounds, which have been recently identified as hERG blockers, had been attributed to maternal toxicity without any valid supporting evidence. New information also suggests that conventionally designed embryo–fetal developmental toxicity studies are not optimal to detect the full spectrum of teratogenicity for hERG blockers, and may fail to identify their true teratogenic potential.

In the adult, the hERG channel is the most important channel responsible for cardiac repolarization in humans, dogs, and rabbits, but not in adult rodents (rats and mice). The hERG channel opens to conduct a specific potassium current (I_{Kr}). Blockade in sensitive species results in a dose-dependent prolongation of the action potential duration in cardiac myocytes (seen as QT interval prolongation on ECG) and is associated with a risk for irregular heart beat (cardiac arrhythmia) and death. The hERG channel is ubiquitous, in contrast to most other ion channels. At the time of the presentations, more than 230 compounds in a variety of pharmacological classes, including antihistamines, antidepressants, macrolide antibiotics, antipsychotics, antiepileptics, and fungicides, have been shown to inhibit this channel

(Polak et al., 2009). Some drugs with negative risk/benefit ratios have been withdrawn from the market, while many others are available for restricted use, with a warning for QT prolongation/cardiac arrhythmia in their product labeling. There are also other compounds which block hERG only at high concentrations with a high benefit/risk clinical ratio.

The heart of the embryo and embryonic cardiomyocytes in all studied species (human, rabbit, rat, and mouse) is very susceptible to I_{Kr}-blocking drugs (Abrahamsson et al., 1994; Wang et al., 1996; He et al., 2003; Danielsson et al., 2007). Despite very high doses, hERG blockers fail to induce arrhythmia and death in adult rodents. However, exposure of the rodent embryonic heart to increasing concentrations of hERG blockers produces cardiac arrhythmia at low doses during a sensitive period between GD10-14 (Spence et al., 1994; Webster et al., 1996). Potent hERG channel blockers designed to inhibit the hERG channel, such as dofetilide, almokalant, and ibutilide, produce high post-implantation embryonic death with little or no incidence of malformed fetuses in conventional repeat-dose embryo–fetal toxicity studies in rodents (Marks and Terry, 1996; Webster et al., 1996; Sköld and Danielsson, 2000). However, when these drugs are administered on single gestation days during the period when rat embryos are sensitive to hERG blockade (GD10, 11, 12, 13, or 14), they induce a high incidence of stage-specific external, visceral, and skeletal malformations almost identical to the pattern of malformations induced by hypoxia (Webster et al., 1996; Wellfelt et al., 1999; Sköld et al., 2001). Studies using the hypoxia probe, pimondiazole, which binds to hypoxic areas in embryonic tissues with oxygen tension below 10 mm Hg, show that a single teratogenic dose of a hERG blocking drug causes severe embryonic hypoxia (Danielsson et al., 2003, 2005; Nilsson et al., 2010). The reason for the disparity in the observed fetal adverse effects between conventional repeat dosing and single dosing is that an embryo may be able to survive a single exposure to a teratogenic dose while repeat dosing often leads to death of the embryo as a result of repeated periods of arrhythmia and hypoxia.

In contrast to the adult rat, the adult rabbit is a sensitive animal model to predict potential compound-related QT prolongation and arrhythmia in adult humans. A consequence of this sensitivity is that the rabbit, which is commonly used as a second species in embryo–fetal toxicity studies, tolerates much lower doses of hERG channel blockers than the rat. Numerous compounds (drugs, fungicides used as pesticides) inhibit the hERG channel with increasing dose, and this is most likely one major reason why the signs of maternal toxicity occur at lower dose levels in rabbits than rats. Furthermore, single-dose studies of hERG blockers with slow dose escalation indicate that the rabbit embryonic heart is more sensitive than the adult rabbit heart. Hypoxia-related embryonic death and typical hERG-related malformations occur in such studies at doses that do not cause maternal cardiac arrhythmia and death (Danielsson et al., 1992; Sköld and Danielsson, 2001).

There are examples when teratogenicity has been missed in conventional embryo–fetal toxicity studies where effects such as massive embryonic death and growth retardation have been attributed to maternal adverse effects (Karlsson et al., 2007). The propulsive drugs cisapride (primarily acting on 5HT-4 receptors in the gastrointestinal tract) and the antihistamine astemizole are both potent I_{Kr} blockers. In rabbits dosed from GD6-19, both drugs caused dose-dependent embryonic death. In rats dosed from GD6-16, the dams tolerated high doses (160 mg/kg/day cisapride) (Sköld et al., 2002). At doses inducing massive embryonic death, but no/few defects in the few surviving fetuses, the dams showed slight decreases in maternal body weight gain. However, in follow-up studies in rats with cisapride (Sköld et al., 2002) and astemizole (Karlsson et al., 2007), embryonic arrhythmia and typical hypoxia-related defects were produced at single doses on GD13 that were 50% lower than the high dose in conventional embryo–fetal studies. Without a proper understanding of the underlying mechanisms and complementary single-dose studies, there is a risk for arriving at misleading pregnancy information; the embryonic death would mask the teratogenicity in rats while malformations and embryonic death observed in rabbits would be attributed to nonspecific maternal toxicity.

As shown in a recent review based on findings in the open literature and public databases (Karlsson et al., 2007), many pharmaceuticals with an intermediate potential to block I_{Kr} (e.g., chlaritromycin, telithromycin, chlomipramine, imipramine, citalopram, and phenytoin) cause dose-dependent increases in embryonic death, growth retardation, and teratogenicity in rats and/or rabbits. Decreased maternal body weight gains and/or maternal symptoms due to primary pharmacological effects have been proposed to explain the observed fetal adverse effects. In order to minimize the potential for misleading risk assessment and pregnancy labeling, it is recommended to conduct single-dose studies in rats (e.g., on GD11 and/or GD13) at increasing doses as a complement to conventional embryo–fetal toxicity studies for hERG channel blockers (Danielsson et al., 2007; Karlsson et al., 2007). Cardiovascular defects, especially ventricular septal defects but also absence, transposition, and/or abnormal vessels (e.g., overriding aorta or reduced aorta with reduced pulmonary trunk), seem to be the easiest defects to be induced after administering a single dose in rats (on GD10, 11, 12, or 13 which corresponds to weeks 5–9 in human pregnancy) (Sköld et al., 2001). It seems that the rhythm abnormalities per se are sufficient to induce heart anomalies, while longer and more severe epidsodes of hypoxia are required for other abnormalities to manifest (e.g., oral clefts and brachydactyly). It may be of particular interest to apply the single dose approach for older pharmaceuticals with signals of human cardiac teratogenicity where the hERG blocking potential was discovered after many years of clinical use. Examples of such I_{Kr} blocking drugs include chlomipramine (Källen and Otterblad Olausson, 2006), erythromycin (Källen et al., 2005), and some selective serotonin reuptake inhibitiors (Klinger and Merlob, 2009; Merlob et al., 2009). The single-dose approach also has the advantage that higher doses can be tested without producing severe signs of maternal toxicity or high incidences of embryonic death, which otherwise may occur after repeated dosing with I_{Kr} blockers.

Maternally mediated effects. There are also different types of drugs causing embryonic hypoxia and malformations via pharmacological action on the maternal side. Vasodilating drugs (e.g., feldopine,

nifedipine, and nitredipine) are potent anti-hypertensive agents that produce digital defects in rabbits (Danielsson et al., 1989, 1990) and rats (Yoshida et al., 1988). The underlying mechanism is most likely diversion of the blood from central compartments (e.g., the pregnant uterus) to peripheral vascular beds and decreased uteroplacental blood flow. Vasoconstricting drugs (e.g., epinephrine, vasopressin, ergotamine, nicotine, misoprostol, and cocaine) cause hypoxia-related malformations, particularly digital defects preceded by hemorrhage in the rat (Webster and Abela, 2007). Mechanistic studies support the hypothesis that vasoconstriction of the uterine arteries and decreased uteroplacental blood flow as a likely mechanism. In this context, it is worth noting that an adverse fetal effect should be characterized as developmental toxicity even if it is maternally mediated. However, such maternally mediated effects may or may not be relevant to humans due to differences in dose, exposure, species, etc.

Maternal hemorrhage can cause embryonic hypoxia, as demonstrated in mice by repeated blood withdrawal, leading to decreased fetal body weight, increased resorptions, digital defects, and cleft palate. Hypovolemia and hypoperfusion of the uterus and placenta were proposed as likely mechanisms for these effects (Fawcett et al., 1998). Anticoagulants at higher doses than the therapeutic level can lead to maternal blood loss, altered hematology parameters, and severe anemia. In such cases, it is reasonable to assume that the increased embryonic death, decreased fetal body weights, and increased hypoxia-related malformations may be related to the hypovolemia.

Exaggerated Pharmacology versus True Toxicity: Biologics—M. Rocca

Some degree of maternal toxicity is required in DART studies, with reduced maternal body weight gain and/or food consumption being the most frequently used endpoints. However, some test articles (e.g., some biologics) do not cause these effects. Additional endpoints to consider are the target tissues identified in repeat-dose toxicity studies, as well as the evaluation of the expected and exaggerated pharmacology. This may mean adding endpoints such as hematology or histopathology to a developmental toxicology study (Martin et al., 2009). Examples were given of test articles that produce the following situations: classic maternal toxicity, expected pharmacology, exaggerated pharmacology, and no toxicity.

Palifermin (Kepivance®) is a recombinant human keratinocyte growth factor and illustrates a biologic producing classic maternal toxicity. The expected pharmacological action of keratinocyte growth factor is to promote the proliferation, differentiation, and migration of epithelial cells. The toxicity identified in repeat-dose studies in rats included hypertrophy/hyperplasia of the gastrointestinal tract and enlarged liver, with corresponding changes in clinical chemistry. Therefore, clinical chemistry and histology endpoints were added to the embryo–fetal toxicity study in rats. In this study, pregnant rats were treated with palifermin (100–1,000 μg/kg/day intravenously from GD6-18). Maternal effects included classic maternal toxicity of decreased body weight gain and food consumption (1000 μg/kg/day) and the expected toxicity identified in repeat-dose studies (enlarged liver and associated changes in clinical chemistry parameters [all doses], thickened stomach [1,000 μg/kg/day], and mammary gland hyperplasia [all doses], as well as increased postimplantation loss [1,000 μg/kg/day]). Therefore, the maternal NOAEL was less than the low dose of 100 μg/kg/day. Fetal effects included decreased body weight and decreased ossification of the cervical centra and sternebrae at 1,000 μg/kg/day; these findings were attributed to intrauterine growth retardation. The developmental NOAEL was the mid-dose of 300 μg/kg/day.

Romiplostim (N-plate®) is a thrombopoietin receptor agonist that illustrates compounds producing exaggerated pharmacology. Romiplostim is expected to increase platelet counts. In an embryo–fetal toxicity study in rats (10–100 μg/kg subcutaneously every other day from GD6-18), there were no effects on maternal body weight or food consumption. However, mortality during blood collection, enlarged spleens, and dose-related increases in platelets were observed. These findings were all attributed to exaggerated pharmacology and were considered adverse; therefore, no maternal NOEL was established. No effects were observed on fetal viability, body weight, or abnormalities, although there were dose-related increases in platelets. The fetal NOAEL was the high dose, 100 μg/kg.

Natalizumab (Tysabri®) is a humanized IgG4 monoclonal antibody against α4 integrin and illustrates compounds producing expected pharmacology. The intended pharmacological effect of Natalizumab is to block lymphocyte trafficking across the endothelium into the parenchyma; therefore, maternal and fetal hematology was added to the developmental toxicity study design (Wehner et al., 2009). In the embryo–fetal toxicity study in cynomolgus monkeys, 3–30 mg/kg natalizumab was infused intravenously every other day from GD20-70. There were no effects on maternal body weight or food consumption, but dose-related increases in white blood cell parameters were observed (expected pharmacology). No effects were observed on fetal viability, body weight, or fetal abnormalities. However, dose-related increases in fetal white blood cell and nucleated red blood cell parameters were observed. As the only effects were the result of expected pharmacology, and they were not considered adverse, the maternal and developmental NOAELs were 30 mg/kg.

Conatumumab is an investigational fully human monoclonal antibody agonist of Death Receptor 5 (DR5), and is an example of compounds producing no toxicity. Conatumumab induces apoptosis in a variety of transformed human cell lines and tumor xenograft models, but is not expected to affect normal cells. It has similar sequence, expression, binding, and function at human and cynomolgus monkey DR5. In an embryo–fetal toxicity study in cynomolgus monkeys (0–300 mg/kg/week intravenously from GD20–50), no effects were observed on maternal body weight, food consumption, or other parameters. In addition, no effects were observed on fetal viability, body weight, or abnormalities. The maternal and fetal NOELs were the high dose of 300 mg/kg.

Therefore, the maternal effect of bioloics may range from classic toxicity to no toxicity. Alternative endpoints should be considered for dose selection and study interpretation, such as target tissue effects and pharmacology.

Retrospective Analysis of Developmental Toxicity Studies in Rat and Rabbit: Value of Rabbit—G. Janer

Developmental toxicity studies are conducted to identify hazards for developmental toxicity, and to establish a NOAEL for use in risk assessment. The way these goals are addressed and their regulatory consequences differ among regulatory frameworks (i.e., chemicals vs. pharmaceuticals, EU vs. US). This section refers to the European regulatory framework for chemicals and particularly to Classification and Labeling (C&L) (EEC, 1967; EC, 2008). In this framework, classification and labeling for developmental toxicity is based purely on hazard identification.

The criteria that this Directive establish for the classification of a substance as toxic to reproduction, when classification is based on animal data, are that: ''data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects.''

The animal developmental toxicity studies that are usually available for the hazard evaluation of a substance consist of rat and rabbit developmental toxicity studies, as recommended by most regulatory test guidelines. The use of rat and rabbit in developmental toxicity testing is supported by several reviews that suggest that a rat embryo–fetal toxicity study alone would miss many teratogenic substances. For example, Hurtt and colleagues (2003) reported that out of the 20 teratogenic substances that had been tested in both rat and rabbit, only 60% would have been identified as teratogenic if a rat study alone (or a rabbit study alone) had been available. A similar conclusion can be drawn from an extensive review by Schardein (2000). The review included 1169 chemicals tested in both rats and rabbits, 257 of these were considered by the author as teratogenic. Out of this subset of substances, 77% were considered teratogenic in the rat studies, but not in the rabbit studies; and 54% were considered teratogenic in the rabbit studies, but not in the rat studies.

These and other existing reviews have rarely taken into consideration whether or not the adverse effects on development were a secondary non-specific consequence of maternal toxicity. In addition, some looked at teratogenicity in a strict sense and did not consider other types of developmental toxicity. Other reviews included studies of heterogeneous designs. All these factors might have confounded the real impact of a developmental toxicity study in a second species in the classification outcome.

The presenter and colleagues selected all substances that were considered in the above quoted reviews to have a positive developmental toxicity study in the rabbit and a negative developmental toxicity study in the rat. Summary data available for developmental studies in these substances were evaluated using the criteria of the European Directive for C&L. It was concluded that, for most of these substances, the apparently discordant outcomes from the rat and the rabbit studies would have led to the same outcome for classification. First, a large proportion of these substances had rabbit studies that showed clear maternal toxicity at the doses where developmental toxicity was found. In these studies, maternal toxicity had, at least partially, contributed to developmental toxicity. Although mechanistic information was not available to assess cause–effect relationships, it is very unlikely that these substances would have been classified as developmental toxicants under the mentioned directive. Discrepancies between rat and rabbit studies for another set of substances were due to the fact that Schardein's review only looked at teratogenicity. For these substances, other clear manifestations of developmental toxicity occurred in the rat (e.g., embryo or fetal lethality) that would have led to classification even if the rabbit study would not have been available.

The presenter and colleagues also searched for summary data on developmental studies for substances classified by the European Directive for C&L as developmental toxicants, for which rat and rabbit embryo–fetal toxicity studies were available for at least one route of exposure (54 substances). For these substances, the possible classification outcome was estimated considering only the rat developmental toxicity study. Following the criteria established in this Directive, 44 out of the 54 substances (81%) would probably have been classified if only the rat study had been available. For three substances that were reviewed, neither the rat nor the rabbit developmental studies would have led to their classification as teratogens. For the remaining seven substances, the rabbit study would probably have been needed for classification. For several of the latter substances, developmental toxic effects were observed in the rat study, but they co-occurred with maternal toxicity and we did not have sufficient information to elucidate whether developmental toxicity had or had not been a consequence of maternal toxicity. If those data had been available to the reviewers, the proportion of discordant studies between rat and rabbit might have been even lower.

In summary, when following the criteria established by the European Directive on C&L, the rabbit embryo–fetal toxicity study had a lower impact on classification and labeling than previously believed. The developmental toxicity endpoints considered, the evaluation of the dependence of maternal toxicity, and the homogeneity of study designs greatly influenced the interspecies differences in terms of classification. Establishing the relationship (or the lack thereof) between maternal and developmental toxicity was a key factor in this classification process, but it was very challenging due to the lack of mechanistic data. Indeed, the conclusions were based on the relative severity of developmental and maternal toxicity rather than on a mechanistic basis.

Postnatal Consequences of Maternal Toxicity—A. Scialli

Some degree of maternal toxicity is required by regulatory testing guidelines, but should not produce death or severe suffering. Maternal parameters that are monitored include clinical signs of toxicity, behavioral changes, and, most frequently, reduced body weight gain. As discussed in greater detail in Neil Chernoff's presentation above, a review of 125 studies from the NTP, Chernoff and colleagues (2008) found that the maternal LOAEL was

determined solely by reduced weight gain in 60–84% of mouse, rat, and rabbit studies. The maternal LOAEL was determined by reduced weight gain plus other toxicity in 86–94% of the studies, while the fetal LOAEL was determined by reduced weight, with or without other toxicity, in 82–87% of rat and mouse studies. In addition, decreased maternal food consumption correlated with decreased maternal weight gain in 87.5% of the studies in which it was measured. Decreased food consumption and decreased weight gain can be due to a smaller litter size or lower litter weight, unpalatable feed, anorexia, sedation, and other causes related to altered maternal homeostasis. The consequences of decreased maternal weight gain are decreased fetal weight.

Maternal starvation, such as was experienced during the Dutch Hunger Winter (1944, 1945) (Smith, 1947), results in decreased birth weight if it occurs during the third trimester of pregnancy. In addition, a study by Hales and colleagues (1991) indicates a high correlation between low birthweight and subsequent glucose intolerance in adult men. Similar results were reported in offspring from the Dutch Hunger Winter if their mothers experienced starvation during the third trimester (Painter et al., 2005; Rooseboom et al., 2006). In addition, other effects observed in adult offspring whose mothers experienced the famine included microalbuminuria, an increase in obstructive airway disease, a more atherogenic lipid profile, an increased incidence of breast cancer, and a higher incidence and earlier age at onset of coronary artery disease; no effects on blood pressure were noted (Rooseboom et al., 2006). Underfeeding of pregnant rats has been shown to increase caloric intake, blood pressure, and plasma insulin concentrations in their offspring after birth and into adulthood (Vickers et al., 2000).

Mechanisms that have been proposed to account for these findings include deficient tissue development (e.g., nephrons), impaired imprinting, or exposure to stress hormones (e.g., cortisol) (Langley-Evans, 2006). Severe maternal stress during pregnancy has also been associated with an increase in congenital malformations arising from contributions from the neural crest cells (Hansen et al., 2000). Therefore, decreased nutritional intake and increased endogenous cortisol/corticosterone should be avoided during pregnancy.

Several maternal diseases have been associated with adverse neonatal outcomes, including diabetes mellitus (malformations, growth abnormalities [too large or too small], stillbirth) (Weintrob et al., 1996; Leguizamón et al., 2007), chronic hypertension (impaired fetal growth) (Podymow and August, 2007), and systemic lupus erythematosus (impaired fetal growth and fetal heart block) (Jaeggi et al., 2010; Smyth et al., 2010). The extent to which maternal illnesses in humans are analogs of drug- or chemical-induced maternal toxicity in experimental animal studies is not known but is consistent with the concept that the best environment for growing a healthy baby is a healthy mother.

Regulatory Perspectives/Case Studies

US—K. Davis-Bruno and W. Harrouk. Standard developmental DART study designs are provided in ICH S5A (ICH, 2005) and include separate studies of fertility and early embryonic development in one species, embryo–fetal development in two species, and pre- and postnatal development in one species to cover all phases of the reproductive cycle. Two case studies were presented to illustrate the issue of maternal toxicity in data received in support of new drug applications to the US FDA.

Case study #1: Treatment of cystic fibrosis.[1] No test article-related effects were observed in the rat fertility study. However, in the embryo–fetal toxicity study in rats (dosages = 30–300 mg/kg/day from GD6-17), a dose-dependent decrease in maternal body weight gain (−2 to −40% vs. control) was observed in parallel with decreased food consumption, as well as increased post-implantation loss (8 vs. 4% in control) at 300 mg/kg/day. Decreased fetal body weight (−10.5 to −18% vs. control) and increased skeletal variations (incomplete ossification, rib misalignment, and rudimentary ribs) were also observed at ≥100 mg/kg/day. In an embryo–fetal toxicity study in rabbits (dosages = 25–100 mg/kg/day from GD7-20), mortality and abortion in two does and one doe, respectively, occurred at 100 mg/kg/day. In addition, maternal body weight (−5%) and food consumption (−53%) were decreased compared to controls, while post-implantation loss was increased. Dosage-dependent decreases in fetal body weight were observed at 50 and 100 mg/kg/day (−2 to −10% vs. control), with single incidences of visceral or skeletal defects at all doses. Are the fetal findings in either or both species attributable to maternal toxicity? FDA generally looks at fetal findings that occur in the absence of significant maternal toxicity since they are considered most relevant to the clinical situation where maternal toxicity is unlikely to be encountered at therapeutic doses.

Case study #2: Treatment of hyperlipidemia (statin). Lovastatin is a lipid-lowering drug that crosses the placenta and is secreted in milk at approximately 2-fold plasma concentrations. In embryo–fetal toxicity studies in rats (dosed from GD6-20), maternal toxicity was observed at dosages ≥400 mg/kg/day, resulting in forestomach hyperplasia. In addition, maternal weight gain and food consumption were decreased at dosages ≥100 mg/kg/day. The maternal NOAEL was 80 mg/kg/day (60-fold exposure margin vs. clinical exposure). Fetal/neonatal findings in rats were observed at clinically relevant exposures. At a 5-fold exposure margin, fetal/pup mortality and decreased body weight were observed. At a 6-fold exposure margin, developmental delay (righting reflex and negative geotaxis), decreased auditory startle response, reduced latency in swimming and open field testing, and incomplete skeletal ossification were observed. At a 25-fold exposure margin, skeletal alterations (supernumerary and wavy ribs) and incomplete skeletal ossification were observed.

Fetal/neonatal toxicity was observed in the absence of maternal toxicity, and consisted of mortality, skeletal malformations, and developmental delays. Fetal findings occurred at or near clinical exposures were:

- rat (<1-fold exposure margin): skeletal, neural tube, gastroschisis, and behavior/learning effects;
- rabbit (5-fold): visceral variations and delayed ossification;
- mouse (2-fold): skeletal and visceral malformations.

[1]The name of the drug is not mentioned since this drug is still in the developmental phase.

The mechanism of action of statins is to inhibit HMG-CoA reductase, thereby decreasing the formation of cholesterol and steroids. In rat embryos, it is known that the source of cholesterol is the yolk sac or placenta and there is little de novo cholesterol synthesis. Co-administration of lovastatin with mevalonate or cholesterol attenuates the more severe fetal malformations, whereas the wavy ribs and incomplete skeletal ossification persist. In addition, evidence of maternal toxicity remains. These findings support the hypothesis that fetal toxicity seen in DART studies with Lovastatin is related to pharmacologically mediated disruption of cholesterol biosynthesis.

The embryo–fetal findings are reflected in the product label. No well-controlled studies have been conducted in pregnant women. Post-marketing activities have shown human fetal adverse effects, when exposure has been established during the first trimester but a cause–effect relationship has not been demonstrated due to the limited available data. Animal data demonstrate fetal/neonatal adverse effects without obvious maternal toxicity. No clinical benefit has been shown for the temporary treatment of pregnant women; therefore, use of lovastatin is contraindicated during pregnancy.

EU—U. Wändel Liminga. As extensively discussed in a publication by Rogers et al. (2005), our ability to interpret developmental toxicity in the presence of maternal toxicity would be greatly improved by additional research in two areas: (1) Identification of syndromes that occur at maternally toxic dosages irrespective of the causative agent, and (2) Identification of measures that better characterize the health status of the mother, and thereby better define maternal toxicity.

The influence of maternal toxicity can be reflected in product labeling, as illustrated by the examples of calcium antagonists (e.g., nifedipine and felodipine). In the Summary of Product Characteristics, the label indicates that studies in animals have demonstrated reproductive toxicity, consisting of embryotoxicity and teratogenicity at maternally toxic dosages. Therefore, nifedipine is contraindicated during pregnancy. DART studies with calcium antagonists in rats and rabbits have shown various adverse effects (e.g., prolonged duration of pregnancy, difficulty in labor, and impaired development of distal phalanges, presumably due to decreased uteroplacental perfusion). Those are not considered the result of a direct teratogenic effect, but have been interpreted as secondary consequences of the pharmacodynamic effect of these compounds.

From a regulatory perspective, Sponsors should be able to support claims that developmental toxicity is due to maternal toxicity or that the findings have no relevance for humans (e.g., provide mechanistic or other data to support the claim). These data are rarely available.

Three examples were provided to illustrate how maternal toxicity in DART studies may influence risk assessment and EU regulatory recommendations regarding the clinical use of a pharmaceutical; only one of these examples has been published and is named below.

In the first example, retarded ossification of single bones was observed at the high dose in a rat embryo–fetal toxicity study, which was attributed by the Sponsor to reduced fetal body weights and marked maternal toxicity. In addition, there were increased incidences of malformations (external, visceral, and skeletal), retarded ossification, and sternebral fusion in a rabbit embryo–fetal toxicity study. The Sponsor considered the rabbit findings to be common malformations that correlated with reduced fetal weight and were only seen at clearly maternally toxic dosages. None of the observations were considered indicative of primary teratogenicity. However, the regulatory assessment determined that the data did not support the Sponsor's claims. Malformations occurred in normal weight fetuses from dams without clear signs of toxicity. It was believed that the decreased fetal weight could be secondary to toxicity causing the malformation. In any case, the findings were observed at clinical exposure levels, which was of serious concern. Accordingly, a very strict label was indicated.

The second example concerned lenalidomide (Revlimid®), which was approved for the treatment of multiple myeloma. Lenalidomide is a thalidomide analogue that is 10–1,000 times more potent in vitro (European Public Assessment Report, updated May 17, 2010). There is no evidence for qualitative differences in pharmacology. In an embryo–fetal toxicity study in New Zealand White rabbits at lenalidomide dosages of 3–20 mg/kg/day or thalidomide at 180 mg/kg/day during GD7-19 (Christian et al., 2007), thalidomide was selectively toxic to development (including characteristic limb and other dysmorphology). Lenalidomide was maternally toxic at dosages $\geq$10 mg/kg/day as shown by body weight loss or decreased weight gain and food consumption and one abortion (20 mg/kg/day). Developmental toxicity was observed at $\geq$10 mg/kg/day, and consisted of decreased fetal body weight and increased post-implantation loss and fetal variations. The Sponsor concluded that the maternal and developmental NOAELs were 3 mg/kg/day, and that lenalidomide affected embryo–fetal development only at maternally toxic dosages. No fetal malformations were attributed to lenalidomide. During the approval process, the Committee for Medicinal Products for Human Use (CHMP) requested a study in a sensitive species as a post-approval commitment since the available data were not sufficiently reassuring that this drug has a potentially different developmental risk profile than thalidomide (European Public Assessment Report, updated May 17, 2010). Very strict risk minimization procedures were put in place for women of child-bearing potential. In the embryo–fetal toxicity study in monkeys at dosages up to 4 mg/kg/day, lenalidomide caused malformations that were similar to those produced by thalidomide in the same study (short limbs; bent digits, wrist and/or tail; supernumerary or absent digits). Therefore, lenalidomide is considered teratogenic, and a birth control program was maintained.

In the third example, a monoclonal antibody caused an increased incidence of abortion/embryo–fetal death in an embryo–fetal study in cynomolgus monkeys. Placental transfer of IgG is low or absent during the first trimester, increases around GD60, and is highest during the third trimester. Therefore, the abortions occurred when fetal exposure was nearly absent, indicating they were most likely due to maternal effects by an unknown mechanism. The product label indicates that women of child-bearing potential must use effective contraception during and up to 6 months after treatment, and this compound should not be used during pregnancy unless clearly necessary.

These examples demonstrate that most recorded maternal parameters are too general to establish maternal toxicity. In addition, few mechanisms of teratogenicity or developmental toxicity are clearly identified. Therefore, Sponsors usually fail to demonstrate a link between maternal toxicity and specific developmental effects. There is a strong need for mechanistic data to support assumptions of a causal relationship between maternal and developmental toxicity, and to aid in risk assessment.

WORKSHOP DISCUSSION SUMMARIES

The workshop discussions are summarized below on the basis of two broad themes: (1) Maternal toxicity as the basis for dose selection in embryo–fetal toxicity studies and (2) Maternal toxicity as a confounder in identifying developmental toxicity. The first theme addresses regulatory guideline requirements in considering the criteria to be used for dose selection, while the second theme covers the investigations of a causal relationship between maternal toxicity and developmental toxicity.

Maternal Toxicity as the Basis for Dose Selection in Embryo–Fetal Toxicity Studies

How much maternal toxicity can be tolerated without confounding developmental toxicity evaluations? While the testing strategies may differ depending on the regulatory guidelines, chemical class, and industry pertaining to a specific chemical, the overall objective is one of detecting potential hazards (i.e., hazard detection). Factors that confound interpretation of results can affect the ability to fulfill the study objectives. Marked maternal toxicity can perturb maternal physiology and homeostasis and, therefore, may affect the developing embryo/fetus. However, the expectation has been that minimal maternal effects (e.g., transient effects on maternal body weight or food consumption, decreases in maternal body weight gain of less than 10% relative to concurrent control animals) will have no adverse effects on embryo–fetal development.

For all practical purposes, the study sponsor defines maternal toxicity. This can be based on traditional endpoints such as maternal clinical observations, body weight, or food consumption. However, this could also be based on pharmacologic effects on target organs, such as the heart, in the case of pharmaceuticals. In any case, it is important to remember that the standard regulatory test designs are, in fact, screens. Special study designs might be required to evaluate the role of suspected maternal toxicity on embryo–fetal outcomes observed using the standard study designs.

Workshop participants believed that, if appropriate, maternal toxicity can provide an explanation for some developmental effects. There seems to be a false expectation by chemical and pharmaceutical companies that regulatory authorities would not label test articles/test materials as ''developmental toxicants'' or ''teratogens'' if maternal toxicity is proposed as the cause of embryo–fetal findings. However, for such assertions to be accepted, they must be supported by data, especially mechanistic data. For some regulators, embryo–fetal findings are important regardless of any relationship to maternal toxicity. This is especially true if the LOAEL or NOAEL for the test article/test material is decreased based on the DART study findings.

Several workshop participants indicated that the current maternal parameters are insufficient to adequately define maternal toxicity. Therefore, attributing embryo–fetal effects to maternal toxicity requires an appropriate investigation in evaluating additional parameters to reach a conclusion on cause and effect relationships. Although maternal weight is an easy and direct parameter to measure, it is too often the only parameter used for defining maternal toxicity due to the lack of additional maternal endpoints, which are selected based on knowledge of the mechanism of action (or pharmacology) of the compound in question. For many chemicals where such information is not known, maternal weight may be the first or only indicator of maternal toxicity.

One proposal was to evaluate the sensitive parameters identified in general toxicology studies and to assess those in pregnant animals in DART studies. Historically, DART studies focused on identifying potential developmental toxicity because maternal toxicity would have been characterized previously in general toxicology studies, since regulatory guidelines do not explicitly require extensive evaluation of maternal toxicity. Unless additional studies have a high probability of success in ''saving'' the compound, industry is often reluctant to conduct additional testing because of resource and time constraint considerations. A risk versus benefit analysis of all available data is then important for labeling.

- Are the current maternal endpoints sensitive enough?

There was a consensus that current endpoints are considered sensitive enough, although additional endpoints might be useful. For example, dam necropsy data could be given more consideration. Pharmacokinetic data are useful and are often available for pharmaceuticals, but are rarely available for pesticides and other chemicals. Alternatively, data from structurally or pharmacologically similar compounds might be useful in addressing the issue of maternal toxicity on a case-by-case basis. Several workshop participants commented that maternal body weight and food consumption data are generally sufficient for establishing maternal toxicity for pharmaceuticals. There was concern by some attendees that evaluating other endpoints might drive the maternal NOAEL lower.

The workshop participants were reminded that studies required by regulatory guidelines are only the first step in hazard identification. Ideally, effects observed in these studies should have a mechanistic explanation for a proposed causal relationship to the test article/test material. Therefore, many regulatory test guidelines suggest a mechanistic follow-up to the screening studies to address the relevance of the findings for humans.

- Is it necessary to confirm pharmacodynamic/toxic effects from other studies in DART studies?

A weight-of-evidence approach is preferred when evaluating the results of DART studies. Therefore, one should evaluate all available data to interpret the extent of maternal toxicity, including pharmacodynamics and general toxicology data.

- What level of exposure margin is sufficient?

Most workshop participants had an idea of what exposure margin was sufficient to convince them that higher exposures were not necessary for test articles/test materials with limited toxicity in DART studies. However, this comfort level varied widely (from 20 to 100 × human exposures). Some participants also believed the exposure margin should be chemical and class-specific. In addition, the exposure margin could depend on the severity of the observed embryo–fetal effect, where a low exposure margin could be permissible for a low severity effect. Evaluation of all available data is necessary (weight-of-evidence approach). Currently, there are no regulatory guidelines on acceptable exposure margins for DART studies, although the ICH M3(R2) guideline, which suggests an exposure margin of 50-fold, could be acceptable for the high dose for general toxicology studies with pharmaceuticals (ICH, 2009). The ICH S5A guideline states that "under most circumstances, 1 g/kg/day should be an adequate limit dose" (ICH, 2005).

- Are there any additional endpoints that would be useful or more useful?

One example was given for monitoring glucocorticoids for stress response if cortisol or a pharmacologically related compound was being administered. Otherwise, measuring glucocorticoid levels was not considered to be a useful biomarker for maternal toxicity. However, monitoring clinical pathology (hematology and clinical chemistry) parameters, as is done for general toxicology studies, could be useful, especially for evaluating the potential confounding influence of exaggerated pharmacology. There did not appear to be any consensus among the workshop participants whether the addition of clinical pathology parameters would increase the sensitivity of DART studies, although the maternal NOAEL could decrease based on these parameters compared with those derived from more traditional maternal endpoints. Maternal necropsy data should also be evaluated more carefully when considering the potential effects of maternal toxicity. Affected endpoints from shorter term general toxicity studies (e.g., 2- or 4-week studies) may provide a starting point for discussions on appropriate parameters to monitor for the evidence of maternal toxicity in embryo–fetal toxicity studies.

Workshop participants did agree that decreased maternal body weight gain or slight decreases in maternal body weight could lead to decreased fetal body weight/growth retardation and evidence of delayed ossification and minor skeletal defects, but not to major fetal malformations. A causal relationship between this degree of maternal toxicity and embryonic death was considered questionable.

Have we adequately defined acceptable levels of maternal toxicity (e.g., a maternal maximum tolerated dose [MTD]), beyond which study results may be compromised? In other words, what level of maternal toxicity is acceptable? Workshop participants agreed that it is not necessary to exceed the maternal MTD in embryo–fetal toxicity studies. An example of exceeding the MTD is the occurrence of maternal mortality. Regulatory guidelines specify that the highest dose should produce minimal maternal toxicity. Some participants noted that a 5% decrease in body weight gain could be adverse in a general toxicology study with a group size of 10 rodents. In the experience of some workshop participants, a 10% threshold is considered toxicologically meaningful for general toxicology studies. While the same criteria could apply to DART studies, there was no consensus on what percentage would be sufficient. On the other hand, a decrease in body weight gain of 20% was considered excessive for most test articles/test materials (compounds designed to produce weight loss would be an exception).

In the absence of target organ toxicity, reversible pharmacologically mediated effects would not be considered evidence of maternal toxicity. This is sometimes the case with biologics. In some cases, it may be possible to begin dosing the animals prior to mating to allow time for adaptation to adverse effects on maternal body weight.

For compounds with no or little toxicity, consider alternative endpoints (target tissue effects and pharmacology) in dose selection and study interpretation? Dose selection based on an exposure margin approach similar to the 50-fold exposure margin accepted for general toxicology studies under ICH M3(R2) was considered appropriate for pharmaceuticals. Even if toxicity was observed at higher than 50-fold exposure margins, it was felt there would be little clinical relevance. The workshop participants believed that the same rationale applies to the high dose to be tested in DART studies when little toxicity is observed. The DART community will need to follow up on the exposure margin concept for it to be accepted by regulatory authorities (perhaps as an addendum to ICH S5). Biologics present difficulties since targets may not be expressed in test species; this issue also applies to viral vectors and other new therapeutic interventions where no receptor is involved. Dose justification needs to be clear because exposure margins may be altered if clinical dosages are increased.

Workshop participants considered maternal toxicity to be indicative of an MTD, above which no useful scientific information for hazard identification would be obtained. Several participants considered maternal toxicity to be an indicator to stop dose escalation, but generally do not consider maternal toxicity as a reliable explanation for developmental toxicity.

On the other hand, pharmacologic targets need to be acknowledged when evaluating certain classes of pharmaceutical compounds. For example, weight loss drugs are designed to cause body weight reductions. In such cases, decreased body weight gain must be pronounced (e.g., greater than 20%) to be considered evidence of "maternal toxicity." It was emphasized that global changes in food intake for any DART study could be tested in an appropriately designed pair-feeding study (with pair-watering). The use of "altered" or "targeted health" animal models may be required for testing some biologics. Some participants advocated the use of disease models for safety testing instead of using healthy animals to better replicate the human situation.

Several workshop participants expressed the opinion that endpoints should be selected on a case-by-case basis depending on what is known about the test article/test material, including any class effects. Evaluation of target organs identified in general toxicology studies was considered important. It is not sufficient to note effects

such as decreased maternal food intake. It is important to understand the underlying cause for the decreased food consumption, as this could provide an explanation for developmental toxicity other than being vaguely linked to maternal toxicity. Several participants emphasized that not all developmental effects can be attributable to decreased maternal food intake.

Discussions of compound-related effects on maternal weight generally apply to rodents because weight loss is common during certain periods of pregnancy in some rabbits. When rabbits go ''off feed,'' ketosis can develop quickly, leading to a rapidly worsening cycle of reduced food intake and ketosis, with profound effects on maternal body weight and health status.

Maternal Toxicity as a Confounder in Identifying Developmental Toxicity

What do animal studies predict about the relationship of maternal toxicity and fetal abnormalities? Only strong teratogens are likely to be detected using the current embryo–fetal toxicity study design. Embryo–fetal toxicity studies are not designed to elicit malformations because the dosing period is prolonged, but evidence of possible developmental toxicity (e.g., decreased fetal survival, fetal body weight effects, increased frequency of developmental variations) can be obtained. Excessive developmental toxicity leading to increased embryo–fetal death can ''mask'' the presence of malformations. Administration of higher doses for shorter or defined periods of time during organogenesis (i.e., critical ''window'' dosing) is a better approach for elucidating the teratogenic potential of a given compound.

There was some discussion of using zebrafish or other nonmammalian alternatives to screen for developmental toxicity, but most participants still rely on testing in mammalian species as the gold standard for DART testing. This is especially true for meeting current regulatory testing requirements.

- Would knowledge of the mechanism(s) help?

It was acknowledged that adverse fetal effects can be induced by exaggerated pharmacologic action. However, increased incidences of malformations or embryo–fetal death cannot be attributed to slight changes in maternal body weight or nonspecific clinical signs. Including a statement that adverse fetal effects were observed only at dosages causing signs of maternal toxicity generally is not acceptable unless supporting or correlative evidence is provided. Embryo–fetal effects caused by exaggerated pharmacology, such as cardiac arrhythmia in the embryo exposed to hERG blocking drugs resulting in embryonic hypoxia, do have clinical relevance and, therefore, a weight-of-evidence evaluation should include general toxicology and safety pharmacology data. The burden of proof is on Sponsors to support statements that embryo–fetal effects are caused by maternal toxicity and/or exaggerated pharmacology, especially in the case of malformations. The expectation would be to provide mechanistic or other supporting data.

- Use existing data from other studies or generate new data?

When available, regulatory authorities consider data from other studies or mechanistic data to be useful.

Is it necessary to distinguish direct versus indirect embryo–fetal effects in animals when predicting human risk? There are three possible effects of exposure to test articles/test materials: direct effects on the mother and the conceptus, direct effects on the mother and indirect effects on the conceptus, and direct effects on the conceptus only.

Workshop participants noted that transient fetal body weight changes in animals may be clinically relevant because low-birth-weight babies often have problems later in life. In addition, a compound that decreased fetal weight, for example, without affecting the dam would be of great concern. On the other hand, compounds that are strongly biologically active in adult animals could be expected to reach the conceptus. Therefore, such a compound could exert direct toxicity on both the mother and conceptus, although it is acknowledged that there could be indirect effects on the conceptus mediated via maternal physiology.

- Would knowledge of the mechanism(s) help?

Some workshop participants consider exaggerated pharmacology to be equivalent to toxicity, while others make a distinction between the two because any findings attributed to exaggerated pharmacology might have a mechanistic explanation. In either case, workshop participants consider the findings to be adverse.

- Use existing data from other studies or generate new data?

Mechanistic data are often used to try to explain adverse DART findings, and are considered useful by regulatory authorities. Mechanistic studies usually are performed on a case-by-case basis.

Is developmental toxicity observed with maternal toxicity an adverse effect? There are well-known cases (e.g., fetal alcohol syndrome, effects of maternal smoking, and exposure to methyl mercury) where some degree of maternal toxicity may occur with adverse embryo–fetal effects. In other cases, desirable pharmacologic effects in humans (e.g., anticoagulant therapy) may lead to adverse effects in normal animals during preclinical testing. In each of these cases, the fetal effects would be considered adverse.

Workshop participants believe that there is a difference between scientific and regulatory needs concerning hazard identification and mechanistic information. Companies that produce commodity chemicals pick them on the basis of physical/chemical properties, whereas pharmaceutical compounds are usually selected based on biological activity. Regulatory participants in the workshop discussion emphasized that Sponsors usually conduct mechanistic studies to explain/dismiss adverse DART findings. In the case of pharmaceuticals, the findings would be included in the label if no scientifically justified mechanistic explanation could show that the findings were irrelevant to human health.

Several workshop participants expressed the opinion that mechanistic data would be needed to support any proposed cause–effect relationship between maternal toxicity and developmental toxicity. Pair-feeding (and pair-watering) studies were discussed as a possibility for addressing the effect of decreased food intake on maternal body weight in cases where that was the only

parameter that constituted ''maternal toxicity,'' but that this model would require validation. Some participants also noted that many companies contract DART studies but may not want to conduct the mechanistic studies necessary to address the role of maternal toxicity in observed embryo–fetal effects. Factors influencing industry decisions include meeting strict compound development timelines, regulatory testing timelines, or resource constraints. A comprehensive evaluation of all affected fetal parameters is essential in determining whether a compound is a developmental toxicant, regardless of any potential influence of maternal toxicity. Some attendees believe that effects on fetal body weight and skeletal ossification are not consistently taken into consideration. Other workshop participants consider decreased fetal body weight that occurs with decreased maternal food intake to be a secondary effect; such an outcome would not be considered evidence of developmental toxicity.

- If decreased fetal body weight is due only to decreased maternal food consumption, is the compound a developmental toxicant?

Decreased fetal body weight that occurs secondary to decreased maternal food consumption, with no other adverse maternal findings, generally would not be considered evidence of compound-specific developmental toxicity. However, such a conclusion must be based on experimental evidence demonstrating reduced fetal body weight in those litters from mothers with decreased food consumption (i.e., examination of the direct relationship based on data from individual animals, not on group mean data).

Pair-feeding studies were discussed as a way to provide valuable information about the role of decreased maternal food consumption in causing decreased fetal body weights. A number of investigators have conducted feed restriction studies to evaluate the potential effects of maternal body weight loss or decreased weight gain on embryo–fetal development (Lederman and Rosso, 1981; Matuzawa et al., 1981; Clark et al., 1986; Petrere et al., 1993; Solomon et al., 1997; Cappon et al., 2005; Fleeman et al., 2005). These studies generally reported only a reduction in fetal body weight with associated skeletal ossification delays, and generally were not associated with malformations, even at levels causing up to 15% maternal body weight loss. Abortion was observed in rabbits following marked feed restriction (i.e., 15 g feed/day). There is less correlation between decreased maternal weight and decreased fetal body weight in rabbit studies because the study design includes a relatively long treatment-free period following the dosing period (e.g., after GD6-19) where fetal weights normally increase rapidly during the last trimester of gestation (Cappon et al., 2005). Rodent studies have a relatively short treatment-free period following the dosing period (e.g., after GD6-17 for rats). It was suggested that pair-watering should be used in pair-feeding studies, as fluid intake has important consequences for body weight and physiologic homeostasis.

As fetal body weight is usually considered the most sensitive developmental endpoint in developmental toxicity assays, variance in fetal body weight was discussed. Control fetal weights (and those from other groups, if they were unaffected by treatment) are expected to have relatively low variability. A significant dosage-related increase in some common effects (variations) may be a signal for developmental toxicity.

Regarding data interpretation, fetal body weights at the NOAEL in an embryo–fetal toxicity study (e.g., in mice) may be lower than those of controls. While these decreased fetal body weights may not be statistically significant relative to controls, they may be toxicologically meaningful. In such cases, the true developmental NOAEL would be lower than might otherwise be concluded if this is not taken into consideration.

What additional maternal endpoints would help construct a weight-of-evidence approach to determine compound relationship to developmental toxicity? Maternal body weight was considered one of the most important endpoints for environmental chemicals, but not necessarily for pharmaceuticals. This illustrates a basic difference between data packages for chemicals versus pharmaceuticals, where there is usually more information available for pharmaceuticals. All available data should be used in evaluating the cause–effect relationship of test articles for developmental toxicity.

Some participants consider that a compound is a developmental toxicant if fetal body weight is decreased while maternal food intake is also decreased. It is the responsibility of the Sponsor to prove that decreased maternal food intake was responsible for the changes in fetal body weight. The ultimate recipients for developmental toxicity information are patients and physicians for pharmaceuticals and workers/consumers for chemicals, not regulatory authorities. Therefore, it is essential to not only identify a DART hazard but also to fully explain any cause–effect and risk–benefit relationships. Accordingly, the effects of maternal toxicity need to be separated from those due to developmental toxicity.

For pharmaceuticals, it was proposed that an exposure margin approach would be one way to avoid the issue of maternal toxicity as a confounder. Under the revised ICH M3(R2) guidelines for general toxicology studies, a 50-fold exposure margin over human exposure is considered adequate. As noted previously, the exposure margin approach is not currently recognized for DART studies under ICH.

What data are needed to better elucidate a potential causal relationship between maternal and developmental toxicity to aid in risk assessment?

- Weight-of-evidence approach?
- Mechanistic data? What kind?

From a regulatory standpoint for hazard identification, mechanistic studies do not always help unless they can demonstrate that a finding in DART studies is not relevant for human risk assessment. Several participants expressed the opinion that the mechanism of developmental toxicity is of no consolation to mothers of malformed children. In such a view, developmental toxicity is still apparent even if it is due to maternal toxicity. From a regulatory perspective, the presence of maternal toxicity and developmental toxicity does not change the level of concern for human risk. It is essential to demonstrate that a developmental effect is not relevant to humans and not simply that it is due to maternal toxicity.

Would a more detailed evaluation of maternal toxicity improve the classification process based on the performance of a developmental toxicity study in only one species? There is a fundamental difference in safety information obtained for pharmaceuticals versus chemicals because exposure data are usually available for pharmaceuticals, but rarely for chemicals. Therefore, it is believed that two species are necessary to address interspecies pharmacokinetic differences in predicting human risk, especially for chemicals. Where sufficient data are available, especially for pharmaceuticals, it may be possible to use one species to predict human risk. In addition, only one relevant species may be available for some biologics.

If a test article is a clear teratogen in one species, it may not be necessary to test in a second species. However, if no developmental toxicity is observed in one species, a second species should be tested.

CONCLUSIONS AND RECOMMENDATIONS

While the goal of developmental toxicity testing is one of hazard identification, the application for risk–benefit purposes seems to depend on the test setting (i.e., academic, industry, or government lab), industry (e.g., chemical vs. pharmaceutical), and regulatory authority. Hazard identification for chemicals places less emphasis on maternal toxicity as a confounding variable relative to the observation of developmental toxicity, regardless of the cause. For pharmaceuticals, there is a greater emphasis on requiring mechanistic explanations of cause–effect relationships for maternal toxicity as an explanation for observed developmental toxicity, especially malformations. However, there is a general expectation that Sponsors should provide appropriate and convincing mechanistic evidence showing that developmental toxicity observed in animal models is not relevant for risk assessment consideration for human exposures to the test article (pharmaceutical) or test material (chemical) in question. For chemicals, physical/chemical properties and limited maternal data (e.g., clinical signs, body weight, and food consumption) are usually available to evaluate potential maternal toxicity, whereas knowledge of the chemical structure, pharmacodynamic and pharmacokinetic properties, and other biologically based information are frequently available for evaluating maternal toxicity parameters in nonclinical studies with pharmaceuticals.

The following recommendations are based on the outcome of the discussions at the workshops:

1. A comprehensive evaluation of all available data from general toxicity studies, range-finding DART studies, class effects, structure–activity relationships, exposure studies, etc. is essential for good dose selection for definitive DART studies. The intent is to avoid marked maternal toxicity leading to mortality or decreased body weight gains of $>20\%$ for prolonged periods.

   (a) For biotherapeutics, where the maternal effects may range from classic toxicity to no toxicity, it is advisable to evaluate alternative endpoints for dose selection and data interpretation (e.g., target tissue effects and pharmacology).

   (b) Consider evaluating additional maternal parameters based on effects and/or target organs observed in short-term (e.g., 2- or 4-week) general toxicity studies.

2. Evaluate all available data to determine a cause–effect relationship for developmental toxicity.

   (a) Consider conducting a pair-feeding/pair-watering study as a follow-up.
   (b) Include an evaluation of the individual data demonstrating maternal toxicity in the mother with adverse embryo–fetal outcomes in the litter associated with the affected mother.
   (c) Consider conducting single-dose studies at increasing doses as a complement to conventional embryo–fetal toxicity studies for certain classes of compounds that affect the hERG channel.

3. Sponsors should support statements that embryo–fetal effects are caused by maternal toxicity and/or exaggerated pharmacology, especially in the case of malformations.

   (a) Provide mechanistic or other supporting data.
   (b) Establish the relevance of the DART findings in animals for human exposures.

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REFERENCES

Abrahamsson C, Palmer M, Ljung B, et al. 1994. Induction of rhythm abnormalities in the fetal rat heart. A tentative mechanism for the embryotoxic effect of the class III antiarrhythmic agent almokalant. Cardiovasc Res 28:337–344.

Cappon GD, Fleeman TL, Chapin RE, Hurtt ME. 2005. Effects of feed restriction during organogenesis on embryo-fetal development in the rabbit. Birth Defects Res B Dev Reprod Toxicol 74:424–430.

Chernoff N, Setzer RW, Miller DB, et al. 1990. Effects of chemically induced maternal toxicity on prenatal development in the rat. Teratology 42:651–658.

Chernoff N, Rogers EH, Gage MI, Francis BM. 2008. The relationship of maternal and fetal toxicology bioassays with notes on the biological significance of the "no observed adverse effect level". Reprod Toxicol 25:192–202.

Christian MS, Laskin OL, Sharper V, et al. 2007. Evaluation of the developmental toxicity of lenalidomide in rabbits. Birth Defects Res B Dev Reprod Toxicol 80:188–207.

Clark RL, Robertson RT, Minsker DH, et al. 1984. Diflunisal-induced maternal anemia as a cause of teratogenicity in rabbits. Teratology 30:319–332.

Clark RL, Robertson RT, Peter CP, et al. 1986. Association between adverse maternal and embryo-fetal effects in norfloxacin-treated and food-deprived rabbits. Fundam Appl Toxicol 7:272–286.

Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.

Danielsson BR, Reiland S, Rundqvist E, Danielson M. 1989. Digital defects induced by vasodilating agents: relationship to reduction in uteroplacental blood flow. Teratology 40:351–358.

Danielsson BR, Danielson M, Reiland S, et al. 1990. Histological and in vitro studies supporting decreased uteroplacental blood flow as explanation for digital defects after administration of vasodilators. Teratology 41:185–193.

Danielsson MK, Danielsson BR, Marchner H, et al. 1992. Histopathological and hemodynamic studies supporting hypoxia and vascular disruption as explanation to phenytoin teratogenicity. Teratology 46:485–497.

Danielsson BR, Sköld AC, Johansson A, et al. 2003. Teratogenicity by the hERG potassium channel blocking drug almokalant: use of hypoxia marker gives evidence for a hypoxia-related mechanism mediated via embryonic arrhythmia. Toxicol Appl Pharmacol 193:68–176.

Danielsson BR, Hohansson A, Danielsson C, et al. 2005. Phenytoin teratogenicity: hypoxia marker and effects on embryonic heart rhythm suggest an hERG-related mechanism. Birth Defects Res A Clin Mol Teratol 73:46–153.

Danielsson BR, Danielsson C, Nilsson M. 2007. Embryonic cardiac arrhythmia and generaton of reactive oxygen species: common teratogenic mechanism for I_{Kr} blocking drugs. Reprod Toxicol 24: 42–56.

European Public Assessment Report on revlimid. August 14, 2008; updated May 17, 2010. Available at: http://www.ema.europa.eu/humandocs/Humans/EPAR/revlimid/revlimid.htm

Fawcett LB, Buck SJ, Brent RL. 1998. Limb reduction defects in the A/J mouse strain associated with maternal blood loss. Teratology 58: 183–189.

Fleeman TL, Cappon GD, Chapin RE, Hurtt ME. 2005. Effects of feed restriction during organogenesis on embryo-fetal development in the rat. Birth Defects Res B Dev Reprod Toxicol 74:42–49.

Franklin JB, Brent RB. 1964. The effect of uterine vascular clamping on the development of rat embryos three to fourteen days old. J Morphol 115:273–290.

Garofano A, Czernichow P, Breant B. 1998. Postnatal somatic growth and insulin contents in moderate or severe intrauterine growth retardation in the rat. Biol Neonate 73:89–98.

Grabowski C. 1970. Embryonic oxygen deficiency—a physiological approach to analysis of teratological mechanisms. In: Woollam DHM, editor. Advances in teratology. Vol. 9. London: Logos Press Ltd. p 125–169.

Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals, M3(R2). International Conference on Harmonization (ICH). Step 4 version, June 11, 2009.

Guidance to Industry: Detection of Toxicity to Reproduction for Medicinal Products & Toxicity to Male Fertility, S5(R2). International Conference on Harmonization (ICH). Amended Step 5 version, November 2005.

Hales CN, Barker DJ, Clark PM, et al. 1991. Fetal and infant growth and impaired glucose tolerance at age 64. Br Med J 303:1019–1022.

Hansen D, Lou HC, Olsen J. 2000. Serious life events and congenital malformations: a national study with complete follow-up. Lancet 356:875–880.

He J-Q, Ma Y, Lee Y, et al. 2003. Human embryonic stem cells develop into multiple types of cardiac myocytes: action potential characterization. Circ Res 93:32–39.

Hood RD, Miller DB. 2006. Maternally mediated effects on development. In: Hood RD, editor. Developmental and reproductive toxicology, a practical approach. 2nd ed. Boca Raton: CRC Press. p 93–124.

Hurtt ME, Cappon GD, Browning A. 2003. Proposal for a tiered approach to developmental toxicity testing for veterinary pharmaceutical products for food-producing animals. Food Chem Toxicol 41:611–619.

Jaeggi E, Laskin C, Hamilton R, et al. 2010. The importance of the level of maternal anti-Ro/SSA antibodies as a prognostic marker of the development of cardiac neonatal lupus erythematosus a prospective study of 186 antibody-exposed fetuses and infants. J Am Coll Cardiol 55:2778–2784.

Källen B, Otterblad Olausson P. 2006. Antidepressant drugs during pregnancy and infant congenital heart defects. Reprod Toxicol 21:221–222.

Källen BA, Otterblad Olausson P, Danielsson BR. 2005. Is erythromycin therapy teratogenic in humans? Reprod Toxciol 20:209–214.

Karlsson M, Danielsson BR, Nilsson MF, et al. 2007. New proposals for testing drugs with I_{Kr}-blocking activity to determine their teratogenic potential. Curr Pharm Des 13:2979–2988.

Kavlock RJ, Chernoff N, Rogers EH. 1985. The effect of acute maternal toxicity on fetal development in the mouse. Teratog Carcinog Mutagen 5:3–13.

Khera KS. 1984. Maternal toxicity—a possible factor in fetal malformations in mice. Teratology 29:411–416.

Khera KS. 1985. Maternal toxicity: a possible etiological factor in embryo-fetal death and fetal malformation of rodent-rabbit species. Teratology 31:129–153.

Khera KS. 1987. Maternal toxicity in humans and animals: effects on fetal development and criteria for detection. Teratog Carcinog Mutagen 7:287–295.

Klinger G, Merlob P. 2009. SSRIs and heart defects in neonates. Br Med J 339:b4288.

Langley-Evans SC. 2006. Developmental programming of health and disease. Proc Nutr Soc 65:97–105.

Lederman SA, Rosso P. 1981. Effects of obesity, food restriction and pregnancy on fetal and maternal weight and on body composition in rats. J Nutr 111:2162–2171.

Leguizamón G, Igarzabal ML, Reece EA. 2007. Periconceptional care of women with diabetes mellitus. Obstet Gynecol Clin North Am 34:225–239.

Marks TA, Terry RD. 1996. Developmental toxicity of ibutilide fumarate in rats after oral administration. Teratolgoy 54:157–164.

Martin PL, Breslin W, Rocca M, et al. 2009. Considerations in assessing the developmental and reproductive toxicity potential of biopharmaceuticals. Birth Defects Res B Dev Reprod Toxicol 86: 167–203.

Matuzawa T, Nakata M, Goto I, Tsushima M. 1981. Dietary deprivation induces fetal loss and abortion in rabbits. Toxicol 22:255–259.

Merlob P, Birk E, Sirota L, et al. 2009. Are selective serotonin reuptake inhibitors cardiac teratogens? Echocardiographic screening of newborns with persistent heart murmur. Birth Defects Res A Clin Mol Teratol 85:837–841.

Nilsson MF, Danielsson C, Sköld AC, et al. 2010. Astemizole: Improved methodology for identifying the teratogenic potential in early drug development of hERG channel blocking drugs. Reprod Toxicol 29:156–163; Epub 2010 Feb 6.

Organization for Economic Cooperation and Development (OECD). 2001. OECD Test Guideline for the Testing of Chemicals. 414 Prenatal Developmental Toxicity Study. Available at: http://oberon.sourceoecd.org/vl = 5117074/cl = 13/nw = 1/rpsv/cgi-bin/fulltextew.pl?prpsv = /ij/oecdjournals/1607310x/v1n4/s14/p1.idx

Organization for Economic Cooperation and Development (OECD). 2007. Draft guidance document on mammalian reproductive toxicity testing and assessment. OECD Environment, Health and Safety Publications, Series on Testing and Assessment, No. 43. Available at: http://www.oecd.org/dataoecd/5/61/39813058.pdf

Painter RC, Roseboom TJ, Bleker OP. 2005. Prenatal exposure to the Dutch famine and disease in later life: an overview. Reprod Toxicol 20:345–352.

Petrere JA, Rohn WR, Grantham LE, Anderson JA. 1993. Food restriction during organogenesis in rabbits: effects on reproduction and the offspring. Fundam Appl Toxicol 21:517–522.

Podymow T, August P. 2007. Hypertension in pregnancy. Adv Chronic Kidney Dis 14:178–190.

Polak S, Wisnowska B, Brandys J. 2009. Collation, assessment and analysis of literature in vitro data on hERG receptor blocking potency for subsequent modeling of drugs' cardiotoxic properties. Appl Toxicol 29:183–206.

Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

Rogers JM, Chernoff N, Keen CL, Daston GP. 2005. Evaluation and interpretation of maternal toxicity in Segment II studies: issues, some answers, and data needs. Toxicol Appl Pharmacol 207: S267–S374.

Rooseboom T, de Rooij S, Painter R. 2006. The Dutch famine and its long-term consequences for adult health. Early Hum Dev 82: 485–491.

Schardein JL. 2000. Chemically induced birth defects. 3rd ed. New York: Marcel Dekker, Inc.

Sköld AC, Danielsson BR. 2000. Developmental toxicity of the class III antiarrhythmic agent almokalant in mice. Adverse effects mediated via induction of embryonic heart rhythm abnormalities. Arzneimittel-Forchung 50:520–525.

Sköld AC, Danielsson BR. 2001. Developmental toxicity in the pregnant rabbit by the class III antiarrhythmic drug sotalol. Pharmacol Toxicol 88:34–39.

Sköld AC, Wellfelt K, Danielsson BR. 2001. Stage-specific skeletal and visceral defects of the I_{Kr}-blocker almokalant: further evidence for teratogenicity via a hypoxia-related mechanism. Teratology 64: 292–300.

Sköld AC, Danielsson C, Linder B, Danielsson BR. 2002. Teratogenicity of the I_{Kr}-blocker cisapride: relation to embryonic cardiac arrhythmia. Reprod Toxicol 16:333–342.

Smith CA. 1947. The effect of wartime starvation in Holland upon pregnancy and its product. Am J Obstet Gynecol 53:599–608.

Smyth A, Oliveira GH, Lahr BD, et al. 2010. A systematic review and meta-analysis of pregnancy outcomes in patients with systemic lupus erythematosus and lupus nephritis. Clin J Am Soc Nephrol 0:CJN.00240110v1–CJN.00240110.

Solomon HM, Wier PJ, Fish CJ, et al. 1997. Spontaneous and induced alterations in the cardiac membranous ventricular septum of fetal, weanling, and adult rats. Teratology 55:185–194.

Spence SG, Vetter C, Hoe C-M. 1994. Effects of the class III antiarrhythmic dofetilide (UK-68,798) on the heart rate of midgestation rat embryos, in vitro. Teratology 49:282–292.

Stein AD, Ravelli AC, Lumey LH. 1995. Famine, third-trimester pregnancy weight gain, and intrauterine growth: The Dutch Famine Birth Cohort Study. Hum Biol 67:135–150.

US Environmental Protection Agency (EPA). 1991. Guidelines for developmental toxicity risk assessment. Fed Regist 56:63798–63826.

US Environmental Protection Agency (EPA). 1998. Health effects test guidelines. OPPTS 870.3700. Prenatal Developmental Toxicity Study. Available at: http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series870.htm

US Food and Drug Administration (FDA), Center for Food Safety and Applied Nutrition. 2000. Toxicological principles for the safety assessment of food ingredients. *Chapter IV.C.9.b. Guidelines for Developmental Toxicity Studies*, Redbook 2000.

Vickers MH, Breier BH, Cutfield WS, et al. 2000. Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nuturition. Am J Physiol Endocrinol Metab 279:E83–E87.

Wang L, Feng ZP, Kondo CS, et al. 1996. Developmental changes in the delayed rectifier K^+ channels in mouse heart. Circ Res 79:79–85.

Webster WS, Abela D. 2007. The effect of hypoxia in development. Birth Defects Res C Embryo Today 81:215–228.

Webster WS, Brown-Woodman PD, Snow MD, Danielsson BR. 1996. Teratogenic potential of almokalant, dofetilide, and d-dotalol: drugs with potassium channel blocking activity. Teratology 53:168–175.

Wehner NG, Shopp G, Oneda S, Clarke J. 2009. Embryo/fetal development in cynomolgus monkeys exposed to natalizumab, an α4 integrin inhibitor. Birth Defects Res B Dev Reprod Toxicol 86: 117–130.

Weintrob N, Karp M, Hod M. 1996. Short- and long-range complications in offspring of diabetic mothers. J Diabetes Complicat 10:294–301.

Wellfelt K, Sköld AC, Wallin A, Danielsson BR. 1999. Teratogenicity of the class III antiarrhythmic drug almokalant. Role of hypoxia and reactive oxygen species. Reprod Toxicol 13:93–101.

Yoshida T, Kanamori S, Hasegawa Y. 1988. Hyperphalangeal bones induced in rat pups by maternal treatment with nifedipine. Toxicol Lett 40:127–132.