Defending the Indefensible?

Indian Scientists Use Outmoded and Discarded Tests

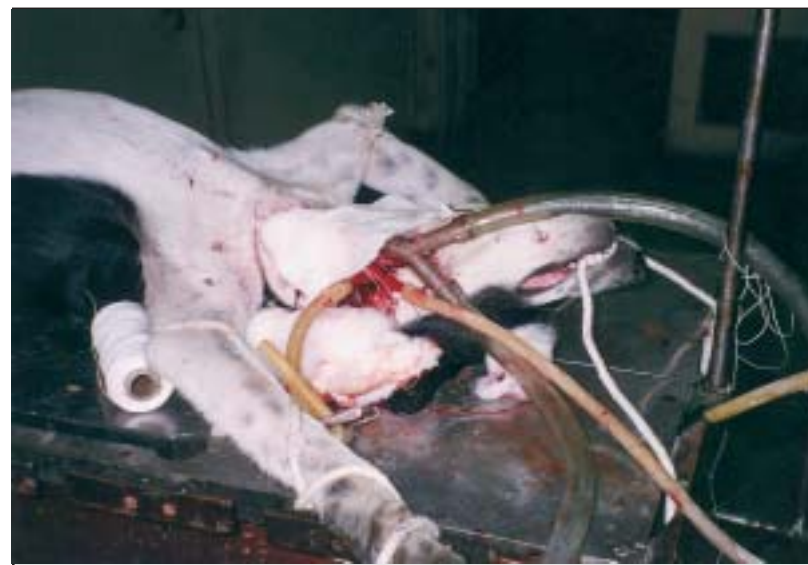
❍ Geeta Sheshamani

Due to several advances in the methodology of international scientific research, there has been a shift away from the needless use of animals in scientific research. However, despite these developments, certain outdated practices continue in India while the rest of the world has discarded them.

Lethal Dose Experiment

Perhaps the most barbaric tool used to evaluate acute lethality from exposure to a substance or product are the Lethal Dose 50 (LD50) tests. LD50 tests are done to classify substances as being safe to transport, to provide information on acute intoxications, to standardise certain biological products, to set dose levels for subsequent toxicity studies, and finally to provide comparative information on the chemical dose response curve.

An LD50 value is the dose at which 50 per cent of the test animals can be expected to die. In other words, the test material is administered in increasing doses to groups of animals, usually ten males and ten females in each group, until all the animals in the group die or are on their way to dying from the test substance.



This experiment can also be carried on rats, but the size of the dog makes it more "convenient for scientists".

Mortalities are recorded. All animals are "sacrificed" if not already dead and the LD calculated statistically. However the LD50 gives no information on what organic system failure caused the death. The controversy began almost three decades ago in the West because animal welfare organisations, legislators, and toxicologists themselves began to question the ethics of using a large number of animals only to evaluate mortality rate.

However, in India the scientists would have us believe that without permission being granted to test on and kill these 100 to 150 animals, the world would not be a safe place to live in for humans, and all drug development and original research would stop.

Are the results of this test therefore that reliable? Animal testing is assumed to be essential under the

Ranbaxy Laboratories : Beagles used to be born and bred on mesh floors. This practice has now changed following CPCSEA interventions.

sacred concept of drug development and everyone hesitates to question it. After all, enormous quantities of money are spent in "researching" drugs. Regular toxicity studies (the infamous LD50 tests for example) have killed hundreds of animals in the course of often thoughtless completion of "standard protocols" - all done of course to ensure you and I receive safe drugs.

Haffkine Biopharmaceutical, Mumbai: rabbits kept in old rusted cages. No tray below each cage for the collection of feacal matter and urine. Therefore, everything falls on the rabbits in the cages below.

Misgivings among Scientists

Why then is there a growing unrest among scientists the world over that suggests animal tests do not yield the results they are supposed to? Why is there a growing misgiving in the scientific community as scientists fumble with wrong inferences drawn from using animals in research? There is a growing worry that animals housed in artificial, cruelly deprived environments, denied natural foods and exercise, developing bizarre dysfunctions, and psychological stress, are in situations that would definitely interfere with coming to valid conclusions about the hypotheses being researched.

The reasons are several and quite obvious besides the above concerns for the animals themselves, which can be shrugged off as mere sentimentalism. It certainly is a matter of concern when one discovers that the results obtained from animal testing often do not replicate in human beings. Animals do not get AIDs or suffer from hallucinations or problems with blood pressure. The tuberculosis and cancer induced by scientists in animals is quite different from the types of tuberculosis and cancers that affect human beings.

AIIMS: Experiment being carried out on the floor of the animal house, with experimental rat in a bread box.

Scientists acknowledge that animals undergoing toxicity tests used to die not because of the toxicity of the substance being tested but because of the bulk of material the animals are being forced to swallow. It is a fact that if a dog is forced to swallow cupfuls of shampoo, toothpaste or talcum powder, it will die whether or not the substance is poisonous.

Closer to home is a strongly worded and logically argued article by Dr. Leo Rebello, Director of Natural Health Centre, Bombay and President of the Indian Council of Natural Medicine and Research. In that article he points out that "everything is teratogenic if given in the right dose to the right species at the right time. It follows that because all agents have the potential for toxicity in some organism at some dose, the production of positive results in developmental toxicity studies is only a matter of finding a sensitive state in a sensitive species and of using an adequately high dose of the toxicant."

Dr. Vernon Coleman points out that there is a massive difference in the way drugs affect humans and

The hotplate test for checking the analgesic properties of a substance. Mice or rats are placed on a hot plate heated to 55°c and the reaction time of the animal is timed and documented.

animals. *(*See, *"Animal Models and Extrapolatin to Humans"*, Sonya Ghosh in this issue*)*.

Differences in Species

Species to species differences in sensitivity gives the LD50 test little capability for assessing toxicity in humans. Acetaminophen, for example, is fatal to mice at 250 – 400 mg/kg due to liver necrosis, while the LD50 for rats is about 1000 mg/kg with little evidence of liver damage. With such profound differences between rats and mice, extrapolation to humans can have little meaning. A recent multicentre study found that even under the most standardised conditions, the correlation between animal LD50 values and acute toxicity in humans was only 63 percent. "Even if the LD50 could be measured exactly and reproducibly, the knowledge of its precise numerical value would barely be of practical importance because extrapolation from the experimental animals to man is hardly possible," says Dr. Rebello.

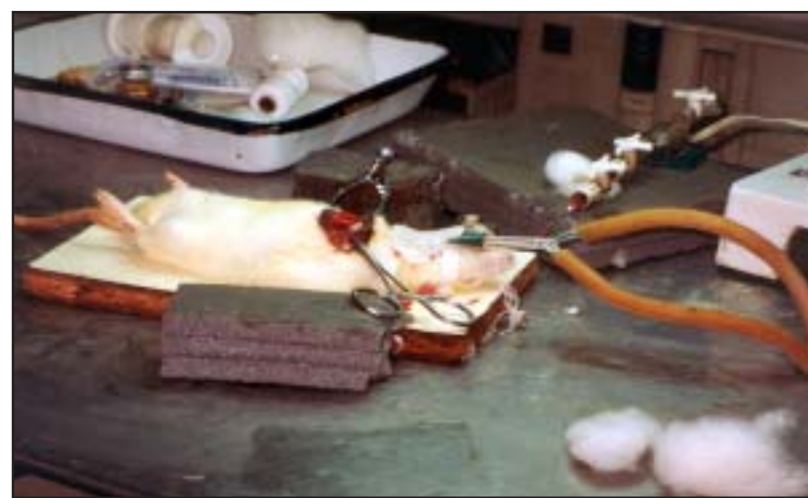
LD50 measures only lethality, ignoring a plenitude of adverse and damaging effects that may not kill but may have terrible non-lethal effects at doses far short of the LD50 dosage. The LD50 measures properties of no significance to man. For example, inosic acid, a flavour enhancer added to food in trace amounts, was found lethal at doses of 20g/kg not from true toxicity but by raising stomach acidity high enough to cause corrosion of the gastrointestinal lining. An equivalent dose in humans would flavour six tonnes of food.

Roughly 80 to 90 per cent of poisonings involve children under five years of age, who commonly react very differently from adults to chemical substances. A study comparing toxicity in newborn and adult animals found large variations due to species–specific developmental patterns that cannot be readily extrapolated to human infants. Fifty per cent of human adult overdoses and 90 per cent of narcotic overdoses involve mixtures of drugs, and often the substances ingested are not known. The LD50 test does not account for drug interactions and is therefore of little use in such cases.

If the LD50 causes acute suffering to animals while producing unreliable and inaccurate results, does it not become a moral and a scientifically ethical duty (towards humans if not animals!) for scientists to choose methods more reliable and accurate?

Convenience, over Science

Mice, rabbits and rats are the commonest species chosen and this is *not* because they are closest to human beings but because they are easy to handle and their use has generated a huge database — not necessarily an accurate database. Thus, when birth defect research is conducted, the scientist pushes for



Monitoring the blood pressure of rat in a terminal experiment.

animals to be used– conveniently omitting to mention that differences in genotypes from one species to another, foetal development, and the relation between chemicals and the foetus differs in each species, and that species-specific differences in placentas affects the results; also that the route of administration is not the human route.

It has been well documented that species, strain, age, along with weight, height, diet, ambient temperatures and housing of the animals all lead to differences in LD50 measurements of differing orders of magnitude. Even factors such as noise, weather, humidity, cycle of light and dark, handling by lab staff can affect the outcome of the testing. A study by the Commission of the European Communities found that LD50 values based on tests of the same substances on the same animals in different laboratories differed by as much as a factor of 12. The quantity measured by the LD50 is not a biological constant and has misleading results.

King Institute, Chennai : rabbit with ears and nose eaten away by infection.

Eyeballs of this rat were removed to enable the investigator at the College of Pharmacy, Delhi University to draw more blood than he could have through the routinely performed tail vein method. The rat is now rehabilitated by the CPCSEA.

Organisations in the USA, such as the Environment Protection Agency (EPA), thze Food and Drug Administration (FDA), the Consumer Product Safety Commission (CPSC), the National Toxicology Programme (NTP), as well as the international organisations OECD (Organisation for Economic Cooperation and Development) and the European Chemical Industry Ecology and Toxicology Centre (ECIET)have consistent policies and recommendations:

a) The LD50 test requires the use of 100 to 150 animals to establish the desired statistical confidence limits of the dose which sentences them to death to evaluate mortality levels. There are recommended alternatives utilising the principles of reduction and refinement. Existing animal data, prior human experience, and expert opinions, can all be useful.
b) A fixed dose procedure, five animals per dose, is sufficient to produce a range of toxic effects.
c) The classic LD50 test should only be conducted when specifically justified for reasons of scientific necessity.
d) The ECIET Centre insists that a dose above 2g/kg is irrelevant and unnecessary.
e) Use of the Structure Activity Relationships (SAR) to obviate the need for testing on animals.
f) Abbreviated test methods such as the approximate lethal dose must be calculated, multiple endpoint evaluations, gross necropsy, moving average, and reversibility of effects.

Archaic Paradigm

Dr. Ray Greek, Scientific Advisor, NAVS, USA, says: "Relying on animals to predict human response, be it drugs or disease, is an archaic paradigm that is being replaced, in scientifically advanced environments, with modern day research modalities like artificial neural networks, computer and

Pharmacy College, Delhi University: wistar rat with a ruptured stomach after surgery : no post operative care in a filthy cage.

mathematical modeling, pharmaco-genetics, high-tech brain imaging scanners and more. Any nation, institution or organisation that continues to rely on animals will be left in the Dark Ages of biomedical research." Here the right code of ethics and honest science merge. In the end the benefit accrues to us, the public, the patients, the humans. India must not be left behind; we need not be an exception to the new world of science and health care.

Virtually all major breakthroughs responsible for identifying the thalidomide disaster, foetal alcohol syndrome, foetal rubella, and other dangers have relied solely on human data.

Animal tests cost a great deal outside India and animal teratogenicity tests require large investment of time, animals, and money (about $60,000 to test one chemical in rats and rabbits). However, in India, animal life is cheap and, therefore, is too often expended in such testing.

In vitro studies are both more practical and predictable and have none of the inherent problems and inaccuracies of animal testing. They are cheaper, faster and more reproducible than animal tests. *In vitro* screens are successfully used in labs where they assess the relative "toxicity" of chemicals within a family of chemicals defined on the basis of structure, functionality, or pharmacological activity. With the progress in genetic engineering, it is possible to cultivate human and animal cells and this form of testing is more stable and accurate. No single method can be expected to cover the complexity of general toxicity in humans. The MEIC 4 Cell tests gives results with 80 per/cent precision as against 65 per/cent of animal tested data and is the most efficient predictor of human acute and chronic toxicity. But all this will be of no avail if scientists in toxicology are not really convinced of the necessity to reduce the use of animals in their research.

Since November 2000, the OECD and the US Environment Protection Agency abolished the LD50 test and refined alternatives were adopted. The LD50 test was deleted from its manual of internationally accepted chemical test guidelines after December 2002.The use of cell cultures *in vitro* to predict acute poisoning effects has been studied since the 1950's and proven extremely successful.

Guinea pigs fed dry pellets mixed with feacal matter, Maulana Azad Medical College.

Frank E. Barile states: "It is anticipated that cell culture techniques will supplement, support and replace currently used animal testing procedures and will be an important component of a battery of tests to predict human toxicity." For the last two decades scientists have found *in vitro* methods in toxicology a perfect substitute for animal tests or tests where repeated doses of a substance or chemical compound is

Bengal Chemicals, Kolkata : unhygienic and primitive condition of the bleeding room for horses.

given to check acute toxicity or subtle toxic effects and recovery from chronic toxic insults. Since 1987 international laboratories have developed and evaluated the success of various tissue culture tests for these same purposes. The Multicentre Evaluation for In *Vitro* Cytotoxicity Program (MEIC) is organised by the Scandinavian Society of Cell Toxicology to coordinate international laboratory testing of chemicals for the purpose of developing *in vitro* alternatives. These best tests mimic chronic human exposure to toxic chemicals as encountered in environments or in occupational settings. Sweden's Dr. Bjorn Ekwall and his colleagues at MEIC have tested 50 chemicals with 61 different *in vitro* assays and demonstrated that these non-animal human cell line tests are more predictive of human toxicity than animal testing. They gave results with 80 per cent precision, as against 65 per cent accuracy using animals for such testing.

In India, however much the large companies cry out that animal welfare issues are holding back the development of the pharmaceutical industry, the fact is that the sale of bulk drugs which require little or no research have been the financial mainstays of Indian pharmaceutical companies. The prospect of enforcement of the Product Patent Regime starting in 2005 has startled them, forcing them to focus on fine tuned independent research. But whereas the West has an open mind to new scientific ideas and earns considerably from this research, India is unprepared for these changes.

Research institutions in the West, as early as 1960's, began to strive for alternatives to animal testing through computer simulation to arrive at results by far more consistent and accurate methods. The quality of research improved; they produced better drugs. Indian companies will now have to follow the WTO (World Trade Organisation) norms. According to Arun Rajan, General Manager, Airtel, Chennai, there is the danger of "a distinct emergence of contract research organisations that will be engaged in conducting clinical research trials for large multinationals who already have and will continue to dominate the pharmaceutical business in India. This will necessarily mean that animal experimentations will be on the increase, as MNC's will now have the singular advantage of using India as a base for animal experiments hitherto banned in the West. This is fraught with danger as this will lead to large scale abuse

Geese at NIV, Pune kept in a waterless tiled basin in an unventilated room. Two basins of dirty stagnant water kept for them to drink. The inspecting team found that no food had been given that day. The birds looked visibly malnourished.

Monkey from NII, Delhi with amputated arm rehablitated with the CPCSEA.

of animal rights in the country."

It is necessary that the advances in scientific protocols for drug testing should be adopted in India also. Guidelines followed in India should also be reviewed to eliminate the necessity for the LD50 test for regulatory testing of chemicals, pesticides and cosmetics. *In vitro* tests should be welcomed by manufacturers of industrial and agricultural chemicals, cosmetic and food industries, and pharmaceutical companies as being more accurate, cheaper and therefore a more thorough evaluation of the safety of their products.

Female rabbits with new borns, no water, no feed. Kept on a dirty straw bedding, food trays have not been cleaned for days.

It would be a terrible irony that, while singing of 'original research,' the most outdated and primitive means of testing and arriving at results should be India's scientific contribution to the world.

Pyrogen Testing

Another area where the use of animals has been greatly reduced is in pyrogen testing. Pyrogen testing is a crucial safety control for drugs as well as innovative high tech products such as medical, cellular therapies and species–specific agents (for example, recombinant proteins). For most biologicals, especially blood-derived drugs, the use of rabbits as the animal for testing still represents the method of choice. It consumes hundreds of thousands of animals every year. This test is laborious, expensive, raises ethical concerns, and cannot be applied to some new products. In recent years, a number of alternative cellular assays have been developed that exploit the human fever reaction, that is, human leukocytes release inflammatory mediators in the presence of pyrogenic contamination. The suggested network brings together the most prominent test systems for transnational comparison and subsequent validation of the most promising models as an integrated goal-oriented problem-solving approach.

At King's Institute Chennai, Vahini-a blind and lame horse was bled 18 litres of blood just before delivery. She died within a month of delivery.

The animal house of Hindustan Syringes & Medical Devices, Faridabad was inspected after reports on television that there was an illegal animal facility being run by the company. The experiments carried out there include tests to ascertain freedom from pyrogenic materials on rabbits and undue/abnormal toxicity tests on mice. Hindustan Syringes, as the name suggests, manufactures biomedical devices such as insulin syringes, cannulas, disposable needles, disposable syringes, surgical blades, infusion sets and glass syringes. The pyrogen test and abnormal toxicity test are part of the required safety testing as per the Drug Control Regulations. The Indian Pharma-copoeia has detailed the experimental requirements for both tests.

Hindustan Syringes is willing to stop these tests if alternatives are found and have already written to their importers for information on alternatives and on the kinds of tests required by them. They have been given information on the test Limulus Amoebocyte Lysate [LAL] which has replaced the

pyrogen test in many parts of the world. From all accounts it appears that the pyrogen test is almost non-existent in the USA and other parts of the world.

The Biomedical Products Manufacturers' Association is of the opinion that these pyrogen and abnormal tests are an unnecessary procedure imposed on them by the Drug Controller. They feel that the sterility tests are stringent enough and once these have been carried out, the animal tests are a formality imposed on them. In fact, in the last 15 years, not a single animal has died in testing which proves that the animal tests are completely unnecessary and there is no need to maintain an animal house. However, until the orders for discontinuance of the tests are issued, certain precautions have to be taken in carrying out the existing procedures.

Sheep fully alive have their skulls drilled with a hole to inject the rabies virus. The sheep languish in pain and suffer paralysis for 6-7 days because their brains are harvested while they are fully alive and conscious. All this to produce the outdated and high risk neurogenic anti-rabies vaccine banned in most countries.

Anti-Rabies Vaccine

Despite government agreement in principle that the Neural Tissue Anti Rabies Vaccine (NTV or ARV) should be banned immediately, nothing concrete has been achieved on this front. Rabies is a dreaded zoonotic disease caused by a neurotropic virus and has a 100 percent fatality rate. Human beings (as well as animals) bitten by dogs, rats, monkeys and some other animals have necessarily to be treated with ARV.

There are two types of vaccines available in India. The conventional Semple's vaccine, also known as the 5 per cent sheep brain suspension BPL inactivated Nervous Tissue Vaccine (NTV) and the Tissue Culture Vaccine (TCV).

It is shocking that more than 20 years after WHO recommended the worldwide ban on the use of the Neural Tissue Anti-Rabies Vaccine and recommended a switch over to the Tissue Culture Anti Rabies Vaccine, the Ministry of Health continues to fund and encourage the production and use of NTV and has still not formulated a plan for its banning.

An overcrowded insufferably hot humid sheep enclosure without ventilation . The floor is wet and smeared with feacal matter.

Countries all over the world have banned the use of sheep brain ARV and have switched over to the production and use of TCV. The only countries continuing with the NCV vaccines are India and Tunisia. The Vaccine Board meeting held on September 9, 1999 presided over by the Director General of Health Services, decided that the production of sheep brain ARV would be phased out and the Tissue Culture Vaccine would be introduced. However, no time frame was given. Even today there are nine biological production centres producing 35,000 litres of anti-rabies vaccine

from sheep brain and 4.5 lakh people are vaccinated every year. Sheep brain NTV is produced by drilling a hole in the brain of the sheep while it is still alive. The sheep eventually suffers a slow death as it becomes increasingly paralysed. The brain is then used for NTV production. Over 30,000 lambs are sacrificed on this account.

There are many complicated and painful side effects of the NTV, which affects at least one in every 200 patients. Dr. Balaraman, Former Chief of Medicine, Madras Medical College says "more than 100 patients line up for the vaccine every day. Admittedly, it has helped a lot of people so far. But it had adverse effects in nearly 70 per cent of patients. It is also painful."

The NTV induces neurological complications 4 to 14 days after the first injection involving a course of 7 or 14 injections. NTV also induces development of anti-bodies to myelin basic protein (MBP) leading to immuno alergogenicity. Thus the use of NTV has a high risk of adverse reactions that result in the same number of fatalities as would occur if NTV were not used! Ultimately the choice to the victim between the side effects of the vaccine and death due to the dreaded disease is very narrow.

The number of dropouts during the course of vaccination with NTV is also high as the vaccine causes painful swellings in the abdomen (near the navel) at the site of administration. This in turn leads to high risk of disease and the recipients of NTV suffer mild to severe local or systemic reactions of a transient or permanent nature.

Today, the people receiving NTV are mainly those living below the poverty line and those who seek treatment at government hospitals. They are compelled to receive NTV since most government hospitals do not stock TCV, and thus patients have to undertake the risk of these extreme side effects.

These are the reasons why the rest of the world has shifted over to the Tissue Culture Vaccine. Another reason is that the NTV has to be administered for 14 days while the TCV is administered for only 5 days. "And, unlike the former, this drug is 100 per cent effective and all private medical practitioners opt for it", says Dr. Balaraman.

The reasons for banning the production of NTV are justified. The alternative safe and effective Tissue Culture Anti-Rabies vaccine is available in India as is the technical infra-structure and expertise. Three biological centres are producing TCV and through them the vaccine is already in the market for use by the fortunate who are in the care of private consultants.

The National Dairy Development Board (NDDB) is presently producing four million vials and has the production capacity to meet the requirements of 14 million for the entire country. Even now, the Government is not taking action to ban the production of sheep brain vaccine completely. They claim that the TCV is too expensive – but the NDDB is willing to reduce its price. The Supreme Court of India in its interim orders dated February 15, 2002 has asked the Centre to ban the neurogenic vaccine. Realising the responsibility to their people, several state governments including Karnataka, Tamil Nadu and Kerala have already banned the use of sheep brain vaccine in their states.

Yet the Ministry of Health and Family Welfare, in sheer disregard and callousness towards the health and survival of rabies victims who get treated at government hospitals, continues to encourage the use of the dangerous NTV resulting in thousands of unrecorded deaths across the country. ❑

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Dissection Banned in Senior Secondary Schools

• Following the High Court Judgement of May 19, 1997, Mr. G. Balasubramaniam, Director (Academic), Central Board for School Education issued a circular on April 12, 2000, stopping, with immediate effect, all dissection of animals in Biology practicals in the CBSE curriculum.

• In February 2001, Mr. M. K. Kaw, Secretary, Ministry of Human Resource Development, wrote to the Education Secretaries of all the States requesting them to instruct all schools to dispense with the dissection of animals and to find alternative methods of teaching.

• The State Governments of Delhi, Gujarat, Mizoram, Tripura, Punjab, and UP have banned dissection. Lakhshadweep has asked the Kerala Government to delete dissection as the schools at Minicoy, Androtт and Kadmat are affiliated to the Kerala State Board. Tamil Nadu and West Bengal have teken up this matter and Chief Minister of Orissa, Mr. Naveen Patnaik, assured the Animal Welfare Board Vice Chairman that Orissa would ban the practice.