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Future of developmental toxicity testing

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Abstract

Protection of developing offspring from the potential adverse effects of in utero exposures is an important aspect of the safety profile for medicines, chemicals and physical agents. The standard safety test design for this type of endpoint was developed in the mid-1960's and has changed little in the ensuing half-century. While embryo-fetal development studies have done a good job of protecting pregnant women and their babies, the tests are labor intensive, time consuming and use a large number of test animals. The advent and exploitation of the technology revolution has changed much of what we know about biology and has the potential to streamline the decision process concerning the identification of substances that may be teratogenic hazards. These welcome changes will save animals and expenses in addition to shortening the time to some decisions about whether or not to develop a new medicine or how to protect workers form potential occupational exposures. Data from informatics and computational approaches will inform hypothesis-driven studies to incisively address safety issues. Despite the impressive advances in the world of virtual biology, the anatomic, physiologic, and pharmacologic complexities of the entwined maternal-placental-fetal unit as well as the varied potential mechanisms for compensation in reaction to challenges are not yet able to be sufficiently well-modeled to enable a conclusion of safety. For at least the next few decades, the pregnant mammal will remain the final linchpin in teratology testing.

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In order to discuss the future of developmental toxicity testing, it is important to briefly review the history of this relatively new science. In particular, the trajectory of various trends (scientific, regulatory, and political) over the past few decades can provide some indication of the outlook for developmental toxicity testing over the next one or two decades.

Prior to the rise of the information age and the advent of computers and the internet, the general public and much of the medical community believed that human offspring developed within a shielded habitat provided by the uterus. This privileged location was judged to be a fortress against the assault of toxic environmental exposures. This concept was falsified abruptly and dramatically during the period of 1959–1961 when as many as 10,000–12,000 babies whose mothers had taken the seemingly innocuous morning sickness medication thalidomide were born with severe heart, limb and ear malformations.

This unfortunate episode became the clarion call to design and require toxicity tests that would protect pregnant women and their offspring from such tragedy. Over the next few years, the most prominent scientists who worked in the fields of reproduction and teratology participated in a series of working groups with scientists from pharmaceutical companies and the Food and Drug Administration (FDA) to design safety tests that would predict and eliminate dangerous substances before such substances would be given to pregnant women or those who might become pregnant. The results of these endeavors were captured in a 1966 document from FDA (known as the Goldenthal Guidelines) that provided guidance for the safety testing of new drugs for potential reproductive effects [1]. The contents of this document outlined a safety program for reproductive health and formed the basis for Segments I (fertility and early development), II (embryo-fetal development; teratology), and III (peri- and postnatal study) reproductive safety studies. The core concepts of these 50-year old guidelines are still being followed. This program of experiments is complex, labor intensive, time-consuming and expensive. For instance, the teratology study alone typically entailed the use of >1200 animals (including the fetuses) that are examined in detail.

Coincident with the thalidomide episode, James G. Wilson and other scientists were already investigating the causes of congenital anomalies. In 1959, Wilson assessed the state of the science at the time and published a list of 5 foundational principles of teratology. Over the next 14 years, he would edit, amend, and augment this list that was eventually published in his book, *Environment and Birth Defects* [2]. In 1960, with Josef Warkany and F. Clarke Frazier (two other prominent leaders in the nascent field of teratology), he

founded the Teratology Society. This Society gathered together those with common goal of preventing birth defects, including basic scientists from academia, clinical scientists and physicians, scientists from the pharmaceutical and chemical industries, and epidemiologists. Indeed, this eclectic assemblage served as the source for many of the members of Goldenthal's work groups.

Within the next two decades, scientists searched in vain to discover a biochemical/pharmacological/toxicologic mechanism to explain how thalidomide exerted its devastating effects. There was, however, limited success elucidating the teratologic modes of action of other molecules, and teratologists began identifying multiple mechanisms that underlay the problem of congenital malformations. This was driven in part by the inquisitive nature of university scientists and also by the needs of regulatory scientists and chemical/pharmaceutical manufacturers, both of whom were faced with an enormous (and growing) number of new chemical entities that needed to be tested with limited budgets and constrained schedules. These constraints continue to haunt those who are responsible for assuring the health and safety of pregnant women and their unborn offspring.

Among the approaches taken to attempt to understand how teratogens affected embryonic development was the identification of final common pathways. This concept was based on the observation that embryos appeared to respond to teratogenic insults in a limited number of ways that were manifested as a few discrete adverse effects (such as cell death or vascular disruption) that preceded the development of malformations.

Simultaneously, the federal government expanded its interest in protecting the public from exposure to potentially toxic substances in the environment. The United States Environmental Protection Agency (EPA) was formed in 1970 and soon became involved in the assessment of industrial chemicals and pollutants for adverse health outcomes, including teratology. The mission of the EPA greatly increased the number of chemical substances that needed to be tested.

Over the next three decades, our knowledge about developmental toxicity bourgeoned and the regulatory perspective regarding the assessment of developmental toxicity data broadened. We came to understand that development is not confined to the prenatal period and that we must be aware of exposures during any stage of development. We also discovered that adverse impacts of exposures during development can be manifested much later during one's lifetime and may take the form of functional changes that are not apparent based on visual observation of the animals only (e.g., [3]). Additionally, the Agencies that are charged with regulating substances used for distinct and diverse purposes often approach developmental toxicity findings in different ways. Thus, the allowable exposures for a life-saving medicine may differ from the exposures allowed for an environmental contaminant or a food additive. Overall, this situation has resulted in a great expansion in the number of industrial and agricultural chemicals that need to be tested and the complexity of the test designs to be used such as the extended one-generation reproductive toxicity test (OECD 443) with its multiple arms for assessing endocrine and immunological endpoints [4].

While the design of the whole animal developmental toxicity tests appeared to have been effective in protecting us from another major episode of preventable birth defects, the system is not perfect. It is costly in terms of funding, animals and effort. Attempts to streamline this system resulted in several creative new test systems that were advanced with the hope of accelerating the time needed to complete the tests and reducing the number of agents that needed to be put through the standard series of tests. These included such methods as limb bud culture, the hydra assay, FETAX, and eventually, whole embryo culture.

Each of these types of testing systems had differences from a pregnant mammal. Limb bud cultures involved the growth in culture medium of a limb bud (or limb bud cells) that had been removed from rat embryos [5,6]. The limb bud culture lacked an operating vascular system, but was able to develop cartilaginous rudiments of the limb skeleton; nevertheless, the rudiments were not normal in appearance and ossification did not take place, which limited its ability to predict generalized developmental toxicity. Hydrae are freshwater coelenterates that regenerate when dissociated by re-aggregating into "artificial embryos" [7,8]. This test system was quick and inexpensive, but the animals are invertebrates and lack a relevant mammalian metabolizing system, which diminished their relevance for intact mammals. The FETAX system is based on the tetraploid South African clawed frog (*Xenopus laevis*) [9,10]. While this system involves vertebrate embryos, it also lacks both a maternal presence/placenta and a mammalian metabolism system. The whole embryo culture system meets many of the challenges presented by the previous systems (e.g., [11,12]). It is typically a rat or mouse conceptus that is removed intact from the uterus at day 9 or 10 of gestation and cultured in carefully adjusted medium for 72 h or longer. The time of culturing covers most of the period of organogenesis. However, nutrition comes from the inverted yolk sac membrane (a structure that does not exist in humans) by means of diffusion from the culture medium. The maternal influence is not present, nor is an operating chorioallantoic placenta. Mammalian metabolism has been added to the system by the inclusion of preparations such as the S9 fraction of a homogenate from (usually) rat liver. All of the above mentioned test systems have exposure concentrations and durations that