EXPERIMENT : 10

TERATOGENESIS

Teratology is the study of environmentally induced congenital anomalies. A teratogen is an agent, which by acting on the developing embryo or fetus, can cause a structural anomaly. To date, very few drugs are proven teratogens. However, malformations induced by drugs are important because they are potentially preventable.

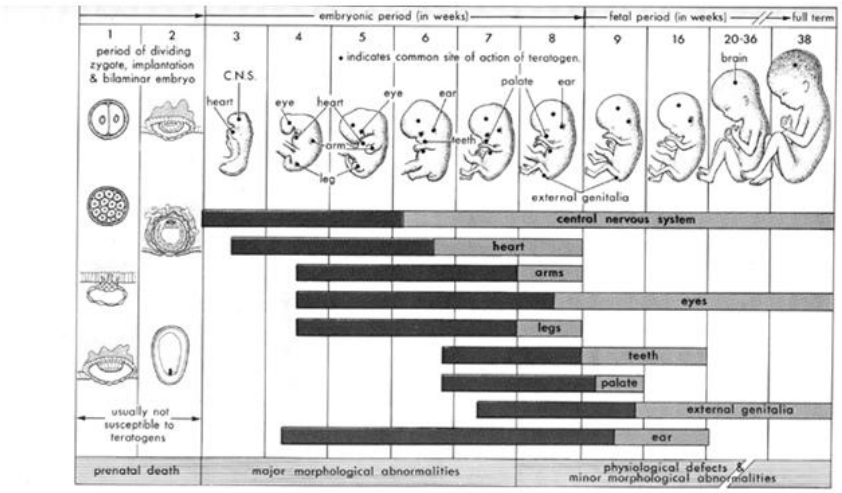

Figure.1 Critical periods of human development during which time teratogens are most effective. Black areas show the most critical spans of time and denote the major morphological abnormalities most likely to occur

Timing of embryonic and foetal development

The effect produced by a teratogenic agent depends upon the developmental stage in which the fetus is exposed to the agent. Several important phases in human development are recognized:

- The time from conception until implantation known as the "all or none" period, when insults to the embryo are likely to result in death of the conceptus and miscarriage (or resorption), or in intact survival. At this stage, the embryo is undifferentiated and repair and recovery are possible through multiplication of the still totipotential cells to replace those which have been lost. Exposure of embryos to teratogens during the preimplantation stage usually does not cause congenital malformations, unless the agent persists in the body beyond this period.

- The embryonic period, from 18 to 54-60 days after conception is the period when the basic steps in organogenesis occur. This is the period of maximum sensitivity to teratogenicity since not only are tissues differentiating rapidly but damage to them becomes irreparable. Exposure to teratogenic agents during this period has the greatest likelihood of causing a structural anomaly. Since teratogens are capable of affecting many organ systems, the pattern of anomalies produced depends upon which systems are differentiating at the time of teratogenic exposure.

- The foetal phase, from the end of the embryonic stage to term, is the period when growth and functional maturation of organs and systems already formed occurs. Teratogen exposure in this period will affect foetal growth (e.g., intrauterine growth retardation), the size of a specific organ, or the function of the organ, rather than cause gross structural anomalies. The term foetal toxicity is commonly used to describe such an effect. Of particular interest is the potential effect of psychoactive agents (e.g., antidepressants, antiepileptics, alcohol and other drugs of abuse) on the developing central nervous system, which has led to a new field of behavioural teratology.

Many organ systems continue structural and functional maturation long after birth. Most of the adenocarcinomas associated with first trimester exposure to the synthetic estrogen, diethylstilbestrol, occurred many years after the exposure (transplacental carcinogenesis).

The FDA has established five categories to indicate the potential of a drug to cause birth defects if used during pregnancy. The categories are determined by the reliability of documentation and the risk to benefit ratio. They do not take into account any risks from pharmaceutical agents or their metabolites in breast milk. The categories are:

Table 1. FDA Drug Risk Classification

Category	Description
A	Controlled studies in humans show no risk to the fetus
B	No controlled studies have been conducted in humans; animal studies show no risk to the fetus
C	No controlled studies have been conducted in animals or humans
D	Evidence of human risk to the fetus exists; however, benefits may outweigh risks in certain situations
X	Controlled studies in both animals and humans demonstrate fetal abnormalities; the risk in pregnant women outweighs any possible benefit

Category A
Adequate and well-controlled studies have failed to demonstrate a risk to the fetus in the first trimester of pregnancy (and there is no evidence of risk in later trimesters).

Category B
Animal reproduction studies have failed to demonstrate a risk to the fetus and there are no adequate and well-controlled studies in pregnant women.

Category C
Animal reproduction studies have shown an adverse effect on the fetus and there are no adequate and well-controlled studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential risks.

Category D
There is positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience or studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential risks.

Category X
Studies in animals or humans have demonstrated fetal abnormalities and/or there is positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience, and the risks involved in use of the drug in pregnant women clearly outweigh potential benefits.

Some Known Teratogens

• **Radiation**	• **Infections**	• **Maternal & Metabolic Imbalance**
Atomic weapons	Cytomegalovirus	Alcoholism
Radioiodine	Herpes simplex virus I and II	Amniocentesis, early (before day 70 post conception)
Therapeutic radiation	Parvovirus B-19 (Erythema infectiosum)	Chorionic villus sampling (before day 60 post conception)
• **Drugs and Environmental Chemicals**	Rubella virus	Cretinism, endemic
ACE inhibitors (benazepril, captopril, enalapril, fosinopril, lisinopril, moexipril, quinapril, ramipril, trandolapril)	Syphilis	Diabetes
	Toxoplasmosis	Folic acid deficiency
	Varicella virus	Hyperthermia
	Venezuelan equine encephalitis virus	Myasthenia gravis
		Phenylketonuria
		Rheumatic disease
	Possible Teratogens	Sjögren's syndrome
	Binge drinking	Virilizing tumors
Aminopterin	Carbamazepine	
Androgenic hormones	Colchicine	
	Disulfiram	**Unlikely Teratogens**
Busulfan	Ergotamine	Agent Orange
Chlorobiphenyls	Glucocorticoids	Anesthetics
Cigarette Smoking	Lead	Aspartame
Cocaine	Primidone	Aspirin (but aspirin in the 2nd half of pregnancy may increase cerebral hemorrhage during delivery)
Coumarin anticoagulants	Quinine (suicidal doses)	
	Streptomycin	
Cyclophosphamide	Vitamin A (high doses)	Bendectin® (antinauseant)
Diethylstilbestrol	Zidovudine (AZT)	Electromagnetic waves
Etretinate	Zinc deficiency	Hydroxyprogesterone
Fluconazole (high doses)		LSD
		Marijuana
Iodides		Medroxyprogesterone
Isotretinoin (Accutane)		Metronidazole
		Oral contraceptives
Lithium		Progesterone
Mercury, organic		Rubella vaccine
Methimazole		Spermicides
Methotrexate (methylaminopterin)		Video display terminals
		Ultrasound
Methylene blue (via		

intraamniotic injection) Misoprostol Penicillamine Phenytoin Tetracyclines Thalidomide Toluene (abuse) Trimethadione Valproic acid		

Principles of Teratology

Teratogens act with specificity in that they produce specific abnormalities at specific times during gestation. For example, thalidomide produces limb phocomelia, while valproic acid and carbamazepine produce neural tube defects. Other teratogens are associated with recognizable patterns of malformations, for example, phenytoin with foetal hydantoin syndrome and coumarin anticoagulants with foetal warfarin syndrome (see proven teratogenic drugs in humans for description of the above). Teratogenic specificity also applies to species, for example, aspirin and corticosteroids have been found to be teratogenic in mice and rats but appear to be safe in humans. Thalidomide, on the other hand, was not shown to be teratogenic in rats, a tragic fact that resulted in significant human morbidity.

Teratogens may demonstrate a dose-effect relationship. At low doses there can be no effect, at intermediate doses the characteristic pattern of malformations will result, and at high dose the embryo will be killed.

A dose-response may be considered essential in establishing teratogenicity in animals, but is uncommonly demonstrated in sufficient data among humans. A threshold dose is the dosage below which the incidence of adverse effects is not statistically greater than that of controls. With most agents, a dose threshold for teratogenic effects has not been determined; however they are usually well below levels required to cause toxicity in adults.

Teratogens must reach the developing conceptus in sufficient amounts to cause their effects. Large molecules with molecular weights greater than 1,000 do not easily cross the placenta into the embryonic-foetal bloodstream to exert potential teratogenic effect. Other factors influencing the rate and extent of placental transfer of xenobiotics include polarity, lipid solubility and the existence of a specific protein carrier.

Evaluation of Drugs for Potential Teratogenicity in Humans

All new drug applications are filed by the United States Food and Drug Administration (FDA) including data from animal developmental and reproductive-toxicological studies. Although major new teratogenic drugs in humans have been predicted from animal studies, there are problems in extrapolating animal data to humans. Animals have a different "gestational clock" to humans, there is marked interspecies variability in susceptibility to teratogens and no experimental animal is metabolically and physiologically identical to humans. Animal

studies are important because, in some instances, they have shed light on mechanisms of teratogenicity and because when an agent causes similar patterns of anomalies in several species, human teratogenesis should also be suspected.

For obvious ethical considerations, no studies of teratogenicity are conducted during embryogenesis in humans. The studies are, therefore, either retrospective in nature (case reports, case-series and case-control studies), or prospective cohort studies, where a specific maternal exposure in question is ascertained during pregnancy and the pregnancy outcome is evaluated and compared to a control group. Retrospective case-control studies are less costly and easier to conduct but they have other weaknesses such as the inaccuracy of data collected from medical records and recall bias. For the rare malformation/rare exposure, the case report method is commonly used to suggest association, but case reports are unable to prove or disprove teratogenicity, nor can they give estimation of teratogenic risk. Human teratogenicity is supported by:

1. A recognizable pattern of anomalies.
2. A statistically higher prevalence of a particular anomaly in patients exposed to an agent than in appropriate controls.
3. Presence of the agent during the stage of organogenesis of the affected organ system.
4. Decreased incidence of the anomaly in the population prior to the introduction of the agent.
5. Production of the anomaly in experimental animals by administering the agent in the critical period of organogenesis.

There are some important factors on applying teratogenecity tests;

- The type of the animal
- The number of the birth
- The way, the dose and the frequency of the application
- To analyze the fetus: growth, behaviour, biochemical and physiological development

Advantages of the Chick Embryo Test:

- It is important for the statistical analysis that many numbers of chicken embryos can be used for this assay
- It is economic and fast
- The stage of the development of the chick embryo is well known
- The chick embryos are very sensitive to Thalidomide as humans.
- It is a selective method

Disadvantages of the Chick Embryo Test

1) *Mammalian fertilization* and *pregnancy* are different from chick's anatomically and physiologically due to the fact that chick embryo doesn't have placenta.
2) Chick embryo is non-specifically sensitive to lots of compound resulting in false positive results.

3) There are some pharmacokinetic differences for the injected substances because of the structure of bird eggs.
Egg injection can be done by two methods:
1) The method of injection to egg sac or air sac before incubation:
The teratogenic effects of Thalidomide, pesticides and different food additives were investigated by using this method.
2) Injecting compound to embryo during incubation:
The compound whose teratojenic effect is investigated is injected to 48 hours or 72-74 hours incubated embryos, egg sac, air sac or amniotic fluid.

Optimum injection time is third and forth days to investigate teratogenic effects of medicine in chick embryo. After the injection; on the 14., 15. and 17. days of incubation, eggs are opened and examined. Teratogenic potential of compounds are evaluated according to early deaths, late deaths with or without malformations and births and lives with malformation.

Purpose of the Test

After applying an organophosphorus pesticide Diazinon to chick embryo; in the active phase of organogenesis (between second and forth days of growing), embryotoxic effects are occurred such as developmental disorders, malformation and death.

Required Tools, Materials and Solution

Fertilized chicken egg (40)	Petri dish
Methanol	Scissors
Diazinon solution	Pliers
Injector	Ruler
Pin	Weighing machine
Sticking plaster	Incubator (37.5-38 °C, %80 moisture)
Light source	Filter paper

Experimental Procedure

1. The air sac of each egg is marked by using light source due to using injection technique to their air sac. A hole is punched by tap.
2. There are 5 groups. Control and methanol groups includes 4 eggs, the others includes 8 eggs. Nothing applied to the first group. Methanol which is used as the solvent to prepare Diazinon solution is applied to the second one. Different concentrations of Diazinon solutions are applied to the third, fourth and fifth groups slowly. The injection volume is 50 µL.

Group	Number of Egg (n)	Injected Solution
1 (control)	4	0
2	4	Methanol
3	8	100 µg/mL Diazinon solution
4	8	200 µg/mL Diazinon solution
5	8	400 µg/mL Diazinon solution

3. Eggs are incubated until the 16-17. days of growing in an incubater, 37.5-38 °C - %80 moisture (14-15 days after injection).
4. Eggs are turned in the vertical direction by using spike as an axis.
5. At the end of incubation, eggs are cut along the longitudinal axis carefully by using scissors and emptied slowly to a petri dish.
6. Both the alive and dead embryos and the weight and length of the embryos are determined.
7. Malformations are investigated in alive embryos. Pubescence disorders, malformations in amniotic fluid, malformations of head structure, growth disorders might be observed.