



# REPROTOX®

## IN A NUTSHELL



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### Why Experimental Animal Studies?

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A patient asks you whether her exposure to a medication during pregnancy will hurt the baby. You go to REPROTOX® or another good source of information. The only available data come from rats and mice. How can you use information from rats and mice when you are counseling men and women?

You might think that only human studies can give useful data on human risk. But, what if there are no human data, or what if there are human data that you can't interpret? We don't test new drugs in pregnant women, so there are no human data for new drugs or for recently introduced chemicals. Even when products have been around a long time, human data may raise questions that experimental animal studies can clarify (see Table).

#### Reasons for experimental animal studies

There are two reasons to do

reproduction studies in laboratory animals.

The Food and Drug Administration and the Environmental Protection Agency may require experimental animal studies prior to approval of a drug or chemical for marketing. These regulatory studies are performed according to detailed guidelines, which have been standardized to meet the requirements of regulatory agencies in several countries. But even before a company submits regulatory studies to a government agency, it may use these studies to decide how much time and money to invest in a potential new product. For example, a company may have a series of related compounds that might be worth developing as pesticides. Experimental animal studies may be used to decide which appear to be the least toxic and therefore the most promising.

The second reason to do experimental animal studies is to

evaluate mechanisms of toxicity and to answer specific questions about the effects of a drug or chemical. For example, exposing pregnant rodents to ethanol causes abnormal embryo development, as does heavy alcohol use in humans. Investigators wanted to know whether ethanol itself causes embryo damage or whether a metabolite such as acetaldehyde causes the damage. By administering acetaldehyde to pregnant animals in amounts similar to the exposures expected after drinking ethanol, it could be determined that the developmental effects of ethanol were largely due to the ethanol itself rather than to metabolites. These mechanistic studies are useful in understanding the action of existing chemicals or in designing new, safer chemicals.

#### How are experimental animal studies done?

Let's say that a prospective new

Problem with human data	Experimental Animal Solution
1. Not enough reproducing people exposed	1. Studies on adequate number of animals
2. Imprecise dose estimates (people don't always know their exposures)	2. Controlled dosing
3. The presence of disease (for example, a study on insulin might be confounded by the effects of diabetes,)	3. Use of healthy animals
4. Other confounding factors (cigarettes, alcohol, nutrition)	4. Controlled exposures and diet
5. Genetic variability	5. Homogeneous animal strains
6. Imprecise determination of outcomes, which may come from birth certificates or incomplete medical records	6. Uniform, detailed evaluation of birth defects and other end points

medication is being tested in rats for adverse pregnancy effects. How do investigators decide how much medication to give and when to give it?

It isn't clear how best to dose a rat to make it a good model for effects in humans. If the planned human dose of a new medicine is 2 mg/kg (about 120 mg in a pregnant woman), will the same weight-adjusted amount (about 0.5 mg in a rat) be the right dose? The answer depends on how the rat absorbs, distributes, and metabolizes the medication.

In order to test a reasonable dose range, preliminary studies test what dose will cause toxicity in an adult rat. It is common to aim for a dose that produces a small decrease in the amount of weight gained by a pregnant female. This dose is selected in a subsequent developmental toxicity study as the highest dose to be tested, because the investigator knows that, no matter how the rat absorbs and metabolizes the chemical, this dose has been shown to have at least some effect in the species. The lowest dose tested is one at which there is no toxicity, and is often similar to the dose planned for humans based either on body weight, surface area, or serum concentration. A third dose, between these two doses, is also selected. In this way, the new chemical can be tested over a range of doses extending from the clearly nontoxic to the clearly toxic.

The dosing period is selected based on the reproductive events of interest. For pregnancy studies, dosing often begins with the onset of pregnancy, although older designs began dosing on about day 6 to permit implantation to occur without interference. Other designs include treatment for some weeks prior to mating (to evaluate fertility effects) or for three weeks after delivery (to evaluate lactation

effects). Multigeneration tests are also performed, in which parents are exposed from before mating through pregnancy and lactation, and then offspring are exposed until they, too, grow up and mate.

The end points in these experiments depend on the study type. If congenital anomalies are the focus of the experiment, the offspring are delivered by hysterotomy on the last day of pregnancy and are dissected in detail. Hysterotomy is used rather than natural delivery because rodent mothers will eat abnormal young, preventing them from being evaluated. Other end points in a teratology study include the number of offspring in a litter and their weights. Fertility studies will use different end points. For example, females may be evaluated 2 weeks after mating for the number of implantations in the uterus. Normally, a lab rat will have a litter of 10 or more offspring after a single mating. Fewer implanted embryos in the uterus may mean there was an effect of treatment on fertility, or that early embryos died before implantation. After implantation, embryos may die and be resorbed by the uterus; resorption is the rodent analog of miscarriage. After treatment of male animals, sperm can be evaluated for number, motility, and morphology. Histologic evaluation can be performed on the gonads and reproductive organs in both sexes.

### **Are these studies reliable?**

We have about 50 years' experience with the routine use of experimental animal studies in reproductive toxicology. Do these studies protect our patients?

It is not easy to come up with a report card on these tests. Here's why:

*1. No test can categorize compounds as safe or unsafe.* People who want animal tests to give an answer like "teratogenic" versus

"nonteratogenic" are disappointed. Toxicity always depends on dose. X-ray exposure, for example, increases the risk of abnormal human embryo development at 50 rads or higher. Exposure to lower doses (equivalent to most diagnostic x-rays) does not increase the risk of abnormal development. So x-ray, independent of dose, is neither teratogenic nor nonteratogenic.

*2. Past studies gave little attention to internal dose.* When a chemical is given to a pregnant rat by stomach tube, it must be absorbed to have a chance of reaching the embryo. In addition, the chemical may need to be metabolized to an active form. In the past, scientists paid attention only to the dose put into the animal's stomach or injected into its peritoneal cavity, not the amount or form of the chemical that got to the embryo. This problem led to the erroneous belief that thalidomide is not teratogenic in rats. In fact, thalidomide is not well absorbed by the pregnant rat when the chemical is given by stomach tube. If thalidomide is injected intravenously in pregnant rats, malformations occur in the offspring just as they do in rabbits, monkeys, and humans.

*3. Experimental animal study results are applicable only to the end points that are evaluated.* When pregnant rodents are dosed with a chemical throughout pregnancy, organ systems that develop after birth may not have an opportunity to be affected. Kidney and genital development, for example, occur after birth in rodents, and medications (ACE inhibitors, estrogens) that affect these systems need to be given during the neonatal period in order to be tested completely. Some experimental study designs, therefore, may miss important effects.

In spite of these limitations, the fact remains that all exposures known to cause abnormal embryo

development in humans have experimental animal models. (In some cases, models were developed only after a human effect had been suspected, because the experimental animal model needed to be designed to include exposure during a sensitive time period).

In fact, rather than underestimating risk, experimental animal studies may overestimate it. Sometimes when an experimental animal study suggests a risk of toxicity after an exposure, there may not be a risk at all.

Why do these studies raise a false alarm about risk? It may be because such high doses, much higher than human exposures, are given to experimental animals. In the face of such high doses, maternal illness may cause abnormal pregnancy outcome, or unreasonably large amounts of the test chemical may be delivered to the embryo. It is a basic principle of toxicology that all substances are poisonous if taken in a high enough dose.

### **Can experimental animal studies be used in counseling?**

Experimental animal studies can *always* be used in counseling. Even when there are human data, the experimental animal studies can be considered as supportive. After all, the pregnant rat is a complex biologic system, with genetic and physiologic processes that have parallels to those of humans. If there is a discrepancy in the sensitivity of two species to the action of a chemical, the scientific response is to look for an explanation for the discrepancy, not just to ignore it.

Often, experimental data are the only data available for use in counseling. How can we best counsel people if the only available data are from experimental animal studies?

Because all exposures that affect embryo development in humans

have also been shown to do so in experimental models, we can get a sense of the risk of an exposure to humans based on experimental data. FDA regulations call for pregnancy testing of drugs in at least two species, one of which is not a rodent. For most medications, testing is performed in rats and rabbits. No medications have spared embryo development in two species and then caused damage in human embryos. Counselors don't guarantee the safety of any drug, but medication that has been adequately tested and not found to produce adverse embryo effects is unlikely to have surprise adverse effects in humans. Concerned consumers may be reassured to know that a medication found to be uniquely harmful in human pregnancy would be big news, indeed.

When interpreting adverse effects that have been reported in experimental animals, the type of effect and the exposure level at which it occurred are important. If the adverse effect is a small decrease in fetal weight in the face of a decrease in maternal weight gain, one interpretation is that the lack of congenital anomalies is reassuring, given that the maternal-fetal system has been sufficiently stressed that anomalies ought to have appeared. Delayed ossification of fetal bones also signifies stress on the maternal-fetal unit, not abnormal development.

If there are frank malformations in the experimental animal study, the kind of malformation can be meaningful. Some strains of laboratory mice are susceptible to cleft palate, and clefting in the presence of severe maternal stress is common. If food consumption and pregnancy weight gain are substantially decreased in the mothers after treatment with a high dose of the test chemical, cleft palate in the offspring may be a consequence of the stress. Rats may respond to

similar stresses with anophthalmia.

Other malformations can have a different meaning. Limb reduction defects in rodents are often a sign that embryo development has been disrupted. This kind of abnormality is of particular concern if it occurs at doses that are relatively low compared to maternally toxic doses and compared to anticipated human exposure levels. In fact, a medication that produces dose-related limb abnormalities in experimental animal studies is unlikely to be brought to market.

### **Risk and Uncertainty**

Counseling is a matter of communicating risk and uncertainty. Risk and uncertainty come in degrees; it is unusual to have a risk that is absolute or uncertainty that is absolute. Consider how you might counsel a pregnant woman who has been exposed to a medication for which there are human data showing risk. Let's take as examples therapy with phenytoin, associated with about a 10% risk of major congenital malformation, valproic acid, for which the risk is about 1%, and lithium, for which the risk is somewhere between zero and 1%, depending on whom you ask. In each case, there is substantial uncertainty about whether the particular pregnancy at issue will be affected: counseling gives only a likelihood estimate, not a yes or no answer. There may be antenatal tests that reduce uncertainty, but only the birth of the baby resolves all questions.

When a medication has been studied only in experimental animals, there may be substantially more uncertainty than when the medication has been *well-studied* in humans, but substantially less uncertainty than when the medication has not been studied at all. Consider these two hypothetical cases:

1. Medication A has been used in

10 human pregnancies. One baby had a post-auricular skin tag. The other 9 were normal. Experimental animal studies showed an increase in neural tube defects in mice at maternally toxic doses.

2. Medication B has been tested in mice, rats, and rabbits up to a dose that caused a 10% decrease in maternal weight gain. There were no increases in adverse pregnancy outcome (malformations, fetal death, growth impairment, and so on). There are no human data.

Which medication is safer? The answer is, we don't know for sure. But we recognize that the 10 human cases for Medication A don't make it safer or better. The experimental animal data for Medication B are far more reassuring than they are for Medication A. We know of no medications like Medication B that turned out to produce birth defects in humans when used in therapy; the same cannot be said for Medication A.

The key to counseling may be to give up the idea that we are supposed to tell people which exposures are safe and unsafe. Safe and unsafe are the answers people may want, but they are usually not the answers that science can give. Instead of trying to come up with a black-or-white answer (safe or unsafe), we should try to do a better job of communicating risk and uncertainty, the subject of the next *Reprotox in a Nutshell*.

REPROTOX® in a Nutshell is a supplement to the REPROTOX program for residents. Make sure all your colleagues have registered for their free access to REPROTOX. Contact Tony Scialli ([ascialli@sciences.com](mailto:ascialli@sciences.com)).

In future issues:

Risk and uncertainty

What's so important about dose-response relationships?

How do birth defects occur?

What about workplace exposures?

We would love to hear your ideas for articles—[ascialli@sciences.com](mailto:ascialli@sciences.com).