Chapter 7

The Use of Animals in Testing

Distress caused by the Draize eye test is sometimes so acute that rabbits do ***scream out in pain.***

Close-Up Report
Humane Society of the United States
1985

Laws should neither push the science where it is not yet ready to go nor hold the science to procedures that have been modified or replaced.

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Chemical Manufacturers Association
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Testing for the safety or efficacy of a substance or product accounts for a major use of animals as defined in this assessment, most of which are rats and mice (see ch. 3). Of these, probably the largest portion are used in developing drugs. A significant portion are also used to test other substances—pesticides, industrial chemicals, and consumer products—to assess possible toxicity and to establish conditions under which they can be used safely.

Research and testing have been differentiated for purposes of this assessment, but the boundary between them is not sharp. From the standpoint of developing alternatives, a key difference is that a particular test maybe performed for hundreds, perhaps thousands of substances and use hundreds or thousands of animals, whereas a given research method will be used on far fewer. As a corollary there are far more research procedures than testing procedures from which to choose, Furthermore, individual researchers are much more likely to develop their own methods than are those conducting testing. These differences make the task of developing alternatives more manageable for testing than for research.

Testing for efficacy has some attributes of research and some of toxicity testing. A particular protocol may be used on a small number of substances and is likely to be tailored either to the application or to the family of substances being tested. Experimenters testing for efficacy need to have a better understanding of the mechanisms by which a particular effect occurs than those testing for toxicity, primarily because efficacy testing is closely related to the physiological mechanisms that the new drug may affect, whereas toxic effects may be quite independent. Finally, an important distinction of efficacy testing is that the animals used would ordinarily be diseased.

Other kinds of tests include those for safety other than for toxicity, as in testing of diagnostic techniques or quality control tests in the manufacture of medical devices. These have endpoints even more specific than those for toxicity, and are thus good candidates for the development of alternatives (see ch. 8).

Toxicity testing is the focus of this and the following chapter for three reasons. First, this is an area of animal use in testing in which the government has great influence on nongovernmental activities. Second, these tests are used in a more routine fashion than are tests for efficacy or general safety and therefore have a greater tendency to lag in the application of state-of-the-art technology. Third, toxicity tests include methods that have attracted the largest political attack.

All substances can be toxic at some exposure level, even water. Conversely, even substances known to be highly toxic maybe harmless at low doses or under certain circumstances. Determining the hazard to humans requires information about the potential hazard and the expected level of exposure, resulting in an estimate of the probability that a substance will produce harm under certain conditions (8). This assessment of risk is a scientific endeavor, whereas the management of risk is a sociopolitical one (31,36).

Although toxicity data on humans are invaluable in conducting risk assessments, they are usually unavailable. Some information comes from epidemiologic studies or episodes of accidental human exposure. Most often, however, testing on animals is used. An appropriate weight is given to the following factors on a case-by-case basis, considering the seriousness of the hazard and the kind of assumptions needed to estimate risks to humans:

- the relationship between dose and response;
- the effects at the molecular, cellular, organ, organ system, and whole-organism levels;
- conflicting results between studies and possible explanations for the conflicts;
- the effects of structurally similar substances on humans or animals;
- any known metabolic differences between humans and the test species that could affect the toxic response; and
- statistical uncertainties and difficulties in extrapolating to a low dose (55).

TESTING METHODS

Toxicology as a science began in the 16th century and has advanced with the growth of the chemical, pesticide, drug, and cosmetic industries. The concept of protecting the public from harmful effects of chemicals dates back to laws of ancient civilizations that made it illegal to adulterate the food supply (25). The importance of toxicology to public health has received considerable attention in the United States since the 1930s. Public awareness of the value of toxicological testing has also been furthered by disasters such as Minamata disease (methyl mercury poisoning in Japan), the thalidomide tragedy, and, more recently, the development of cancer in those exposed to diethylstilbestrol (DES) in utero.

Designing a Test

There are two approaches to toxicology-mechanistic and descriptive—and these affect the design of experiments and the choice of biological end points to be measured. Mechanistic toxicology focuses on the chemical processes by which a toxic effect occurs and relies heavily on the techniques of physiology, biochemistry, and analytical chemistry to monitor these processes. A simple example of this approach would be a series of experiments showing that a certain substance is metabolized in the liver, that one of the byproducts of metabolism happens to be a potent liver carcinogen, and that liver cancer typically follows administration of that substance. Mechanistic tests are custom designed and are closely related to research. They can contribute greatly to the design and interpretation of descriptive tests, Mechanistic toxicology plays a major role in the development of methodologies that could replace whole-animal testing.

Descriptive toxicology deals with phenomena above the molecular level and may rely heavily on the techniques of pathology, statistics, physiology, and pharmacology, e.g., the evaluation of changes in the appearance of an organ or its constituent cells, the presence of tumors, or signs of irritation. This approach does not necessarily require an understanding of the mechanisms by which toxic effects occur, although if mechanistic information were available, it would be used. In terms of the test substance and species in the preceding hypothetical case, descriptive toxicology would show that a certain substance causes liver cancer in a particular species within a certain time. It might also show the approximate relationship between the substance dose and the incidence of the liver cancer. Regulatory schemes requiring testing most often rely on descriptive toxicology.

Mechanistic toxicology provides an approach to extrapolation from one species to another based on known similarities and differences in physiology. The closer the test animal is biologically to humans or the greater the number of species in which the effect is detected, the more likely it will occur in humans as well. The reliability of extrapolations from descriptive experiments is greatly enhanced when mechanistic information is also used. Similarly, the use of mechanistic information in the design of descriptive tests contributes greatly to the reasonableness of any later extrapolation to humans if human toxicity data are lacking.

Most state-of-the-art toxicological tests require whole animals. Although in vitro alternatives are being developed (see ch. 8), different end points would be measured. For example, whole animals will probably continue to be needed to look for effects in previously unknown target organs, to evaluate effects that represent an interaction of multiple organ systems, to monitor metabolism and pharmacokinetics, or to evaluate healing or diminished responsiveness to the toxic substance. Thus, whole-animal use is unlikely to stop entirely in the foreseeable future.

Choice of Species and Strain

In 1$_{10}$4, the Food and Drug Administration (FDA) was still using human employees to test food preservatives (e.g., boric acid, salicylic acid, their derivatives, and formaldehyde) for toxicity (25). Use of animals remained limited until a few decades ago, when breeding technology provided large numbers of animals with carefully controlled genetic characteristics, thus allowing toxic effects to be more easily detected than had previously been the case. Animal use has grown with increas-

ing demands by the public for safe and effective products.

The most appropriate animals are ideally those that, for the substance being tested, predict the human response most accurately. There is no other animal wholly identical to humans in terms of toxic effects. The choice of animal is influenced by known similarity to humans for the organ system or mechanism of interest, as well as convenience of breeding or purchasing, familiarity with the species, existing data, lifespan, ease of handling under experimental conditions, cost of obtaining and maintaining, litter size, and gestation period. Rodents have been used extensively, as have rabbits, primates, and dogs.

Rodents have been used in almost all carcinogenicity testing despite the fact that such tests are the most difficult to extrapolate to humans. Mice and rats have been used because their lifespan is short, they are small and easily handled, and they have a number of metabolic pathways and pathological responses similar to those of humans. Some specially developed strains are sufficiently susceptible to cancer that test groups can be small. These factors contribute greatly to the economic feasibility of conducting carcinogenicity testing with rodents. Extensive experience in using them, and in using particular strains, is often an important reason for continuing their use (55). A large amount of data are already available on spontaneous tumors at specific organ sites (l).

Although rodents are routinely used for many kinds of tests, other animals maybe used for specific reasons. For example, the rabbit is used for eye irritation tests because it has large, easily manipulated eyes and because its eyes have many characteristics found in human eyes (19). Hens have been shown to be a good model for delayed neurotoxic effects of organophosphorous compounds (12).

Dose Levels and Route and Duration of Exposure

The way in which exposure to a substance occurs can affect the kind and severity of toxic effects. For example, if a chemical does not present a hazard when applied to skin because it is not absorbed, it may nonetheless be very toxic if taken orally. When the route of exposure does not affect the portion of the dose taken up or its distribution in the body, testing might be done in the manner most easily controlled. For other than the most preliminary tests to characterize toxicity, most would administer the substance by the same route as would occur in the course of accidental exposure or use by humans. Sometimes the palatability, volubility, stability, or volatility of a substance will determine which routes are feasible.

Certain tests, such as the acute toxicity for a single exposure, are used as inexpensive screening tools for estimating the relative hazard presented by a substance. As discussed later in this chapter, the acute toxicity test known as the LD_{50} is used in classification schemes for the transportation or disposal of chemicals. Acute toxicity testing might also be used to determine the risks of one-time exposure, as might occur in an accident. Ordinarily, the duration of exposure in an animal study is greater (at least in proportion to the lifespan) than the exposure period for which data will be used in extrapolating the risks to humans.

The dose levels administered depend on a variety of factors. On the one hand, it is not possible to detect long-term effects if the dose is so large that many animals die before the end of the test. On the other hand, administered doses representative of human exposure levels may not produce detectable effects with what may be considered a reasonable number of test animals. Generally, three dose levels are used; they are chosen so as to span the range of responses from a “no-observed-effect level” to fully observable toxic effects,

For carcinogenicity and other long-term testing, the highest dose should be one that will produce measurable toxicity without significantly altering lifespan. Other levels may depend on whether the carcinogenicity is being looked for in combination with chronic toxicity (55). The lowest dose could be one for which there are no observed effects or it might be related to the level of estimated human exposure (38).

Another approach is to choose doses that will yield levels in the blood similar to those expected for humans. Although this is perhaps a more realistic test, effects may be more difficult to detect. In addition, the criterion of similarity may require

more than one administration per day because metabolic rates and excretion rates tend to be faster in small animals than in humans (24).

Statistical Considerations

To obtain valid results, an experiment must be designed so that what is measured provides useful and sufficiently accurate information. Statistical methods allow a scientist to estimate the minimum number of test animals from which conclusions can be drawn to estimate the reliability of any conclusions. Statistical analysis can help reduce the number of animals needed for a particular test procedure.

To allow for the unexpected (including death, illness, or error), the number of animals used always exceeds the minimum number needed to detect expected effects reliably. Determining that minimum number of animals is more difficult for longer tests, both because the passage of time makes the probability of something going wrong during the experiment increase, and because certain problems are more likely to occur as the animals age.

Another factor affecting the number of animals needed is the variability in the sensitivity of individual animals to the substance involved. Thus, as few as 6 animals might be used for an eye irritation test or 10 per dose level for an acute toxicity test. In carcinogenicity or teratogenicity testing, many of the animals maybe unaffected by the test substance, and 100 animals may be needed for each dose level.

Most species experience some cancer and other diseases during their life. Any measurement of incidence as it relates to the dose given must be taken against this background incidence, which is gauged in an (untreated) control group. Control groups may also be important if a test substance is being carried in a particular vehicle needed to administer the test substance, such as in solution with another chemical, that is not itself being tested (vehicle control group). The sensitivity of the test animals to a substance known to be toxic may also be measured for comparison (a positive control group). Because there are so many variables that can influence a test, toxicologists consider it vital that the control and test groups be drawn from the same pool of animals and be tested concurrently.

Any experiment suffers from experimental error, of which there are three sources: the natural variation due to differences among test animals, the variation in experimental conditions, and error arising in measurement. Determining the amount of error is crucial to drawing reliable conclusions from experimental results, but it is also important to keep the error as low as possible by controlling conditions carefully. Differences among test animals are controlled by using genetically similar and sufficiently large groups for each condition. Even minor environmental factors can influence toxic response (15,23). Sources of measurement error depend on the measurement technique and the equipment.

Use of Standardized Test Methods and Guidelines

Testing methodologies are standardized to control experimental variables, thus allowing results to be easily compared. Methodologies may become standardized through round-robin testing in many labs, through publication and imitation, and through development by recognized organizations or agencies. Methodologies or guidelines are published by the Food and Drug Administration, the Environmental Protection Agency (EPA), the Organization for Economic Cooperation and Development (OECD), the National Cancer Institute, the American Society for Testing and Materials, the American National Standards Institute, the British Standards Institute, the International Agency for Research on Cancer, and others (see app. A for information on FDA, EPA, and OECD guidelines).

The most important reason to strive for compatibility among guidelines is to avoid the need to repeat identical tests to satisfy particular requirements of various governments and agencies. Compatibility can also avoid nontariff trade barriers, Any government that would like to change its testing requirements to further the cause of animal welfare needs to consider the effects of its policies on testing in other countries.

Pharmacokinetics

Pharmacokinetic studies provide information about the mechanisms of absorption, about a substance's distribution among the various body compartments, and about metabolism and elimination. They facilitate the interpretation of results from other tests and their extrapolation to humans because the distribution and elimination of a foreign substance will often explain its toxicity or lack thereof.

Absorption of a substance into the body can occur by a variety of routes. If exposure is by inhalation, absorption can occur in the lungs, in the pathways leading to the lungs, and sometimes in the gastrointestinal tract. If exposure is by mouth, absorption would occur as the substance passes through the gastrointestinal tract. What is not absorbed is excreted in the feces, With dermal exposure, the substance must be absorbed through the skin. If exposure is via injection into a body cavity, the substance cannot be removed without the involvement of other parts of the body.

Once a substance is absorbed, it maybe excreted unchanged. Excretion could be through the skin, in the urine, feces, semen, or breast milk, or, if it is volatile, in exhaled air. It might also be stored in tissues, organs, or body fluids, perhaps for the life of the organism. A substance might also be chemically modified until it can be excreted or until the body is unable to metabolize it any further. This metabolism normally takes place in the liver, the site where detoxification of substances takes place. A test substance or its metabolic products can react with the chemicals that make up the body, perhaps resulting in toxic effects.

Pharmacokinetic studies are usually conducted through the sampling of body fluids, both those that are excreted (urine, saliva) and those that are not (blood, cerebrospinal fluid). Tissue samples are often taken, although normally not until the end of a study (4).

Acute Toxicity Tests

Acute toxicity testing is used to detect the toxic effects of single or multiple exposures occurring within 24 hours. These are frequently the first tests performed in determining the toxic characteristics of a substance and may serve as a basis for classification or labeling or for concerns about accidental exposure. The results are used to establish toxicity relative to other substances, to determine specific toxic effects, and to provide information on the mode of toxic action and the relationship between dose and adverse effects. Results may also help in designing long-term tests.

One of the most common acute toxicity tests is the LD_{50} (from Lethal Dose for 50 percent), developed in 1927 for comparing batches of dangerous drugs (52). The LD_{50} is calculated to be the dose, within statistically established confidence intervals, at which half the test animals can be expected to die upon exposure to a test substance. A substance is administered once by the oral, dermal, or parenteral (injection into a vein or the body cavity) route or it is inhaled. The animals, usually rodents, are observed for 14 days and then sacrificed so that their organs and tissues can be evaluated for gross changes. Other measurements and observations can be added to increase the amount of information this test provides.

A related procedure is the limit test. A high dose is given, often 5 g/kg body weight (54); if no animals die, the test ends. This is based on the assumption that if an organism is not killed by an extremely large dose, it does not matter what dose it takes to actually cause death. Other tests using fewer animals have been devised and are receiving growing acceptance (see ch. 8).

Acute toxicity testing has its limitations, particularly because the end point is death. Death can come about in many ways and the mechanism is not conveyed in the numerical value of an LD_{50}. In addition, the results may vary greatly both among and within species, with the animals' sex, age, and diet, and with other test conditions. Acute toxicity testing, although not necessarily the classic LD_{50} procedures, will continue to be of interest because there are many substances for which the toxic effects of acute exposure are quite different from those produced by chronic exposure (8). It may also continue to be used in selecting doses for long-term studies. Nonetheless, circumstances may be identified in which acute toxicity testing is not needed because other tests more relevant to the use should be performed. The Toxicity Committee of the Fund for the Replacement of Ani-

reals in Medical Experiments recommended that study of the consequences of not acquiring knowledge of acute toxicity of products be undertaken and that in the case of products such as drugs, LD~Otests should be replaced by acute toxicity tests that emphasize the nature of the effects observed (18).

Skin and Eye Irritation/Corrosion Tests

Irritation is the production of reversible tissue damage such as swelling, while corrosion is the production of irreversible tissue damage. Skin and eye irritation tests normally involve acute exposure. Repeated exposure can be used to test for allergic reactions, which involve the organism's immune system, and cumulative effects. Skin irritation studies are used to initially characterize a substance's toxicity and to develop precautionary information for situations in which human skin or eye exposure is possible.

Although it is not yet possible to reliably predict the degree of irritation or corrosion a substance will cause, a considerable body of knowledge exists. The factors that determine damaging effects to eyes or skin are:

- intimacy and duration of contact,
- physical properties that determine the amount of penetration, and
- the reactivity of the substance with tissues (10).

Intimacy is affected by both the ability of the substance to spread over the surface (such as soaps or detergents) and its concentration. Penetration of the skin or other membranes is greatest in substances with small molecular size and with abilities to mix with both water and oil. A substance that can react with proteins and enzymes in tissues is especially damaging if it can penetrate to the delicate structures of the eye (50).

Skin irritation tests are usually conducted on rabbits, guinea pigs, rats, and mice, although other mammals may also be used. The test substance is applied to a small area of skin from which the fur has been clipped or shaved and maybe held in place with a dressing. Using untreated skin of the same animal for comparison, the degree of redness or blistering is scored at intervals (e.g., 38,54).

There are many similarities between the skin cells of humans and other mammals, but there are important differences as well. For example, there are structural differences that affect permeability (32). Animal models have been shown to be particularly poor in the evaluation of mild irritants (27). The extrapolation of animal models is further complicated by large differences in the race, age, and skin condition of humans (21,26,58).

The method most commonly used to evaluate eye irritation is the Draize test, which has remained largely unaltered since it was introduced more than 40 years ago (9). A single dose of a substance is applied to one eye of at least three adult rabbits. The other eye remains untreated. The degree of irritation or corrosion to the cornea, iris, and conjunctival is scored by comparison with standard pictures over a period of 3 days. The rabbits may be observed for 3 weeks to determine whether the effects are reversible.

A substance shown to be highly corrosive to skin will be highly irritating to the eye and thus might not be tested. Similarly, a substance with a pH of 2 or less (strongly acid) or 11.5 or more (strongly alkaline) is assumed to be highly irritating or corrosive to skin or eye and need not be tested (38,54). The cornea tolerates substances with a pH ranging from 3 to 11 variably, with the severity of a reaction depending in large part on a substance's ability to affect protein structure or function (17,35).

Repeated-Dose Toxicity Tests

Humans are often exposed repeatedly to a substance and this does not necessarily cause the same effects as an acute, one-time exposure. Chronic toxicity effects differ from acute toxicity ones when the test substance or its metabolizes accumulate in the organism to a toxic level or when it causes irreversible toxic effects that accumulate with each administration (8). Rats are most frequently used, and testing in a second, nonrodent species, usually a dog, is also common.

Repeated or prolonged exposure to the test substance is used in chronic, subchronic, and short-term toxicity tests. The term chronic generally

refers to tests with exposure for at least 1 year or most of the lifetime of the test species. Subchronic usually refers to tests of intermediate duration-3 to 6 months. Short-term repeated dose toxicity tests last from 2 to 4 weeks.

Some have suggested that there is little to be gained by exposures of more than 6 months duration for chronic toxicity testing (18)34). One commentator has argued that studies of 3 to 6 months are easier to interpret because the complicating effects of aging are avoided (44)45). Another finds longer tests necessary for detecting effects that occur only late in life or for which cumulative toxicity is an important consequence (42).

Throughout repeated-dose testing, animals would be observed for general appearance, respiratory problems, central and peripheral nervous system function, coordination, and behavioral changes. During and following the course of exposure, observations are made of hematology (hematocrit, white cell count, platelet count, clotting factors), ophthalmology, electrolyte balance, carbohydrate metabolism, liver and kidney function (as determined from concentrations of certain substances in the blood), body weight, and the appearance of lesions. After the animals have been sacrificed, observations are made of body surfaces, orifices, cavities, and organs. Microscopic examinations are made of selected tissues and organs, of gross lesions, and of organs that changed in size. one technique used in repeated dose toxicity testing to determine whether the toxic effects are reversible is to give a satellite group the highest dose of the test substance and then give the animals time to recover before sacrificing them.

Carcinogenicity

Cancer is a major human health concern, striking one out of four and killing one out of five Americans (53). Consequently, carcinogenicity is an important animal test. Detecting human carcinogens presents special problems because a latency period of 20 years or more can occur. Animal testing, particularly in rodents, is useful because the latency period for tumor formation is much shorter (1 to 2 years for rodents), thus allowing potential human carcinogens to be detected during testing and before use, at which point they could become major public health problems. It is also much easier to control the animal environment than the human environment, and therefore to investigate causal relationships.

Although many human carcinogens were discovered without animal testing, several have been identified by first using such tests, e.g., DES, vinyl chloride, and bis(chloro-methyl) ether (55). Animal use has its limitations; many substances cause cancer only in certain species. The known human carcinogens benzene and arsenic have never proved to be animal carcinogens. Hundreds of substances have been identified as carcinogens in tests with one or more animal species but not in humans, in part because of insufficient human epidemiologic data and in part because some of them undoubtedly do not cause cancer in humans (41). Nonetheless, the use of animals in testing for carcinogenicity is widely endorsed (55).

Carcinogenicity testing is more costly and requires far more animals than other tests. Chronic toxicity testing may use about 160 rats and 32 dogs, whereas carcinogenicity testing would use about 400 rats and 400 mice. (In order to economize, carcinogenicity testing and chronic toxicity testing are often combined.) Cancer is easy to detect if tumors are visible, but it can only be detected in its early stages by microscopic examination of multiple samples of 30 or more tissues and organs that may appear normal. Typically, 500,000 data points must be analyzed (41).

These large numbers of animals and multiple data points are needed for statistical reasons. Cancer has a high background incidence and large variations from animal to animal, making it difficult to establish that cancer was caused by the test substance. The higher the incidence of spontaneous cancers, the more difficult it is to establish a link between cancer and the test substance. For example, if the background rate of cancer is 10 percent and the common criterion for statistical significance of 0.05 is used, the number of animals required to detect carcinogenicity in 90 percent of the tests is as shown in table 7-1. As can be seen, if a test substance causes cancer in 80 percent of the animals, 48 animals are needed to demonstrate carcinogenicity. If the incidence is only 15 percent, over 3,000 animals are needed. It has been suggested that the background incidence could be re-

Table 7.1 .—Number of Animals Needed to Detect Carcinogenicity in 90 Percent of All Tests for a Statistical Significance of 0.05

Rate of incidence caused by test substance (percent)	Number of animals (3 dose levels plus control group)
80	48
60	84
40	184
20	1,020
15	3,304

SOURCE: Adapted from I.F.H. Purchase, "Carcinogenicity," *Animals and Alternatives in Toxicity Testing,* M. Balls, R.J. Ridden, and A.N. Worden (eds.) (New York: Academic Press, 1983).

duced, and the sensitivity of the method thereby improved, if animals were not kept under conditions that aggravate cancer (excessively nutritious diet, little exercise, and isolation) (42).

Developmental and Reproductive Toxicity

The effects of chemicals on human reproduction are difficult to assess because of the complexity of the reproductive process and the many kinds of insults that can be inflicted before reproductive maturity as well as during fetal development (8). Reproductive functions that can be harmed by foreign substances include the storage and maturation of the germ cells, fertility (including factors that affect sperm maturation and implantation of the fertilized egg), and the development of the fetus. Possible toxic effects to the fetus include birth defects (teratogenicity), low birth weight, abnormal gestation time, and prenatal or postnatal death (7).

There are a variety of experimental protocols by which these effects can be determined in animals. Some involve more than one generation; others involve evaluation of a fetus before birth. Exposure to a substance can start before the female ovulates or as late as some specific stage of fetal development. Exposure can be chronic or acute. The great variety of procedures available can lead to a certain amount of overlapping testing (2).

Rats and rabbits are the most commonly used species. Mice and hamsters and other mammals are used as well. Three dose levels are normally used, the highest of which causes minimal toxicity in the adult female. Groups of about 20 pregnant females are typically used. In the OECD Testing Guidelines, if no teratogenic effects are observed at a dose of 1,000 mg/kg body weight, other dose levels are not necessary (38).

Neurotoxicity

Neurotoxicity (damage to the nervous system) is observed in acute and chronic testing, but the range of neurotoxic effects is so great and the signs so varied that special tests for damage to the nervous system are sometimes warranted, Neurotoxic effects that tend to be associated with acute exposure are functional, sometimes reversible changes in the nervous system that might not involve structural damage or degeneration. Most chronic neurotoxic effects do involve structural changes or degeneration and are not readily reversible (6). The type of neurotoxic effect tends to depend on the size of the dose and the duration of exposure (46).

There are many types of nerve cells, each performing special functions. Damage can occur to the functioning of the cell itself, to its connections to other nerve cells or to muscle cells, or to the supporting cells. Neurotoxicity can be manifested in the following ways: motor disorders such as weakness, lack of coordination, paralysis, tremor, convulsions, or slurred speech; sensory disorders such as numbness, pain, or auditory, olfactory, or visual deficits; disturbances of autonomic function such as sweating, incontinence, vomiting, impotence, or tear formation; increased state of excitability such as hyperactivity, irritability, or euphoria; impairment of short-or long-term memory, disorientation, or confusion; sleep disorders; psychiatric disturbances; impaired temperature regulation; or alterations in appetite, or weight gain or loss (6).

More than any other kind of toxicity test, neuro - toxicity does not lend itself to standard procedures or in vitro tests because the range of effects is so broad. There are considerable differences among species, and little standardization of tests across species has occurred. Neurotoxicity tests would typically follow acute or chronic toxicity ones in which neurotoxic effects had been observed or were suspected (6).

Mutagenicity

A mutation is a permanent change in a gene that is passed along to any descendants of the cell. Thus, mutations in germ cells will be passed along to offspring. If recessive, the mutation will not be observed in the offspring but will become part of the gene pool from which future generations will draw. If the mutations dominant, it may be lethal to the developing fetus or it might affect the offspring in a variety of ways, including impairing its fertility. If the damage is to a somatic cell, the mutation could lead to cancer or, in a developing fetus, birth defects.

There are several nonanimal and in vitro tests based on mammalian or human cells that would be considered alternative mutagenicity tests (see ch. 8). There are several whole-animal tests as well. One is the dominant lethal assay, in which a male is exposed to the test substance and then mated with an untreated female. Part way through the pregnancy, the female is killed and the number and condition of the fetuses observed. Another is the heritable translocation assay, in which the male progeny of treated males are mated with untreated females and the effect on fetuses determined. The mutations transmissible to the next generation are of special interest because of their implications for the human gene pool (5).

The in vivo sister chromatid exchange and mouse micronucleus tests rely on microscopic examination of the chromosomes themselves after the test substance has been administered to the whole animal. In vitro versions of these techniques also exist (see ch. 8). Changes can be observed using a microscope. Host-mediated assays are a hybrid of nonanimal and whole-animal techniques in which the test substance and a micro-organism are administered to an animal and the effects on the micro-organism determined (5).

Current Trends

Many factors are likely to influence testing practices in the near future. Public pressure to use alternatives to whole animals, increasing costs of using animals, and improvements in toxicological methods are likely to reduce the use of some tests, such as the LD_{50} and the Draize eye irritation tests. This pressure is also likely to result in changes in some existing tests in order to reduce animal suffering.

These developments could bring about a review of current legal requirements for testing, perhaps reducing the amount of testing per chemical and the number of animals per test. Such a review, as well as advances in the state of the art, might better tailor testing to the substance being examined and to the circumstances of human exposure. On the other hand, the number of substances being tested could increase with greater regulatory or product liability requirements, with greater funding available for testing, or with less expensive tests available.

Interpretation and extrapolation of test results to humans can be expected to improve as the mechanisms of toxic responses are better understood. Increasing use of pharmacokinetics and mechanistic studies is likely to result in improved designs and better selection of tests.

THE ROLE OF GOVERNMENT IN TESTING

The Federal Government and each of the States are involved in testing in a variety of ways. Perhaps the most important are various explicit and implicit requirements for testing under existing statutes. Another area is the funding of research and development leading to new methods (see ch. 12). Yet another is the funding of toxicological testing, conducted primarily by the National Toxicology Program (NTP), supported largely by the National Institute of Environmental Health Sciences. This program, chartered in 1978, is a cooperative effort among agencies within the Department of Health and Human Services (see chs. 11 and 12).

Four principal Federal agencies have a significant role in animal testing for regulatory purposes: FDA, EPA, the Consumer Product Safety Commission (CPSC), and the Occupational Safety and Health Administration (OSHA). Other agencies whose regulatory activities affect animal use include the

Centers for Disease Control (CDC), the Department of Transportation (DOT), the Federal Trade Commission (FTC), and the US. Department of Agriculture (USDA). Animal testing is also funded by the Department of Defense.

Testing is covered by several types of statutes and regulations. Most common are laws that require a product to be safe and effective. Given the state of currently accepted technology and practice, such a statute implicitly (although not explicitly) calls for animal testing. Such tests are routinely expected as an indication of meeting the standard of product safety and effectiveness. A second stimulus for animal testing involves premarket approval. Under this authority, testing with animals is explicitly required by regulations of the agency involved. Or, animal testing may be explicitly required by statute, as in the case of the Federal Hazardous Substances Act administered by CPSC. As a practical matter, it makes little difference whether the tests involving animals occur under implicit or explicit statutory or regulatory authority: The procedures used are quite similar.

The specific tests performed and the methodologies used may be dictated by informal or formal requirements of the agency. These may take the form of promulgated regulations, published guidelines, unpublished guidelines, or customary practices. Some guidelines and the use of specific tests are accepted internationally (see app. A.)

With these general principles in mind, this discussion summarizes current Federal regulatory requirements relating to testing with animals (see also app. B). This review is not intended to evaluate the justification of such testing, only to describe its scope and magnitude. It is meant to provide sufficient background to permit an evaluation of the reasons testing is conducted and of the regulatory needs that any alternatives to such testing must satisfy.

Food and Drug Administration

FDA is responsible for administering several statutes that regulate animal and human food, animal and human drugs, medical devices, cosmetics, color additives, and radiological products. This regulation takes place primarily under the 1938 Federal Food, Drug, and Cosmetic Act as amended (21 U.S.C. 301 et seq.) and the Public Health Service Act of 1944 (42 U.S.C. 200 et seq.).

FDA evaluates each product on a case-by-case basis. The exact testing regime is determined by considering the type of product, the method of exposure, the amount and duration of intended use, and the potential hazards associated with the specific product. In support of its regulatory responsibilities and to assure quality testing, FDA has issued standards for good laboratory practice (see ch. 13) and has developed guidelines and testing protocols. Although some special guidelines or testing protocols are established for specific products, most tests are the same as or similar to the toxicological tests used by other agencies. Appendix A lists the types of tests used.

The National Center for Toxicological Research (NCTR) in Jefferson, AR, and the National Toxicology Program are the research and testing arms of FDA. Although NCTR and NTP have no direct regulatory responsibilities, they provide information needed to evaluate the safety of chemicals. Research that involves the use of animals or alternative methods includes studies of effects of low-dose, long-term exposure to chemicals; development of new methodology to investigate toxic effects; study of biological mechanisms of toxicity; and investigation of methods for estimating human health risks using experimental laboratory data.

The misbranding or adulteration of virtually any product regulated by FDA is prohibited. In addition, testing is required both to substantiate labeling claims and to demonstrate safety. These requirements should be assumed to apply to the substances and products discussed in this section unless otherwise stated.

Food for Humans

Under the law, a food additive is defined as a food substance that is not “generally recognized as safe” (as defined in the Federal Food, Drug, and Cosmetic Act) and that has not previously been approved as safe by FDA or USDA between 1938 and 1958. No such additive may be used until it has been subjected to extensive toxicity testing, a food additive petition has been submitted to FDA, and FDA has approved the additive as safe and promulgated a food additive regulation governing its use.

The safety of an additive is established by evaluating data from combinations of tests. The amount of testing that must be performed is determined by the amount of information already available and the degree of toxicological concern. Guidelines have been developed ***(Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food (56),*** known as the Red Book) that contain detailed procedures adequate to meet minimum requirements. However, manufacturers are permitted to modify the testing as they deem necessary as long as the data are equal to or better than what would be derived by using the guidelines.

Food safety has been important to FDA since the early 1900s. However, the use of animals to test food additives was not begun until the passage of the 1954 Pesticide Chemical Amendments and the 1958 Food Additive Amendments. The most famous amendment, sponsored by Delaney, required that any additive that induces cancer in animals or in humans be banned.

Drugs for Humans

FDA regulates all human drugs, including biological ones. The 1938 amendments of the Federal Food, Drug, and Cosmetic Act require drug manufacturers to submit evidence to FDA that a new drug is safe prior to commercialization. Safety evaluations are primarily based on preclinical animal testing and subsequent clinical testing in humans. In 1962, amendments to the act required that the effectiveness of a new drug also be demonstrated, and this is accomplished through clinical testing.

The requirements to use animals to test new human drugs depend on the proposed scope of clinical investigation and on the drug's anticipated use, Determining the best procedures for testing is complex because of the variation that exists in the use and activity of drugs. Testing must be tailored to each drug and specific requirements are determined by considering the route of administration, the target population, the length of treatment, and the relationship of the drug to others already in use. In addition to the formal procedures required under the Good Laboratory Practices regulations (see ch. 13), guidelines are available to aid manufacturers in designing test protocols. Manufacturers commonly discuss their programs with FDA before and during testing, as well as afterward.

Guidelines are available for tests required for drugs intended for oral, parenteral, dermal, inhalation, ophthalmic, vaginal, and rectal uses, and those used in combination. Duration of proposed human administration is a major factor for determining the particular animal test species, the number of animals, and the duration of the test.

Biological products—any virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, or allergenic product used to prevent, treat, or cure human diseases or injuries—are regulated under the Public Health Service Act and the Federal Food, Drug, and Cosmetic Act. As with drugs, before a new vaccine or allergenic can be marketed, the manufacturer must provide test data to show that the product is safe and effective. FDA Center for Drugs and Biologics licenses the product and the manufacturing facility. For some products, tests are performed on each batch to assure that standards of potency and safety are met prior to release. For most of these, requirements are specified in the Code of Federal Regulations.

Food and Drugs for Animals

Food for pets, food-producing animals, and any other animal is subject to the same basic regulatory requirements as food for humans, with the addition of testing in the target species.

The Federal regulation of animal drugs, medicated feeds, and feed additives began under the 1938 act. The 1968 Animal Drug Amendments consolidated animal food and drug laws, keeping the 1962 standard for safety and effectiveness. The basic intent of these statutes and their resultant regulations is to avoid using substances that may leave harmful residues in animal products intended for human consumption, and to avoid harm to food-producing and other animals.

FDA regulates all animal drugs except those derived from living matter (biologic), which are regulated by USDA. Animal drugs may not be misbranded or adulterated. Testing is done to substantiate labeling claims and to prove safety. A "new

animal drug" is defined as one not "generally recognized as safe" and effective. It must be tested to demonstrate both safety and effectiveness before marketing is permitted.

Medical Devices

Extensive regulatory provisions relating to the safety and effectiveness of medical devices for humans were enacted in 1976 (21 U.S.C. 321). For devices available before then, FDA may at anytime require that proof of safety and effectiveness be submitted. For post -1976 medical devices that are substantially equivalent to those for humans before 1976, the same rule applies. But for those not substantially equivalent, testing must be undertaken to prove both safety and effectiveness, a premarket approval application must be submitted to FDA, and FDA must approve the device as safe and effective before it maybe marketed. Because of the diversity of medical devices, the testing required is tailored specifically to the product involved and there are relatively few guidelines.

As the materials involved and methods of application are often unique, determining the safety of medical devices from the standpoint of toxicity presents special problems. Consequently, recommendations for specific tests are based on an evaluation of the following factors:

- the population for which the device is intended, with special reference to the target group's age and sex, and the benefit to be derived;
- the intended use of the device and its potential to contact the body or, for leachable or absorbable materials, to be distributed in the body;
- the location of the device in the immediate vicinity of various organs that might be adversely affected by its presence;
- the size of the device and the amount of leachate potentially available to the body; and
- chemical or toxicological information suggesting the potential for adverse toxic effects, such as when a leachable substance belongs to a chemical family that contains compounds with known potential for these effects.

Requirements for testing ophthalmic devices and products, color additives used in devices, and female contraceptive devices are more standardized. For color additives used in devices, the same types of tests are recommended as for color additives used in foods. For female contraceptive devices, the requirements are the same as those used for contraceptive drugs.

Medical devices for animals may not be misbranded or adulterated either. Testing can involve animals and is undertaken to substantiate labeling claims and safety. The law does not require premarket approval of such devices, however.

Cosmetics

Although the law prohibits misbranding or adulteration of cosmetics, FDA has no statutory authority to require testing of cosmetics for safety (other than their color additives) before they are marketed. However, animal testing is commonly undertaken to substantiate labeling claims and, by regulation, FDA has stated that any cosmetic with an ingredient that has not been substantiated for safety or that itself has not been substantiated for safety in its final product form must bear a prominent label declaration that the safety of the product has not been determined.

Color Additives

The law requires that any color additive used in food or drugs for animals or humans, in medical devices for humans, or in a cosmetic must be proved safe; must be the subject of a color additive petition filed with FDA; and must be determined by FDA to be safe before it is used (2 1 U.S.C. 321 et seq.). Color additives in use at the time of the enactment of this provision in ***1960*** have been placed on a provisional list and are subject to the same requirements for testing and approval as post-1960 color additives.

Radiological Products

The law authorizes FDA to regulate the emission of radiation from electronic products through the establishment of performance standards and a program of research and other activities to minimize human exposure. Testing on electronic product radiation is undertaken both in relation to proposed and promulgated performance standards and to determine other aspects of potential hazard for humans from such emissions.

Environmental Protection Agency

In fulfilling its statutory responsibilities, EPA uses toxicity data derived from animal testing in a variety of ways. EPA has the authority to require such data be submitted under laws it administers, but data are obtained through other means as well. They are submitted voluntarily by those who conduct or sponsor testing and are obtained from the open literature, from other government agencies, through contracts and grants, and from EPA laboratories.

This section describes the regulatory programs for which animal testing data are needed and the authorities under which existing data or testing can be required. (EPA's testing guidelines are described in app. A.)

Pesticides

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (Public Law 92-516, 7 U.S.C. 136 et seq.) is designed to protect human health and the environment from adverse effects of pesticides while allowing the benefits of their use. This is done by granting or denying registrations; approving labeling; setting maximum residue levels on or in raw agricultural commodities; and establishing procedures for safe application, storage, and disposal. In registering the approximately 50,000 formulations of "pesticide products," EPA uses comprehensive registration standards that include animal testing data, as well as physical properties, analytical methods, and descriptions of manufacturing and use conditions.

EPA also relies on animal toxicity data when it issues emergency exemptions, experimental-use permits, and temporary tolerances for experimental purposes in response to unexpected and temporary food or health emergencies. Emergency exemptions may be granted to State or Federal agencies for uses not included in the registration. Experimental-use permits allow large-scale testing of new pesticides or new uses of a registered pesticide.

The Agency's Data Requirements for pesticide Registration specify the kinds of material that must be submitted to EPA to support registration of each pesticide under Section 3 of FIFRA. EPA uses the information to determine the identity and composition of pesticides and to evaluate their potential adverse effects and environmental fate. Tests are either '(required" or "conditionally required" depending on such factors as the results of preliminary tests, whether the pesticide use is for a food crop, whether the use is experimental, where and how the pesticide is to be applied, and the fate of the pesticide residue. Certain tests are required for new products, and guidelines for conducting these tests have also been developed (40 CFR 158, 49 FR 42856). Many are conditionally required through "tiered testing," whereby the results of the first tier of tests determine the need for additional ones. Three tiers have been described.

There is some flexibility in the application of these testing requirements, but EPA is to be consulted if test protocols other than those described are to be used. Additional flexibility in the testing requirements is available through EPA's procedures for waivers and for minor uses (40 CFR 158).

Virtually all data are submitted in the context of obtaining, maintaining, or renewing a registration. Another requirement is that the registrant must submit any health or safety information that would be of interest to EPA regarding a registered pesticide. This includes the submission of ongoing or completed studies for pesticides subject to registration standards, cancellation, or review; incidents involving adverse effects to human or nontarget organisms resulting from exposure; or incidents regarding lack of efficacy that could indirectly pose a hazard to human life.

Industrial Chemicals

The Toxic Substances Control Act (TSCA) (15 U.S.C. 2601) authorizes EPA to regulate chemical substances that present an "unreasonable risk" of injury to health or the environment and to require the reporting or development of data necessary for EPA to assess risks posed by a given substance. Toxicological testing data derived from animals form the basis for risk assessment and subsequent regulatory actions taken by EPA in implementing TSCA.

If a chemical substance presents an unreasonable risk, EPA can regulate its manufacturing, processing, distribution in commerce, use, or disposal.

Such regulatory actions would be based on toxicity data and exposure data, as well as on data regarding the beneficial uses of the substance. Regulation can be in the form of prohibiting or limiting certain actions, requiring warnings or instructions for use, or requiring the submission or retention of certain records.

If EPA has reason to believe that a substance presents an unreasonable risk but the agency lacks sufficient information to make such a finding, it can require reporting of existing toxicity or exposure data. EPA can also require that a substance be tested in animals for specific toxic effects.

Under TSCA, EPA has authority to require testing of industrial chemicals if testing is needed to perform a risk assessment. To aid in identifying relevant chemical substances, TSCA authorized an interagency testing committee to make suggestions. EPA must consider these suggestions and either initiate rulemaking or publish reasons for not doing so.

TSCA requires that 90 days before the manufacture or import of a "new" chemical (a chemical not on the TSCA Inventory of Chemical Substances) can begin, a Premanufacture Notification must be submitted to EPA. The submitters must provide all information in their possession or control related to health or environmental effects or to exposure. EPA can also require hazard or exposure information for substances already in commerce.

Air

The Clean Air Act (42 U.S.C. 7401 et seq.) requires the Federal and State Governments to take certain actions to improve or maintain the quality of ambient air. Animal testing data support various activities under the act. EPA designates certain substances as "criteria pollutants" and establishes national standards for ambient air based on toxicity and other concerns. Under Section 112, EPA also designates certain very toxic pollutants as "hazardous" and establishes standards for their emission or other control.

For registrations of any fuel or fuel additive, the EPA Administrator may require the manufacturer to conduct tests to determine whether there are potential short- or long-term health effects. Tests may be for acute effects, chronic effects, immunotoxicity, carcinogenicity, teratogenicity, or mutagenicity.

Radiation

EPA's authority over radiation was delegated in the President's Reorganization Plan of 1970 (35 FR 15623), under which EPA makes recommendations to other Federal agencies (the Nuclear Regulatory Commission, the Department of Energy, and OSHA) regarding acceptable levels of emissions for the byproducts of producing fuel-grade uranium and from other low-level wastes. Most of the data used to develop regulatory standards were gathered from humans inadvertently exposed to radiation, but data from animals are used for genetic and other effects, dose-response relationships, and metabolism.

Water

The Clean Water Act (33 U.S.C. 466) requires Federal and State efforts to restore and maintain the integrity of U.S. waters. Data needed to fulfill these requirements are obtained primarily from testing fish and other aquatic organisms.

The 1977 amendments to the act listed toxic substances that are commonly referred to as the 126 priority pollutants, primarily because of their toxic effects on humans and animals. These are controlled through nationally uniform limitations on the effluents containing them. Water Quality Criteria have also been promulgated for permissible ambient concentrations of these substances and are used to establish State water quality standards. Other toxic chemicals will also be regulated under the Clean Water Act.

The Clean Water Act calls for National Water Quality Criteria to be derived. The complete data set is developed by conducting a series of acute and long-term bioassays using organisms from at least eight different families. Acute tests are required on a salmonid, another family belonging to the class Osteichthyes (bony fish), and another representative of the phylum Chordata. The long-term tests required are chronic tests with one species of fish and a bioconcentration test with one aquatic species,

In 1982, EPA published a Water Quality Standards Handbook that provides guidance for developing site-specific water quality criteria that reflect local environmental conditions based on toxicity testing in fish.

The Safe Drinking Water Act (42 U.S.C. 300) is designed to protect public drinking water supplies through minimum national standards that are implemented by the States. Under this act, EPA also regulates the underground injection of fluids and other imminent or substantial hazards to drinking water. In addition, health advisories are prepared on specific problems.

Primary drinking water regulations are developed for certain contaminants that may have adverse effects on human health, Maximum contaminant levels are established or health advisories published using mammalian testing data.

EPA's authority over groundwater is based on a number of the laws that the agency administers. The management of groundwater is a joint Federal and State responsibility, but EPA provides technical assistance to State agencies and prepares advisories dealing with common problems that endanger groundwater. To some extent, these support activities rely on toxicity data.

Because groundwater is the source of drinking water for about half the U.S. population, the identification and characterization of groundwater problems is an important part of the drinking water program. Over 700 synthetic organic chemicals have been identified in various drinking water supplies. Some epidemiologic evidence is available, and more is being collected to help characterize the toxicity of these contaminants, but animal testing data are mainly used.

Solid Waste

The Resource Conservation and Recovery Act (RCRA) (Public Law 94-580, 48 U.S.C. 6901) protects public health and the environment by controlling the disposal of solid waste and by regulating the management and handling of hazardous waste materials. EPA is authorized to develop regulations governing the generation, transportation, treatment, storage, and disposal of hazardous wastes. These regulations, in addition to State laws on waste, are enforced by the States.

Animal testing is used to identify hazardous wastes. Toxicity is one of the criteria. RCRA regulations list chemicals that have been determined to be hazardous and processes that are presumed to generate hazardous waste. Analytical procedures for determining the contents of waste are also described, as are criteria for determining whether the contents are toxic or otherwise hazardous. When information does not exist for certain wastes, EPA must develop it. RCRA does not require those who generate hazardous waste to test the toxicity of the waste.

Because RCRA deals with solid waste, the predominant health problems arise from the leaching of waste from disposal sites. EPA is in the process of selecting and validating tests for characterizing waste. These will look for acute and chronic effects on aquatic animals, primarily fathead minnows. Partial or full life-cycle bioassays and fish bioaccumulation tests will also be required. The potential hazards to humans are characterized with several mutagenicity tests.

Data from tests with humans and animals are used under RCRA to develop "acceptable daily intake" levels that are regulated under the act. Because of the nature of exposure to these wastes, data from short-term and dermal tests are not used.

Superfund

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) (42 U.S.C. 9601), known as Superfund, authorizes the Federal Government to clean up or otherwise respond to the release of hazardous substances or other pollutants that may endanger public welfare. The most significant activity under CERCLA, from the standpoint of animal testing, is the designation of hazardous substances. Substances designated as hazardous under certain sections of other laws (TSCA, the Clean Air Act, the Clean Water Act, and RCRA) are also considered hazardous under CERCLA, and the EPA Administrator is to designate specific amounts of hazardous substances to be "reportable quantities, " based in part on toxicity data.

One activity under CERCLA that diminishes the need for animal testing (because it assembles data on humans) is the compilation of a Toxic Substances

and Disease Registry under the Department of Health and Human Services. This registry will track persons exposed to hazardous substances, along with the medical testing and evaluation that follows the exposure.

Consumer Product Safety Commission

The CPSC administers the Consumer Product Safety Act (I5 U.S.C. 401 et seq.), the Federal Hazardous Substances Act (15 U.S.C. 1261 et seq.), the Poison Prevention packaging Act (15 U.S.C. 1471 et seq.), and the Flammable Fabrics Act (15 U.S.C. 1191 et seq.).

The Consumer product Safety Act empowers CPSC to prevent unreasonable risks of injury from consumer products. Included are both the risk of acute and chronic toxicity and the risk of physical injury. Under this statute, industry regularly conducts animal toxicity testing to determine the safety of consumer products.

The Federal Hazardous Substances Act provides for the regulation of hazardous substances used in or around the household. These are defined as any substance or mixture that is toxic, corrosive, flammable, or combustible, that is an irritant or a strong sensitizer, or that generates pressure through decomposition, heat, or other means, if such substance may cause substantial personal injury or illness during customary or reasonably foreseeable handling or use. Unlike its usual method of letting a regulatory agency or the manufacturer determine what kind of testing is needed to determine safety, in this act Congress defines a "highly toxic" substance in terms of the results of the LD_{50} test and requires certain labeling when the LD_{50} is less than 50 mg/kg body weight, 2 mg/l of air inhaled for an hour or less, or 200 mg/lcg of dermal exposure for 24 hours or less. Although the act does not literally require that these tests be done, a manufacturer cannot know whether they are in compliance with the act unless they perform the tests. CPSC has issued regulations regarding testing requirements needed to determine whether a substance is a skin or an eye irritant (16 CFR 1500).

The Flammable Fabrics Act authorized regulation of wearing apparel and fabrics that are flammable. Industry regularly conducts animal testing to determine the toxicity of substances applied to fabric in order to reduce or eliminate flammability.

Department of Labor

The Occupational Safety and Health Act of 1970 (29 U.S.C. 651 et seq.) requires the National Institute for Occupational Safety and Health (NIOSH) to conduct health hazard evaluations of the workplace (see section on Centers for Disease Control).

A goal of the act is that no employee suffer diminished health as a result of conditions in the workplace. To this end, employers have a duty to communicate safety information about substances present in the workplace through labels, material safety data sheets, and training. Most safety testing is done with animals.

Under the Federal Mine Safety and Health Act of 1977 (30 U.S.C. 801 et seq.), employers must determine whether substances found or used in mines are potentially toxic at the concentrations at which they occur.

Department of Transportation

The Hazardous Materials Transportation Act (49 U.S.C. 1801 et seq.) requires that any materials shipped in interstate commerce be properly labeled and contained in a manner reflecting the degree of hazard present. DOT requires that acute toxicity studies be carried out on substances not already classified or for which toxic effects to humans or test animals are not already known. A substance would be treated as a class B poison (and thus as presenting a health hazard during transportation) if its administration to 10 or more rats at a single dose of a specified amount (orally, dermally, or by inhalation) killed at least half the animals within 48 hours. Analogous authority exists for the U.S. Coast Guard under the Dangerous Cargo Act (46 U.S.C. 179) and the Ports and Waterways Safety Act (33 U.S.C. 1221 et seq.).

Department of Agriculture

USDA administers the Virus-Serum-Toxin Act of 1913 (21 U.S,C. 151 et seq.), under which it licenses animal biologics. The regulatory requirements are similar to those administered by FDA for other animal drugs. Animal testing is undertaken to substantiate labeling claims for animal drugs and to prove their safety. The testing required by USDA for proof of safety and effectiveness of these animal biological drugs is extensive.

Under a series of statutes, USDA exercises close inspection authority over the processing of meat, poultry, and eggs for human consumption. These statutes prohibit any misbranding or adulteration, Testing is required to substantiate labeling claims. Although most safety issues are handled by FDA, testing may also on occasion be required by USDA to demonstrate safety under particular conditions.

USDA administers a number of statutes designed to control and eradicate disease in plants and animals. This authority extends from research through to control of interstate and foreign transportation. Substantial testing is undertaken by USDA in pursuing these statutory mandates.

Centers for Disease Control

The Public Health Service Act (42 U.S.C. 201 et seq.) authorizes CDC to take appropriate action to prevent the spread of communicable disease. Pursuant to this authority, CDC regulates any agent that could cause such illnesses. CDC uses animal data to determine the agents that should be regulated.

Under the authority of the Occupational Safety and Health Act of 1970, the National Institute for Occupational Safety and Health, a component of CDC, develops and periodically revises recommendations for limits of exposure to potentially hazardous substances or conditions in the workplace. When morbidity cannot be explained on the basis of current toxicological knowledge, NIOSH must design toxicological investigations to discover the cause. Such occupational hazard assessments are based on data on humans and animals collected by NIOSH.

Federal Trade Commission

The Federal Trade Commission Act (15 U.S.C. 41 et seq.) prohibits any advertisement that is misleading in a material respect. FTC has adopted the position that an advertiser must have adequate substantiation for any claims relating to safety or effectiveness. Thus, manufacturers and distributors regularly test their products, using data on humans and animals to substantiate their claims.

STATE USES OF ANIMAL TESTING DATA

States engage in a variety of regulatory activities that rely directly or indirectly on animal testing data. One of the most important longstanding uses is the registration of pesticides. Air, water, and waste have also been the subject of State legislation in recent years. State laws often use animal testing data for the identification and classification of substances for control. Several States have also enacted right -to-know laws that may give people greater access to testing data, although such legislation does not necessarily affect the amount of testing done.

Pesticide Registrations

All States are required to register pesticides un - der Section 24 of FIFRA. Most States have 5,000 to 10)000 pesticides registered and grant 5 to 10 emergency exemptions per year. As part of the registration process, States receive animal testing data for evaluation. Much of the time, the information is required only in summary form, unless the State specifically requests the raw data. The data are usually obtained directly from the registrant to avoid possible delays or confidentiality

problems. Although States generally rely on EPA's assessment of data for registration purposes, they regularly review it for emergency exemptions and special local needs (22).

California and Florida have the largest pesticide programs. These States also have the authority to require additional testing (e.g., field testing locally). In addition, California also recently passed a law giving its Director of Food and Agriculture the authority to require data for which EPA has granted a waiver or exemption (e.g., experimental-use permits). California law also requires that data gaps for 200 pesticides be filled and that the first report of an injury to a worker exposed to a pesticide be reported to the Health Department (California Food and Agriculture Code, Div. 7, ch. 2).

Identification and Classification of Toxic Substances

Identification and classification of substances is an important function in most environmental laws. Such activities take place under each Federal environmental statute. Coordination among offices in EPA or with other agencies is common. State agencies also coordinate these activities with their Federal counterparts.

Sometimes, Federal law or regulations are simply adopted by a State and remodified. For example, certain provisions of the New York and Florida regulations governing hazardous wastes incorporate, by reference, EPA regulations appearing at 40 CFR 261 and its Appendices (New York Compilation of Rules and Regulations, Title 6, ch. 366). These regulations list hazardous waste and their constituents, provide analytical procedures to determine the composition of a waste so that it can be classified, and provide for variances from these regulations that may be granted by EPA's Administrator. Much more common are statutes that incorporate Federal laws and regulations and that add other requirements or combine Federal requirements in new ways.

The Wisconsin Pollution Discharge Elimination Law (Wisconsin Statutes Annotated, ch. 147) adopts EPA effluent limitations, effluent standards, and prohibitions. In addition to substances already regulated by EPA, Wisconsin effluent limitations apply to all toxic pollutants "referred to in table 1 of committee print number 95-30 of the Committee on Public Works and Transportation of the U.S. House of Representatives." Additional pollutants are to be identified under Section 147.07 of the Wisconsin law.

The Colorado Hazardous Waste Management Regulations (Code of Colorado Regulations, Title 5, ch. 1007) adopt EPA toxicity provisions of 40 CFR 261 but include "any other substance which has been found to be fatal to humans at low doses, or in the absence of human data, has an oral LD_{50} in the rat of 50 mg/kg or less, an inhalation LC_{50} (lethal concentration) in the rat of 2 mg/l or less, or a dermal LD_{50} in the rabbit of 200 mg/kg or less ."

The Texas Water Quality Acts (Texas Water Code, Title 2, chs. 5, 26, 30, 313) use several Federal laws to classify a substance as hazardous: CERCLA; the Water Pollution Control Act; the Solid Waste Disposal Act; the Clean Air Act; and TSCA. If it is hazardous under any one of these laws, it is hazardous for purposes of Texas law.

Under Oregon Hazardous Waste Management Regulations (Oregon Administrative Rules, ch. 340, div. 62, 63), a substance is considered toxic if it is a pesticide or pesticide manufacturing residue and has one of the following properties:

- oral toxicity in a 14-day test with an LD_{50} less than 500 mg/kg,
- . inhalation toxicity over 1 hour with an LC_{50} less than 2 mg/l gas or 200 mg/m^3 dust or mist,
- dermal toxicity over 14 days with an LD_{50} less than 200 mg/kg, or
- aquatic toxicity over 96 hours at an LC_{50} less than 250 mg/l.

It would also be considered toxic if it contains a carcinogen identified by OSHA at 29 CFR 1910.93(C).

Washington Dangerous Waste Regulations (Washington Administrative Code, Title 173, ch. 303) require the polluter to use EPA toxicity information, EPA's Spill Table, NIOSH's Registry of Toxic Effects of Chemicals (see ch. 10), and any other reasonably available sources to determine if a pollutant is toxic. Carcinogens are identified by an International Agency for Research on Cancer finding that a substance is a positive or suspected human or animal carcinogen. Additional criteria are provided

in the Toxic Category Table, which contains five categories of hazards based on an LC_{50} test for fish, an oral LD_{50} for rats, an inhalation LC_{50} for rats, and a dermal LD_{50} for rabbits.

Some State laws do not explicitly provide for harmonization with Federal requirements regarding the identification and classification of toxic substances. Under the California Air Pollution Laws (California Air Pollution Control Laws, 1979 Edition), the California Air Resources Board and the State Department of Health Service are to prepare recommendations for substances to be regulated and to consider all relevant data. State officials may request information on any substance under evaluation, although they do not have the authority to require testing. However, any person who wishes the board to review one of its determinations must specify additional evidence that is to be considered. Similarly, the California Hazardous Waste Control Act (California Health and Safety Code, Div. 20, chs. 6.5, 1039; California Administrative Code, Title 22, div. 4, ch. 30) directs the California Department of Health Services to prepare lists of hazardous waste and extremely hazardous waste and to develop regulations for their management.

PRODUCT LIABILITY CONSIDERATIONS

Toxicological testing and research play an important role in the law of product liability. Manufacturers are responsible for knowing what dangers their products may present and must pay for any damages these products cause. Animals are used to discover possible dangers, and courts may award damages to a party whose injuries could have been prevented with additional testing or research (see ch. 11).

This discussion of product liability law focuses primarily on drugs because animal use plays such an important role in determining safety. Drugs are also an interesting case study because they are reviewed for safety and effectiveness by the Food and Drug Administration before they are marketed, and yet satisfying FDA's testing requirements does not necessarily fulfill the manufacturer's duty to test.

The Manufacturer's Duty to Produce a Safe Product

In general, a manufacturer has a duty to produce a safe product with appropriate warnings and instructions. This is based on an individual's responsibility to exercise care to avoid unreasonable risks of harm to others. The duty extends to all persons who might foreseeably be injured by the product manufactured. Under the Uniform Commercial Code—a law governing commercial transactions involving goods, which varies only slightly from State to State—failure to produce a safe product results in liability for the manufacturer for the damages thereby caused.

Generally, an injured plaintiff must prove that the drug in question was unreasonably dangerous, that the defect existed at the time the drug left the manufacturer's control, that the consumer was injured or suffered damages from the use of the drug, and that the defect in the drug was the proximate cause of the injury (13,37).

product liability law inmost jurisdictions follows the "strict liability" standard—that is, no matter how careful a manufacturer is, it is liable for injuries caused by its products. Some jurisdictions only hold the manufacturer to a high standard of care, and many that do have strict liability standards also have exceptions.

One exception is for drugs that are necessary but that cannot be made safe. Some have a high risk of harmful side effects but treat conditions that are even more harmful if left untreated, such as rabies (57). (Conversely, when the advantages a product offers are small, such as where vaccines were combined instead of using multiple injections, the manufacturer is more likely to be held liable (51).) Another exception is for products for which no developed skill or foresight could have avoided the harm (14). Even though a toxic effect might not have been tested for using existing methods with animals, a manufacturer must not ignore in-

juries its product may cause after marketing (13). Similarly, if a new test becomes available, the manufacturer may be required to use it (14,29,47).

Methods of Testing Required

A manufacturer must normally use the safest and most effective testing method available. Thus, when monkeys provided the only reliable means for testing polio vaccine, they had to be used to test individual batches of drugs, despite the difficulty and expense of obtaining them (20). Although no cases could be found pertaining to drugs, this standard might not apply when testing is impractical in relation to the risk of harm (30,48).

Testing must reflect conditions of actual use as closely as possible. Thus, where the drug DES was to be used on pregnant women, the manufacturer should have tested pregnant animals and was held liable for cancer in offspring (3). Several smokers have tried to recover from cigarette manufacturers. They have been denied recovery to date because when they started smoking, the risk of cancer had not been demonstrated (28,40)43).

A judge or jury would normally decide whether testing was adequate, but if there was a failure to comply with regulatory requirements, this would normally prove insufficient testing (16)33,39). However, compliance with such requirements would not prove that testing was adequate (14).

In addition to examining what tests were done, the judge or jury might look at the adequacy of the test protocols themselves. For example, the injured plaintiff might argue that the number of test animals was not large enough to determine if a risk was presented (11) or that the conditions under which the drug was tested did not represent actual use conditions (49,51).

SUMMARY AND CONCLUSIONS

The most widespread kind of testing with animals is conducted for the elucidation of toxicity from drugs, chemicals, and so forth. Toxicology has advanced with the growth of the synthetic chemical industries and the use of chemicals in consumer products. Toxicological testing is used in the assessment of hazards and the management of health risks to humans. The use of animals for such testing did not become common until a few decades ago; it now accounts for several million animals per year.

Many toxicological tests are standardized to aid in the comparison of results and because they have been shown to be acceptable tools for measuring certain phenomena. Most of the standard tests are descriptive in that they indicate an end result but do not necessarily elucidate the processes leading to it. Knowledge of the mechanisms by which a toxic effect occurs allows much greater reliability in extrapolation to humans.

The design of a test involves many trade-offs. The choice of species is affected by its physiological similarity to humans, its cost and availability, and the amount of data for other substances available for comparison. The route of exposure, duration of exposure, and size of doses are affected by the possible nature and extent of exposure in humans, by the dose needed to produce a measurable toxic effect, and by convenience. Expected variability in the toxic response governs the numbers of animals used.

Commonly used tests include the following:

- acute **toxicity—a** single dose at high enough concentrations to produce toxic effects or death, often used to screen substances for relative toxicity;
- eye and skin irritation—usually a single exposure, generally used to develop warnings for handling and predict accidental exposure toxicity;
- repeated-dose chronic toxicity—repeated exposure for periods ranging from 2 weeks to more than a year, used to determine the possible effects of long-term human exposure;
- carcinogenicity—repeated exposure for most of lifespan, used to detect possible human carcinogens;
- developmental and reproductive toxicity–

a variety of exposures to determine the possible production of infertility, miscarriages, and birth defects;
- neurotoxicity--a variety of doses and routes to determine toxic effects to nerves, with toxic end points such as behavioral changes, lack of coordination, or learning disabilities; and
- **mutagenicity—a** variety of methods for determining if genetic material of germ or somatic cells has been changed.

To aid in the design of tests and in the extrapolation of results to humans, studies are sometimes done to determine the mechanisms by which toxicity occurs or to characterize the processes by which the test substance enters, is handled, and leaves the body.

The Federal Government has considerable impact on testing practices through a variety of laws and regulations. Sometimes testing is required for premarket approval; more often, it is implied by requirements for safe and effective products. In only a handful of instances, such as the Federal Hazardous Substances Act administered by the Consumer Product Safety Commission and the Hazardous Materials Transportation Act administered by the Department of Transportation, do Federal statutes explicitly require animal testing.

The four agencies with the largest roles are the Food and Drug Administration, the Environmental Protection Agency, the Consumer Product Safety Commission, and the occupational Safety and Health Administration. FDA uses animal testing data in the approval of food additives, drugs, biologics, medical devices, and color additives for humans and animals. EPA and State Governments use such test results in the registration of pesticides and the regulation of industrial chemicals, as well as in the protection of water and air and in the regulation of waste disposal. CPSC relies on animal data in identifying and regulating risks to consumers, while OSHA indirectly uses them in requiring employers to maintain a safe workplace.

Testing also plays an important role in the liability of a manufacturer for unsafe products. In most States, a manufacturer is responsible for any injuries arising from use of its products, regardless of how much testing was done. Exceptions may be made where suitable tests do not exist or the product is known to present risks but those risks are preferable to the harm that would occur without the product, as in the case of rabies vaccine.

Despite the problems of extrapolating to humans and other shortcomings of animal testing techniques, the use of animals in testing is an integral part of the Nation's attempt to protect human health. Ideally, as the practice of toxicology advances, there will be less emphasis on numerical values in certain tests and more consideration of the mechanisms by which toxic effects occur.

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