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NEWSLETTER

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# Chulabhorn Research Institute

## INTERNATIONAL CENTRE FOR ENVIRONMENTAL AND INDUSTRIAL TOXICOLOGY (ICEIT)

CRI's ICEIT has been designated as a
"UNEP Centre of Excellence for Environmental and Industrial Toxicology".

# Princess Chulabhorn Distinguished Lecture Series: Nobel Symposium


*The inauguration of the Princess Chulabhorn Distinguished Lecture Series on 14 July 1993 at the Queen Sirikit National Convention Center commemorated the 36th birthday anniversary of Professor Dr. Her Royal Highness Princess Chulabhorn. The programme on this auspicious occasion included lectures by three Nobel laureates: Professor D.H.R. Barton, Professor B.S. Blumberg and Professor R. Huber.*

The Chulabhorn Research Institute and the Ministry of University Affairs, with generous support from both public and private sectors, have established an endowment fund to ensure the continuation of the Distinguished Lecture Series in the future, as a tribute to the work of Her Royal Highness Princess Chulabhorn. The lecture series will provide an opportunity for Thai scientists to learn from the research work of internationally renowned scientists in chemistry and the medical sciences and will serve to encourage and inspire younger generations of Thai scientists.

## Opening Address

*In her opening address, Her Royal Highness Princess Chulabhorn expressed gratification that nowadays both the public and private sectors are increasingly aware of the importance of science and technology.*

*Development in science and technology is essential because it is an important factor in improving the economic status of the country and uplifting the quality of life of the people. Science is international, so the exchange of knowledge and experience at the international level is essential for the development of science and technology.*

## Nobel Symposium Programme

The three distinguished speakers, all Nobel laureates, work in the areas of chemistry and medical science. Professor D.H.R. Barton from the Department of Chemistry, Texas A & M University, U.S.A., who received the Nobel Prize in chemistry in 1969, spoke on the topic of *"How to Win a Nobel Prize – A Personal Case Study"*. Professor R. Huber from the Max Planck Institute Fur Biochemie, Munchen, Germany, who received the Nobel Prize in chemistry in 1988, spoke on the topic *"Protein Crystallography at the Interface of Chemistry, Physics and Biology"*. Professor B.S. Blumberg, Master of Balliol College, Oxford University, United Kingdom, who was awarded the Nobel Prize in Medicine in 1976 for the discovery of Australia antigen and its impact on the understanding of the biology of hepatitis B virus, gave his presentation on the topic of *"Hepatitis B Virus and the Prevention of Primary Cancer of the Liver"*.

# Achieving Acceptable Air Quality

*Control of motor vehicle emission is an essential factor in efforts to abate urban air pollution.*

*In the United States, the 1990 Clean Air Act Amendments represent a further attempt to set and enforce vehicle emission standards.*

### Motor Vehicle Emissions

Auto emissions control has a long history. Exhaust emission standards for new cars were first set in 1968 (1965 in California), after which the standards for exhaust emissions became steadily more strict every couple of years until the early 1980s. The strategy adopted to minimize smog was major reductions in unburned HC emission with lesser reductions in $NO_x$. The strategy was chosen in part from our assessment of how the photochemical smog chemistry responds to changes in HCs and $NO_x$ as well as from the technical feasibility of reducing HCs relative to $HO_x$.

Unburned carbon-containing compounds in the exhaust are fuel HCs and partial oxidation products that escape burning during the normal combustion events that occur in each cylinder of the automobile spark-ignition engine. Carbon monoxide emissions are significant when the engine is operated under fuel-rich conditions; that is, when the air in the fuel-air mixture that enters the engine cylinder is insufficient to convert all the fuel carbon to $CO_2$. Rich mixtures are used as the engine approaches wide open throttle because they give the highest possible power from the engine. They also help with combustion stability during engine warm-up and, in older cars, at idle. Oxides of nitrogen are formed from nitrogen and oxygen in the engine cylinder at the high temperature air mixture and are fixed from the reformation of oxygen and nitrogen by the rapid cooling of the exhaust gases.

For the last 18 years, catalytic converters in the engine exhaust system have been used to achieve the large additional reductions in emissions required to meet federal standards. In current new vehicles, a properly working catalyst reduces the emissions of each of the three pollutants – HCs, NOx, and CO-that leave the engine's cylinders by a factor of 5 to 10 before the exhaust enters the atmosphere. However, it has taken two decades for the combined catalyst and engine technology to reach this point.

The 1990 amendments, the subject of intense debate over costs, benefits, and regional differences, will continue the downward trend in standards for new car exhaust emissions; mandate fuel improvements to reduce vehicle emissions further; impose more stringent requirements on stationary sources, both large and small; and introduce specific controls on power plants to reduce acid rain. California has set in place a program of future emissions standards more stringent than those in the 1990 Clean Air Amendments that are designed to go by stages to low emission levels, including a requirement that 10% of the new cars in the year 2003 have "zero emissions".

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*(Abridged from: "Achieving Acceptable Air Quality: Some reflections on Controlling Vehicle Emissions" J.G. Calvert, J.B. Heywood, R.F. Sawyer, J.H. Semfield in Science Vol.261 2 July 1993)*

# TOXICITY TESTING IN ANIMALS

*The controversy over in vitro versus in vivo toxicity testing has attracted much public interest and concern. Scientists themselves are divided on the issue. We present a summary of the views of Dr. J. Fentem, Scientific Liaison Officer at FRAME – Fund for the Replacement of Animals in Medical Experiments – and Professor M. Balls, Director of the FRAME research programme at the Department of Human Morphology, Medical School, University of Nottingham, United Kingdom. Their article entitled "In Vitro Alternatives to Toxicity Testing in Animals" was published in the March 1992 issue of Chemistry & Industry.*

The following excerpts are from Dr. Fentem and Professor Balls' article:

"We are constantly exposed to an increasing number of chemicals, including pharmaceuticals, food additives, household products, agrochemicals, cosmetics and toiletries, as well as industrial chemicals and environmental pollutants.

Currently, the manufacturers of these substances are legally required to carry out extensive toxicity testing, using large numbers of animals (mainly rats and mice), on the premise that this provides the best method available for evaluating the potential human hazard of a particular chemical. In Great Britain alone, in 1990, more than 3.2 million scientific procedures were undertaken on living animals, of which 558,000 (17 per cent) were toxicity tests. And this is against a background of increasing concern among the general public regarding the use of animals in testing.

The need for alternatives to toxicity testing in animals is evident on ethical grounds alone, since, as practiced, much toxicity testing requires adverse effects to be produced in animals, often leading to pain and distress. However, there are also scientific and logistic arguments for adopting non-animal contexts for experimentation. There is increasing scientific criticism of the current dependence of toxicity testing on animal procedures, most of which have never been formally validated, particularly because of the known differences in chemical-induced toxicity between species, which may be manifested as major differences in tissue-specific toxicity or differences in the magnitude of response. Moreover, results of animal toxicity studies are of questionable relevance to human risk assessment and can often be misleading. Alternative methods are generally less expensive, time-consuming and labour-intensive than traditional animal tests. An important alternative procedure is the use of *in vitro* methods in toxicity testing.

During the past twenty years, *in vitro* techniques have become increasingly popular research tools. Mammalian cells and tissues are being used as alternatives to living animals because of lower costs, shorter durations of experiments, dissatisfaction with poor correlations between laboratory animal and human data, and mounting pressure from the general public to reduce the number of animals being used in experiments.

Of the wide range of *in vitro* techniques that have so far been developed, tissue culture methods have the greatest potential with regard to toxicity testing. Cell culture systems are ideally suited to investigation of the molecular, cellular and physiological mechanisms of chemical-induced toxicity, which cannot be readily investigated *in vivo*, for target-organ and target-species toxicity studies, and for answering specific questions about toxic effects. Cell cultures can be established from various organs, such as the liver, kidney, lung and brain, in order to examine possible tissue-specific toxicity, and tissues can be taken from a wide range of possible target species, which is particularly relevant when investigating the potential toxicity of pesticides, environmental pollutants, and veterinary products.

Thus, the main justification for developing *in vitro* toxicity tests is that they will make toxicology a more scientifically-based activity. Perhaps the greatest advantage of *in vitro* toxicity testing is that human tissue can be used, thereby obviating the need for extrapolating data from laboratory animals to man, and making the results obtained of direct relevance to human risk assessment. The use of primary cultures of human cells involves a whole range of ethical, safety and technical problems, but these are gradually being overcome.

If *in vitro* alternatives in combination with other non-animal methods are properly developed and validated, the information they provide will be no less useful or reliable than that currently obtained from animal toxicity tests."

**To provide our readers with an indication of the range of different view points that are held on this important topic. Chulabhorn Research Institute Newsletter invited Dr. Harri Vainio and Dr. Douglas McGregor of the WHO International Agency for Research on Cancer, for their view on this contentious issue.**

Dr. Vainio and Dr. McGregor provided the following view:

"There are strong pressures driving towards the discovery and validation of alternatives to the use of animals in toxicology. In carcinogenicity testing in particular, the emphasis has been on saving time and money since, for each substance tested, both of these are used in enormous quantities. However, quicker and cheaper must be

*(Continued on page 8)*

# Centre Page Interview

***The Centre Page Interview by Dr. Mathuros Ruchirawat and Professor M.R. Jisnuson Swasti, members of the ICEIT Newsletter Editorial Board, highlights the views of Professor Barton, Professor Blumberg and Professor Huber, the three Nobel laureates working in the areas of chemistry and medical science.***

## Dr. Mathuros to Professor Barton

***Question:***

*In developing countries like Thailand with limited resources, the question of priority between basic and applied research has often been raised. What is your view on this?*

Well, this question is not limited to developing countries. It applies to all countries all over the world. You always have to strike a balance between pure and applied research. The most important thing in developing countries is to do basic research in areas that are relevant to the overall economic development of the country. That is to say you don't want to spend your money on grandiose projects but on more practical things like examining the fauna and flora and marine natural products for useful drugs. That is potentially something that could be very profitable.

***Question:***

*Since chemistry plays a crucial and fundamental role in every form of development, what are the most important issues in the field of chemistry in general and organic chemistry in particular that need intensified research?*

Well, in the whole field of chemistry the problem of the 100% yield in chemical reactions always remains with us. If we could run chemical reactions with a 100% yield of the desired product we would reduce pollution problems enormously; and in fact in real chemical manufacture you will find that this remains an unsolved problem.

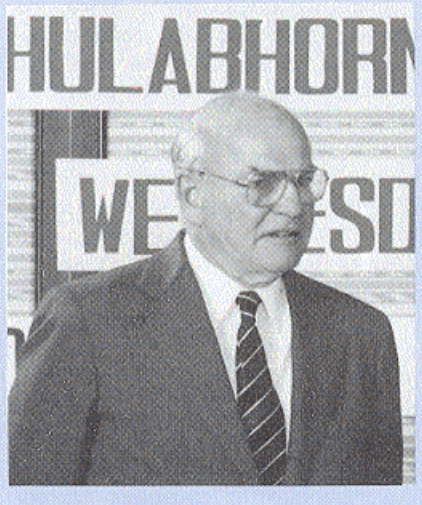
## Dr. Mathuros to Professor Blumberg

***Question:***

*Is there any correlation between developmental patterns, environmental quality and human health?*

There are, of course, many such connections, particularly for infectious diseases. If I can restrict my comments to the hepatitis viruses; at least two of the hepatitis viruses, hepatitis A and hepatitis E are transmitted by contaminated water and food, that is water and food consumed under unhygenic living conditions, so improved development is usually accompanied by these improvements and subsequent improvements in health.

Hepatitis virus B and hepatitus virus C are transmitted by blood, for the most part in transfusions or by needle injection. In the case of virus B, the virus is usually transmitted within the family, particularly by mothers to their children, and it is also sexually transmitted. It has now been



shown in several countries that childhood vaccination programmes have remarkably decreased the infection.

***Question:***

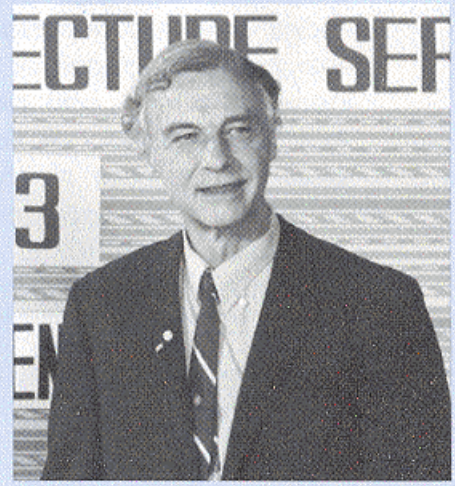
*At present how many types of hepatitis are there, and what are the prospects for prevention and a cure for them?*

There are five identified viruses: A, B, C, D and E, as they are designated. There are probably also one or more others that have not yet been identified, but that are known to exist. It's possible to protect against hepatitis virus A by protection against contamination of water and food, as I have already mentioned.

In addition, a vaccine is available for hepatitis A and injections of gamma globulin can also prevent it for a period of 7 months.

The hepatitis B vaccine, when administered as a part of a universal programme in particular, can markedly decrease the probability of new infection. The vaccine is not useful in people who are already chronically infected, but it is useful particularly when given to new-born children. And, I know that in Thailand the programme for childhood vaccination is now in progress and let's hope that it will be universal within a few years.

Of course, the problem of the cost of the vaccine is a considerable one, but our experience has been that in the last few years the cost of the vaccine has dropped dramatically, and I would have thought that it will reach appropriate levels in due course. It already has in many places. The protection of blood supply against hepatitis B has been in place for quite a few years now.



## Professor M.R. Jisnuson to Professor Huber

***Question:***

*In your lecture on protein crystallography, you alluded to some of the potential applications of this technique. Would you like to elaborate?*

I'm interested in the three dimensional architecture of proteins. Proteins are the basic components of living organisms. When we move, when we speak, when we think, proteins are involved. We also know the function of these proteins depends on their three dimensional structure. So, in order to understand life, we have to know the three dimensional structure of these important components.

Now, apart from their importance for life, proteins are also important in some technical processes, for instance, in the food industry. So we can help in improving proteins for industrial purposes by studying their structure and suggesting changes to them which we can make now with modern molecular biology. More importantly, we can help in the design and development of drugs. Once we have identified the protein molecule involved in a disease, we can study its structure, we can study its interaction with a drug and can suggest how to improve the drug, how to make it more specific, more discriminatory. There is direct application of what we do, particularly in the field of medicine.

***Question:***

*With the current manpower shortage in science and technology in Thailand, young people now are less interested in science. Is there anything you could suggest we might do to promote the interest of the younger generation so that they become involved in science?*

This is clearly not only a problem in Thailand. It is one in my country (Germany) as well. We have to do all we can to promote interest in science. We have to make people aware that the survival of mankind depends on science. How should we solve the overpopulation problem, how should we solve the food problem, how should we solve the energy problem, but with science? This is what we have to emphasize.

In addition, I would like to emphasize the beauty of science; the beauty concerning my field of large molecular structures, of protein structures with their delicate architecture, and before that, the beauty of crystalline materials, their shining faces, their sharp edges. I like them very much and I would like to have young people sharing my enthusiasm for the beauty of science.

# The Ozone Backlash

*While evidence for the role of chlorofluorocarbons (CFCs) in ozone depletion grows stronger, researchers have recently been subjected to a backlash of public criticism of their theories. This backlash graphically demonstrates the problems of doing research on a politically charged issue while there are still many scientific uncertainties. The gap between the present danger of ozone depletion little or none of which can be attributed to rising ultraviolet radiation at Earth's surface – and the possible danger in the future, had not the Montreal protocol been passed, provides plenty of room for a wide range of opinions on how much concern is warranted.*

Much of the better public debate over ozone depletion has centered on the claim that CFCs pale into insignificance when compared with natural sources of chlorine in the stratosphere. Many argue that chlorine could not be depleting ozone as atmospheric scientists claim, because the natural sources such as seawater, biomass and volcanoes have existed since time immemorial, and the ozone layer is still there.

The volcano theory was first proposed in a 1980 *Science* paper by the late David Johnston, a volcanologist with the U.S. Geological Survey. Johnston estimated the chlorine emitted by a 1976 eruption of Mount Augustine in Alaska, and concluded that it pumped 175,000 tons of hydrogen chloride (HCl) into the stratosphere. He then suggested that "the eruption of the Bishop Tuff from Long Valley Caldera, California, 700,000 years ago .... may have injected 289 millions tons of HCl into the stratosphere, equivalent to about 570 times the 1975 world industrial production of chlorine in fluorocarbons".

Atmospheric researchers argue, however, that even Mt. Erebus, the volcano blamed as the source of chlorine for the Antarctic ozone hole, although 14,000 feet high, is still several kilometers below the base of the stratosphere in Antarctica. And Erebus does not erupt explosively, which is a necessary condition to lift chlorine from volcanoes into the stratosphere.

Indeed, so far, expeditions that have brought back direct experimental data on volcanic emissions into the stratosphere suggest that volcanoes play a relatively minor role.

Bill Mankin and Michael Coffey, both of the National Center for Atmospheric Research, sampled emissions from El Chichon after its 1982 eruption. According to Mankin, they saw a "significant increase in HCl in the stratospheric cloud, roughly 40% above the background level". This represented a 10% increase in global stratospheric chlorine at a time when the stratospheric HCl budget was increasing by 5% each year. Thus, El Chichon seems to have advanced chlorine build-up in the atmosphere by just 2 years.

Similar measurements were attempted after Mount Pinatubo erupted in April 1991, but according to Mankin, the nature of the cloud from Pinatubo made the measurements more difficult than those from El Chichon. Nonetheless, he says, Pinatubo appeared to have emitted an amount of HCl, "perhaps less than, perhaps comparable to El Chichon".

For the global picture, atmospheric researchers point to measurements from the ATMOS instrument, which flew on the space shuttle in 1985. The instrument precisely determined the total chlorine content in the stratosphere by making measurements of 30 molecular signatures, including the major CFCs, as well as their sinks and sources. According to Curtis Rinsland of NASA Langley, the measurements showed that chlorine is bound up in CFCs at lower levels of the stratosphere and in the predicted by products of CFC breakdown, HCl and

# THE ROLE OF IE/PAC

UNEP's Industry and Environment Programme Activity Centre (IE/PAC) was established in Paris in 1975 to bring industry, governments and non-governmental organizations together to work towards environmentally sound forms of industrial development. IE/PAC seeks to:

- define and encourage the incorporation of environmental criteria in industrial development;
- help formulate policies and strategies for sustainable industrial development and facilitate their implementation;
- promote preventive environmental protection through cleaner production and other pro-active approaches; and
- stimulate the exchange of information on environmentally sound forms of industrial development

## IE/PAC AFTER UNCED

1992 proved an eventful year for the UNEP Industry and Environment Programme Activity Centre (EI/PAC). The United Nations Conference on Environment and Development (UNCED) held in Rio de Janeiro in June, was a watershed event for all concerned with sustainable development.

The post-Rio period led IE/PAC to review its strategy for the implementation of UNCED's agenda for action, 'Agenda 21', with the help of an advisory group fromed by its partners in industry, government and non-governmental organizations (NGOs).

'Cleaner production', a term coined by IE/PAC when it launched its Cleaner Production programme in 1990, has now entered the sustainable development lexicon. This programme was strongly supported in Agenda 21, and also received backing from participants at a Ministerial Meeting held in October.

The APELL Programme (Awareness and Preparedness for Emergencies at Local Level), now in its fifth year, also received acknowledgement in Agenda 21. The programme, developed with the support of the international chemical industry and governments, aims to minimize the occurrence and impact of industrial accidents. It holds international and regional workshops to train people to implement the programme, and disseminate information through a network of APELL focal points.

Seventy-two national governments now have APELL focal points and IE/PAC provides APELL information to the focal points which in turn ensure its dissemination to appropriate agencies nationwide.

In just one year since its launch in 1991, the OzonAction programme for the implementation of the Montreal Protocol has expanded rapidly, with Country Programmes now underway in 25 countries. Training and information exchange have brought practical assistance to industries and governments around the world, helping the world move closer to reaching the targets set for the phase out of ozone-depleting substances.

In addition to its sector-specific activities, IE/PAC has been active in networking and outreach activities with partners, raising awareness, and initiating and encouraging action in a wide range of cross-sectoral areas.

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Adapted from UNEP IE/PAC ACTIVITY REPORT 1992, Jacqueline Aloisi de Larderel, Director.

hydrogen fluoride (HF), at higher levels. This is exactly what the ozone theory predicts.

Further studies done from the Kitt Peak Observatory, by Rinsland and his colleagues, and from a base in the Swiss Alps by Rodolphe Zander, an atmospheric physicist with the University of Liege, and his colleagues, document the rise in HCl and HF over the past 20 years for Kitt Peak, and 40 years for the Swiss station. Both show a steady atmospheric increase of the two molecules, with HF rising at a consistently higher rate than HCl. Whereas HCl does have some natural sources, HF is produced almost entirely by photodisassociation of CFCs.

Such data leaves the critics with little basis for claiming that the ozone layer has long withstood high levels of chlorine without harm.

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(Adapted from Science Vol 260, II June 1993)

OZONACTION

## INFORMATION EXCHANGE

IE/PAC's OzonAction Information Clearinghouse (OAIC), established to transfer information on policy and technical options for the phase out of controlled Ozone-Depleting Substances (ODS), contains: descriptions of alternative technologies; a database of ODS-reduction products and services; national and corporate programme summaries; a calendar of ODS-reduction events; an international directory of ODS-reduction experts; abstracts of ODS-reduction documents; a message centre; and news bulletins describing the latest worldwide developments in ODS-reduction.

During 1992, OAIC responded to more than 500 queries from both developed and developing countries. The OAIC computer system was continually improved and updated, and an OAIC *User Guide* was drafted for inclusion in the *OAIC Information Networking Kit,* to be published in early 1993. Four issues of the quarterly *OzonAction* newsletter, which reports on the latest news, technology updates and initiatives undertaken by countries and organizations implementing the Montreal Protocol, have been published in Arabic, Chinese, English French and Spanish since March 1992, and distributed to 15,000 individuals and organizations. Five technical brochures, part of the series "Protecting the Ozone Layer" have been published or are soon to be published in English, French and Spanish, concerning: *Refrigerants; Solvents, coatings and adhesives; Fire extinguishing substances; Foams and Aerosols, sterilants, carbon tetrachloride and miscellaneous uses.*

# International Course on the Detection of Health Hazards in Human Populations Exposed to Mutagens and Carcinogens

**Chulabhorn Research Institute**
Bangkok, 15-26 November 1993

The International Agency for Research on Cancer and the International Programme on Chemical Safety are jointly organizing a course on the Detection of Health Hazards in Human Populations Exposed to Chemical Mutagens and Carcinogens, with the collaboration of the Chulabhorn Research Institute, Bangkok.

The course is intended for public health and research workers involved in activities aimed at identifying and reducing the health hazards that the urban and rural populations may face in South-East Asia. Both toxicologists and epidemiologists may be involved in this field, and the course should strengthen the interdisciplinary understanding of all involved. The course will comprise plenary lectures in the mornings, with participants selecting study groups or laboratory practicals in the afternoons. Practicals will involve hands-on training in carcinogen-protein/DNA adduct detection and PCR techniques to detect both specific gene mutations and polymorphisms in carcinogen metabolism.

Specific topics to be covered in the course programme include:

- Basic mechanisms in mutagenesis and carcinogenesis
- Dosimetry of chemical carcinogen exposures: approaches examples
- Assessing individual susceptibility to carcinogen exposure:
  - Polymorphisms in carcinogen metabolism
  - DNA repair
- Markers of biological effects
- Cytogenetic surveillance of populations at risk
- Viruses and cancer in South-East Asia
- Parasites and cancer in South-East Asia
- Oesophageal cancer
- Liver cancer
- Lung cancer
- Qualitative and quantitative risk assessment: basic principles and concepts
- Identification and assessment of cancer risks

**SCIENTIFIC PROGRAMME COORDINATORS:**
DRS HARRI VAINIO AND CHRISTOPHER WILD
(International Agency for Research on Cancer, Lyon, France)

## TOXICITY TESTING IN ANIMALS

*(Continued from page 3)*

accompanied by at least a maintenance of reliability if the non-animal (or fewer animals) alternative is to be acceptable.

Carcinogenesis is a complex, multi-step, multifactorial process which occurs in animals (human and non-human); it does not occur *in vitro*. The strength of *in vitro* tests supposedly predictive of this process is their simplicity, directed as they are at some important link in the chain of events beginning with normalcy and health and ending with malignancy and death. Such investigations often hold mechanistic significance and do help us to understand and evaluate the process better. But, the source of their strength is also their weakness. It is currently impossible to model the complex interactions of the biological machinery used by mammalian systems by other than experimental animals or man. No single cell is typical of an entire organism and no enzymic reaction can model for more than itself.

Tests using *in vitro* systems may be useful in setting priorities for testing in animals, but so far they are useful only to warn us of a hazard: absence of responses in the predictive tests is an unreliable indicator of safety. Where animal tests for carcinogenicity could be failing us – and it is not clear to what extent this is so – is in the manner in which we design them (rather than in the response of the animals) and interpret the results. There is little doubt, however, that the complexities of pharmacokinetics, metabolism and pharmacodynamics as they occur in mammals during carcinogenesis can be examined constructively only in mammals. One should continue to use *in vivo* data to protect public health and to reduce the burden of cancer from exposures to factors known to cause these diseases in humans and in animals. The cascade of events collectively termed carcinogenesis may eventually be deciphered fully enough to allow better predictions of carcinogenic risks to humans – at the moment, however, we are not yet there."




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