Seminar in Occupational Medicine

Toxicology: Assessing the Hazard

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At the start of any discussion of the fundamentals of toxicology, it is necessary to make a distinction between toxicity and hazard. Toxicity is an inherent property of a compound, like its melting point or boiling point. Hazard, on the other hand, is the likelihood that a compound may cause harm under its intended use. The function of the toxicologist is not simply to determine the toxicity of a compound but rather to perform the infinitely more difficult task of assessing its hazard. How does he go about this task, and what are the things he has to take cognizance of in assessing toxicity?

Shown in Table 1 are some of the factors that a toxicologist has to consider. Was the dose large, or was it small? Was the route of administration appropriate? After all. you can easily drown in water, but that route of administration is not appropriate for assessing the hazard of drinking water or washing your hands with it. What was the frequency and duration of the administered dose or doses? What species of animals was used? In the carcinogenesis experiments on trichloroethylene reported by the U.S. National Cancer institute, liver tumors were produced in mice but not in rats. This raises the question of whether man is more like the rat, the mouse, the guinea pig, the hamster, or the pig. In terms of Vitamin C requirements, he is more like the guinea pig, which can't synthesize Vitamin C, while both the rat and the mouse synthesize Vitamin C in their intestinal tracts. On the other hand, many people believe the skin of the pig is histologically more like that of the human than other species, and have, therefore, recommended the pig for skin toxicity studies. What was the age of the animals used? This can be important because very young animals may not yet be producing certain detoxifying enzymes and, as a result, may be sensitive to some materials that do not affect adults who have these enzymes in place. What was the sex of the animals? Returning to the trichloroethylene experiment mentioned above, the male mice developed liver tumors in a statistically significant increased number of animals (about 45%) but in the female mice *(6%* liver tumors) the results were barely significant, if at all. Were the toxic effects local (such as an ulcer produced at the site of application) or were they systemic, damaging some organ (liver or kidney) distant from the site of application? Finally, were the effects acute or chronic? Thus, high concentrations of sulfur dioxide produce an acute inflammation of the trachea or bronchi from which you either die or recover fully. Repeated low concentrations may cause emphysema which is a debilitating long-lasting condition.

Dose-Response Curve

One of the first things a toxicologist does is determine a dose-response curve. A typical dose-response curve is shown in Fig I. If deaths are the response being determined. the results determine the LD_{50} of the compound, that is the dose that kills 50% of the animals. Generally the toxicologist will plot these results on log-probability paper which tends to convert the data into a straight line as shown in Fig 2. This is really just a convenience to make it easier to determine what the 50% response point is. But the toxicologist is not satisfied in knowing only the LD_{50}. He is interested in knowing the slope of the dose-response line. Thus in Fig 3, Compound 1 would be more hazardous than Compound 2, even though they both have the same LD_{50}. The reason for this is that to go from an LD_0 to an LD_{100} with Compound 1 requires a much smaller increase in dose than with Compound 2. Perhaps doubling the dose with Compound 1 may kill all the animals, but doubling it with Compound 2 may cause only a 5 or 10% increase in deaths. In assessing hazard the toxicologist wants to know the slope of his dose-response or LD_{50} line.

Genetic factors must be taken into consideration by the toxicologist as shown in Table 2. In the case of isoniazid, a well known antituberculosis drug, the Oriental acetylates it much more

Table 1 — Toxicity and Hazard.

Dose	Species	Local
Route	Age	Systemic
Frequency	Sea	Acute
Duration		Chronic

Table 2. — Genetic Factors Influencing Dose.

Accumulation of Chemical — Isoniazid Acetylation
Prolongation of Action — Succinylcholine Hydrolysis
Hypersensitivity to Drug Action — Primaquine Hemolytic Anemia

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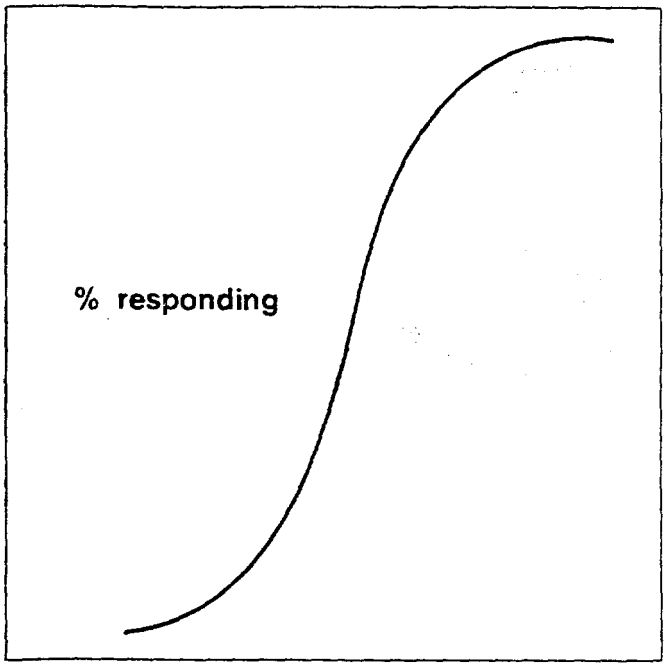

Fig 1. — Typical dose-response curve

rapidly than the Occidental Since acetvlation is a detoxifying mechanism, the Oriental is much less prone to get toxic eftects, such as liver damage or neuropathy than the Occidental Other examples of genetic factors that may influence toxicitv are shown in Table 2 In Fig 4, an attempt is made to show the influence of genetic effects on an experiment In the upper left-hand corner, numbers of deaths are plotted against dose for a genetically homogeneous population When this is plotted as cumulative deaths versus dose the usual dose-response curve shown in the upper right-hand corner is obtained If a genetically non-homogeneous population is being dealt with. it is as if there were two separate populations each responding in its own way A plot of numbers of deaths versus dose would give a curve like the one in the lower left-hand corner The initial small bump represents the genetically susceptible group, while the second large bump represents the rest of the population If this is plotted as cuinuiative deaths versus dose, a skewed dose-response curve as shown in the lower right-hand corner is obtained The toxicologist

Table 3. — Termination of Toxic Effect.
Excretion (permanent)
Metabolic transformation (increases or decreases toxicity)
Storage (deposition)

Table 4. — Classification of Toxicity Influencing Factors.
Factors Related to the Toxic Agent
Chemical composition (pH choice of anion etc)
Physical characteristics (particle size *method* of formulation etc.)
Presence of impurities *a* contaminants
Stability and staage characteristics of the toxic agent
Solubility of the toxic agent in biologic fluids
Choice of the vehicle
Presence of excipients adjuvants emulsifiers surfactants binding agents mating agents coloring agents flavoring agents preservatives antioxidants and other intentional and nonintentional additives

Table 5. — Classification of Toxicity Influencing Factors.
Factors Related to the Exposure Situation
Dose concentration and volume of administration
Route. rate and site of administration
Duration and frequency of exposure
Time of administration (time of day season of the year etc)

Table 6. — Classification of Toxicity Influencing Factors.
inherent Factors Related to the Subject
Species and strain (taxonomic classification)
Genetic status (littermate siblings multigeneration effects etc)
Immunologic status
Nutritional status (diet faclors state of hydration etc)
Hormonal status (pregnancy etc)
Age sex body weight and maturity
Central nervous system status (activity crowding handling presence of other species etc.)
Presence of disease or specific wgan pathology

must know not only what the LD_{50} is, but also what the slope and shape of the dose-response curve look like.

Influences on Toxicity

The termination of a toxic effect can occur through several mechanisms as shown in Table 3. A material may be excreted either unchanged, as much of the inhaled benzene is excreted in the expired air, or changed, as urinary phenol. The compound may undergo metabolic transformation in the body which may serve either to increase or decrease its toxicity. Vinyl chloride is not carcinogenic itself, but after it is metabolized in the body, the chloroethylene oxide metabolite is carcinogenic. Finally. materials may be removed from the circulation and stored in relatively unreactive regions of the body. DDT is stored in the fat and lead in the bones. As long as they remain in the fat or bones, they produce no harm, but if mobilized out of these storage areas at

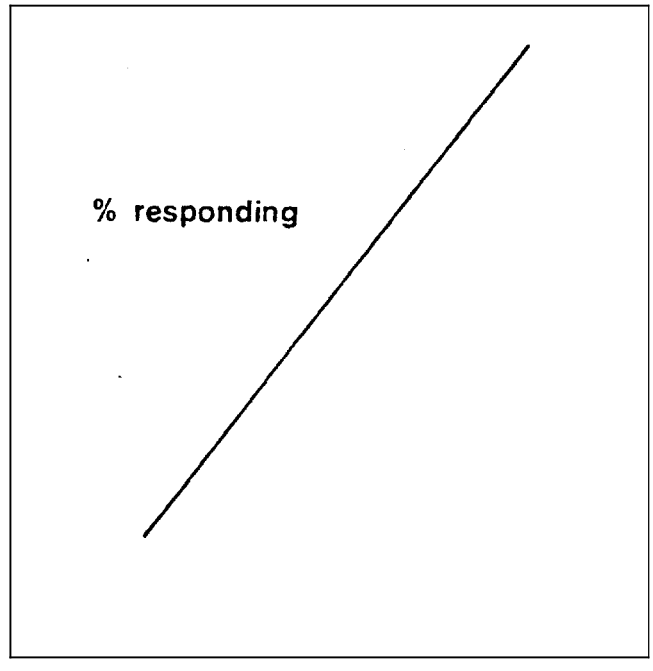

Fig 2. — Dose-response curve converted to straight line.

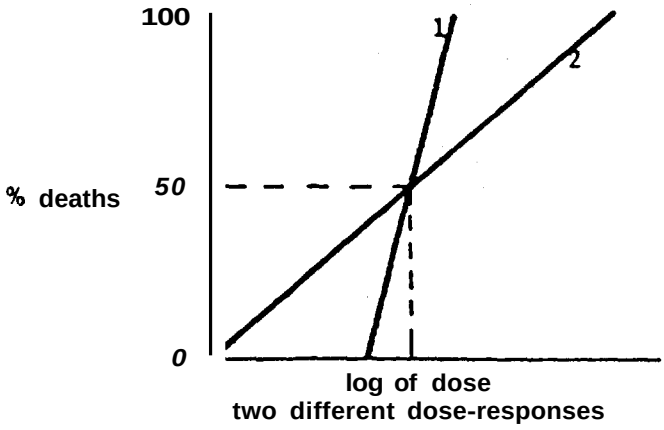

Fig 3. — Comparison of dose-response curves for 2 different compounds.

Table 7. — Classification of Toxicity Influencing Factors.

Environmental Factors Related to the Subject
Temperature and humidity
Barometric pressure (hyper- and hypobaric effects)
Ambient atmospheric composition
Light and other forms of radiation
Housing 3rd caging effects
Noise and other geographic influences
Social factors
Chemical factors

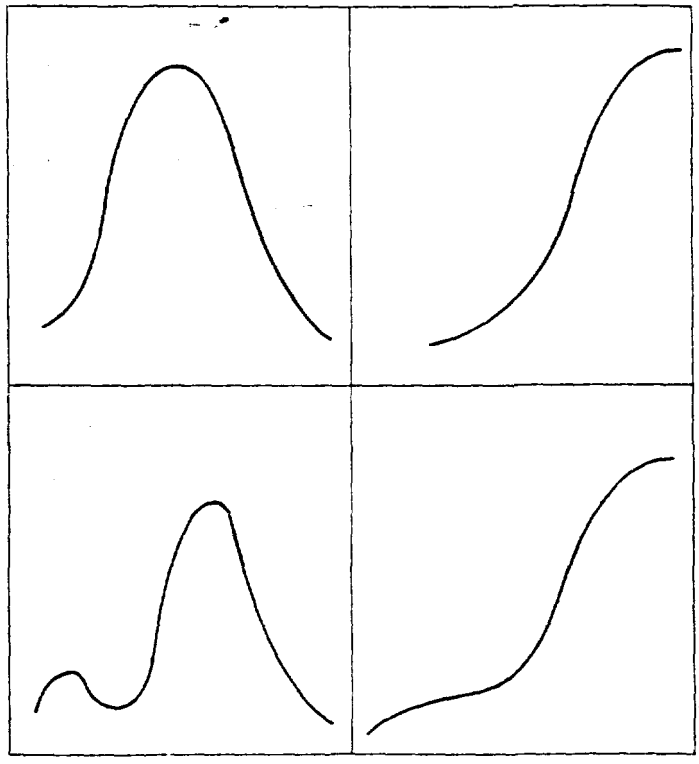

Fig 4. — Influence of genetic factors on an experiment.

some future time, they may then again create trouble. in removing stored lead from bone with EDTA, caution must be exercised or acute lead intoxication can be produced. Acute lead encephalitis has been produced or exacerbated in children treated with EDTA.

In Fig 5 (adapted from reference 1, p. 271, a schematic of various body components is shown and glancing at this can indicate the importance of the route of administration. Ingested materials pass through the liver and may undergo metabolic transformation. On the other hand. inhaled materials may enter the blood stream directly, bypassing the liver. and be excreted by the kidney in the urine unchanged. The effects produced by the same compound may be quite different depending on the route ot administration.

In Table 4 are listed a series ot additional factors that may influence toxicity. The pH may affect toxicity. Large particles perhaps cannot be inhaled. Impurities or contaminants may be most important. The highly toxic dioxin, present as an impurity, was largely responsible for certain of the toxic effects ot 2,4,5-T. Sometimes the compound mav break down in storage to form either more toxic or less toxic compounds It is essential to know what happens to a compound in storage Carcinogenic oils are kept in brown bottles and stored in dark cabinets because we know that ultraviolet light mav destroy polynuclear aromatics.

Other factors that may intluence results are shown in Table 5. Most of these have already been discussed. The time of day when a material is administered may intluence results because of diurnal variations in the animals.

Still further factors related to the animal which may atfect results are shown in Table 6 Some of these have been discussed already. it is well known that mice put on a restricted caloric intake do not grow tumors very well. Further, if animals are too crowded, they may become belligerent, and in some cases become lonely if they are housed alone Thus, in our carcinogenesis experiments, we usually house several animals in a cage because they groom each other and keep themselves more free of parasites If housed alone.

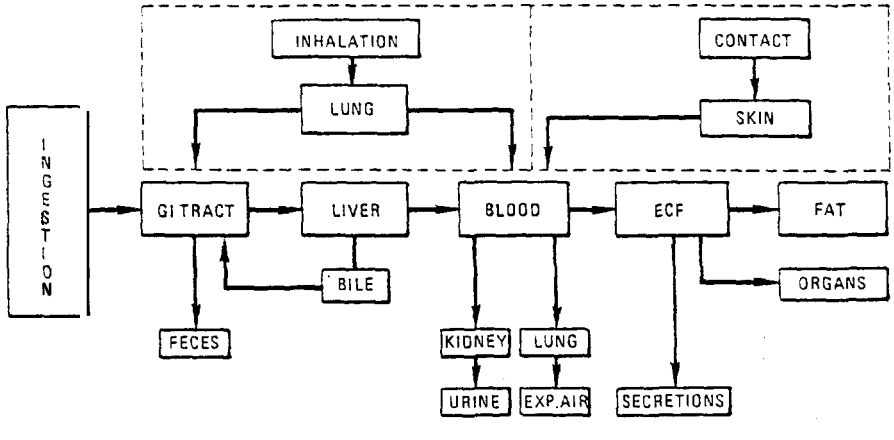

Fig 5. — Schematic of various body components.

Table 8. — Interlaboratory Variability — Oral Dosing.

Laboratories Involved	
American Cyanamid	Kodak
Atlas	Bio-Test
Dow	Shell
duPont	Carbide

Chemical Tested	
Diethanolamine	Isophorone
Butyl cellosolve	Ethylene glycol
Propylene oxide	1-Hexanol
2-Ethylhexanol	2 4 Pentanedione
di-n-Butylamine	Acetonitrile

they sometimes become so covered with parasites that their general health is affected and the results become less clear-cut If animals are handled too much, or too roughly, they become unhappy and may not give consistent results.

Finally. other factors in the environment of the animals may profoundly affect the results of an experiment as shown in Table 7. Animals are usually housed in temperature and humiditv controlled rooms If their quarters get too hot or too cold, the results of an experiment may be invalidated In fact, in manv animal quarters, back-up systems for controlling temperature and humidity are available so that if the main system breaks down, a whole experiment will not be lost When animals have been on an experiment for a year or more, several hundred thousands of dollars have been invested in them, so it is essential that precautions be taken to see that nothing invalidates the experiment. If an inhalation experiment is being performed, it may be necessary to clean-up the air first, by filtration or other means, because the ambient air in the vicinity of the laboratory may be so polluted as to be more toxic than the material being tested. There are many factors that must be given careful consideration by the toxicologist in designing and carrying out his experiments. (Tables 4, 5, 6 and 7 have been taken from Reference 1, p 134.)

Test Repeatability

Finally, let us examine how reproducible toxicity testing is under the best of circumstances A series of laboratories. shown in Table 8, did an LD_{50} determination on a series of compounds Each of these laboratories is a very good and highly respected toxicological laboratory The compounds selected for test, ten in all, are shown in Table 9 It was agreed in advance that each laboratory would conduct the test as shown in Table 10 In other words, methods were standardized insofar as possible, even to the time of day the animals would be dosed The results are shown in Table 11 With Compound 7, the laboratory reporting the highest LD_{50} (i.e., the least toxic) had a value 1.3 times that of the laboratory reporting the lowest LD_{50} (most toxic) This was a

Table 10. — Interlaboratory Variability — Oral Dosing.

Method — Reference Procedure 1
Male Wistar rats (Manor Farms 150-200 g)
Overnight fast dosed 8 10 am 14 d observation
Groups of 5 2X dosing factor (undiluted)
Purina Lab Chow der
LD50 calculation by moving average tables

Table 11. — Interlaboratory Variability — Oral Dosing.

Ratio of Highest to Lowest LD50					
Sample	1	2	3	4	5
Ratio	1 60	2 35	2 52	167	209
Sample	6	7	8	9	10
Ratio	2 07	1.30	4 98	283	192

Absolute value 0 2 to 8 0 ml/kg

pretty good agreement. On the other hand, with Compound 8, one laboratory found it five times less toxic than another laboratory. Thus. under the very best of circumstances, the variability between laboratories may be as much as five-fold. With this in mind, quality control checks should be done periodically with toxicological laboratories. The same compound under two different code numbers can be sent to the laboratory. In this way, the laboratory unknowingly tests the same compound twice and a check can be made on how well they are able to repeat themselves. At other times, a compound already tested in one laboratory is sent to a different laboratory and the same test is performed. If the results are fairly comparable, one can have greater confidence, but if a wide difference shows up, then a quandary develops to try to understand which was, in truth, the real result. (Tables 8, 9, 10 and 11 have been adapted from Reference 2.)

Summary

Some of the fundamentals of toxicology that are used by the toxicologist in assessing the toxic properties of compounds have been discussed. It is easy to see that a large amount of keen professional judgment must enter into the toxicologist's decisions. It is not a job that can be done by an amateur, even though some well meaning lay and political associates may believe they are capable of doing so. The toxicologist's job is not finished when he has determined the toxicological properties of a compound. He then has to assess the hazard of the compound, an infinitely more complex and complicated task. In the end, as stated by Dr. Mrak, "There are no harmless substances, there are only harmless ways of using substances" (quoted in Reference 1, p. 11).

References

1 Toxicology The Basic Science of Poisons L J Casarett and J Doull (Eds) New York Macmillan Publishing Company Inc 1975 768 p

2 Weil CS and Wright GJ Intra- and interlaboratory comparative evaluation of single oral test *Toxicol Appl Pharmacol* 11 378-388 1967