



BIOLOGICAL TEST FOR TOXICITY

- Toxicology has been defined as the study of the effects of chemical agents on biological material with special emphasis on harmful effects. There is ample evidence to indicate that every chemical is capable, under some conditions, of producing some type of effect on every biological tissue. Toxicology tests are therefore the tests that define the conditions that must be present when a biological cell is affected by a given chemical, and the nature of the effect that is produced.



Why do toxicity testing ?

- There is a practical need to obtain as much information as possible about the effects of chemicals exposure can occur through direct industrial or domestic occupational contact, through contact with the clothes or devices you wear, the food, liquid and drugs you eat and the atmosphere you breathe.
- It is necessary to not only identify possible toxicant but also obtain assurance that the chemical soup we do live in has no direct or insidious indirect detrimental effects. SO some toxicology testing needs to be done especially on chemicals which are intentionally administered to humans such as food additives, food substitutes or drugs.




What do we use as a test subject?

- Ideally humans but this data is usually limited
- mammalian species in vivo
- non-mammalian species
- cell cultures




How do we test ?

- Since all effects of chemicals on living systems are not necessarily harmful effects, a principal function of toxicology tests is to identify clearly those chemicals capable of producing serious harm to living systems.



General principles

- In order for a chemical to produce a biological effect, it must come into immediate contact with the biological cells (or receptors) under consideration.
- For each chemical there exists a quantity below which it produces no detectable effect on all biological systems, and a quantity at which it produces a significant effect on all biological systems. Between these extremes lies the range of quantities at which each chemical will exert a significant effect on some types of biologic systems.

- 
- Cells have similar functions and similar metabolic pathways in various species generally will be affected similarly by a given chemical entity.
 - Small changes in the structure of a chemical agent may greatly influence the biological action of the agent.




Animal toxicology tests

- ▶ Acute tests
- ▶ Subtoxic
- ▶ Chronic
- ▶ Special



Special tests

- ▶ For potentiation with other chemicals
- ▶ For effects on reproduction
- ▶ For teratogenicity
- ▶ For carcinogenicity
- ▶ For mutagenicity
- ▶ For skin and eye effects
- ▶ For behavioural effects
- ▶ For immune effects



Acute Toxicity Tests

- ▶ The purpose of the test is to determine the symptomology consequent to administration of the chemical and to determine the order of lethality of the chemical
- ▶ Method:
 - Administer chemical at different doses and determine lethality of dose. Graph as dose-response curve for lethality. Determine LD50 ie dose at which 50% of test animals are killed.
 - Two species (usually rats and mice)
 - Two routes of administration (one by intended route of use)



Prolonged Toxicity Tests

- ▶ One of the objectives is to attempt to demonstrate some form of toxic effect at least in the high-dose group. If a drug, pharmacological effects are evaluated; if a food additive, prolonged toxicity test is usually followed by a chronic toxicity test.
- ▶ Method:
 - Administer chemical at a dose which can be given daily
 - Duration – 3 months, daily dosing
 - Two species (usually rats and dogs)
 - Three dose levels
 - Route of administration according to intended route of use



Chronic Toxicity Tests

- ▶ Primary reason is to demonstrate first, the absence of toxicity when the doses involved represent some practical concentration and, second, the carcinogenic potential of the chemical. Every effort is made to use the highest dose schedule for the maximum no-effect dose.
- ▶ Method:
 - Administer chemical at a dose which can be given daily
 - Duration – 2 to 7 years depending on species
 - Species – selected from results of prior prolonged tests, pharmacodynamic studies on several species of animals, possible single dose human trial studies. Otherwise use two species.
 - Minimum of two dose levels
 - Route of administration according to intended route of use



Teratogenic tests

- ▶ Primary reason is to detect dose of chemical at which abnormalities are induced if administered during pregnancy.
- ▶ Method:
 - Test chemical is administered daily
 - two species, one not a rodent
 - usually 3 doses, the highest dose is not seriously toxic to the mother and the lowest dose is without determinable effect on the mother.
 - Route of administration according to intended route of use




Reproduction tests

- ▶ Primary reason is to detect chemicals which can induce infertility.
- ▶ Method:
 - Test chemical is administered daily to group of animals from the time they are weaned
 - animals are allowed to breed (endpoint 1: fertility and mating behaviour)
 - neonates are examined (endpoint 2: foetal development)
 - mothers are allowed to rear young (endpoint 3: lactation and rearing)
 - Some of this litter is allowed to reproduce (without dosing, endpoint 4: mutagenic effects)



Mutagenicity tests

- ▶ Mutagenesis is the induction of alterations in DNA in either somatic cells or germ cells.
- ▶ Somatic cell mutations may lead to cancer
- ▶ Germ cell mutations may lead to abnormalities in the offspring or foetal death.

- 
- ▶ Submammalian species such as bacteria, are frequently preferred as large populations can be tested very rapidly. Large populations are needed in order to detect a small increase in the mutation rate. These tests can also detect what type of genetic damage is induced by the chemical i.e. Point mutation, chromosomal damage etc.
 - ▶ However, the problem is that bacteria don't metabolise chemicals in the same way as mammals. This has been partially overcome by culturing bacteria with mammalian metabolising systems eg. Liver cells.
 - ▶ The most common mammalian test is the dominant lethal assay.



Dominant lethal assay

- ▶ Method:
 - Male mice are treated with a subtoxic dose of the chemical
 - mice are mated with untreated females.
 - If a mutation occurred in his sperm then some of his offspring will be heterozygous for that mutated gene. If it is lethal then it will be noticed as an increase in foetal deaths.



Carcinogenicity tests

- ▶ First some terminology.
 - Tumour: an abnormal mass of tissue
 - Benign tumour: mass is identical to the tissue it is growing in, only occupies space i.e. does not invade surrounding tissue
 - Malignant tumour: mass is different from surrounding tissue, invades surrounding tissue, can develop secondary growth (metastases) in other parts of the body.
 - Chemical that produces tumour: tumorigenic
 - Chemical that produces malignant tumour: carcinogenic



Factors to consider

- ▶ When tested in laboratory animals, a carcinogenic agent may:
 - increase incidence of tumours seen in the normal population
 - induce the occurrence of novel tumours
- ▶ What does an increased incidence of benign tumours in the experimental animal say about the human risk of developing malignant tumours?

- Tests are interpreted in terms of effect of the chemical on the incidence of occurrence of tumours in experimental as compared to control animals.
- Most of the tests are performed over the life time of the test animals therefore rats and mice are commonly used.
- Because these tests are so long, in vitro tests have been developed as a primary screen. The most common in vitro test is the Ames Salmonella Test.

Ames Salmonella Test

- Ames developed a mutant strain of Salmonella that requires histidine to be added to its culture medium in order to grow.
- Method:
 - The mutant salmonella are grown in media without histidine.
 - Test chemical is added to growing bacteria
 - In the presence of a mutagenic chemicals, the mutant Salmonella tends to mutate back to its original strain. So if there are any colonies growing after the test, then this indicates that a mutation has occurred.

Extrapolation of test results to humans

- After these various tests are performed we will have a value at which we get a positive result in a certain number of animals. When risk assessment is based on extrapolation of data from animal experiments to humans, it is performed by making two assumptions.
- All toxicities with the exception of mutagenesis and carcinogenesis occur only when the body load of the chemical exceeds a "threshold" level.
- In the case of mutagenesis and carcinogenesis the dose-response relation is linear to a virtual zero dose; there is no threshold involved.

- Risk estimation is mandated by Federal Health legislation eg. For carcinogenicity tests, rodents must be fed the test chemical in their diet or water 5 days per week for 2 or more years ie the life time of the animal.
- The data can then be used to estimate a lifetime dose of the test chemical that is legislated as being "safe".
- For a carcinogenic chemical in humans this is usually a level which is estimated to cause one cancer in 100,000 to 1,000,000 exposed humans above the normal background rate of cancer.

Maximum Tolerated Dose

- It is not economically feasible to test chemicals at low doses because enormous numbers of animals would be required to detect statistically significant increases in cancer incidence.
- Therefore, higher doses are used to determine whether a chemical is a carcinogen.
- This dose is known as the MTD (maximum tolerated dose)
- To estimate human risk at exposures to the chemical that are frequently thousands of times lower than the MTD, a linear extrapolation is used.
- However, near-toxic doses can frequently cause cell division which itself can increase the chance of mutations and tumors.

Dose-Response Relationships

- Perhaps most controversial is the basic question of the methods used to estimate human risks from animal testing data. A solution proposed in the 1950s was to use a 100-fold safety factor. It was proposed to:
 - determine a threshold dose level, beyond which a substance was toxic for animals
 - Then divide that amount of the substance by 10 because of the assumption that some people might be more sensitive to the substance than animals.
 - then divide the smaller level by 10 again, since some people might be more sensitive than other people.
 - The result is a 100-fold lower amount of the substance as an acceptable exposure level for people.