DISCUSSION

Dr David Basketter

Dr Griffith, much of the information you presented in your talk used the parameter 'mean time to recovery'. What was the basis for the selection of that parameter?

Dr Jack Griffith

The selection of this parameter is based on the fact that, other than in control experimental exposures of the eye, there is usually very little quantitative information that can be extracted from human experience. Consumer estimates of foreign material entering the eye are probably grossly exaggerated because there is no basis for measurement. Secondly, these episodes usually do not involve any medical examination so there is no record of the condition of the eye at the time of the incident. However, time to recovery can be recalled with some certainty. Furthermore, follow-up of these episodes allows a record to be kept of the time taken for healing to be completed. This is usually the only quantitative measurement that can be obtained.

Professor Paul Turner (Chairman, CoT)

If you take a group of 20 people and introduce into their eyes the same concentration of drug in the same vehicle, you will get 20 different responses in terms of tolerance. Also, the same degree of corneal or conjunctival damage will be associated with different responses in each individual. Does this apply to laboratory animals?

Dr Jack Griffith

Yes, I believe it does. It is interesting that people have a wide range of responses to what they claim is painful.

Dr Philip Botham

Dr Griffith, returning to the low-volume comparison conducted under the auspices of HSE, I realize that you are limited by chemicals for which there is little data on their potential to cause human effects, but there is another important consideration; if a regulatory authority is able to accept data based on the low-volume procedure and to allocate a chemical the appropriate EEC risk phrase, for example, then, if nothing else, we are satisfying our obligation to reduce the suffering of animals. For this reason alone ICI believes it is justified in continuing the development of this test.

Dr Jack Griffith

I was not intending to be critical of that point.

Dr Diana Anderson (BIBRA)

Dr Basketter and Professor Turner, if the results from the joint study on the lymph-node assay are satisfactory, would the UK authorities see this as an acceptable alternative?

Dr David Basketter

This is an important question that we are currently considering. I think it will be a long time before we are able to use this assay to present evidence to the regulatory authorities that a new chemical is *not* a sensitizer. It will

be easier to present evidence in support of a positive classification.

Professor Paul Turner
The CoT is not a pro-active committee, that is we would need to be persuaded by industry that their test is successful. I think that this will take a long time, and there will probably be another chairman of the CoT by then!

Dr Klara Miller (BIBRA)
In guinea-pig testing it is sometimes very difficult to differentiate between chemicals that may be irritants and those that may be sensitizers. The group working on the lymph-node assay is hoping that the test will be able to distinguish between irritancy and induction of an immune response.

Dr David Basketter
That is one of the positive aspects of the lymph-node assay. From Unilever's experience I think that our guinea-pig tests are very reliable indicators of sensitization potential. If experiments are done properly it should be possible to distinguish for most materials between allergy and irritation, because the tests should be below the irritation threshold.

Dr Philip Botham
From the standpoint of risk assessment, a test that establishes dose response is highly desirable.

Dr Klara Miller

Also in favour of the lymph-node assay, is that coloured substances are sometimes difficult to assess in the guinea-pig test, where the endpoint relates to cell proliferation and the colour of the substance is not taken into account.

Professor Paul Turner

Dose-response data is very important, and more should be made available to regulatory bodies.

Dr Ralph Peacock (Lilly Research)

Dr Basketter, in your lymph-node test you showed an excellent correlation with 'physicochemical calculations'. What were these?

Dr David Basketter

I was actually correlating guinea-pig test results, not local lymph-node test results, with physicochemical parameters. These parameters have been described by Roberts and Williams. David Roberts described the relative alkylation index of a material, that is its ability to behave as a nucleophile. If you take aspects of reaction rate and lipophilicity as measured by the partition coefficient, and then also consider the doses used in the guinea-pig assays, then you can build a composite parameter and obtain the type of correlation that we have shown.

Dr Gareth Blaine (Consultant)

I hope that alternative tests, such as the lymph-node assay, do not fall into the same category as mutagenicity tests seem to have done; some regulatory authorities have built into their guidelines a whole series of mutagenicity tests that do not decrease their requirements for testing in whole animals, especially carcinogenicity testing. I hope that this does not happen with the Draize eye test.

DISCUSSION PART TWO

Dr Gareth Blaine

Dr Gangolli, I am struck by the difference between your findings and those of S.R. Walker and C.E. Lumley (*Regul. Toxicol. Pharmac.* 1986, **6**, 66-72) who analysed a number of short-term and long-term studies on products provided by the pharmaceutical industry. Can you comment on my suspicion that pharmaceutical products, which by definition have a pharmacodynamic activity (lowering blood pressure or affecting some other organ system), might be different from products from the chemical industry, which would not be expected to have a pharmacodynamic effect.

Dr Sharat Gangolli

A wide variety of chemicals was examined in our survey, and some of them were toxicologically active. We were anxious to confirm the supposition that all the required information can be obtained from a 90-day study. It is possible that all relevant effects might be picked up in a study continued for 6 months, but unfortunately the NTP reports do not comment on this.

Dr Francis Roe (Consultant)

Ideally, a long-term study should be conducted first, and then, after careful evaluation of the results, one should ask whether the changes found could have been picked up earlier. But this is not what happens. Unfortunately, it is not common for researchers to look back at the results of short-term studies when they unexpectedly encounter evidence of effects in long-term studies. If they did so - knowing now what to look for - they might find that mild effects had been overlooked in the earlier short-term studies. My second point is that I do not see how this question can be considered in isolation. Almost anything that disturbs physiological status or hormonal status is liable to have an effect on the incidence of ageing-related disease and of neoplasia. A requirement of a Regulatory Authority to test a chemical at toxic doses is a requirement to cary out tests in physiologically abnormal animals and any adverse effects seen might be indicative of the toxicity of the chemical. Alternatively they may be non-specific consequences of physiological dissaray. A system of testing that does not require exposure to unrealistically high and toxic doses, would cut down the risk of encountering non-specific effects of this latter kind. Arguably the top-dose level tested should be based not on the maximum tolerated dose level, but on levels that relate realistically to human exposure levels.

Dr Sharat Gangolli

The NTP was committed to carrying out carcinogenicity studies from the beginning, and the subacute and subchronic studies were used as dose-range-finding studies to establish the no-effect level. Doses selected for the long-term studies were well below those that produced toxic effects in the 90-day studies, so that the argument about disturbing physiological processes does not really hold. One would have expected the same effects as seen at 90 days, but at a lower dose and after

a longer period, but instead an entirely new range of target-organ effects was observed. It is possible, however, that if the 90-day studies had been conducted more carefully and more detailed investigations had been carried out, these effects might have been picked up.

Professor Paul Turner

Does anyone have any comments on Mr van den Heuvel's paper?

Dr Philip Botham

One of the critical features of the fixed-dose procedure is the ability to determine signs of toxicity at different levels. Can you comment on this Mr van den Heuvel?

Mr Michael van den Heuvel

The pioneers of the fixed-dose procedure have always had to face the challenge that apparent toxicity is not a discrete endpoint like death. However, toxicologists are required to distinguish between compound-related effects and background effects in traditional toxicity studies, so I think it is likely that properly trained toxicologists will be able to identify signs of compound toxicity at different levels in the fixed-dose procedure.

Panel: Professor Paul Turner (Chairman, CoT) [Panel Chairman]; Professor John Daniel (EUROBEST Associates); Dr Sharat Gangolli (Director, BIBRA); Dr Jack Griffith (The Procter & Gamble Co.); Professor Michael Sharratt (BP); Dr Philippe Shubik (Green College, Oxford); Mr Michael van den Heuvel (Department of Health).

Dr Philippe Shubik

My first point relates to skin irritation and effects on the eye. I try to avoid using the term irritation because it describes a multitude of events. For those who are concerned with the practical outcome of irritation studies, more knowledge is required of the mechanism so that different effects can be identified and 'irritant' compounds classified. Appropriate methods are needed to detect different endpoints such as erythema or oedema. The same applies to carcinogenesis; there is at present a drive to investigate mechanisms of carcinogenicity so that this knowledge can be brought to bear on regulatory decision-making. Since carcinogens act in entirely different ways it is inappropriate to view all carcinogenic effects collectively.

Secondly, I was delighted to hear Dr Gangolli refer to John Barnes' statement (Barnes J.M. and Denz F.A. *Pharmac. Rev.* 1954, **6**, 191-242) that "There is very little useful information to be extracted from chronic toxicity studies beyond the first three to six months". However, it should be borne in mind that many of the chronic studies conducted in the 1950s were poor and the NTP probably has not helped very much. We now have a huge mass of data emerging from the NTP on

which IARC bases its classifications, but what we need is more emphasis on tightening up the definition of carcinogenicity, and improved subacute studies to establish mechanisms. In addition, there should be a greater concentration on research in combination with testing.

Professor Michael Sharratt

Over the past few years toxicologists have paid a lot of attention to developing consistency of results between laboratories, rather than to developing a knowledge of mechanisms. Consistency between laboratories may be achieved, but unless tests actually predict what will happen in man, they are virtually useless. Some progress has clearly been made in relation to eye irritation; in occupational health we ask what happens when a material comes into contact with the human eye, and will the ordinary first-aid measures (washing out with water) actually protect the eye and save a person's vision?

There is still a long way to go in the interpretation of skin irritation studies, largely because they have been conducted in a routine manner, without any thought as to the pathological changes. Again, in industry we are far more interested in the long-term effects of repeated exposure, than a result showing redness on rat or rabbit skin after a single exposure. So we need more predictive methods in skin irritation. It is encouraging that the Magnusson-Kligman test is becoming less and less popular--it produces much distress in animals. The mouse test, however, sounds encouraging. A

major problem for industry is that there is no test available that detects pulmonary sensitization, although I know that some work is being done on this at BIBRA and ICI.

I think the pioneers of the fixed-dose procedure should be applauded. I am disappointed, however, that the LD_{50} is so widely used in the classification of materials for the potential dangers to man, and in transport and the workplace. The LD_{50} tells you very little about risks in the workplace, instead a thorough knowledge is needed of the physiological and pathological changes brought about by a material. We do not have this information for the majority of chemicals. I agree with Professor Shubik that we need to understand what is actually happening in animals and how this is relevant to man before we can use the data properly.

Professor John Daniel

Those who have been associated with toxicology for the last 20 ~~twenty~~ years or so will realize that the science has not really progressed in the last decade. We have not made the advances that John Barnes and Leon Golberg would have hoped for. Dr Gangolli illustrated both the strengths and weaknesses of the current practices, he showed that it is relatively easy to identify a hazard and highlighted the difficulties in using such data for valid risk assessment. These two points are incompatible with current design. But we must ask what is the justification for rejecting a process that seems to have worked reasonably well over the past 20-30 years? The answer to this is provided in part by cases where drugs have been

withdrawn because of adverse effects that could not have been predicted by animal studies either because the techniques were not available or because the process was not understood. So we need to consider how we can improve the current approach to hazard evaluation and risk assessment. Furthermore, so that we, and consumers, may have greater confidence in safety testing, our programmes should have more direction. For instance there should be more emphasis on quantitative structure-activity relationships; we need to know the significance of effects occurring in a six-month or two-year study. In addition, observed effects in acute toxicity studies should be examined more closely. I should like to see more tests of function introduced, as opposed to the plethora of laboratory assays currently in use, which have limited value. I should also like to see more intense use of a smaller number of animals, including if necessary a reversibility phase. Ultimately, use of safety factors should be avoided; it is too easy to say that application of a hundred-fold or a thousand-fold safety factor will produce a safe limit, and I do not think this is where the future of toxicology lies.

Dr Frank Fairweather (Chairman, BIBRA Council)
A major problem with alternative tests lies in convincing the regulatory authorities of their feasibility. How can we persuade the authorities to accept well validated alternative tests?

Mr Michael van den Heuvel

Acceptance by regulatory authorities takes a long time, but it is encouraging that there is now an OECD procedure that may help to speed up the process. A proposal can be put to the OECD for inclusion of a test in the internationally agreed guidelines. If the test is accepted by all 26 OECD countries this will put pressure on regulatory authorities to approve it. The question of international acceptance of new techniques is to be addressed by a group sponsored by the CEC.

Dr Jack Griffith

From a practical point of view, if a country is to accept a test it really needs to be actively involved in its development. It can be difficult to gain the attention not only of regulatory authorities but also of other research groups, especially when those research groups may be competing. Additionally, industry-wide acceptance must be obtained since it is ultimately industry that will be regulated. There must be a consensus between industry and toxicologists on the scientific process before a proposal can be put to the regulatory authorities. In the case of the low-volume eye test we have had support from the UK and Switzerland but negative responses from the USA, probably because we did not do sufficient groundwork in obtaining agreement between the regulators and industry.

Professor Paul Turner

Dr Sharratt, from the point of view of the HSE is there any way in which we can expedite the acceptance of some of these new procedures?

Professor
~~Dr~~ Michael Sharratt

I do not know. I think the new procedures are good ones in that they reduce the numbers of animals used, and encourage researchers to obtain the maximum information from those animals that are used. My only criticism is that acute toxicity is still the most poorly studied area, whereas a lot of effort goes into studying short- and long-term toxicity, teratogenicity etc. From an industrial point of view acute toxicity really does need to be well studied. We need to know what effects occur at all dose levels and what pathological and functional changes occur. Industrially, the lethal dose is relatively unimportant.

Philippe
Dr Shubik

In the USA, particularly in the FDA, there is a great desire to move ahead and change things, but the Agency requires independent advice in the form of a committee, and this is extremely difficult to appoint with the present political pressures.

Dr Diana Anderson

The fixed-dose procedure has a far more comprehensive data set than the low-volume eye test, and yet it still has not been accepted by regulatory authorities. How large does the data set have to be before international acceptance is obtained? In

addition, Dr Sharratt has said that more mechanistic information about chemicals is required. How can we bring these two elements together and obtain acceptance from governments?

Professor Paul Turner

I consider that it is much easier now for us to incorporate requests for mechanistic work into our requests for further work. Dr Roe, as a former member of the CoT do you agree with this?

Dr Francis Roe

Yes, this general trend has been particularly noticeable over the last eight years or so, though more so in the UK than in the USA.

Dr Jack Griffith

In industry, we are so involved with the regulatory apparatus that we do not have the opportunity to concentrate on pure research in toxicology. Secondly, all the toxicology we do is open to public view and so studies for the sake of furthering the science, or for the sake of mechanisms, especially in animals, is subject to criticizm and public outcry.

Professor Paul Turner

That is a rather discouraging approach. I did not agree with Professor John Daniels' remark that no drugs have been withdrawn for reasons that could have been picked up in animals. I can think of some examples from companies that are represented here today. In addition, some drugs have been

factor approach for micronutrients, and an alternative approach for macronutrients including novel foods. I should like to ask Dr Roe whether the long-term dietary study in rats had a defined objective, that is, were you looking at particular parameters like hormonal levels in blood, or dietary contaminants, or was it a large exploratory study looking at the effects of different diets?

Dr Francis Roe

We already knew that restricting the diet to about 80% of the amount that ad libitum-fed rats eat would have a dramatic effect on age-related diseases and endocrine tumours in rats, so our objective was to investigate whether these trends would be produced by altering the diet formula, by reducing the energy value of the diet or by restricting the daily period of access to diet. Interestingly, it was a low-nutrient, high-fibre diet that gave rise to the excess incidences of uterine tumours and mesenteric lymph node tumours which I referred to earlier. At present we are at a loss to explain these findings. We have in store samples of the diets for possible future analyses over and above those that have already been carried out. However, at present we have no idea as to what to analyse them for. In direct answer to your question, we did collect clinical chemistry, urinalysis and circulating hormone data and will, in due course, be looking carefully at these.

Dr Philip Botham

I must emphasize that there are some enlightened industrial laboratories, and I would include ICI among these, that see great benefit in mechanistic studies, especially in the study of carcinogens, particularly non-genotoxic carcinogens. Presentation of such studies to regulatory authorities has helped to put into perspective the results of the more traditional toxicity tests.

Dr Gareth Blaine

withdrawn when this could have been avoided if the sponsor had paid more attention to the known toxicology of the compound, and simple pharmacokinetic principles. I think it is rather sad if a company does no more than basic toxicology.

Professor
~~Dr~~ Michael Sharratt

I am afraid that industry is shooting itself in the foot. For example, renal tumours were found in some industry inhalation studies in which the animals were overdosed with gasoline. It took a great deal of mechanistic work to show that this finding was probably 'irrelevant to man'. The same situation occurred in a series of studies with materials like kerosine, in which skin painting produced skin tumours. Again, a great deal of mechanistic work was required to confirm that these materials almost certainly do not present a carcinogenic hazard to man. Unless industry modifies its strategy it will continually see severe restriction on materials that really do not require such action.

Dr Francis Roe

I have seen a number of promising drugs abandoned because the company felt that it would cost more to obtain approval than to investigate the mechanism of toxicity. With several colleagues I have recently completed a 1200-rat study investigating different diets, and have obtained some startling results. One fairly ordinary kind of diet quite unexpectedly greatly increased the incidence of mesenteric lymph-node haemangiomas and haemangiosarcomas. Perhaps even more significant is that the same rather ordinary diet produced a highly significant excess of adenocarcinomas of the uterus. Such findings would 'kill' any prospective drug stone dead! So I make the plea that toxicologists should take far more seriously then at present the influence of type and quantity of diets fed to animals under experiment. Under conditions of ad libitum feeding it is not the toxicologist but the test animal who decides how much it eats, and the more it eats the sooner it will develop ageing-related diseases and cancers and the earlier it will die.

Professor Paul Turner

Dr Gangolli where can we obtain funding to follow up these important findings from Dr Roe?

Dr Sharat Gangolli

I cannot give you a quick answer to that. However, there are two problems that tend to confound the situation. First, commercial exploitation requires rapid clearance of a product through regulatory hurdles by means of a series of quick cheap tests. Secondly, good laboratory practice presents a number of problems that may lead to inflexibility. I would suggest that since industry has the commercial imperative it should pay for these studies.

Professor Paul Turner

Dr Basketter, would you like to respond on behalf of industry?

Dr David Basketter

I agree that it would be of great value to carry out a detailed follow-up on the study described by Dr Roe, but I suggest that those who might be able to offer the financial resources are not present at this meeting!

Professor Paul Turner

Industry is astonishingly slow at times to carry out even modest studies, which will inevitably be required later on, and which become more expensive as time goes on.

Dr Anthony Yardley-Jones

There may be many of us who consider that our toxicity testing programme could be improved, but the reality is that decisions have to be made about compounds every day. Is the battery of tests that industry has at the moment adequate for realistic risk assessment?

Dr Philippe Shubik

I do not think these tests are adequate. By accepting the simplest tests, illogically based mathematical risk assessment and empiricism at every stage, we become targets for extremists, and the situation will worsen if this procedure is allowed to continue. The public are constantly told that substances are highly toxic when in most instances there is very little information on the nature of their toxicity. This arises from the acceptance of simple procedures that satisfy the regulators and keep industry happy.

Professor John Daniel

Over the next few years a variety of biotechnological food products is likely to emerge, and we will need a new framework for testing the safety of these products, especially those that are based on the use of recombinant DNA technology. Ultimately, we will need a dual set of standards; the safety

Dr Shubik appeared to be saying that current toxicity testing is of no value. The public are unaware that many potential drugs are screened out before they ever reach the market, and so they are not able to judge how they have benefited from the toxicologist's work. Dr Roe, surely any problem with a diet would show up as a difference in the results between treated and control groups?

Dr Francis Roe

In many rat studies there is virtually 100% incidence of pituitary tumours and this could be prevented by dietary restriction, but industrial and contract laboratories continue to overfeed rats. Can you think of any human population that shows 100% incidence of pituitary tumours?

Dr Philippe Shubik

I was not saying that current toxicity testing is of no value; some kind of toxicological evaluation has to be done, and the current programme is obviously useful. But to codify these tests into law so that they cannot be modified in the future is not a sensible way to proceed. The point is that research should be carried out alongside test procedures for verification. The results generated by standard tests should be used to greater effect for research purposes. Dr Roe has illustrated how much can be achieved with a large study. Secondly, I have never implied that industry is doing nothing. I am well aware of the important work of companies like ICI and Procter & Gamble.

Professor Vincent Marks (University of Surrey)

The trouble with many studies is that inbred strains are often used, so it is possible to get a 100% incidence of a lesion. This does not reflect the human situation, in which you have many different genotypes. Genotypic variation in humans accounts for the fact that it is impossible to achieve 100% safety, and until we get this message across to the public we will be looking for the perfect test forever.

Dr Francis Roe

Our study was conducted in an outbred strain, and my remarks about pituitary tumours therefore apply to outbred strains. It is very easy to blame the genes, which are certainly not unimportant, but the vast majority of the variation we are seeing is environmental, and in the case of rats the cause is overfeeding combined possibly with lack of normal sexual activity.

Professor Vincent Marks

I agree that it is easy to blame the genes, but it is also just as easy to conclude that effects are entirely due to environmental influences. In fact the two forces interact with each other.

Professor
~~Dr~~ Michael Sharratt

The tests that are laid down by government agencies are really pilot studies and they should be recognized as such and not treated as an end in themselves. They will often show up peculiarities that need to be followed up, but at present

toxicologists continually carry out tests and turn out reports without giving much thought to the observations that are made.

Dr Philippe Shubik

The problem for the regulators is that they are presented with a certain amount of information upon which they must base their evaluations and make a decision. They may request more data, which might become available months or years later, but in the meantime they have to make decisions.

Dr Diana Anderson

Industry and regulators are very loath to move away from standard protocols because the historical data base is then altered, and effects might be found for which there is no reference point.

Professor John Daniel

It is the responsibility of industry when it markets new products to demonstrate that they are safe, and in addition to the tests required by the regulators industry very often does an enormous amount of extra work to satisfy itself of the safety of its products. However, it tends to follow a well trodden track from acute through to subacute toxicity and then teratology and chronic toxicity, whereas I would favour looking at, for example, retrospective pharmacokinetic data so that predictive toxicology plays a greater part. In this way the use of excessive doses can be avoided, and certainly in the USA the regulatory bodies are very receptive to this type of approach because it helps them make a more valid judgement.

Dr Rod Morrod (ICI plc)

We have heard much of this discusion many times before, and I think the attainment of more sophisticated toxicology will depend not only on financial resources but also on the availability of highly trained toxicologists. We have a number of centres of excellence in toxicology in the UK, and we need to set up a committee to decide policy for the direction of future research.

Dr David Gompertz (HSE)

The MRC is to set up a committee on environmental and occupational toxic hazards, chaired by Dame Barbara Clayton. I believe it will address some of these issues.