

CHEMICAL SAFETY AND ANIMAL TESTING

A REGULATORY SMOKESCREEN?

A British Union for the Abolition of Vivisection (BUAV) report
by Dr Gill Langley MA, PhD (Cantab), MIBiol, CBiol



The British Union for the
Abolition of Vivisection



European Coalition to
End Animal Experiments

INTRODUCING THE BUAV AND THE EUROPEAN COALITION TO END ANIMAL EXPERIMENTS

FOREWORD

The British Union for the Abolition of Vivisection (BUAV) is the world's leading organisation campaigning peacefully to end all animal experiments. As Chair of the European Coalition to End Animal Experiments¹, the BUAV liaises with key animal groups across Europe to co-ordinate campaigning initiatives and ensure that laboratory animals are high on the European political agenda. As a founding member of the International Council for Animal Protection in OECD Programmes (ICAPO), the BUAV joins with animal protection groups across Europe, the United States and Japan to ensure that laboratory animals have an effective voice within the Organisation for Economic Co-operation and Development, as it co-ordinates international testing guidelines that affect laboratory animals around the world.

The Way Forward – Action to End Animal Toxicity Testing

The BUAV's Harmful If Swallowed campaign was launched in 2000 to oppose the mass poisoning of millions of laboratory animals in cruel and scientifically dubious toxicity tests for the EU Chemicals Policy. Whilst fully supporting the aim of the proposed Chemicals Policy – to improve chemicals regulation and to protect humans and the environment from harmful substances – we do not believe that animal testing is the most humane or the most reliable method to achieve that goal.

In 2001 the BUAV published *The Way Forward: a non-animal testing strategy for chemicals*². This ground-breaking document has inspired a major debate within Europe about the current and future potential of non-animal toxicity testing, and has challenged worldwide regulatory systems that rely on outdated and cruel animal tests. As well as highlighting the scientific failings of animal toxicity testing, *The Way Forward* calls for targeted funding of non-animal test development and the immediate introduction of further non-animal tests, both within the EU and internationally.

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SUMMARY

In this report we set out five key reasons why a system of chemical control which relies on laboratory animal testing cannot be precautionary or responsive. There are highly significant inherent weaknesses in animal toxicity testing which cannot be overcome. A regulatory system which depends on trying to interpret vast quantities of animal test data will fail environmental and human health, as well as waste limited resources.

This BUAV report uses a number of chemical case studies to illustrate our five key criticisms of the role of animal tests in regulatory decision-making. The lessons of asbestos, tributyl tin, brominated flame retardants and bisphenol A point to the need for a new approach. We propose a strategy that dispenses with animal testing, and instead combines non-animal, human-based methods of testing with contemporaneous, technologically-advanced programmes for monitoring environmental and human exposures to chemicals.

We suggest that, together with the precautionary principle, information on exposure and toxicity from these two broad approaches would underpin a regulatory system that could identify and control toxic chemicals in a timely manner.

INTRODUCTION

For several decades, assessments of the hazards and risks of chemicals have depended heavily on animal test data. Under the new European chemicals strategy, animal test data currently continues to underpin efforts to predict the safety of chemicals to humans and the environment.

However, the BUAV believes that by producing results of indeterminate reliability and relevance, animal test results act as a 'smokescreen', hiding the weakness at the heart of chemical regulation and allowing the continued marketing of chemicals that should be banned. Instead, hazard and risk assessment could and should involve a precautionary approach based on non-animal testing methods plus pro-active programmes of human and environmental monitoring.

In this report we use historical chemical case studies to argue against any reliance on animal test results in assessing whether chemicals pose a risk to the environment or to human health. We explain how laboratory animal data have hindered regulatory decision-making, leading to postponement of chemical controls in some cases and unnecessary controls in others.

Ten ways animal test results may hinder chemical regulation

1. The response to a chemical in the animal species/strain/gender used differs from that of humans or another test species.
2. The absorption, distribution, metabolism or excretion of a chemical differ between species.
3. The tissue effects are not the same at the macroscopic or microscopic level as in humans or are seen in different organs.
4. Differences at the anatomical, physiological, cellular, subcellular, or receptor levels cause varying susceptibilities to toxicity.
5. The dose required to produce toxic effects in animals may never be reached in humans.
6. The target dose in humans cannot be achieved in test animals or the test is not sensitive enough.
7. The potential synergy between many chemicals to which humans are exposed cannot be studied in animal tests.
8. The test animals (i.e. inbred, genetically identical rodents) neither represent normal animals of their species nor the human population of concern.
9. The experimental conditions may differ from test to test.
10. The experimental conditions are inappropriate to the human situation.

Based on Table 2 in Gad, SC (1990). Model selection in toxicology: Principles and practice. J. Am. Coll. Toxicol. 9:291-302.

RISK ASSESSMENT

It is often claimed that animal tests are needed to provide a range of hazard data for a chemical, including dose/response relationships, knowledge of its absorption, distribution, metabolism and excretion, and mechanisms of toxicity. These data underpin the risk assessment process where decisions are made about how a chemical should be classified and labelled, how it should be handled and how its uses should be controlled (or banned). Animal tests are conducted primarily to predict human health hazards, but some are also carried out for environmental hazards (e.g. bioaccumulation, toxicity to fish).

Hazard assessment is usually considered to be the most 'scientific' part of the process, while risk assessment is more of an 'art', involving the weighing of dissimilar and sometimes contradictory information. Results are often not clear cut and judgement is required to reach a regulatory decision.

For example:

- Regulators have to judge carefully the many instances where results from different tests, especially animal tests, conflict.
- They attempt to give appropriate 'weight' to the different kinds of evidence, e.g. animal test data versus human data.
- If a chemical is hazardous at higher doses they must try and estimate a 'safe' threshold, if any, for exposure.
- Regulators try to assess whether a hazardous chemical poses a real risk, for example on the basis of likely exposure of humans and wildlife.
- They are frequently expected to make these judgements in the absence of measured exposure data for humans and wildlife.

Applying modern non-animal techniques to toxicology, such as those outlined in The Way Forward, would greatly improve the feasibility of testing the chemical backlog in a reasonable timespan, and enhance the quality and relevance of data available for decision-making on all chemicals.

Such non-animal techniques include:

- human cells and tissues in culture,
- reconstituted tissues (i.e. complex tissues 'rebuilt' in the test tube from simple cells),
- in vitro (test-tube) studies with enzymes and proteins,
- receptor-binding studies,
- changes in gene expression in vitro (e.g. toxicogenomics),
- computer models such as quantitative structure-activity relationships³ and physiologically-based biokinetic models⁴
- ethically conducted human volunteer studies.

In this report we introduce the need for contemporaneous monitoring programmes, which will identify, at an early stage, the build-up and/or toxicity of chemicals both in the environment and in the tissues of humans.

Animal tests for predicting human health hazards are costly and time-consuming, cause suffering to thousands of animals and have many scientific weaknesses. Here we critically address those weaknesses and describe individual cases where animal test data have failed to support - or have definitely hindered - optimal regulatory decision-making.

There are many reasons why the reliance on animal test results can hinder the process of risk assessment. Here we focus on five key issues (which are described in detail in the next sections):

1. Animal tests: problems with extrapolation to different species, breeds and genders.
2. Animal tests: problems with using small animals and large doses.
3. Animal tests: problems with dosing routes.
4. Animal tests: problems with multiple chemical forms.
5. Animal tests: reliance on animal data has displaced the role of monitoring programmes.

ANIMAL TESTS: SECTION 1

PROBLEMS WITH EXTRAPOLATION TO DIFFERENT SPECIES, BREEDS AND GENDERS

Different species (human and non-human), breeds and even genders of animals frequently vary in terms of their sensitivity to toxic chemicals, as well as in rates of chemical absorption and excretion, and rates and routes of metabolism. These variations are due to evolutionary differences in anatomy, physiology, pharmacology, biochemistry and metabolism, and are the single greatest weakness of animal tests for assessing human health hazards.

Regulators admit that because of the uncertainty of the relevance of animal test results, when setting safe exposure levels for people they have to use 'uncertainty' factors. These factors, applied to what appears to be the highest safe dose in animals, are 10-fold or 100-fold (or more) reductions to try and ensure human safety. Often there is a 10-fold reduction in dose to try to account for species differences, and another 10-fold reduction to try to account for the different sensitivities of individual humans. On the basis of only animal data, the estimated uncertainty factor is always a guesstimate and the approach represents an unscientific 'catch-all' effort to deal with the inherent limitations of animal tests.

As a recent European Commission report on risk assessment⁵ acknowledges, "The replacement of a default value for either interspecies differences or human variability by a quantitative chemical-specific adjustment factor requires experimental data generated from in vivo or in vitro studies **in humans**" (our emphasis).

Human data, such as from human volunteer tests or monitoring programmes, eliminate the need to adjust for species differences. In vitro tests using human cells also avoid the species problem and, in contrast to animal tests, can be designed to take account of different human susceptibilities.

CASE STUDY - Bisphenol A

In 1986, on the basis of 90-day repeat dose tests in rats, a safe daily human intake of the chemical bisphenol A was calculated. This was done by dividing the 'no observed adverse effect' dose in rats by an uncertainty factor of 500⁶. This high figure was intended to compensate for considerable uncertainties in the animal data,

including the species differences seen.

Bisphenol A is currently suspected of causing endocrine disruption (hormonal changes) but animal tests are failing to clarify the situation. Three different breeds of rats - Wistar, Sprague-Dawley and Da/Han rats - responded differently to bisphenol A in a non-validated test for assessing endocrine disrupter effects (the uterotrophic assay)⁷. Sprague-Dawley rats seem to be the most sensitive breed for oestrogenic effects in this assay, but less sensitive according to multi-generation reproductive studies (see also Section 5). In contrast, mice appear to be less sensitive than rats to uterine effects, but more sensitive with respect to subtle alterations in reproductive development⁸. No-one knows if humans will react in the same way as any particular breed of mouse or rat - or none, and none of these tests address important issues of varying susceptibility between different groups in the human population.

Scientists and regulators are perplexed by the variety of results obtained from different animals and at different doses. Referring to endocrine disrupters, Professor Frederick vom Saal at the University of Missouri-Columbia was quoted by *Nature*⁹ as saying:

"The evidence is that there can be as much as a 1,000-fold, or greater, range of responses to these chemicals in different strains of mice. The regulatory default assumption of a ten-fold correction or safety factor for genetic variability is completely out of touch with the data."

A partial explanation for genetic variation in susceptibility to bisphenol A is the rate and route of metabolism in the body. Bisphenol A is inactivated by metabolism in the liver, which clears the chemical from the bloodstream. Studies were done in rats to measure how quickly this took place, but it was not known whether rats were good 'models' of humans.

The essential 'gold standard' data were provided by a single low-dose study in human volunteers¹⁰. This indicated "major species differences in the disposition of bisphenol A." Specifically, in rats the metabolite of bisphenol A re-circulates between the liver and intestine, slowing its excretion from the body. In humans, bisphenol A is rapidly metabolised and excreted some three times faster than in rats.

CASE STUDY - Asbestos

The toxic effects of asbestos in humans include asbestosis, lung cancer and mesothelioma, and were discovered entirely through human studies (see Box below).

Many animal tests of asbestos have been conducted, including 20 carcinogenicity tests alone. The most commonly used species is the rat, but the significance of the test results to humans has been debated and disputed for decades. In 1995, a paper¹¹ re-analysed rat and human data and concluded that humans are 300 times more susceptible than rats to mesothelioma from inhaled asbestos fibres. This led the scientists to conclude "...that inhalation studies in rats are not sufficiently sensitive for the detection of hazards and risks to humans exposed to man-made fibres".

Another review of asbestos toxicity in rodents stated, "In addition, significant dissimilarities exist in the deposition pattern between rats and humans... Inhalation experiments with rats need fibre exposure concentrations over 100 times higher to match the lung cancer risk of asbestos workers, and about 1,000 times higher to reach the same mesothelioma risk"¹².

One explanation for these species differences is that even if rats and humans inhaled the same dose of toxic particles, their actual exposure would not be the same¹³. This is due to differences in:

- methods of breathing (rodents breathe only through their noses and develop more nasal damage, but less lung damage, than humans),
- the geometry of the airways (branching patterns, diameters and angles),
- the speed of air movement in and out of the lungs, and
- whether or not particles are deposited in regions where the tiny hairs (cilia) can clear the airways.

Hamsters have also been used in asbestos studies. They are even more resistant than rats to lung cancers from long-term asbestos inhalation¹⁴, although more sensitive to mesotheliomas¹⁵. As well as anatomical variations, there are more subtle underlying cellular and molecular reasons for species-specific responses, and many animals' lives have been spent in trying to discover them. Hamsters react to asbestos fibres with a milder lung inflammation and maintain their

anti-oxidative defences more effectively than do rats¹⁶.

Even after all this time, animal test results for asbestos are still being debated and 'safe' exposure limits constantly undergo revision by regulators. It is pertinent that the US Department of Health and Human Sciences recently recommended¹⁷ that human studies are needed to overcome the difficulties with interspecies extrapolations: "...additional research on deposition and clearance of asbestos fibers in humans may help to properly address this issue." Moreover, applying a precautionary approach, instead of continuing to study a chemical that clearly poses an unacceptable hazard to the human population, would prevent waste of resources as well as waste of animal life.

Asbestos – human versus animal data and regulatory control

The damaging effects of asbestos to humans were discovered and elucidated entirely through the study of exposed populations, from 1900 to the present day. These studies, numbering more than 45 in total, yielded dose/response data (often sought from animal tests) for different forms of asbestos.

During the 1940s and 1950s in the USA, the asbestos-manufacturing industry attempted to refute the human evidence of health effects. The first personal injury claim in the UK was launched in 1967, and was successful in 1971.

Interestingly, the first of more than 20 animal carcinogenicity tests of asbestos also dates from 1967. For many decades the significance of animal test data was unclear, as rats appeared to be less sensitive than humans to asbestos.

Regulations to control human exposure to asbestos were not introduced until 1969 in the UK and 1971 in the USA. These are constantly reviewed and updated, but there is still no consensus on how to extrapolate results across the species boundaries.

CASE STUDY - Coumarin

Coumarin is a natural substance and is used as a fragrance in cosmetic, toiletry and household products. It exhibits dramatic species differences both in metabolism and in toxicity. The major pathway of metabolism in humans is 7-hydroxylation, and the human body converts 79% of coumarin to this inactive metabolite. But in baboons the equivalent figure is 60%, in rats less than 1% and in mice it is variable¹⁸. In complete contrast to humans, the major metabolic pathway in rats and mice actually converts coumarin to a toxic metabolite.

Coumarin has been tested on mice, rats, hamsters, dogs, baboons and other species. In tests for toxicity and carcinogenicity on mice and rats, the organs mainly affected were the liver and lungs, with tumours forming at higher doses¹⁹. Hamsters appear to be resistant to coumarin toxicity.

In the 1970s coumarin was classified as a class I carcinogen on the basis of tests in rats and mice. The realisation that humans metabolise coumarin in a qualitatively and quantitatively different way to rodents caused this classification to be altered, and it is not now considered to pose a cancer risk for people. However, given the uncertainty of animal tests, there is absolutely no guarantee that this classification is correct.

ANIMAL TESTS: SECTION 2

PROBLEMS WITH USING SMALL ANIMALS AND LARGE DOSES

Most animal tests use small-bodied, short-lived animals (i.e. rodents), but the results are intended to be applied to large-bodied, long-lived animals (i.e. humans). The use of surrogate species creates several difficulties relating to experimental design and interpretation of data.

The maximum lifespan of a laboratory mouse is about two years and that of a rat about three years, although there is variation between breeds. The maximum lifespan of a human is somewhere around 100 years. A human weighs about 70 kilos and can be up to 2.2 metres tall. Rats tip the balance at less than half a kilo and their body length is about 0.1 metre.

Scaling up from small animals to larger humans

Adjusting for differences in rates (e.g. metabolic rates) or sizes between test animals and humans is called scaling. Scaling is important in toxicology because the time course of the disposition of the chemical in the body profoundly affects its toxicity. Each step in the scaling process adds a degree of uncertainty to the final outcome.

In the past, lifespan potential was used to scale animal test results to humans. Some scientists have proposed that scaling should be in proportion to the product of brain weight and clearance from the body²⁰. Indeed, different test parameters (such as metabolism or distribution) may actually require different scaling factors.

Today, scaling is usually on the basis of comparative body weight or body surface area, but this is entirely empirical (see Box below). Sometimes scaling from rodents to humans is done on the basis of the ratio of body weights raised to the power of 0.667. Others have suggested using 0.75 as the exponent, as this better reflects differences in basal metabolic rate between rodents and humans²¹.

Measure for measure – which scaling factor to use?

- The human lifespan is 4.4 to 66 times longer than that of common test animals.
- The heart of the mouse circulates its whole blood volume around

its body every minute. The human heart takes 20 times this long.

- The larger the animal, the larger the number of susceptible cells which can interact with a toxic chemical.
- The body surface areas of a mouse, rat and human are, respectively, 46.4 cm², 516.7 cm² and 18,000 cm², i.e. ratios of 1:11:388.
- The body weights of a mouse, rat and human are, respectively, 20 g, 400 g and 70,000 g, i.e. ratios of 1:20:3,500.
- Rates of cell division in rats and mice are about double those in humans. Cell division rates underlie many responses to toxic chemicals, such as the cellular immune response and genotoxicity.

Based on: Gad, SC (1990). Model selection in toxicology: Principles and practice. *J. Am. Coll. Toxicol.* 9:291-302.

However, available evidence shows that predicting from animal tests the clearance of a chemical in humans on the basis of body weight ratios does not give reliable results. An error rate of more than 30% is seen for drugs²². There is even less certainty with chemicals because, unlike drugs, there are seldom clinical trial results to confirm or contradict the animal test data.

Computer models are under development which, on the basis of in vitro data on human enzyme systems, can predict the rate of clearance of a drug, and could be applied to chemicals. For example, a modified form of the computer system called Simcyp has so far produced results which are more predictive of human drug clearance than scaling up from animal test data²³. The model also allows prediction of individual variations in drug clearance. Mathematical models of this kind, combined with in vitro data, could replace animal testing and improve results.

Unrealistic megadoses used in animal tests

The fact that small animals have short lifespans means that test animals are never exposed to chemicals for as long as humans may be. It takes time for certain effects, especially tumours, to develop, adding further difficulties to extrapolation from rodents to humans.

Animal tests for carcinogenicity cannot be made sufficiently sensitive to detect small increases in risk associated with low doses of chemicals, without using many thousands of animals per test - which would be unethical as well as impractical in terms of cost and time. Thus tests are conducted at unrealistically high doses in an effort to maximise the sensitivity of the experiment. A similar problem arises with some genotoxicity tests on animals, and the same megadose

solution is applied. In vitro methods of testing are, of course, much more sensitive to chemical effects.

Even in acute toxicity tests (whole body, single dose), where the purpose is to try to predict target organ toxicity and toxic mechanisms for humans exposed at much lower doses, the study may be considered suspect by scientists and regulators if all the animals in the highest-dose group survive²⁴. In the case of people and wildlife, exposure to toxic chemicals will very seldom approach these large test doses.

Using megadoses in animal tests creates a number of problems of data interpretation. Assumptions are often made that the dose/response relationship for chemicals will be linear, so that extrapolation 'backwards' to lower doses is feasible. This has led to many different mathematical models but estimates from them vary, and there are large margins of error and uncertainty²⁵. Some of the problems encountered include the fact that, at chemical megadoses, enzymes may become saturated, the chemical may be metabolised to forms which would not occur at lower doses, detoxification mechanisms may become depleted or saturated, bioavailability may be different due to local effects (e.g. gut irritation) and organ systems may be overwhelmed. Ironically, these arguments are often put forward by those using animal tests to undermine the validity of positive results, and prevent decisive regulatory action on harmful chemicals.

CASE STUDY - Tooth whiteners

The use of hydrogen peroxide to bleach discoloured teeth dates back at least one hundred years. Hydrogen peroxide has been tested on animals, including rats, mice, guinea pigs, hamsters, rabbits and dogs, from the 1970s onwards. In the 1980s, the US Food and Drug Administration said that there was "insufficient" evidence of carcinogenicity in humans, and the International Agency for Research on Cancer stated that there was "limited" evidence of carcinogenicity in animals.

A commercial tooth whitener product based on the release of hydrogen peroxide was introduced in about 1990, but within two years the UK government tried to prohibit it. According to expert scientific witnesses, Dr Kelleher and Dr Roe, who testified in the subsequent court case, the government was reacting to fears based on animal tests that the tooth whitening product, called Opalescence, was carcinogenic²⁶.

Hydrogen peroxide and species differences

The intestines contain an enzyme called catalase, which destroys hydrogen peroxide. Low levels of catalase could increase the risk of intestinal damage, and possibly of cancer.

Mice, who develop tumours when dosed with hydrogen peroxide, have levels of catalase 70-1000 times lower than rats.

Levels of catalase in the human intestine are 2-27 times higher than those in mice (depending on the breed), which may offer humans some protection against toxic effects of hydrogen peroxide.

Benign and cancerous tumours had been found in the intestines of mice exposed to hydrogen peroxide in their drinking water - but two studies using rats found no such effects. Kelleher and Roe argued that the results of animal tests were inapplicable to people, largely because of the unrealistic megadoses used.

After several years of disagreement and litigation, commercial tooth whiteners are now in use again, although there is still uncertainty about safe dose levels of hydrogen peroxide in humans. If efforts to understand the hazards had prompted good-quality human studies of tooth whiteners in use, instead of endless animal tests, the resulting data would not have been confounded by megadose problems.

CASE STUDY - Water chlorination by-products

In 1992, human population studies had suggested that some of the chemical by-products of water chlorination may have adverse effects on human foetal development. These early studies suggested increased risks of retarded foetal growth, low-birthweight babies and birth defects.

Water chlorination hazards

Drinking water is chlorinated as a means of disinfection. In the 1970s it was discovered that chemicals ('chlorination by-products') form as a result of reactions between chlorine and

other chemicals in the water. It is now known that there are hundreds of such chemicals, and many are believed to be hazardous. This is a very important public health issue, as huge numbers of people are at risk even if the hazard is low.

In 1993, the US Environmental Protection Agency (EPA) co-authored a review of the human and animal evidence for reproductive toxicity caused by chlorination by-products²⁷. Several animal tests had already been done to assess the toxicity of chlorination by-products on reproduction and foetal development, mainly in rats and primarily in one breed of rat. The tests pointed towards some chlorination by-products causing birth defects in rats, and possible toxicity to reproductive organs in adult rats and mice.

The EPA report highlighted that the animal studies were difficult to interpret because the tests had been conducted with high doses (several orders of magnitude higher than concentrations in drinking water) of individual chemicals. Very few studies had looked at the potential toxicity of drinking water itself, while many chemical by-products had not been tested at all.

The EPA concluded that further human studies were essential and that more animal tests should be conducted. They said that "The issue of extrapolation of animal findings at high dose levels to humans where exposure is at very low concentrations warrants further comment", and suggested a special focus on extrapolating high-dose to low-dose situations. Unfortunately, the means of doing this reliably has still not been developed. Ten years later, developmental toxicity studies in rats are still being undertaken and the doses used continue to be unrealistically high. A study published in 2003 concerned a single chemical known as MX and involved more than 100 female rats²⁸. Regulatory decisions on safe levels of chlorination by-products are still hampered by reliance on animal tests, rather than on non-animal testing methods and modern studies of human populations.

Difficulties in interpreting megadose data from animal tests became apparent in the case of another chlorinated chemical. Trichloroethylene (TCE) is an industrial solvent and degreasing agent and is found as a contaminant of drinking water. In 1993 its regulation was based on a linear extrapolation of high doses tested on rodents to risks for humans at low doses. However, the

mechanism of carcinogenesis is non-linear in rodents, requiring very high doses sufficient to cause cells to die. Cancerous tumours arose from repeated cycles of cell death and regeneration.

Scientists argued that this toxicity was not related to dose in the usual way, but represented a threshold effect: "We conclude that the assumptions underlying current regulations are not applicable to TCE. Instead of a straight line extrapolation model, a threshold model may be more appropriate"²⁹.

Chlorination by-products, megadoses and cancer risk

Chloroform is one of the chlorination by-products found in drinking water. In the early 1990s the EPA imposed very strict standards on levels of chloroform in water, based on a study in which mice developed liver cancer after being given massive daily doses of the chemical.

More animals had to die before it was realised that the toxic effects of such high doses could not be interpreted at low doses. In the mid-1990s the EPA acknowledged the limitations of standard rodent carcinogenicity tests, and it accepted the crucial need for analysis of chemical structure/activity relationships and mechanistic studies – which can be conducted without using animals.

"EPA's new emphasis on molecular data is based on a growing body of evidence that extrapolations from megadoses can provide a misleading picture of the effects of low-level exposure."

Stone, R (1995). A molecular approach to cancer risk. *Science* 268:356-357.

ANIMAL TESTS: SECTION 3

PROBLEMS WITH DOSING ROUTES

In animal toxicity tests, the standard routes of administration of test chemicals are by oral dosing (by stomach tube, or in food or water), inhalation, injection into the abdomen (intra-peritoneal) or injection under the skin.

The route of chemical administration can have dramatic effects on apparent toxicity. For example, an orally administered chemical that is highly absorbed from the gut into the bloodstream has the potential to affect every organ in the body, but it is also immediately exposed to the metabolic activity of the liver. This may neutralise the chemical or convert it into a more toxic form.

A chemical injected into the abdomen or under the skin is not immediately exposed to the liver's metabolic activity. If it is toxic, it can therefore cause adverse effects at lower doses than the same chemical applied orally. Tests where a chemical is administered to animals by different routes may therefore yield different dose/response curves and different measures of toxicity. When this occurs, further studies are carried out to characterise both the absorption of the chemical by the different routes, and the route and rate of metabolism by the liver. There is no guarantee that the same values for absorption and metabolism will be found with a different test species or, indeed, in humans.

Some animal tests use inappropriate dosing routes which are not applicable to the human exposure situation. For example, tests³⁰ on animals to see if a chemical causes genetic damage often involve injecting the chemical directly into the abdomen. The effect of the chemical is assessed by looking at cells from the bone marrow. However, this route of exposure is irrelevant to the human situation and it can be unclear whether the chemical actually penetrates to the bone marrow. This increases false negative results³¹. Moreover, even when tests use the oral route, there can be differences in the results depending on the precise means used (see Box below). Results obtained using inappropriate dosing routes produce particularly irrelevant data that can only help to delay regulatory action.

Food additives, dosing routes and carcinogenicity

Many food additives are synthetic chemicals which are tested on animals for carcinogenicity. The oral route of dosing is recommended because humans are exposed to these chemicals in their food.

There are three common ways of dosing animals by mouth: by stomach tube, in water or in feed.

The US National Toxicology Program data show that only one of 18 food chemicals mixed in food caused cancer in rodents, but 16 of 23 food chemicals given by stomach tube caused rodent cancers.

“Human experience with known carcinogens such as tobacco, asbestos, and benzidine convinces us that environmental carcinogens constitute a real threat to human health, although predicting human carcinogens from rodent tests involves a number of uncertainties.... In the interests of public safety, a serious effort should be made to resolve the questions surrounding the presence of chemicals identified as rodent carcinogens in our food.”

Johnson, FM (2002). How many food additives are rodent carcinogens? *Environ. Mol. Mutagen.* 39:69-90.

CASE STUDY - Bisphenol A

Scientists and regulators are still struggling to understand the conflicting data for bisphenol A derived from animal tests (see Sections 1 & 5). Consequently, long-term reproductive toxicity tests using rats are being repeated by several laboratories, each test starting with as many as 240 adult animals.

One of the most recent reports of a three-generation reproductive study of bisphenol A used oral dosing and found no toxicity at low doses³². The authors reviewed other studies that showed toxic effects when bisphenol A was injected at low doses under the skin, although when it was given by mouth it was only toxic at much higher doses. This was explained by the fact that bisphenol A given by mouth is metabolised to a non-toxic compound. However, without information about how humans absorb and metabolise the chemical, the rat results shed little light on human risk assessment.

Interpretation was aided enormously by a single study in human volunteers³³ which showed significant species differences. In adult humans, bisphenol A is rapidly metabolised to the same non-toxic form but this occurs three times faster than in rats. This case study illustrates why conflicting results from animal tests using different administration routes delay effective regulation, and why ethically-conducted human volunteer studies should be the scientific approach of choice.

CASE STUDY - Tooth whiteners

Tooth whiteners act by the bleaching effects of hydrogen peroxide. They are applied in the form of bleaching strips, or as a gel contained in a custom-made 'tray' which fits into the mouth.

Many animal tests have been conducted on tooth whiteners, using varying routes of exposure and studying different endpoints, and the results have proved highly controversial (see Section 2 for example).

On the basis of an acute toxicity study in rats, scientists conducted a risk assessment for humans and concluded that people using tooth bleaches could be exposed to enough hydrogen peroxide to cause stomach lesions³⁴. However, other scientists refuted the test results, claiming that the route of administration had distorted the results³⁵. Rats had been dosed by stomach tube, and the critics stated that:

"...their findings are difficult to interpret in relation to the use of carbamide peroxide for tooth whitening... the method of administration served to maximise exposure of the [stomach tissues] by avoiding dilution by saliva or food. Common sense dictates that it is the concentration of hydrogen peroxide that comes into contact with particular tissues and not the total dose on a mg/kg body weight basis that determines manifestations of local toxicity."

Contradictory conclusions were drawn by other researchers in the case of carcinogenicity tests of hydrogen peroxide. They criticised a carcinogenicity study in which mice were dosed with hydrogen peroxide via their drinking water, and developed lesions in their small intestines. They said that due to the chemistry of dilute hydrogen peroxide solutions (and to species differences in the stomach and intestines), it was unlikely that the swallowed hydrogen peroxide even reached the animals' small intestines³⁶. They also argued that the mice drank less water than usual (because of the presence of the chemical in their water) and that the intestinal damage resulted from abrasion when they ate their dry food pellets. The data obtained from these tests has brought us no nearer to understanding the actual effect of tooth whiteners on people using products currently marketed as 'safe'. Arguments about inappropriate dosing routes have undermined a more precautionary approach.

ANIMAL TESTS: SECTION 4

PROBLEMS WITH MULTIPLE CHEMICAL FORMS

Many chemicals exist in several related forms (e.g. asbestos) or are a single member of a large series of related chemicals (e.g. polybrominated diphenyl ethers). Routine regulatory animal tests are conducted on a single chemical at a time, because the tests cannot distinguish between chemical effects. Although additional animal tests are sometimes carried out using commercial mixtures of chemicals, the results are then extremely difficult to interpret.

Regulatory decisions on one chemical can be delayed while further animal testing is called for on yet another related chemical. Clearly, in cases where there are dozens or even hundreds of chemicals in the series, regulation cannot and should not be delayed while each substance is tested on animals. In vitro tests should be used, and QSARs applied for chemicals in the series that have missing data. Where hazard information is available for some chemicals, expert judgement should also be used to apply this knowledge to related substances. Evidence of human or wildlife exposure, accumulation of the chemical in the tissues, or evidence of toxicity should invoke the precautionary principle and the chemical/s should be banned.

CASE STUDY - Brominated flame retardants

Brominated flame retardants are ubiquitous chemicals produced in large volumes. One-third consists of polybrominated diphenyl ethers (PBDEs). The total number of possible PBDE forms (congeners) exceeds 200. The PBDEs are closely related and have similar properties, differing in the number and position of bromine atoms in the molecule.

PBDEs have widely contaminated the environment, are persistent and readily accumulate in animal tissues. They have been found in sediments and in salmon, seals, whales and other wildlife species.

The general public is exposed to PBDEs primarily through breathing indoor air and through consumption of contaminated fish. Levels of PBDEs in human breast milk have risen exponentially since 1972, doubling every five years - although this was only discovered in the late 1990s when archived samples of breast milk were tested³⁷. Breast-fed infants are effectively at the top of the food chain and

their exposure to these chemicals is extremely worrying. PBDEs have also been found in the serum and fatty tissues of the general population. Even a complete and immediate ban would not stop levels rising in human and animal tissues for some time.

There are only four published studies looking for toxic effects in people occupationally exposed to brominated flame retardants, and one of these suggested effects on the thyroid gland and on neurological function³⁸. Thyroid effects are an expected outcome of some endocrine disrupter chemicals.

PBDEs are structurally related to known toxins, the polychlorinated biphenyls (PCBs) and the polybrominated biphenyls (PBBs), causing serious concern about likely human and wildlife health hazards. PBDEs are also structurally similar to compounds which are toxic to the immune system.

PBDEs have been subjected to several animal tests, either in the form of commercial products (containing a mixture of chemicals) or as single chemicals. The tests suggest thyroid toxicity, cancers and neurodevelopmental effects: newborn mice showed impaired learning and memory and altered spontaneous movements. Of the latter, the Swedish National Food Administration said³⁹ "...it is not known what these observations of altered spontaneous activity in neonatal mice means in terms of human risk assessment." The same could also be said of many other animal test results.

The PBDEs have not been banned despite the weight of evidence that they are toxic, persistent and bioaccumulative and that humans and wildlife are increasingly contaminated. Rather than continuing with animal tests, invoking the precautionary principle would lead to a ban in these circumstances, especially as alternative flame retardants are available (although more costly)⁴⁰.

Instead, risk assessors point to 'data gaps' that should be filled, calling for more animal studies, including tests on additional chemicals in the PBDE series and more studies of carcinogenicity, immunological toxicity, thyroid function and neurodevelopment^{41,42}. The fruitless search for definitive laboratory animal data thus delays regulatory action, putting humans and the environment at increasing risk.

ANIMAL TESTS: SECTION 5

RELIANCE ON ANIMAL DATA HAS DISPLACED THE ROLE OF MONITORING PROGRAMMES

The range of laboratory-based animal tests for chemical toxicity is ever increasing, but inevitably such tests are only devised to address recognised types of toxicity.

Thus in recent years, efforts have been made to devise valid animal tests for 'new' endpoints such as damage to the adult and developing immune and nervous systems, as well as endocrine disruption. These tests have proved very difficult, slow and costly to devise and to validate, especially in the case of endocrine disruption. In the meantime, wildlife and humans continue to be exposed to potentially toxic chemicals.

The chemical regulation system has always relied heavily on data from laboratory animal tests, but attempting to develop new animal tests retrospectively, sometimes years or decades after chemical exposure first occurred, cannot support precautionary regulation or offer adequate protection.

There are two solutions to this impasse. The first is laboratory-based and involves developing in vitro methods for a wide range of toxic endpoints: those already discovered and those yet to be fully understood. This can be done with human cell cultures in the test tube, because high-throughput techniques such as scanning for chemically induced changes in patterns of gene activity⁴³ (microarray methods) or the production of metabolites (metabonomics) will indicate significant changes in normal cellular function. These tests will be far quicker and less costly than equivalent animal studies, and instead of each test measuring one toxic endpoint, in one test these in vitro methods can 'scan' for changes which would underlie different toxicities.

The second solution (and both are essential) is to instigate widespread real-time monitoring of the environment and of humans. Unlike animal testing for new toxicities, this is a prospective approach which identifies emerging chemicals of concern on the basis of exposure and cumulative effects in people and wildlife⁴⁴.

New pro-active programmes of routine monitoring must be established for human and environmental exposures. They should address at-risk wildlife and human populations as well as the general population. Data analysis must be ongoing, rather than retrospective. The programmes would scan for the build-up of chemicals in human tissues (e.g. urine, saliva, sperm, blood, breast milk) and

in the environment, and these will act as early warning systems for emerging pollutants. Additionally, 'biomarkers' of toxic effects - telltale molecular changes in tissue samples - would flag up any early signs of toxicity.

The development and use of biomarkers for exposure and toxicity are essential. They offer real opportunities to gain human dose/response data necessary for quantitative risk assessment, such as with the analysis of forms of arsenic in the urine of subjects when investigating health effects of arsenic exposure⁴⁵.

Human population studies can be difficult to conduct rigorously, because of confounding factors such as diet, lifestyle, drug habits and use of contraceptives. All of these can be overcome by careful selection of populations and by use of medical records, lifestyle questioning and occupational exposures. Despite their obvious importance and relevance, until now, such studies have been under-developed because of the fixated reliance on animal tests.

CASE STUDY - Tributyl tin

This case study is unusual in that the adverse environmental effects of tributyl tin (TBT) were completely novel and were recognised solely through field studies.

TBT has been very widely used to prevent the growth of unwanted organisms and it is now a global contaminant of the marine environment. Its major applications have been as an anti-fouling agent on the hulls of boats, to stop the growth of crustacea and other organisms, and as a wood preservative. However, it can also be found in paints, carpets, and even in the padding of cycling shorts.

TBT's application in anti-fouling paints dates from the 1960s and by the 1970s it was the most common agent used for this purpose⁴⁶. Gender changes in marine crustacea were first observed in 1970, and by the mid-1970s French fishermen noticed a failure of the oyster beds in Arcachon Bay. Simple correlations began to be made between the observed gender changes and failure of the oyster beds, and areas where large numbers of boats were being treated with TBT anti-foulants. A study in 1981 revealed that TBT accumulated in oysters.

Definitive proof that TBT was responsible was delayed, because analytical techniques were not sensitive enough to measure levels of

TBT in the water. Nevertheless, because of the economic impact of the failing oyster industry in Arcachon Bay, in 1981 the French government imposed a temporary ban on anti-foulants containing more than 3% TBT being applied to smaller boats. At the same time the UK government attempted to persuade the industry to withdraw TBT-based paints, without success. By the mid-80s, techniques of analysis permitted sensitive measurements, providing proof that TBT was the causative agent.

In the late 1980s many countries, including the UK, introduced restrictions, followed by the EU in 1991. Localised concentrations of TBT in the water began to fall rapidly. Subsequently, however, improved environmental monitoring has found continuing problems around the globe, especially in major shipping lanes and with continuing accumulation of TBT in marine animals. An EU directive formally banned the application of TBT paints to all ships as from 2003.

If regulatory action had awaited a full package of laboratory animal test data there would have been few controls on TBT to this day. Toxic effects have been recorded in more than 100 species of marine animals, but building up data from laboratory tests would take many years. TBT has been shown to be a skin and eye irritant and may cause embryo damage in rats, mice and rabbits - but these data only became available in 1992. Reliance on animal test data would have postponed regulation, especially as effects on reproduction involve long-term animal studies whose relevance (to other animals or to humans) is dubious. Regulatory bodies are still discussing the possible risks to human health of TBT exposure by other routes, including food and consumer products⁴⁷.

The lessons to be learned from this case study are that routine monitoring for chemical contamination of the environment should be an ongoing contemporaneous activity, and that sensitive techniques of analysis are an essential part of this process. When a novel chemical is introduced, this should be accompanied by the development of analytical methods appropriate for water, sediment, and human, animal and plant tissues. Observations and samples analysed regularly will provide early warnings of chemical exposure and/or toxicity, and should be followed by precautionary action - as was taken in this case by the French government, albeit for economic rather than environmental reasons.

CASE STUDY - Bisphenol A

Bisphenol A is a widespread industrial chemical used mainly to make polycarbonate plastic (used in spectacle lenses, plastic bottles, plates and mugs and cell phones) and epoxy resins (adhesives, industrial floorings, food can coatings and printed circuit boards).

Concerns have arisen in recent years over the potential endocrine disrupting effects of bisphenol A. Specific tests for detecting endocrine disruptors have not yet been formally validated but numerous reproductive toxicity tests in rats and mice have been conducted. Some adverse effects on fertility, on weight gain of pups and on their development have been reported. However, mice appear to be more sensitive than rats, and the results of studies even in mice have been contradictory. "The implications of these conflicting observations for human risk assessment are difficult to assess at present", according to the 2002 report of the European Commission's Scientific Committee on Food⁴⁸.

Additionally, some animal tests indicated that adverse endocrine effects occurred at unusually low doses of bisphenol A. These were criticised for using 'non-standard' protocols, and in other tests the results were not reproduced. It is becoming apparent that the effects of endocrine-disrupting chemicals vary widely, depending on the species, strain and even sub-strain of animals used, as well as their environmental conditions. This has left regulators in a very difficult position since no-one knows which, if any, of the animals react like humans.

The European Commission's Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) has accepted that for bisphenol A and reproductive toxicity, the uncertainties cannot be resolved by further animal studies⁴⁹. This view is echoed by the director of the Reproductive Toxicology Division at the US Environmental Protection Agency, Robert Kavlock, who said⁵⁰: "I am particularly perplexed by the data for bisphenol A, where some labs see it as a relatively potent oestrogen, and others fail to see much activity. This enigma needs to be understood at the mechanistic level." Genomic and proteomic approaches in human cells and tissues may hold the key.

One of the problems in discovering whether endocrine disruption is having deleterious effects on humans is the lack of effective

monitoring. It has been claimed, for example, that male fertility is declining as evidenced by decreases of sperm number or quality over many years. Although there have been sporadic efforts to study this, properly standardised long-term monitoring has not been carried out.

Britain's Royal Society also concluded that to assess exposure to 'cocktails' of endocrine disrupting chemicals would require "Alternative, indirect approaches based on epidemiological studies of predicted effects in humans, or in wildlife..."⁵¹.

The effective regulation of bisphenol A should not await the development of validated animal tests of dubious scientific value. Chemicals should be tested by existing non-animal methods (such as receptor-binding studies, reporter gene tests and steroid metabolism studies) which would help identify major endocrine disrupting chemicals now. In the meantime, validation of a wider range of non-animal tests should be conducted as a matter of urgency.

Monitoring and analysis of human tissues would provide real (rather than calculated) exposure data for different groups. Only in 2001 was it reported that a sensitive analytical method had been developed for determining bisphenol A in the placenta; the chemical was found in the placenta following normal food consumption⁵². Not until very recently was a study of bisphenol A absorption, metabolism and excretion in human volunteers reported⁵³, with results which "indicate major species differences". Using data of human relevance such as this, controls should be implemented on the basis of the precautionary principle - and without waiting for yet another study in rodents.

CASE STUDY - Brominated flame retardants

As described in Section 4, the PBDE family of brominated flame retardants provides a case study where human tissue monitoring first drew attention to a serious public health risk.

In the late 1990s, several decades' worth of archived samples of breast milk were tested for the presence of PBDEs. It was found that these chemicals had increased in human milk samples at an exponential rate over 25 years⁵⁴. As breast-fed infants are effectively at the top of the food chain, their exposure to the PBDEs is extremely worrying and has now prompted a number of animal toxicity tests.

Although relatively little is yet known about their toxicology, regulation should not await the results of a full programme of animal testing. Even banning the PBDEs now would not stop them continuing to rise in human breast milk and in the environment for several years. If the human milk samples had been analysed as they were collected, instead of up to 25 years later, action could have been taken much sooner. That is why 'real time' monitoring and analysis programmes are essential.

A NEW APPROACH

The current system of controlling chemicals places great weight on animal test results but, far from providing definitive evidence of risks, this reliance has led to delays in classification and control and to misclassification of chemicals.

Tests can be designed to provide masses of data but if the data are irrelevant to real situations, they have no value. As we have explained, uncertainties in animal data include scaling up from small to large animals; extrapolating from megadoses in animals to small doses in humans; and uncertainty factors to account for species differences. Animal test results, far from providing definitive evidence of human health risks, offer regulators endless opportunities to defer controlling or banning toxic chemicals. Because the data are derived from unrealistic test situations, using unreliable test 'models' (other animal species), interpretation is very difficult. Informed by animal test results, regulatory decisions about a chemical risk being postponed by:

- dismissing some laboratory animal evidence of toxicity (because it is conflicting or not reproducible);
- too little reliance on human evidence and an over-reliance on animal data.
- calling for more (and more) animal tests because of a perceived 'data gap', no matter how small;
- proposing more animal tests in different species to see if the 'mechanism' of toxicity might be idiosyncratic to one species, and therefore not applicable to humans;
- calling for new kinds of animal tests to be developed, because of concerns about novel kinds of toxicity.
- getting caught in an animal test trap - chemicals like asbestos are massively 'data rich', including endless animal test results, yet still the precise nature of the risks to wildlife and humans appears unclear.

Animal tests have been integral to regulatory systems for far too long, and have contributed to the current crisis in chemicals management. Far from providing a solution to the threat posed by toxic chemicals, animal tests add to the problem. It is essential for ethical, practical and safety reasons that animal based toxicity testing is consigned to history.

By adopting some key principles and policies, a chemicals strategy can be developed which would put health and environmental issues first and which would enable, rather than disable, regulatory decision-making. These principles and policies include the following:

1. Non-animal methods, preferably based on human cells and computational systems, should be implemented in place of animal tests⁵⁵.
2. New programmes should be established to achieve high-quality human data of exposure and toxicity, from routine monitoring of the general population as well as occupationally-exposed people or those exposed by accidental overdoses or pollution incidents.
3. Similar routine monitoring programmes should be instigated for the environment (i.e. routine sampling of water, sediment, plants and animal tissues).
4. The development and use of sensitive molecular biomarkers to provide early evidence of exposure and toxicity in monitored populations (human and environmental). These would provide an early warning system, as well as dose/response data for emerging toxins and pollutants.
5. Resources must be made available to provide highly sensitive methods of analysis for detecting low levels of toxins in the human population and the environment.
6. Regulatory decisions should be based on these new kinds of data, thus avoiding the pitfalls of interpreting laboratory results from tests on surrogate species of animals.
7. A chemical which is identified as persistent, bioaccumulative or toxic, should be stringently and effectively controlled or banned.
8. Consumers and companies should aim to reduce reliance on potentially toxic substances.
9. The precautionary principle must operate and in cases where there is evidence

to suggest toxicity, bioaccumulation or persistence, regulatory decisions should follow without delay. For example, if a chemical accumulates in breast milk or crosses the human placenta, it should be assumed that this is hazardous to the human foetus and the chemical should be controlled accordingly.

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