What Are the Alternatives?

THE SAME TYPES of alternatives can be used in both research and toxicity testing. These alternatives are in various stages of development and span a wide variety of procedures and systems, including human studies, in vitro techniques, mathematical and computer modeling, use of less sentient organisms, and physical and chemical techniques. We will look at each of these possibilities, then determine how they apply to the Draize and LD50 tests.

Human Studies
Humans are already used extensively in research. For example, 400,000 to 800,000 patients a year are enrolled in organized clinical investigations of drugs and other treatments in the U.S. However, an even greater emphasis on human studies could reduce the demand for laboratory animals. Sick or injured persons could be studied to improve the diagnosis, treatment, and prevention of medical problems. Healthy volunteers could be incorporated into these clinical studies as controls. Healthy volunteers could also be useful in studies that focus on maintaining or improving health, rather than on coping with medical problems.

A second way to conduct human research is to analyze information on large numbers of people to uncover potential relationships between the incidence of disease or injury and people’s habits or environments, such as smoking, drinking, and working in certain occupations. These epidemiological studies are helpful in identifying probable causes of health problems. Similar studies are helpful in identifying promoters of good health. These studies may not convincingly demonstrate a cause-and-effect relationship in some cases; however, they are often helpful in providing clues that focus future research efforts.

The remaining category of human research consists of postmortem studies of cadavers donated to science. These studies are particularly useful in anatomical and transplant research. Cadavers are also sources of transplantable organs.

Cadavers have far more potential in biomedical research than current usage suggests. In fact, postmortem studies could revolutionize research, toxicity testing, and education and thereby greatly reduce our reliance on laboratory animals. The key, according to a physician and an educator and physician, is to use cadavers that are brain-dead but whose physiological functions are sustained by artificial support systems. Known as “neomorts,” these cadavers resemble comatose patients but have
be studied while given emergency treatment. One such center was established at Guy's Hospital in England, where researchers concluded:

Whilst the data from the animal studies required by regulatory bodies provide some basic information of the mechanism of toxicity and relative toxicity, it cannot be assumed that this information will be entirely relevant for man. Furthermore, whilst these studies may give indications as to the appropriate treatment for acute overdose, they are unlikely to indicate the efficacy of treatment. Experience gained from a careful assessment of patients suffering from acute overdose of drugs is potentially much more useful than that obtained from animal tests: 12

- Epidemiological studies have linked genetic damage to a variety of factors, including drugs, metals, industrial chemicals, radiation, tobacco smoke, and alcohol. The evidence is particularly strong for vinyl chloride, alcohol, and tobacco—the higher the dose, the greater the incidence of genetic damage. 11 Genetic damage was assessed by monitoring chromosome breakage in certain blood cells.

Further evidence of the importance of human studies comes from an analysis of Nobel Prizes awarded in medicine or physiology. These prestigious prizes are awarded for outstanding contributions in basic and applied research. Seventy-two prizes have been awarded from 1901 (the year the prizes were initiated) through 1983. Of these, twenty-two (thirty-one percent) involved human studies to some degree, including ten (forty percent) projects that were wholly or primarily conducted on humans.

Despite such accomplishments, not all human studies can be considered alternatives to animal studies. Instead, many human studies are follow-ups to research on animals. Researchers often turn to animals before conducting studies on humans because of ethical and practical problems of studying humans directly. However, findings from animal studies must be verified in humans because they cannot be extrapolated to humans with great accuracy. Given the uncertainties of this extrapolation, follow-up research on humans can truly be regarded as experimental and the human subjects regarded as the last in a series of “guinea pigs.”

Sophisticated new techniques are helping to overcome ethical and practical restrictions that have limited the extent to which humans could be studied directly, without recourse to potentially misleading animal models. For example, remarkable new “imaging” techniques, which can generate visual images of the body’s interior without the need for invasive procedures, are now being used to harmlessly study the human brain in action. One such technique is positron emission tomography (PET): tiny amounts of radioactive chemicals mark areas of interest in the brain, and a brain scanner detects these chemicals and generates pictures or “scans” that show the living brain in action.

PET has recently been applied in the study of Parkinson’s disease, which afflicts 400,000 Americans, mostly the elderly. Sufferers exhibit tremor, muscle rigidity and weakness, and a shuffling gait. PET scans were taken of the brains of volunteers who were known to have used heroin tained with a brain-damaging substance. The scans revealed Parkinson’s-like damage to specific brain cells in the absence of overt signs of the disease. Such signs have begun to appear in persons who first used the heroin two years previously. These studies suggested that exposure to similar toxic substances may predispose people to develop Parkinson’s later in life, when additional brain-cell loss occurs as a result of aging.

This application of PET scans to humans has sparked a revolution in the understanding of Parkinson’s, which has baffled physicians for more than a century. 11 Such studies are pointing the way to human research on the diagnosis, treatment, and prevention of the disease. 13
In Vitro Techniques

There is virtually no field of biomedical research that has not been affected by in vitro technology.

Some human and animal tissues can be removed from the body and studied in vitro (literally, "in glass," i.e., in a laboratory container). In vitro alternatives can be either replacements or reductions. If tissue samples are derived entirely from humans (from biopsies, autopsies, and placentas), then the research is a replacement. If animals are deliberately killed to obtain tissue samples, then the research is a reduction because tissue from a single animal often is enough to substitute for several animals. In vitro studies involving animal tissue can be replacements if the tissue is propagated indefinitely, providing material for study after study.

In vitro techniques include cell culture and organ culture. In cell culture, cells are isolated, maintained, and studied apart from confounding influences of other body systems. Because chemicals of interest can be added directly to the culture, much smaller amounts of chemicals are needed. This sensitivity was the main reason why the National Cancer Institute (NCI) recently launched a $2.5 million screening program for anti-tumor agents. An NCI representative noted that "the materials that we are typically looking for are trace components, so the in vitro model is inherently an insensitive one and we may miss, in most cases, our most interesting lead." Cells to be cultured can first be cloned to achieve genetic homogeneity or be manipulated in other desired ways and then studied.

Although in vitro techniques are ideally suited to studying biological systems in isolation, they can also be designed to reflect interactions between systems. For example, tissue from one organ can be exposed to specific hormones produced by other organs, or a potentially toxic chemical can be incubated with liver cells to determine whether the liver detoxifies the chemical before it can exert any toxic effect on other cells. Although in vitro systems can be made more complicated in this way, the strengths of the in vitro approach are its simplicity and precision.

While it is true that in vitro studies are ill-suited to model complex systems and hence will never fully replace in vivo studies, the converse is also true. In vitro technology can be applied to study virtually any type of cells in the body. The practical problem of not being able to grow specialized cells has now been largely solved.

Examples of in vitro procedures follow:

1. The LAL test, described earlier, is an in vitro test that uses subcellular components obtained from horseshoe crabs to determine whether intravenous fluids will induce fever. This newly introduced test is already being conducted more than a million times annually.

2. A tissue-culture technique has been developed to standardize the potency of rabies vaccine. This vaccine consists of a weakened, live form of the rabies virus. The potency of each batch of vaccine must be standardized so that it is not too strong or too weak. Potency is currently evaluated in an LD50 test on mice, but the twenty-one-day test period makes this test impractical. Confounding factors, such as unrelated deaths and differential susceptibility of animals to the virus, can increase the variability in test results. The alternative, tissue-culture test is as sensitive as the LD50 test and hence will never fully replace in vivo techniques.

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Tissue-culture techniques can also be used to screen substances for their potential as pharmacological agents. According to the National Academy of Sciences, "Major recent advances in our knowledge of the immune system, one can make 200 to 400 cultures from a single mouse. If these same studies were to be conducted in vitro, they would require 200 to 400 mice to achieve the same number of observations.

Cell–culture techniques have recently been applied to behavioral research in studies of the biochemical basis of depression and mania. Human skin cells were used to determine if the cells of manic-depressives and their relatives exhibited biochemical properties markedly different from the cells of persons without a family history of manic depression. One commentator characterized this research as "a step forward, applying to psychiatry the techniques of tissue sampling and cell culture that have been of great value in characterizing molecular abnormalities in numerous medical diseases." Imaginative research such as this expands the scope of in vitro studies beyond what was formerly attempted.

Mathematical and Computer Models

Modern approaches to biomedical research are increasingly incorporating the language of mathematics into their descriptions of living systems. Mathematical approaches to biomedical research are increasingly incorporating the language of mathematics into their descriptions of living systems. Mathematical approaches are being applied in studies of all levels of biological organization, from interactions among molecules to interactions among organisms. These approaches, existing information is used to describe the system under study in mathematical terms. The resulting mathematical model usually is a simplified version of reality but is, nonetheless, helpful in understanding complicated systems, especially those in which several variables influence an outcome.

As an illustration, consider the outcome to be the degree to which various chemicals are toxic. Toxicity is likely to be influenced by several factors, including the size and shape of the chemicals' molecules, the presence of certain reactive groups, the way reactive fragments are linked together, and the chemicals' affinity for fats versus water. Each of these factors can be represented mathematically by one or more variables or "parameters." In this example, toxicity would be modeled on the basis of the chemicals' structure, composition, and physical/chemical properties. Toxicity data on already-tested compounds could be used to help predict the toxicity of unknown compounds. Models such as these are known as structure/activity relationships (SARs) because chemical structure is used to predict activity, in this case, toxicity.

Once mathematical models are formulated, they must be verified to see if they accurately reflect the relationship under study. In toxicity testing, this verification procedure is known as validation. In the area of research, verification usually involves a procedure known as simulation. In a simulation, one or more parameters in the model is changed to determine if the response is similar to that seen in the living system. If dissimilar responses are obtained, the model can be refined or entirely reformulated. Because simulations usually are too complex to conduct by hand, researchers often turn to computers. Computer simulations are useful not only in validating models but also in suggesting new mechanisms and hypotheses for further study.

Modeling is now an integral part of research in many laboratories, particularly in the pharmaceutical industry. Unfortunately, its more widespread application is hampered by a general lack of mathematical and computer skills among researchers and the cost of computer equipment and commercially available programs. NIH has recently taken steps to overcome these problems. It financed the creation of the
Biomedical Simulation Resource at Duke University Medical Center, which makes its facilities for building and examining mathematical models available nationally to biomedical researchers. The resource offers technical advice and access to computers and programs either at the facility or over a telephone data network. Computer models serve at least two general purposes in alternatives research. First, they can substitute for animal tests, in some cases. The extent to which models need to be backed up by animal tests depends on how well the models perform during validation. The better the performance, the less the need for back-up tests. In toxicity testing, models are likely to bring major reductions in animal use because existing information from animal studies on thousands of compounds can be applied toward predicting toxicity of closely related compounds that have not been tested. The outlook is not quite as bright when models are applied in new areas of research, since the results from the simplified models will have to be checked in the far more complex living system.

Second, mathematical models can make animal research more humane by identifying promising avenues of investigation and thereby preventing fruitless animal research or by estimating the toxicities of a closely-related series of compounds, so that only the least toxic compounds will be developed and tested on animals. These functions of mathematical models are illustrated in the following examples and in the following chapter.

- Mathematical modeling has been used to determine the molecular characteristics of cancer-causing chemicals. One hundred and fifty structurally related chemicals were analyzed; each had been found previously to be either carcinogenic or non-carcinogenic in animal studies. The model was an attempt to distinguish between these two sets of chemicals based solely on molecular structure. Using the statistical technique of pattern recognition known as discriminant analysis, researchers correctly classified ninety-seven percent of the compounds. Such studies should encourage further research in predicting toxicity from molecular structure. Success in these endeavors will lead to a decrease in animal use for predicting toxicity.

- The potential value of mathematical modeling to cancer research has also been illustrated by Charles DeLisi and coworkers at the National Cancer Institute. According to a recent article, Dr. DeLisi's computer program...analyzed the response of the immune system to cancer. From information they gave the computer about tumor growth and antibody production, it calculated that the immune system could not only fight cancer growth but stimulate it as well. Researchers know that now, says DeLisi, "but if our model had been around ten years ago, it could have predicted what it's taken scientists countless man-hours and animals to figure out. This is the value of mathematical modeling—it comes up with things that you might otherwise miss." Mathematical modeling of malaria research illustrates the potential value of modeling in guiding research efforts. This modeling was a retrospective analysis of results from the testing of potential anti-malarial drugs. A large-scale testing program had been conducted on mice at the Walter Reed Army Institute of Research. Development of a structure/activity relationship for a certain class of chemicals synthesized early in the program showed retrospectively that further research on this class was futile, yet many other chemicals in this class were synthesized and tested in mice. This analysis suggests that prospective use of mathematical modeling will prevent much futile animal experimentation.

- A computer program developed by thirty scientists at the Los Alamos National Laboratory is an ambitious attempt to duplicate the complex physiological systems of the human body. The program is known as "HUMTRN," short for human transport. It is a data bank that gives simultaneous access to ten million pieces of information on what happens when any chemically identifiable substance is taken into the human body. HUMTRN is dynamic to the point of being programmed to eat, breathe, perspire, defecate, grow, develop sexually, age, work, and die. A scientist associated with the HUMTRN project has called this program "the cutting edge of modeling technology." In one study, HUMTRN suggested that, in most kinds of nuclear accidents, teenagers and young adults would be the highest risk group in suffering long-term effects. The developers of HUMTRN refer to this mathematical model as the "research rat of the future."

Use of Less Sentient Organisms

A simple test for detecting teratogens (chemicals that cause birth defects) has been developed using hydras, tiny aquatic animals related to jellyfish. The test is based on the observation that chemicals that cause birth defects in animals also tend to disrupt normal development in hydras. This test is currently the most promising alternative screen for teratogens.

- Plants may replace animals in tests to detect substances that cause skin damage in the presence of light. Such substances, termed photosensitizers, exert their effects after being ingested or applied to the skin. Laboratory animals, particularly hairless mice, are currently used routinely in phototoxicity tests. The alternative test is based on the observation that photosensitizers inhibit the growth of yeast in the presence of light. The test, developed by F. Daniels, yields results that are similar to those from the mouse test when testing substances that are phototoxic when applied directly to the skin. Other alternative tests need to be developed to detect substances that are phototoxic after being ingested. Further research is needed on Daniels's test to corroborate and extend the encouraging results found to date.

*Research on microorganisms is sometimes characterized as in vitro because these organisms are so small they can be cultured in laboratory containers. A different classification is adopted here in order to emphasize the affinity between research on microorganisms and research on other organisms of limited or no sentience.*
The Ames test uses bacteria to detect mutagens (chemicals that induce genetic mutations). Because mutations are often associated with cancer production, the Ames test is used as a screen for carcinogenicity. This well-researched test is now a classic example of an alternative. It uses a specially prepared strain of the bacterium Salmonella typhimurium. The culture medium is designed so that only bacteria that have undergone certain mutations are capable of growing.

In addition to bacteria, the Ames test also makes use of in vitro culture of liver enzymes. Rats are the recommended source of livers, although human cadavers have potential. Whatever the source, the culture contains the microsomal structures mentioned in the test’s alternate title, the Salmonella/Microsome test. Potential mutagens are incubated with this culture in order to simulate a process known as “metabolic activation,” which normally occurs in the liver (and to a lesser extent in other organs) of intact animals. Unless activated, mutagens might not exert their effects and would thereby escape detection.

The Ames test has been improved continually since its introduction and now gives results comparable to those of animal bioassays. It has the added advantage of being quick and inexpensive. It is widely used as an initial screen, often in combination with other short-term tests, and therefore has reduced the demand for laboratory animals in carcinogenicity testing. A considerable number of mutagens first detected by the Ames test have been shown subsequently to be carcinogenic in animal tests.

About ninety percent of known carcinogens can now be detected by short-term mutagenic testing using batteries of tests. These tests are inexpensive and can be conducted in one to five days.

### Physical and Chemical Techniques

Physical/chemical techniques exploit instruments and chemical procedures, not animals, to analyze the physical and chemical properties of drugs, toxins, body chemicals, and other substances. For instance, high performance liquid chromatography and mass spectrophotometers are physicochemical instruments that accurately isolate, identify, and measure the amount of a given substance in complex biological mixtures. In high performance liquid chromatography, the test substance is forced through a column of silica and different chemicals pass through at different speeds. This characteristic is used to analyze precisely the components of the substance.

Physicochemical techniques are replacements when used instead of animals to assay substances. They are reductions when they perform their analyses better than cruder methods and thereby require fewer animals per experiment. Numerous technical improvements can be considered as physicochemical reduction alternatives. For example, a device is now available that divides a one-microliter sample (which itself is tiny) into one thousand subsamples, each of which can be analyzed biochemically. It is easy to see how the use of such an instrument could reduce the number of animals needed as sources of tissue samples.

- Physicochemical techniques have replaced the use of animals in assays for vitamins A, D, and E and for "biologics" such as the hormone oxytocin. In the case of vitamin D₃, the new technique involves high performance liquid chromatography and provides a simpler, quicker, and cheaper alternative to the animal bioassay. The latter procedure involved inducing a vitamin D₃ deficiency (rickets) in rats and administering D₃-rich substances such as cod liver oil over several weeks—a laborious and time-consuming method.

- Physicochemical techniques have replaced the use of rabbits in human pregnancy tests. Nowadays, one can obtain pregnancy diagnostic kits from the corner drug store. These kits contain simple materials to screen a potential mother’s blood or urine for a chemical associated with pregnancy.

### Other Techniques

Other techniques or systems may be used to replace, reduce, or refine the use of animals in research. These include mechanical models, veterinary patients, and computer-aided drug design.

- **Mechanical Models**: animals are sometimes used to study effects of accidents such as vehicle crashes and specific injuries such as burns. Mechanical models are being developed that might replace animals in these studies. For example, an artificial neck developed by General Motors is being used in car-crash simulation tests, and a human simulator known as Thermoman is being used to test potential burn risks with different garments.

- **Veterinary Patients**: just as clinical studies of humans can reduce the demand for laboratory animals, so, too, can clinical studies of animals. Animals are susceptible to many of the same illnesses and injuries that plague humans. Animals that are already sick could be studied while undergoing treatment, and the resulting knowledge could benefit human health. (Of course, the primary concern in these studies should be the animals.) Clinical studies of animals could reduce the number of laboratory animals that are deliberately sickened or injured in experimental studies.

Prof. Calvin Schwabe, a respected research veterinarian, argues that both clinical and epidemiological studies of animals are being virtually overlooked as potential resources for understanding human diseases. The relevance of spontaneously occurring diseases in animals to medical research on humans is unappreciated. A consequence of this, according to Schwabe, is that most of the research in comparative medicine that is being conducted by physicians is focused upon the potentially least rewarding approach to animal diseases, namely, studying artificially induced...
rather than spontaneous diseases. Veterinarian Michael Fox, in recounting Schwabe’s view, calls for greater collaboration between veterinary and medical researchers.\textsuperscript{40}

\textit{Computer-Aided Drug Design:} discovering new drugs is largely a trial-and-error process, costly in terms of time, money, and animals. It takes eight years, on average, to screen a new substance from the seven thousand to eight thousand novel compounds created each year and to bring it into medical practice.\textsuperscript{46} Fortunately, methods are being developed to replace this shotgun approach with the more directed approach of computer-aided drug design. Three-dimensional computer graphics and the theoretical field of quantum pharmacology are being used in efforts to design drugs with particular specifications. These efforts are based on the lock-and-key mechanism of drug action; that is, drugs must be the right shape and composition in order to “dock” with their targets and trigger their effects. Color graphics help visualize this process.

Although computer-aided drug design is in its infancy and is highly theoretical, there are indications that progress is being made. Several “drug designers” have been included on new drug patents for aid in discovering drugs.\textsuperscript{47} A new drug being tested clinically for effectiveness against high blood pressure was designed with computer methods.\textsuperscript{48} Perhaps it is not surprising, then, that several pharmaceutical companies now employ such “drug designers.”

Much of the work in computer-aided drug design is apparently being conducted in Britain, where it has received some financial support from the Lord Dowding Fund. However, researchers at the University of Pittsburgh are collaborating with British researchers in attempts to use computer-aided methods to design a drug to treat sickle-cell anemia.\textsuperscript{49} New efforts such as these hold great promise for reducing animal use by revolutionizing the process of drug discovery.

\textbf{Case Studies: The LD50 Test And the Draize Test}

MUCH OF THE public outcry against the use of animals in toxicity tests has centered on the LD50 test and the Draize test. It is not surprising, therefore, that much of the research into developing alternatives in toxicity testing has been directed at these two tests. Substantial progress in this research has been made during the last five years.

\textbf{The LD50 Test}

The LD50 test was developed in 1927 to standardize the potency of potentially poisonous substances destined for human use, such as diphtheria toxin, digitalis extract, and insulin. Although not originally designed to do so, the test gradually became incorporated into routine toxicity programs for testing new chemicals. Government regulations in the U.S. and abroad specified the LD50 test for evaluating new drugs, food additives, cosmetics, household products, industrial chemicals, and pesticides. Each year in the U.S., four to five million rats, mice, guinea pigs, and, less frequently, rabbits, dogs, and primates, are subjected to this test.

In the LD50 test, test substances are force-fed, inhaled, injected, or applied to the skin of animals. Of these variants—the oral, inhalation, injectable, and dermal LD50 tests, respectively—the oral LD50 is the most common. It produces signs of poisoning including bleeding from the eyes, nose, or mouth; labored breathing; convulsions; tremors; paralysis; and coma.

The classical LD50 test uses large numbers of animals to derive a numerical index of toxicity (the LD50 value). This approach has two major scientific problems. First, the test is of limited value in protecting human health. This limitation stems primarily from an overemphasis on the LD50 value. Sometimes, little or no additional information (such as poison symptoms, body organs affected, and specific cause of death) is gathered. This important information could be derived from relatively few animals. According to D.V.W. Parke, the “counting of cadavers” should be replaced by full clinical and postmortem studies using fewer animals.\textsuperscript{41}

Even when the LD50 value is supplemented with clinical and pathological information, public health officials can still be at a loss to infer the \textit{maximum safe dose} of the test substance in humans. The LD50 provides the median \textit{lethal dose}, not the safe dose. Moreover, the lethal dose, as ill-suited a measure as it is, still has