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Developmental Toxicity: Extrapolation Across Species

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Summary: There is a pervasive lack of understanding about the definition of a developmental toxicant and the experimental design necessary to differentiate between maternal toxicants and those uniquely, i.e., selectively, hazardous to the conceptus. Thus, the acceptance of the adult-to-developmental (A/D) ratio and its ability to identify those compounds uniquely hazardous to the conceptus has been compromised, as has the extrapolation of hazard across species. The failure to establish a shared understanding is discussed, together with the role of an in vitro screen for determining the A/D ratio. The addition of an environmental exposure factor permitting the extrapolation of hazard assessment across species and the estimation of potential human developmental toxicity is detailed. **Key Words:** Developmental toxicology—Adult-to-developmental ratio—Hazard assessment—Extrapolation across species.

As more women enter the workplace and are exposed to chemicals with unknown toxicities, their reproductive capacity must be protected at least as well as is that of the male. Women may bring a third population, the conceptus, to the workplace. Its normal development will depend to some extent on maternal avoidance of chemical exposures that may place the embryo at risk of abnormal development. To avoid such situations, it is essential to become informed and aware of not only those toxicants uniquely hazardous to the conceptus, but also those that interfere with development only at adult toxic levels. It is essential to make such assessments based on results of animal experiments and to react properly to them.

To achieve these goals, it is appropriate to evaluate current approaches to an assessment of developmental toxicity. A careful exploration must be made of the relationship between animal and human developmental toxicity and consideration given to the degree of similarity of hazard evaluation ratios across species. By these means one can approach estimation of potential human developmental toxicity that includes both hazard assessment, i.e., nature or target organ predilection of the chemical, and also human adult toxicity of thresholds.

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BASIC CONSIDERATIONS

Developmental toxicology is "the study of adverse effects on the developing conceptus from prior to conception through the time of sexual maturation. The major manifestations of developmental toxicity are death/resorption, malformation, runting and/or functional deficit" [U.S. Environmental Protection Agency (EPA), 1986, p. 34030]. Each of these manifestations of developmental toxicity is of equal importance and equal value; therefore, runting (retarded growth) is just as important as malformations when determining the potential of a test article to be a developmental toxicant. As a generalization, it is true that most known human developmental toxicants produce similar effects/types in at least one animal model. However, at the outset of testing, one is not immediately aware of just which animal model is the most similar to humans, and animal/human mimicry cannot be relied upon in a prospective manner.

The types of developmental effects produced in one species may not be exactly the same as those in another exposed to the same chemical, and damage to an animal conceptus indicates the potential for damage to the human conceptus. For instance, consideration of developmental toxicity, rather than malformations alone, would have revealed a dosage-dependent increase in the resorption rate of rats exposed to thalidomide and thus would have raised concerns. Instead, at least in part, because no malformations were seen, it was assumed that there was no human risk. This emphasis on malformations (teratology), with limited consideration of exposure severity, still exists today, promoting the false concept that animal studies are a waste of resources. Some believe that animal models are poor surrogates for humans, exhibiting little similarity in developmental toxicity, as evidenced by the >600 animal "teratogens" in contrast to only 20 or so in humans.

DEVELOPMENTAL TOXICANT VERSUS TERATOGEN

The pervasive lack of understanding about the definition of a developmental toxicant (read "teratogen") has complicated the understanding and acceptance of an experimental design necessary for establishing mammalian developmental toxicity. There are three basic tenets that must be understood and accepted before proceeding to a discussion of any experimental design. First, almost any compound can be teratogenic (read "can become a developmental toxicant"). Karnofsky (1965) stated: "It is proposed as a law, which cannot be disproved, that any drug administered at the proper dosage, at the proper stage of development to embryos of the proper species—and these include both vertebrates and invertebrates—will be effective in causing disturbances in embryonic development" (p. 185). If the dosage, timing, and species are right, developmental toxicity will be produced; thus, every compound has the potential to be a developmental toxicant. Second, if a pregnant animal (human) is exposed to toxic levels of a chemical, the conceptus should also be considered exposed. The adult may recover with no signs of damage, but the conceptus may not be able to withstand or fully recover from the insult. Third, the best technique for the production of malformations is to expose the pregnant animal to a pulse of exposure, i.e., a massive dose for 1–3

days. Such a treatment period is too short to produce obvious maternotoxicity, but the dose level is so high that the conceptus cannot avoid the toxicity and cannot recover.

The key question to ask of a true measure of developmental toxicity is: What would happen if such dosage levels were continued throughout organogenesis? Either the pregnancy would be interrupted, or the dam would become debilitated or die. However, such an experimental design would create an overly complicated testing procedure because numerous 1- to 3-day intervals would be required with multiple exposure levels. To obviate this problem, the generally accepted testing method is to begin maternal exposure at the time of embryonic implantation (the start of organogenesis) and terminate exposure after the palatal shelves fuse (the end of major organogenesis). Such a study is often still called a teratology evaluation [Food and Drug Administration (FDA), 1966, Segment II, Teratology Study], even though this is not the most effective way to produce terata. U.S. EPA guidelines (1986) refer to this evaluation as a test of developmental toxicity. The International Conference on Harmonization (ICH) guidelines (1993) emphasize flexibility and provide guidelines that are not mandatory but rather starting points whose primary objective is to bring to light any indication of reproductive toxicity.

The basic EPA design is outlined in Table 1 (U.S. EPA, 1986). In this type of

TABLE 1. *Developmental toxicity testing protocol*

Phase	Time	Developmental toxicity testing
Acclimation period	Variable number of weeks	No exposure of animals to test chemical
Cohabitation period	Day of mating determined	No exposure of animals to test chemical
Preembryonic period of pregnancy	Day of mating through day 6[a] or 7[b] of pregnancy	No exposure of animals to test chemical
Period of major embryonic organogenesis	Day 6 or 7 through day 15[a] or 18[b] of pregnancy	Groups of pregnant animals exposed to test agent[d]
Fetal period	Day 15 or 18 through day 21[a] or 30[b] of pregnancy	No exposure of pregnant animals to test chemical
Term	Day 22[a] or 31[b] of pregnancy	Sacrifice females (to preclude cannibalization of malformed fetuses) and examine young externally and internally
Lactation and early nurture	Delivery through day 21[c]	N/A

Modified from Johnson (1990).

[a] Rats.

[b] Rabbits.

[c] Postpartum in rats only.

[d] Usually a sham-treated control group and three agent-treated groups are used with 20–25 rats and 15–18 rabbits per group. The dose-levels are chosen with the goal of no maternal or developmental effects in the low-dose group and at least maternal toxicity in the high-dose group (failure to gain or loss of weight during dosing, reduced feed and/or water consumption, increased clinical signs or no more than 10% maternal death).

testing, at least the high-dose exposure must produce signs of materno-toxicity, but no more than ~10% maternal deaths. Such maternotoxicity should be expected to produce developmental toxicity (except in rare circumstances such as with formaldehyde or perhaps xylene in some species). Maternotoxicity is described as altered maternal homeostasis: reduced weight gain at least for an interval during dosing or reduced feed and/or water consumption, death of no more than 10% of the high-dose animals, and/or an increased incidence and severity of clinical signs of distress. If designed properly, the middle dose in such a protocol will produce neither maternal nor fetal affects unless the compound is a selective developmental toxin, affecting the conceptus at exposure levels below those that produce maternotoxicity, e.g., thalidomide.

PROTOCOL PARAMETERS

While Table 1 presents an outline of a developmental toxicity study in rats and rabbits, it does not present the extent of the maternal and fetal data that must be gathered from such a study. To adequately determine maternal toxicity, clinical signs of distress, body weight, and feed consumption must be determined daily during the dosage and postdosage periods. These data are presented as average body weights for each dosage group on days 0 (day of mating), 6 (first day of dosing), and daily thereafter until sacrifice. Maternal absolute feed consumption (g/day) and relative feed consumption (g/kg/day) are reported in the same manner. The data must be collated over these short intervals because often a reduction in weight or feed consumption lasts for <1 week and is masked by a rebound phenomenon that is not apparent when data are collected, examined, and presented only in weekly intervals. No matter how sophisticated the determination of maternal toxicity may become, it must be measured with sufficient frequency to avoid questions of its appearance (or disappearance) during an interval in which the measurement was not taken. The importance of care in the measurement of this parameter cannot be overemphasized.

Failure to consistently evaluate and report maternal weight gain can lead to unnecessary confusion and anthropogenically create confusion when data are compared among tests in multiple species. For example, Solomon et al. (1993) demonstrated, in a study employing four groups of rats exposed to methyl methacrylate, that the interval used to measure body weight changes (ascertainment interval) strikingly altered the statistically significant maternal effect level. When maternal weight gain was measured between days 6 and 20 of pregnancy, a statistically significant maternal effect was present only in the group exposed to 2,028 ppm. With days 6–16 ascertainment, statistical significance existed at 1,178 ppm. When the ascertainment period for maternal weights was days 6–8 of pregnancy (first 2 days of exposure), statistical significance existed down through 304 and 99 ppm.

On the day prior to anticipated maternal delivery, the dams are killed and necropsied, and an evaluation of their reproductive tract and its contents is made. The ovaries of each female are examined for number of corpora lutea, and the uterus is excised and examined for the number and placement of implantation

sites, early and late resorptions, and live and dead fetuses. Uteri from the females that appear nonpregnant are stained or otherwise examined to more fully determine pregnancy status. An early resorption is defined as one in which organogenesis is not grossly evident; a late resorption is one in which the occurrence of organogenesis is grossly evident. Dead fetuses and late resorptions are differentiated by the degree of autolysis present, with marked to extreme autolysis indicating a late resorption. Such data permit the determination of preimplantation loss (number of corpora lutea minus number of implantation sites) and allow it to be separated from postimplantation loss (number of implants minus the number of resorptions and dead pups) induced by the test compound. Live fetuses are weighed, sexed, and examined for runting and external, visceral, and skeletal alterations, which include both malformations and variations.

Malformations are defined as permanent structural changes that may adversely affect survival, development, or function (U.S. EPA, 1986). Other manifestations of developmental toxicity, if significantly altered, are of equal value to malformation. Variations are defined as minor, spontaneous, structural deviations from the normal that may not adversely affect survival or health (U.S. EPA, 1986). Variations may occur as a result of delayed growth (runting) or altered ossification associated with maternal toxicity. This phenomenon is most often observed in the high-dose offspring, if the dosage altered maternal homeostatis. Variations may also be an indicator of embryotoxicity if they show a dose–response relationship either alone or in association with an increased frank malformation rate.

METHODS OF RISK/HAZARD IDENTIFICATION

The most common method for use of data such as those outlined is to divide the no-observed-adverse-effect level (NOAEL) of the most sensitive relevant animal model by 100 to devise a human permissible exposure level. The first 10× reduction is considered to allow for potential interspecies variability and the second 10× to allow for potential intraspecies (human) variability. In instances where the data are inadequate or partially absent, an additional 1–10× reduction may also be made as a modifying factor.

These data can be used also for hazard detection. From guideline-compliant and fully repeatable studies, it is possible to evaluate the degree of hazard potential (priority of concern for developmental toxicity) a chemical may represent. To achieve this, an adult-to-developmental (A/D) ratio can be derived from the standard adult and developmental endpoint assays of effect levels. The NOAELs for the dam and conceptus obtained from a standard protocol (Table 1) are used. The larger a chemical's A/D ratio, the greater is the uniqueness of the embryos' vulnerability to it. For example, 13-*cis*-retinoic acid (Accutane) has an A/D ratio of ~1, indicating that the compound is toxic to the conceptus only at a level that is maternotoxic. On the other hand, thalidomide has an A/D ratio of >30, indicating that this compound is uniquely toxic to the conceptus, damaging its development at dosage levels that are not toxic to the dam.

The A/D ratio is said to show remarkable uniformity from one study to another across the mammalian species used in this type of safety evaluation (Johnson,

1984; Johnson et al., 1982), primarily because the ratio is based on the concept of target organ toxicity and therefore more a function of chemical nature than it is the species tested or the route of exposure. Furthermore, the concept of A/D ratio, which has a notable tendency to be similar between mammalian species, can be predicted in vitro by the fresh water coelenterate *Hydra attenuata*. In this assay, free-living artificial hydra "embryos" are produced from dissociated cells of adult hydra and are exposed to the test compound, as are the adults, according to the methods described by Johnson and co-workers (Johnson et al., 1982; Johnson and Gabel, 1982, 1983) and modified by Mayura et al. (1991). The A/D ratio is calculated in the hydra assay by dividing the adult minimal affective concentration (MAC) by the developmental MAC, both of which are based on standard and objective endpoints of toxicity that occur within a 90- to 92-h period of exposure.

The hydra assay is not intended to be the definitive assessment of developmental toxicity, but rather to assist in prioritization of the need for further study of a compound in mammalian species (Johnson, 1987). It is $\geq$90% accurate in identifying compounds that are uniquely hazardous to the conceptus, and its errors are almost exclusively false positives (Johnson et al., 1988).

It is not surprising that doubts have been expressed concerning the constancy of the A/D ratio calculated across species (Daston and D'Amato, 1989; Daston et al., 1989, 1991; Rogers, 1987; Rogers et al., 1988; Schardein, 1987). However, good examples of different ratios for the same chemicals tested in standard evaluations are rare or nonexistent. In the references cited, there was a notable yet subtle redefinition of how a ratio was calculated, for the same care establishing maternotoxicity was not taken in those studies as is necessary to develop and compare A/D ratios (Jensen et al., 1989; Johnson et al., 1989; Johnson and Newman, 1992); the problem of prescreening for developmental toxicity remains unchanged since it was first clearly spelled out (Johnson, 1980).

Whereas some false positives and false negatives are to be expected, the studies to date where such was supposedly demonstrated are from submammalian forms, i.e., South African clawed frog, fathead minnow, and fruit fly (Daston et al., 1989, 1991). The experimental designs of these studies were quite inadequate to the stated goal of examining the predictability of A/D ratios across mammalian species because of the selection of submammalian endpoints. Other studies (Rogers, 1987; Rogers et al., 1988) reported that in response to the fungicide dinocap, the mouse had an A/D ratio of ~16, whereas the rat and hamster had A/D ratios of 1. However, these authors used erroneously calculated "ratios" from studies in hamsters and rats that had themselves failed to identify an accurate NOAEL for either the mothers or the fetuses. In summary, most of the questions raised so far concerning the constancy of the A/D ratio across species are iatrogenic, arising from inadequate mammalian developmental toxicity protocols in which insufficient data were collected to permit accurate determination of the adult NOAEL.

Maternal weights obtained only at the beginning of dosing and at termination of the pregnancy do not permit determination of an accurate maternal NOAEL. Studies of submammalian forms in which toxic endpoints were not selected on the same basis as were those in the hydra assay will not produce similar A/D ratios.

It is expected that as greater attention is given to the design of mammalian

developmental toxicity protocols, more data will become available from which adult and developmental NOAELs can be derived with greater precision. As preliminary screening techniques evolve, more time should be spent reducing interlaboratory artifacts, and more emphasis placed on the combination of screening procedures while establishing a developmental hazard index, as was reported recently by Mayura et al. (1991). These scientists determined the A/D ratios of selected chlorinated phenols utilizing *H. attenuata* and confirmed these results via extracorporeally maintained postimplantation rat embryos in culture. They concluded from their studies that the toxicity of chlorinated phenols was apparently due to increased chlorine substitution and that (with the possible exception of 3, 5-C_2 P) these compounds are developmental hazards, but only at maternotoxic levels.

HAZARD EXTRAPOLATION ACROSS SPECIES

It must be emphasized that the in vitro techniques for developmental toxicity evaluations can be used only for screening procedures intended to direct attention to those compounds with the greatest developmental toxicity selectivity, i.e., the highest A/D ratios, because these agents may have the greatest potential to injure a conceptus without producing maternal toxicity. As such, the A/D ratio is an effective tool for ranking agents for further testing, but it lacks an environmental exposure component, which is the other paramount consideration of any developmentally toxic exposure.

It is essential to quantify exposure levels in a manner that normalizes them across species, thereby permitting both better predictive ability and extrapolation of hazard assessment across species. This can be achieved if one accepts the idea that there is an exposure level for any given chemical that is toxic to the dam (mother). As the exposure to a chemical increases, a threshold will be exceeded and overt maternal toxicity will occur; i.e., the maternal Systemic effects Toxicity Threshold (SeTT) has been exceeded (Johnson and Newman, 1990). Each chemical has its individual toxic threshold, but human exposure to it can be normalized by determining what percentage of the human maternal SeTT level a particular exposure represents. Thus, the SeTT score of maternal homeostasis provides the second major essential of a developmental toxicity ranking system and normalizes toxic effect levels of one chemical with another.

To estimate potential human developmental toxicity in such a ranking system requires that the A/D ratio be combined with the SeTT score of maternal homeostasis (A/D $\times$ SeTT score). The A/D ratio permits the nature of a chemical to be considered while classifying its potential as a selective developmental toxin. The SeTT score represents the percentage of the maternally toxic level of the chemical exposure in question. Thus, by multiplying the A/D ratio by the SeTT score, the two major considerations in developmental toxicity hazard detection are considered equally (relative selective developmental toxicity potential times the severity of maternal exposure).

The vast majority of the >600 animal developmental toxicants achieved developmental toxicity only because the maternal SeTT score approximated 100. The

major difference between the large number of animal "teratogens" and the relatively few human teratogens is exposure of pregnant animals, at an SeTT score of 100 with A/D ratios near unity. These concepts are used to estimate potential human developmental toxicity of contrasting examples as follows:

13-*cis*-Retinoic acid (Accutane)		
A/D ratio (animal)	=	1
SeTT score (human)	=	100
i.e., the effective human dosage produces signs of hypervitaminosis A toxicity		
Developmental toxicity derivative	=	100
Thalidomide		
A/D ratio (animal)	=	⩾30
SeTT score (human)	=	5 (estimated)
Developmental toxicity derviative	=	⩾150

A human developmental toxicant, therefore, is either a chemical selectively toxic to embryogenesis (large A/D ratio), or it is a chemical with a low A/D ratio occurring at exposure levels near the adult toxic (SeTT) level. Determinations of developmental toxicity that include and consider both agent nature and dose severity avoid the casual and dangerous labeling of a compound as a "teratogen."

Protocols for determining developmental toxicity and examining mechanisms of abnormal embryogenesis are not static, nor should they be. Denial of their value in protecting the human conceptus from developmental toxicants is more a reflection of misunderstanding and misplaced expectations than it is of the failure of developmental toxicologists to think beyond the original experimental design.

The existing U.S. EPA (1986) and ICH (1993) guidelines have much to recommend them, particularly if the investigator is willing to show due diligence and the reviewer unwilling to accept substandard efforts. A/D ratios determined from inadequate efforts to establish maternotoxicity should be suspect, as they are unlikely to be similar across species. It behooves those who use animal data to demand of them all that they can contribute to our understanding rather than accept an incompletely executed or reported study.

Can the sensitivity of the standard in vitro testing protocol(s) be increased? When the data are available, a pharmacokinetic and metabolic profile in both humans and a reasonable human surrogate would greatly assist in clarifying an animal species to be relied upon, as is strongly suggested in the ICH guidelines (1993). A more accurate NOAEL and a more sharply defined A/D ratio would be achieved if four or five dosage levels of the chemical being studied were tested rather than only three. It would be a significant improvement if there were more guidance and acceptance of what constitutes maternal toxicity, because much of the integrity of the A/D ratio depends upon a consistent and accepted evaluation of maternal toxicity.

In the U.S. EPA developmental toxicity guidelines (1986) and the FDA guidelines (1966, Segment II), treatment stops at the end of organogenesis, prior to the

development of organ function, essentially eliminating any effect of the test compound upon this developmental endpoint. The ICH guidelines (1993) provide much more flexibility in their design. In addition, evidence is accumulating that suggests that it is possible to damage the zygote and not only interfere with implantation, but also produce a variety of skeletal alterations (Rutledge and Generoso, 1991; Polifka et al., 1991). Fetal exposure is also known to affect postnatal development.

EPIDEMIOLOGY

The purpose of animal experimentation is primarily to provide information that may be extrapolated to other species including humans. However, adequate assessment in animals of the chemical/environmental interactions within human existence will continue to elude us because of their infinite variations. Epidemiology is best suited to examine this problem. No matter how accurately the developmental toxicologists provide dose–response relationships, improve the assessment of hazard prior to human exposure, or extend the ability to extrapolate animal data to humans, epidemiologists will be essential to any assessment of human developmental toxicity. Epidemiology is ideally suited to an examination of those often delayed developmental toxicities that manifest themselves as subtle central nervous system damage, transplacentally induced carcinogenesis, or premature aging and death, none of which can be adequately determined by the developmental toxicity protocols currently used. Identification of effects of unsuspected chemical or chemical/environmental interactions, particularly of nonpharmaceuticals, when combined with such human variables as age, sex, nutritional status, and genetics requires constant vigilance. The costs of such studies will never decrease, but improved animal studies and more accurate species extrapolation should permit sharper focus of the epidemiologists' efforts.

SUMMARY AND CONCLUSIONS

The assessment of developmental toxicity is not an art, it is a science. As such, it will continue to apply new techniques and new interpretations to the information on mammalian development, thereby increasing the accuracy of hazard assessment and decreasing the risk to the human conceptus. It is up to the scientists working in this discipline to make use of these new techniques, apply good testing procedures, and correctly interpret and apply basic concepts such as A/D ratios. There is no excuse for individuals to ignore or modify a protocol and then claim their results do not match those published previously by others. Developmental toxicologists must remain alert and open to the information provided by other biological scientists, just as the public must gain a better understanding of the value of animal data for reducing human risk of disrupted development.

REFERENCES

Daston GP, D'Amato RA. (1989) In vitro techniques in teratology. *Toxicol Ind Health* 5:555–85.

Daston GP, Rogers JM, Sabourin TD, Versteeg DJ (1989) Interspecies comparison of A/D ratios [Abstract]. *Toxicologist* 9:32.

Daston GP, Rogers JM, Versteeg DJ, Sabourin TD, Baines D, Marsh SS. (1991) Interspecies comparisons of A/D ratios: A/D ratios are not constant across species. *Fundam Appl Toxicol* 17:696–722.

Food and Drug Administration. (1966) *Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use*. Washington, D.C.: FDA.

International Conference on Harmonization (ICH). (1993) International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use.

Jensen M, Newman LM, Johnson EM. (1989) Problems in validation of in vitro developmental toxicity assays [Letter]. *Fundam Appl Toxicol* 13:863–5.

Johnson EM. (1980) Screening for teratogenic potential: are we asking the proper questions [Letter]? *Teratology* 21:259.

Johnson EM (1984) A prioritization and biological decision tree for developmental toxicity safety evaluations. *J Am Coll Toxicol* 3:141–7.

Johnson EM. (1987) A tier system for developmental toxicity evaluations based on considerations of exposure and effect relationships. *Teratology* 35:405–27.

Johnson EM. (1990) The effects of ribovirin on development and reproduction: a critical review of published and unpublished studies in experimental animals. *J Am Coll Toxicol* 9:551–61.

Johnson EM, Gabel BEG. (1982) Application of the hydra assay for rapid detection of developmental hazards. *J Am Coll Toxicol* 1:57–71.

Johnson EM, Gabel BEG. (1983) The role of an artificial embryo in detecting potential teratogenic hazards. *Handbook Exp Pharmacol* 65:335–48.

Johnson EM, Gorman RM, Gable BEG, George ME. (1982) The *Hydra attenuata* system for detection of teratogenic hazards. *Teratogen Carcinogen Mutagen* 2:263–76.

Johnson EM, Newman LM. (1990) Application of the hydra assay and its attendant concepts to considerations of maternal toxicity in developmental toxicity safety evaluation. Proceedings of the Society of Toxicology of Canada, November 28–29, 1989, Montreal, Quebec.

Johnson EM, Newman LM (1992) Difficulties in identifying selective developmental toxicity [Letter]. *Fundam Appl Toxicol* 18:635–9.

Johnson EM, Newman LM, Fu LJ. (1989) Letter to the editor. *Toxicol Appl Pharmacol* 99:173–6.

Johnson EM, Newman LM, Gabel BEG, Boerner TE, Dansky LA. (1988) An analysis of the hydra assay's applicability and reliability as a developmental toxicity prescreen. *J Am Coll Toxicol* 7:111–26.

Karnofsky CA. (1965) Mechanisms of action of certain growth-inhibiting drugs. In: Wilson JG, Warkany J, eds. *Teratology: Principles and Techniques*. Chicago: University of Chicago Press, 185–213.

Mayura K, Smith EE, Clement BA, Phillips TD. (1991) Evaluation of the developmental toxicity of chlorinated phenols utilizing *Hydra attenuata* and postimplantation rat embryos in culture. *Toxicol Appl Pharmacol* 108:253–66.

Polifka JE, Rutledge JC, Kimmel GL, Dellarco VV, Generoso WM. (1991) Skeletal deviations in mice offspring following zygotic exposure to ethylene oxide [Abstract]. *Teratology* 43:444.

Rogers JM. (1987) Comparison of maternal and fetal toxic dose responses in mammals. *Teratogen Carcinogen Mutagen* 7:297–306.

Rogers JM, Barbee B, Burkhead CM, Rushin EA, Kavlock RJ. (1988) The mouse teratogen dinocap has lower A/D ratios and is not teratogenic in the rat and hamster. *Teratology* 37:553–9.

Rutledge JC, Generoso WM. (1991) Congenital defects resulting from mutagen treatments of the mouse zygote [Abstract]. *Teratology* 43:415.

Schardein JL. (1987) Approaches to defining the relationship of maternal and developmental toxicity. *Teratogen Carcinogen Mutagen* 7:255–71.

Solomon HM, McLaughlin JE, Swenson RE, et al. (1993) Methyl methacrylate: inhalation developmental toxicity study in rats. *Teratology* 48:115–25.

U.S. Environmental Protection Agency. (1986) Guidelines for the health assessment of suspect developmental toxicants. *Fed Regist* 51:34028–40.