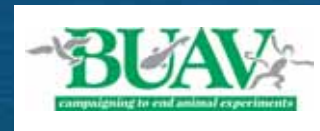


Endocrine disrupting chemicals

A non-animal testing approach



Green Party 
Real Progress



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Endocrine disrupting chemicals – a non-animal testing approach

Foreword by Caroline Lucas MEP



As a Member of the European Parliament, I have become increasingly aware of the threat posed by chemicals in our food, our homes and the environment. Throughout discussions of the European Commission's proposal for a new regulatory regime for chemicals (REACH), I have argued that the protection of human health and the environment is of paramount importance. At the same time, however, I have made the case that this does not have

to lead to yet more cruel, outdated and inefficient animal tests. To the contrary, the new drive for safer chemicals can be used to generate new momentum for the use and development of non-animal testing strategies.

I have therefore commissioned this report in order to explore these issues further, with particular reference to endocrine disrupting chemicals, which currently contaminate our air, water and soil. These chemicals are suspected of causing serious hormonal effects in humans and wildlife. They are found in many consumer goods, such as computers, toys, paints, upholstery, cosmetics and food packaging, as well as in some drugs and foods.

Known as endocrine disruptors (EDs), these substances have been linked to physical malformations in frogs, cancers in whales, infections in seals and polar bears, and sexual abnormalities in shellfish. In humans, endocrine disruptors have been linked to early puberty, neurological changes, declining fertility in men and rising rates of breast and testicular cancer.

Efforts to identify and control endocrine disruptors have been hampered by a lack of

hard evidence of their effects on people and the environment. EDs are not currently listed as 'chemicals of high concern' in the Commission's REACH proposal, but I believe that enough is known about them to warrant their inclusion in this category, and for controlled authorisation or restrictions to be applied on the basis of existing information and further investigation using non-animal tests where necessary.

One serious problem in preventing damage from EDs is that regulatory agencies have overlooked the importance of gaining good-quality population data, and appear to be obsessed with developing costly, time-consuming and unproven test methods using millions of laboratory animals.

This situation is untenable: the tests are not only extremely cruel and wasteful of animal lives, but they are likely to produce, in regulatory terms, 'more of the same' – that is, regulators and industry disagreeing over what constitutes a risk, instead of controlling the substances in a timely way based on the precautionary principle.

Now, while tests for EDs are still under development, we have a unique opportunity to initiate a new, modern testing process, breaking away from traditional animal tests which are ethically unacceptable and scientifically dubious. We must work to

solve the problem of endocrine disrupting chemicals now, not put all our faith into developing further animal tests that are already delaying effective regulation.

Solutions lie in taking a more precautionary approach; in measuring and monitoring chemical build-up in people and the environment, along with studies of reproductive and other problems linked to endocrine disruption. These studies will identify a range of endocrine disrupting effects and, where safer chemicals can be substituted, should lead to the immediate withdrawal of EDs of obvious concern. In cases that are less clear, these approaches must be combined with a programme of humane, non-animal tests, so that endocrine disruptors can be rapidly identified and banned.

This will require the implementation of existing non-animal methods, plus a high-priority programme to develop the required additional techniques. Resources currently being poured into the development of animal tests should be redirected into this programme.

Our priorities must be the protection of human health, the environment and animals in laboratories, with the goal of eliminating toxic chemicals. I hope this report will help achieve these aims.

1.0 Background

Prior to 1981, many thousands of synthetic chemicals were introduced into our daily lives without any clear understanding of the risks they might pose to our health, or to that of wildlife or the environment.

Chemicals may cause toxic effects during their manufacture, transport, use (as chemicals, or in consumer products) or disposal. During the lifecycle of a chemical we may be exposed to it through direct contact (e.g. inhalation or swallowing), or indirectly via contamination of soil, food, air or water.

In the last two decades the introduction of new chemicals has been more strictly controlled, but even so the regulations are inadequate to support a precautionary approach to protecting human health and the environment. New proposals for dealing with old chemicals as well as novel ones are currently being introduced in the European Union. However, the regulatory system still relies heavily on the results of laboratory animal tests, whose relevance and reliability for humans are highly questionable.

Since the 1990s there has been growing concern about a particular kind of risk from some chemicals that may have hormone-disrupting effects, in people and wildlife (such as fish, birds and mammals). They are described as endocrine-disrupting chemicals, or endocrine disruptors (EDs), because of their potential to interfere with endocrine hormones. An enormous programme of animal testing is being proposed to try to identify these chemicals.

2.0 Animal tests – ethically and scientifically unsound

The use of living animals in tests to study chemical safety has a long history, despite the fact that most tests have never been shown to be scientifically valid and indeed some have even failed retrospective validation.

Tests on rats, mice, rabbits, guinea pigs, dogs and monkeys continue on the assumption that they provide sufficiently high-quality evidence on which to base judgements about the safety of chemicals for human use.

That assumption has not been systematically proven. In fact, there is growing and persuasive evidence that animal tests are not reliably predictive for humans. Several analyses, for example of results for carcinogenicity, eye irritation, acute toxicity and skin allergy, have revealed the animal tests to perform poorly, either because of variations in results between laboratories or over time; or because of insufficient relevance to humans^{1,2}.

Extensive testing is also conducted on new chemicals to assess their impact on the environment, particularly wildlife. While it is essential to have a clear understanding of the hazards of chemicals to wildlife, the use of large numbers of animals such as birds, amphibians and fish in painful and usually lethal laboratory tests cannot be ethically justified. Confinement in laboratory conditions stresses animals and this could affect the experimental outcome. Results

from a single species of bird or amphibian also cannot reliably be applied to all birds and amphibians in the wild.

Internationally, but especially in Britain³ and the European Union⁴, legislation controlling animal tests is based on the understanding that other animals, from octopi (in Britain) through fish, amphibians, birds and reptiles to mammals, can and do experience pain, distress, suffering and harm. However, it is simply claimed – even in the absence of proof – that the suffering caused by testing chemicals on animals is justified because the results are considered to benefit humans. Underlying the paradigm of animal testing is a world view that discounts the suffering of individuals of species other than our own.

This point of view is untenable, ethically and scientifically. There is no major biological discontinuity between all humans and all other species in their capacity to experience pain and distress. It is, in fact, a blind, self-serving prejudice that causes the human species always to put its own concerns (whether commercial, health or vanity, etc.) ahead of those of all other sentient animals.

Now, an extensive new programme of animal testing is being introduced in an effort to deal with the risks of EDs. It will cost millions of animals' lives, as well as being scientifically dubious and economically impractical. A better way has to be found to safeguard human and environmental health. Now is the critical moment, as tests are being developed, to

choose methods which are humane and effective

Modern, humane, non-animal tests offer a solution. Using the latest molecular and cell-based techniques, precise data of particular relevance to the species of interest (human or wildlife) could be obtained, more cost-effectively and more quickly than from animal tests. Despite a chronic lack of funding for the development and implementation of non-animal tests, many are already now in use. Now is the moment for a properly-funded, high-priority programme of non-animal test development, to ensure that ED screening can be done effectively and rapidly, to protect people and the environment.

Combined with pro-active and routine monitoring to detect the accumulation of chemicals and their potential effects in human and animal populations, a new and precautionary regulatory system could be established. It is unethical and unscientific not to seize the opportunity to implement these new methods of testing, which cause no animal suffering, while also improving hazard and risk assessment.

3.0 What are endocrine disrupters?

Endocrine hormones regulate all our biological processes, from conception to old age. There are many such hormones including estrogens (female hormones), androgens (male hormones e.g. testosterone), thyroxine and glucocorticoids.

Produced by glands and circulating round our bodies, endocrine hormones help to control our development, growth, metabolism, fertility and reproduction.

Many kinds of potential endocrine disrupter chemicals (EDs) are present as contaminants in the environment (air, soil, water, food) and have built up in the tissues of humans (e.g. in fat and breast milk) and animals (e.g. birds, fish, seals). There are three main ways that EDs can affect the production or action of endocrine hormones in the body:

- Acting as a hormone mimic by attaching to hormone receptors in our cells, causing additional hormonal effects;
- Blocking the normal action of our own hormones by occupying hormone receptors, causing reduced hormone effects;
- Increasing or decreasing the body's own production of endocrine hormones, for example by affecting the enzymes involved.

Any of these influences can, theoretically, lead to a range of adverse outcomes such as early developmental problems, reproductive cancers or changes in fertility (see section 4).

EDs can be natural substances as well as synthetic chemicals. Natural substances include estrogens in cow's milk or phytoestrogens in food plants. Synthetic chemicals include industrial or household chemicals, pesticides, disinfectants, food additives, drugs or waste incineration products (see following page).

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Some synthetic chemicals considered to be endocrine disruptors⁵

Industrial chemicals and consumer products

- Bisphenol A** (plastic dental fillings, coating in food cans)
- Dioxins** (from waste incineration, in metal and paper production)
- Styrene** (in paints, lacquers, paper, food packaging; flavouring agent in ice cream)
- Polybrominated biphenyls** (fire retardants)
- Nonylphenol** (in detergents, paints, contraceptive foams)
- Phthalates** (plasticisers)
- Polychlorinated biphenyls** (plastics, glues, paints)
- Resorcinol** (manufacture of adhesives, dyes and cosmetics)

Pesticides

- Acetochlor** (herbicide used on food crops)
- Alachlor** (herbicide used on food crops)
- Atrazine** (herbicide used on food crops and roadsides)
- DDT** (insecticide; still used in some countries)
- Hexachlorobenzene** (fungicide on seeds and food crops)
- Lindane** (insecticide on seeds)
- Thiram** (domestic and agricultural fungicide)
- Tributyl tin** (anti-fouling paint, wood preservatives, disinfectants)
- Vinclozolin** (fungicide on food crops and ornamental plants)

Synthetic hormones

- Trenbolone** (used in livestock farming)
- Zeranol** (used in livestock farming)
- Diethylstilbestrol** (a human drug)
- Estrogens and progestogens** (contraceptive pill, hormone replacement therapy)

4.0 Uncertainties about endocrine disrupter effects

The endocrine disrupter story has been controversial right from the start. Partly this is due to a lack of knowledge about the real, as opposed to perceived, risks posed to different species and populations.

4.1 Endocrine disrupter effects on wildlife

There are two proven instances where contamination of the environment by EDs has caused gender changes in wildlife⁶. The first involves anti-fouling paints (containing tributyl tin) used on the hulls of ships that caused sexual abnormalities in dozens of species of shellfish. The second case is the feminisation of fish (known as 'intersex') in rivers. This has been attributed to low levels of estrogens, synthetic (e.g. from the contraceptive pill) and natural, excreted by people into sewage.

According to a report from the International Programme on Chemical Safety⁷, although organochlorines such as PCBs have adversely affected reproduction and immune function in Baltic seals, the mechanism of this toxicity is not clear. Eggshell thinning and embryonic abnormalities in some birds of prey are also linked to DDT and PCBs. There are many other cases where EDs are suspected of causing toxic effects in wildlife species including whales, seals, polar bears, alligators, frogs and other amphibians.

4.2 Endocrine disrupter effects and laboratory animals

Some chemicals suspected of being EDs have been tested extensively on laboratory animals and have caused hormone-related changes including:

- Changes in the weight of the uterus (womb) in female animals;
- Changes in the weight of reproductive organs in male animals;
- Alterations in patterns of estrus in female animals;
- Changed rate of sexual development in immature animals;
- Changes in weight gain or development in the offspring of dosed pregnant females;
- Abnormal hormone levels in the bloodstream.

The significance of these effects to the health of laboratory animals is not always known. As critics have pointed out, a slight change in the weight of reproductive organs may not actually be an adverse or toxic effect. Moreover the relevance of such findings to humans, and to likely human exposures, is unknown.

4.3 Endocrine disrupter effects and human health

When it was realised that chemicals could have hormonal effects on wildlife and on laboratory animals, attention turned to possible human health outcomes.

It has been proposed that EDs are linked to

a range of hormonally-related human conditions believed to be on the increase. These include testicular problems (failure of testicular descent, testicular cancer) and declining sperm quality/counts in men⁸; increases in breast cancer in women; early puberty in children; and changes in neurological development and behaviour. There is strong evidence of environmental influences on these conditions and ED chemicals are the prime suspects. However, other factors such as dietary changes or other pollutants could be involved, and in some cases the trends in health conditions do not match the trends in chemical use.

Many EDs, especially those that act as hormone mimics or blockers via hormone receptors, are much weaker than their natural hormone equivalents in the body. Since hormone systems in adults are self-regulating within reasonable limits, some argue that they may adapt to exposure to EDs; but fetuses and infants, whose endocrine systems are under development, are at much greater risk.

The International Programme on Chemicals Safety, drawing on the expertise of more than 60 scientists, reported in 2002⁹ that the evidence for ED-induced effects in humans, especially at low exposures over long periods, is inconclusive and inconsistent. Part of the problem has been an over-reliance on laboratory-based animal tests and a failure to adequately monitor health trends and chemicals accumulating in people. Nevertheless, even though definite causative links have not yet been established, there is

increasing evidence that EDs may be causing serious human health effects.

4.4 Knowledge gaps

The full scale of the ED problem is unknown and it is reasonable to fear that there are other toxic effects that have not yet been identified. There are uncertainties and 'knowledge gaps' of many kinds, which contribute to the controversy surrounding EDs and, importantly, how they should be tested. For example, the knowledge gaps include:

- Does chemical perturbation of hormone systems invariably lead to health problems in humans or animals, and if so, which health problems?
- Are changes in the incidence of human reproductive disorders attributable to EDs, or to other environmental factors?
- What are the current levels of human and wildlife exposure to ED chemicals?
- Is there a threshold exposure to these chemicals below which endocrine disruption does not occur, or is there a risk at any dose?
- Do chemicals that have very low endocrine activity interact with natural hormones in the body, or with each other when mixed (the cocktail effect)?
- To what extent are documented effects of ED chemicals on wildlife actually attributable to endocrine disruption, or to other kinds of toxicity?
- How relevant to humans are effects on wildlife and tests on laboratory animals?

The answers to many of these questions depend on good-quality population studies, in humans and wildlife, to ascertain what toxic effects EDs may be having; and on thorough monitoring of levels of exposure to these chemicals.

5.0 Regulatory reactions to endocrine disrupters

In the mid-1990s, the US government passed laws calling for screening (rapid identification) and fuller testing of pesticides, commercial chemicals and environmental contaminants for possible endocrine disrupting effects. The US Environmental Protection Agency produced a strategy to develop and validate new or modified laboratory tests to identify EDs and their effects. Many of the proposed tests use animals, although some non-animal methods are also included.

The USA's move into a controversial and costly programme of test development for mass chemical screening has been heavily criticised by some scientists and by animal advocates.

Major criticisms are that:

- The scale and nature of the ED problem (for humans and wildlife) is not yet known and therefore it is difficult to design appropriate tests¹⁰;
- The likely toxic effects are not well understood, further hindering appropriate test development¹¹;
- The proposed use of millions of laboratory

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cancer) and declining sperm quality/counts in men⁸; increases in breast cancer in women; early puberty in children; and changes in neurological development and behaviour.

- animals is ethically unacceptable;
- The likely relevance of animal test data to humans and wildlife species is not known¹².

In a telling comment on the US endocrine disrupter testing programme, Britain's Royal Society report¹³ commented that:

"... it will undoubtedly detect endocrine activity, of various sorts, in many chemicals. The difficulty will be in interpreting these data, relating results to the development of policies for future research on EDCs, and subsequently developing legislation to protect human health and the environment."

In Europe, the Commission's 1999 Community Strategy for Endocrine Disrupters¹⁴ prioritised the establishment of a list of a few hundred chemicals suspected of being EDs. Many of these had already gone through animal tests and had already been classified as toxic.

The Commission proposed participating in international efforts to develop and validate new tests for EDs, but felt that existing methods (mainly based on standard animal tests) were adequate to detect the chemicals most likely to affect hormonal systems, i.e. carcinogens, mutagens and reproductive toxins.

Neither the EU nor the USA have been willing to take rapid and precautionary action. Scientists, industry and regulators have failed to prevent widespread ED contamination of the environment and to predict the likely health effects. In the absence of definitive information, it only

compounds the problem to divert time and resources into developing animal tests. On the basis of existing information and the precautionary principle, many suspected EDs should be withdrawn especially where safer chemicals can be substituted.

6.0 The animal testing proposals

Worldwide, a massive programme of ED test development has been underway in the last five years driven by the USA. Many countries are playing an active role via the initiative of the Organisation for Economic Co-operation and Development (OECD).

In 1996 the OECD established an international programme to co-ordinate the development of new test methods, and to advise on a harmonised approach to ED testing and assessment. The USA, European countries and Japan are playing key roles in researching ED tests, although the USA is also continuing with its own federal programme.

The USA has devised a screening and testing programme based on two tiers. Tier 1 tests were originally proposed to be rapid, inexpensive non-animal methods, i.e. high-throughput in vitro (test tube) and computer-based screening methods. However, subsequently the emphasis has shifted towards including more animal methods. The Tier 1 tests are intended collectively to provide a screening system simply to identify chemicals that can interact with hormone systems in laboratory animals (mainly rats)

and species selected to represent wildlife (fish and amphibians).

Chemicals that test positive in the Tier 1 screens will proceed to Tier 2 tests. These are longer-term, more complex methods, also using living animals (rats, birds, fish, amphibians and invertebrates), intended to provide detailed information about the dose/response relationship of the chemical hazards, as well as characterising specific toxic effects.

In particular, chemical toxicity to three endocrine hormone systems is the subject of attention: estrogens and androgens, which control reproduction and gender characteristics; and the thyroid system, which drives early development, growth and metabolism.

Many of the animal test methods being proposed in the USA and the OECD initiatives are similar. Some, such as the rodent uterotrophic assay and the mammalian two-generation reproduction assay, have been in use for years for related testing purposes, but are being modified to detect ED effects. Other tests are being developed de novo, such as the in utero-through-lactation assay and the rodent pubertal assays. Tests on fish, amphibians and invertebrates are also being designed – even though the toxic effects of EDs on invertebrates such as crustaceans are probably not caused by hormone interactions¹⁵.

As it has not been decided yet which combinations of tests will be used, it is difficult to put a precise number on the animals who may die in the ED test

programme when it gets underway. However, a number of estimates have been made. The US Environmental Protection Agency has calculated that screening and testing will involve about 180,000 rats per 1000 test chemicals¹⁶.

A senior British toxicologist, Dr Iain Purchase, calculated that as many as 600,000 – 1.2 million animals (rats, mice, fish, amphibians and birds) could be used for each 1000 chemicals tested. With the US Environmental Protection Agency proposing to screen up to 87,000 chemicals¹⁷, the number of animals used in the worst case could exceed 100 million¹⁸. Even using the EPA prediction per 1,000 test chemicals, 15.7 million rats alone would be used.

Purchase commented that the US batteries of tests had been selected without adequate regard for the numbers of animals likely to be used; in his view, a different testing scheme could at least reduce animal numbers.

“This approach is the most extravagant in terms either of the number of animals used or the efficiency of the animals used. It is, therefore, the least ethical approach from the point of view of animal welfare and should not be approved by the regulatory authorities in the USA or internationally.”

The following Table summarises the key animal tests being developed internationally and estimates the numbers of animals each test will use.

Animal tests under development for endocrine disrupting chemicals

Name of animal test	Purpose of test	Number of animals per test ^{19,20,21,22}
Rodent uterotrophic assay	To detect chemicals that mimic or block estrogens (3-day test)	~50
Rodent Hershberger assay	To detect chemicals that mimic or block androgens (5-7-day test)	~20
Rodent pubertal female assay	To detect chemicals with estrogen or thyroid activity (20-day test)	~30
Rodent pubertal male assay	To detect chemicals with androgen or thyroid activity (20-day test)	~30
Enhanced 28-day rodent toxicity test	Repeat-dose test on adult rodents modified to detect endocrine effects	~40-60
Mammalian two-generation reproduction assay	To detect chemical effects on reproduction and development, through two generations of rats or mice	~3000 (including offspring)
In utero-through-lactation assay	To detect androgen, estrogen & thyroid effects on development of rat pups after chemical dosing in the womb	~3000 (including offspring)
Amphibian metamorphosis assay	To detect chemical effects on tadpole metamorphosis, via the thyroid gland (14-21 day test)	30-100
Fish screening assay	To detect estrogen & androgen effects in fish (14-21-day test)	~200
Amphibian two-generation assay	To detect chemical effects on reproduction and development, through two generations	~2000 (including offspring)
Avian two-generation reproductive assay	To detect chemical effects on reproduction and development in birds, through two generations	~2000 (including offspring)
Fish lifecycle (or two-generation) assay	Chemical effects on reproduction and development in fish, one lifecycle or two generations (9-14 week test)	~2,544 (including offspring)
Invertebrate lifecycle assay	Chemical effects on reproduction and development in crustaceans	~1,311 (including offspring)

6.1 Animal suffering

Animals kept in laboratory conditions are physically and psychologically stressed because they do not have the opportunity to perform normal behaviours. Rats and mice, for example, are kept in tiny overcrowded cages where they cannot exercise or get away from their neighbours. Fish and amphibians are kept in barren conditions. The laboratory environment contains many stressors: lack of mental and physical stimulation, bright lights, loud noises (including ultrasound), strong odours and human handling, to name a few. These factors can cause rises in stress hormones, changes in heart rate and blood pressure, insulin abnormalities, sleep disorders, ulcers and lowering of immune function. Any of these effects can interfere with test results and complicate efforts to interpret them.

During the tests animals may suffer from frequent dosing procedures, as well as from any toxic effects the chemicals may cause. Repeated injections cause bruising and force-feeding can induce accidental lung or throat damage. A number of rats suffered throat perforation and pleuritis after being intubated and force-fed a suspected ED chemical by Canadian government scientists²³.

Some of the proposed tests are lengthy, especially the two-generational assays in which rats are dosed daily for 18 weeks during mating, pregnancy, birth and lactation. Their offspring then go through the same programme of chemical dosing.

The standard test includes a high dose expected to induce toxicity (and inevitably some deaths). Moreover, because many suspect EDs have only weak endocrine actions, some will be tested at very high doses that would cause more general toxic effects such as lethargy, anaemia, diarrhoea, weight loss, fur loss, organ damage, unsteady gait, salivation, tremors, coma or death.

The uterotrophic and the Hershberger assays usually use rats who have been 'surgically prepared', by ovary-removal in females and castration in males. This surgery causes considerable postoperative pain and distress; in addition to any toxic effects of the chemicals.

7.0 Why animal tests obstruct chemical regulation

Animal tests are costly in terms of time, money and animal suffering. They can generate endless amounts of data about chemicals, but due to the inherent limitations of the tests and the problems of species and breed variations, much of the data are uninterpretable when it comes to human safety.

It has been argued by the British Union for the Abolition of Vivisection that animal testing acts as a smokescreen, hiding the weakness at the heart of chemical regulation and allowing the continued marketing of chemicals that should be banned²⁴. Animal-based results, being very difficult to interpret for their human health significance, can

delay regulatory decision-making while yet more testing is called for.

For instance, where animal data conflict, the underlying uncertainties always make it possible to argue, on a case-by-case basis, that a particular toxic result should be discounted in favour of a result that indicates non-toxicity. On the other hand, regulators reluctant to ban or control a suspect chemical on precautionary grounds can delay their decision by requiring another animal test to be conducted. This was the case recently with a flame retardant chemical called deca-BDE.

Deca-BDE has been undergoing tests and assessments for a decade and in 2004 European Union regulators were due to announce their decision. Instead, they asked the manufacturers to conduct yet another test (in rodents) to try and clarify possible effects on the brain and behaviour (in humans). The industry said it was willing to do the test, but insisted that the regulators should give the chemical an interim all-clear to safeguard the 'market' for the chemical. Thus more animals would die for data of uncertain value, another regulatory decision would be postponed and unknown harm may be done to people and the environment.

There are several reasons why tests on other species, mainly rodents, are impossible to interpret with any certainty in terms of human health concerns. These include:

- Differences between species and breeds of animals in the way they absorb, circulate, metabolise and excrete chemicals, and in the actual chemical effects;

- Differences in lifespans and body sizes between rodents and humans;
- Animal tests often use megadoses of single chemicals, while actual human and wildlife exposures are to a 'cocktail' of chemicals at much lower levels, for a longer duration;
- Differences in results depending on how the chemical was administered to test animals (e.g. in food, by force-feeding, or by injection into the abdomen, the bloodstream or under the skin);
- In the case of EDs, animal tests results are also affected by the gender, caging conditions, group sizes, social rank and womb position of the rodents used²⁵.

The animal tests being proposed for EDs are subject to all these criticisms. But in addition, the very process of developing and selecting tests for EDs has deviated from internationally agreed guidelines (see section 8).

7.1 Problems of species and breed variations

Different species and breeds (strains) 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.

In the case of endocrine disruption, there are variations in test results from rats versus mice, and also from different breeds of the same species. As there are variations between different breeds of the same species, how can animal toxicity testing ever be considered a reliable predictor of human reactions?

Is bisphenol A an endocrine disrupter?

Bisphenol A is a ubiquitous chemical used mainly to make plastic bottles, plates, mugs, spectacle lenses and food-can linings. It is suspected of being an ED and has undergone many animal tests.

In the uterotrophic assay for endocrine disruption effects, three different breeds of rats – **Wistar**, **Sprague-Dawley** and **Da/Han** rats – responded differently to bisphenol A²⁶. **Sprague-Dawley** rats seemed to be the most sensitive breed for estrogenic effects in this assay, but less sensitive according to other tests (e.g. the multi-generation reproductive study). In contrast, mice appear to be less sensitive than rats to uterine effects, but more sensitive with respect to subtle alterations in reproductive development²⁷.

In other studies bisphenol A caused uterus and vaginal cells to proliferate, and uterus weight to increase, in **F344** rats but not in **Sprague-Dawley** rats – even at very high doses^{28,29}. No-one knows if humans will react in the same way as any particular breed of mouse or rat – or none.

There are many underlying reasons why EDs would affect different species in varying ways. Whether chemicals bind to important proteins in the bloodstream (called sex-hormone-binding globulins) affects their toxicity and differs between species³⁰. The main enzyme involved in making estrogen in the body, called aromatase, is distributed in different tissues in rodents compared to humans; and is under different genetic control³¹. Yet one of the tests being considered to assess the effect of chemicals on aromatase uses rats.

Estrogen: Positive and negative in mice and rats

Estrogen, the classic female hormone, caused changes in male reproductive organs in **C57BL/6N** mice, in **B6** mice and in **C17/J1s** mice (thus testing positive); but not in **ICR**, **CD-1** or **S15** mice (testing negative in these breeds)³².

Estrogen caused reproductive organ effects in male rats of **F344** and **Sprague-Dawley** breeds (thus testing positive)³³. But when the test endpoint measured was changes in hormone levels, **Sprague-Dawley** rats were sensitive to estrogen (testing positive) but **F344** rats were not (testing negative)³⁴.

In a separate study, the roles were reversed, and estrogen caused hormone changes readily in **F344** rats but not in **Sprague-Dawley** rats³⁵.



The problems of variability in ED test results between different species and breeds of animals is so severe that the US Environmental Protection Agency commissioned a report³⁶, which concluded that for a number of different tests using rats and mice:

“Comparisons revealed a lack of consistency in effects produced by endocrine-disrupting chemicals on endocrine endpoints from strain to strain. Endocrine effects were chemical specific, strain specific, endpoint specific, and, in some cases, laboratory specific. There were more sensitive and less sensitive strains to endocrine-active compounds among outbred and inbred strains, depending on the chemical used and the endpoints evaluated.”

The implications of the US report are that:

- Different species and breeds of the same species vary in their responses to EDs;
- Results differ according to which effect of an ED was measured in a test;
- There is no obviously ‘best’ species or breed of animal to choose for ED tests;
- There is poor consistency in the effects of chemicals believed to be EDs;
- There is insufficient consistency in test results from laboratory to laboratory (using the same test).

DES, atrazine and breed variations

When dosed with an endocrine-disrupting drug called diethylstilbestrol, **ACI** rats experienced a 53% increase in mammary cancer, but not a single **Sprague-Dawley** rat developed these tumours³⁷. This

suggests that **Sprague-Dawley** rats would be more resistant to ED-linked tumours.

However, in complete contrast, atrazine (a pesticide suspected of being an ED) did cause mammary tumours in Sprague-Dawley rats – but not in **F344** rats³⁸.

In a long-term study atrazine disrupted the ovarian cycle in **Sprague-Dawley** rats but not in **F344** rats³⁹. But in another test, Sprague-Dawley rats were little affected by atrazine, which did disrupt the ovarian cycle in **Long-Evans** rats⁴⁰.

As the relevance to humans of animal test results is so uncertain, regulators have to use uncertainty factors when they try to set safe exposure levels for people. The standard factor is a 10-fold reduction applied to the highest ‘safe’ dose seen in animal tests. This 10-fold uncertainty factor was first introduced in the 1960s on the basis of limited data from the field of drug development, and its accuracy has never been proved. The use of a standard uncertainty factor is always a guesstimate – an unscientific ‘catch-all’ attempt to deal with the inherent limitations of animal tests. The same problem arises if toxicologists attempt to use laboratory results from one wildlife species to predict what might happen in another.

Scientists and regulators are particularly perplexed by the variability of results obtained from different animals and at different doses of EDs. Professor Frederick vom Saal at the University of Missouri-Columbia was quoted by Nature⁴¹ 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.”

One of the major human health effects suspected of being caused by EDs is testicular cancer. This cancer originates in males when they are still in the womb; so if EDs are a cause this would occur via exposure of pregnant women. Yet according to Dr Richard Sharpe of the Medical Research Council Human Reproductive Science Unit, Edinburgh, testicular cancer has never been induced in laboratory tests in male rats treated via the pregnant female⁴².

7.2 Problems of size, lifespan and megadosing

Two very obvious differences between rodents and humans are size and lifespan. These are important in the design and interpretation of animal tests, because the time-course of a chemical and its metabolites in the body underpins their toxicity. Accounting for these differences between test animals and humans is called ‘scaling’, which involves a mathematical adjustment usually based on body weight ratios.

However this adjustment is only an approximation and is believed to have a high error rate. For instance, predicting from animal tests how quickly a drug leaves the

human body, using body weight ratios for scaling, has been reported to be in error more than 30% of the time⁴³. There is even less certainty with chemicals because, unlike drugs, there are seldom clinical trial results to confirm or contradict the animal test data.

Scientists have still not agreed on the best method for scaling up from rodents to humans. Different factors would affect the results, because:

- The lifespan ratios of a laboratory mouse, a laboratory rat and a human are 1:1.5:50.
- The ratios of body surface areas of a mouse, rat and human are 1:11:388.
- The ratios of body weights of a mouse, rat and human are 1:20:3,500.

Because of their short lifespans, in laboratory tests rodents are not exposed to chemicals for as long as humans may be. It takes time for certain effects to develop, so toxicologists have tried to compensate by using unrealistically high doses of chemicals to maximise the sensitivity of the tests. However, the species of interest – people and wildlife – are very seldom exposed to such high doses of toxic chemicals. The more usual situation is low-dose exposure over long periods of time.

Using these ‘megadoses’ in animal tests further complicates data interpretation. Assumptions are often made that the dose/response relationship for chemicals will be linear, so that extrapolation ‘downwards’ from higher doses to lower doses is feasible; but it is acknowledged that this introduces

Some limitations of animal tests for endocrine disruptors

Rodent uterotrophic assay

- An increase in rodent uterus weight is of unknown/variable relevance to actual toxic effects (such as reproductive disorders) in rodents^{46,47} – let alone in humans⁴⁸.
- Breeds of rats respond differently to the same chemicals^{49,50}. Which breed, if any, would reflect the human response?
- For weak EDs, very high doses will have to be used⁵¹, but these are of questionable value in predicting effects from ‘real life’ exposures of humans or wildlife.
- The assay is only sensitive to chemicals binding to estrogen receptors. This means that negative results will lead to yet more animal testing for other kinds of ED effects.

Rodent Hershberger assay

- Different breeds of rats have varying sensitivities to chemicals that affect male hormones^{52,53}.
- If male rats are caged in groups, the non-dominant males develop smaller testes and prostate glands – without any chemical exposure.
- Human testes cells (Leydig cells) appear to be much less sensitive to changes in

hormone levels than those of rats. This means the assay would ‘over-predict’ the number of chemicals which may be human EDs.

Rodent pubertal male assay

- Men have a different rate of testicular development relative to puberty compared to rats. Test results from rodents therefore may not be predictive for humans.

Amphibian tests

- Different types of amphibians have differing reproductive systems: results from tests on one type may not be relevant to another⁵⁴.
- Metamorphosis in amphibian tadpoles has been assumed, without evidence, to be similar enough to the development of fetal mammals, including humans, in the womb, to predict toxic effect on the thyroid system in these other species.

Invertebrate lifecycle test

- In crustaceans, the toxic effects of EDs may not involve their hormone systems at all⁵⁵.

One of the major human health effects suspected of being caused by EDs is testicular cancer. This cancer originates in males when they are still in the womb; so if EDs are a cause this would occur via exposure of pregnant women.



significant errors and uncertainties⁴⁴. For example, several reproductive disorders have been induced in male rat pups by dosing pregnant females with suspected EDs called phthalates. However, this only occurred at doses 100-500 times the maximum human exposure levels.

Most suspected EDs are very weak in comparison to natural hormones. Consequently, in animal tests they would need to be used at megadoses to demonstrate any measurable effect, compounding the problems of data interpretation. Exposure to multiple EDs could lead to the 'cocktail effect', in which chemicals – each relatively harmless on its own – may have additive or synergistic effects in people and wildlife. Animal tests are highly impractical for studying the cocktail effect, as acknowledged by the Royal Society, which recommended that indirect approaches based on population studies should be developed instead⁴⁵.

8.0 Validating animal tests – breaking the rules

To prove that any new or adapted regulatory test (animal or non-animal) is suitable for its intended purpose, it must be subjected to a series of detailed assessments involving method optimisation and standardisation, and a validation study involving more than one laboratory. Data from the studies should be reviewed by independent experts and published.

The OECD has developed internationally

agreed guidelines for test method validation^{56,57}, which should demonstrate both:

- the relevance of the tests to the species of interest (whether human or wildlife); and
- the reproducibility of tests when conducted at different times and by different laboratories.

However, it appears that while non-animal tests are being subjected to stringent validation, some of the rules of validation are being broken in the rush to develop ED tests on animals.

8.1 Relevance of endocrine disruption tests is unknown

Firstly, most of the validation studies of animal ED tests intended to predict human health effects are addressing mainly reproducibility, while the critical issue of relevance is being underplayed. The tests measure the capacity of chemicals to interact with hormone systems in rodents and amphibians, for instance, but little evidence has been provided that they will identify effects in humans.

Without clear evidence of adverse health effects caused by EDs in humans, the relevance of an animal test to human health is unknown. Measuring an increase in uterus weight in rodents is certainly an effect of EDs, but is it a toxic effect? And does it predict human responses? This is a typical instance where reliance on animal tests displaces important human- and wildlife-based monitoring studies, which should have

been conducted before a laboratory test programme was developed.

8.2 'Validation' studies premature and improperly conducted

Secondly, ED tests using animals are undergoing 'validation' studies before their purpose has been clearly defined, their methodology standardised or their endpoints properly selected. For example, the first stage of an OECD validation of the amphibian metamorphosis test was due to start in October 2003, before it was decided⁵⁸:

- Which species should be used;
- Which test endpoints should be measured;
- Whether the results would be relevant to other vertebrates;
- Which methodology would be most appropriate;
- Whether non-animal methods could be developed instead.

Another example is that of the rodent uterotrophic assay. A validation study of this test, which used large numbers of rats, was conducted under the auspices of the OECD⁵⁹, but there were several deviations from the OECD's own guidelines. These included⁶⁰:

- Use of surgically prepared rats without scientific justification (surgery causes extra suffering);
- Different breeds of rats were used by

- different participating laboratories;
- Inadequate development of the method before progressing to validation;
- No standard experimental protocol was agreed before validation;
- No satisfactory performance criteria of the test have been established;
- The relevance of the test to human health has (still) not been demonstrated;
- Insufficient test chemicals that act by blocking the body's estrogen (anti-estrogens).

Despite its serious scientific flaws, the scientists who participated in the study have concluded that the rat uterotrophic assay is valid for assessing chemicals for estrogenic and anti-estrogenic effects in humans.

8.3 Lower standards for animal tests?

Thirdly, it appears that non-animal and animal tests may be undergoing validation studies of different stringency. In the USA, for example, the Environmental Protection Agency has arranged for non-animal ED tests to be independently reviewed by a committee called ICCVAM⁶¹, which operates by rigorous internationally-accepted standards. However, for animal tests the Environmental Protection Agency has appointed a board that is expected to demand lower standards for validation⁶². This reflects a presumption among some scientists that animal tests, with which they are more familiar, are inherently more predictive of human effects than test-tube

methods and therefore require less rigorous validation⁶³.

Thus a dangerous set of double standards has appeared, which might delay effective in vitro tests while introducing unreliable assays that will cause suffering to possibly millions of animals and undermine efforts to protect human health.

By ignoring their own recommendations for test validation procedures, the member countries of the OECD (including the USA, Japan and European Union states) could be jeopardising efforts to identify EDs, delaying effective and precautionary regulation, and putting the health of people and the environment at continuing risk. Adoption of scientifically flawed animal tests could give the appearance that endocrine disrupters are being controlled. But what is needed is proactive regulatory decisions, based on the precautionary principle and enabled by clear and relevant test data.

9.0 Identifying endocrine disrupters without animal experiments

The urgent need for more non-animal testing methods for chemicals has been highlighted by several expert bodies. The British Royal Commission on Environmental Pollution⁶⁴ welcomed the phasing out of animal tests for cosmetics and the international drive for non-animal testing methods. It recommended that:

“The Government should press for wider application of this approach, using screening

tests, existing data and computational techniques, together with in vitro studies, to describe the hazards of chemicals in all but exceptional cases.”

In the context of a new EU chemicals strategy, the House of Lords Select Committee on the European Union recommended that⁶⁵– *“Well-funded programmes to develop alternative testing methods must now be at the top of the agenda.”*

It added: *“The White Paper provides a rare opportunity to generate the political will in the EU to promote non-animal testing ... The EU chemicals strategy must be linked to an EU strategy for minimising animal testing. The United Kingdom Government is more likely to be successful in pressing this argument if it has its own well-funded programme for finding alternatives.”*

These arguments are equally applicable to ED testing. There is a range of relevant non-animal techniques, including:

- Receptor-binding studies in the test tube;
- Cells and tissues in culture;
- Test-tube studies with enzymes and proteins;
- Computer models such as quantitative structure-activity relationships (QSARs) and PBPK⁶⁶ models.

These techniques can be developed and adapted to replace the proposed animal tests for EDs. Most of them are especially valuable for their speed and ease of use, and some are high-throughput assays amenable to automation. This is important because the



Exposure to multiple EDs could lead to the ‘cocktail effect’, in which chemicals – each relatively harmless on its own – may have additive or synergistic effects in people and wildlife.

USA plans to screen as many as 87,000 chemicals⁶⁷. As well as being a tragic waste of animal lives, this is an impractical proposition as long as it relies heavily on animal tests, some of which are very labour-intensive and take weeks or months to complete.

Using modern non-animal methods would have a number of scientific, practical and ethical advantages:

- If cells or receptors from the target species are used there is no difficulty of species variations, improving the relevance of results;
- A wider range of wildlife species could be tested if cell cultures were used instead of whole animals;
- Cell and molecular tests provide information on the mechanisms underlying endocrine disruption;
- Lower doses of test chemicals can be used, better mimicking the 'real life' exposures of humans and wildlife;
- Computational and in vitro methods yield faster results than animal tests, so large numbers of chemicals can be tested more quickly;
- Cocktails of chemicals and their combined effects can be studied;
- Animals do not suffer in tests of dubious reliability.

A few of the tests being developed in the US and OECD programmes are based on non-animal methods. These include chemical binding to estrogen and androgen receptors, and studies of enzymes (e.g. aromatase)

involved in the making of endocrine hormones in the body. However, animal assays comprise the bulk of the proposed testing for human health effects and all the testing for effects on wildlife.

9.1 Molecular and cell-level tests for endocrine disrupters

An entirely non-animal approach to ED testing would be based on assessing chemicals for a battery of relevant activities, firstly at the level of receptors, enzymes, proteins, cells and tissues⁶⁸.

These tests focus on a range of chemical actions that could disturb the endocrine system and cause toxic effects, including:

- Interactions with hormone receptors and changes in gene activity;
- Effects on enzymes controlling levels of hormones in the body;
- Binding to bloodstream proteins that transport hormones round the body;
- Effects on growth of target cells (e.g. breast, testis);
- Toxicity to embryonic development;
- Genetic damage (e.g. mutations).

A stepwise strategy should be applied to non-animal testing for EDs. The simplest and cheapest tests are conducted first (e.g. computational QSAR methods), followed by increasingly complex and more informative methods. Each step or tier of the testing programme should be designed to add extra information about the chemical being assessed.

Computational quantitative structure-activity (QSAR) models predict very quickly and cheaply whether chemicals are likely to bind to hormone receptors^{69,70}. Better use of QSARs could save hundreds of millions of euros in the new European chemical testing strategy, according to the European Commission. QSAR results will divide chemicals into those that bind strongly to hormone receptors and those that do not, identifying chemicals that need closer study. QSARs that predict estrogen receptor- and androgen receptor-binding chemicals are already available and capable of ranking chemicals in order of their likely potency^{71,72}. More work needs to be done on QSARs for predicting chemical inhibition of the enzyme aromatase.

Priority chemicals are then tested in a second step, to see if they actually do bind to the body's hormone receptors. These methods use pure receptors (from humans, fish, frogs and insects) in the test tube to study binding with estrogens, androgens and other hormones. Binding tests for human estrogen and androgen receptors are undergoing validation now.

Chemicals of concern would then proceed to cell tests that can determine whether the body's receptors are activated or blocked by a chemical. These tests are available for use now, and can also be improved continuously as data accumulate. There are reporter gene tests and transcription activation assays for chemicals that mimic estrogen and androgens, relevant to humans, fish, frogs, mud snails and other species^{73,74}. There are

also human cell cultures that respond to and identify chemicals that mimic or block receptors for other hormones, including glucocorticoids and progestagens. They are sensitive, reproducible and rapid, and have the potential for testing pure chemicals as well as environmental samples, e.g. polluted water⁷⁵.

Endocrine disrupting chemicals can cause effects by other means than through hormone receptors. Test-tube methods can be used to assess whether they interfere with proteins that carry hormones in the bloodstream, such as human thyroxine-binding globulin⁷⁶. Cell-based tests can determine whether chemicals affect the production of endocrine hormones, for example by measuring the activity of the aromatase enzyme from human placental⁷⁷ cells and other cells in culture. These tests can be used now in a weight-of-evidence approach to predicting endocrine disrupter activity.

The effects of potential EDs on the growth and structure of target tissues, such as breast cells, can also be measured without using animals. The MCF-7 focus assay uses human cell cultures to detect the biological effects of chemicals that mimic or block estrogens^{78,79}. Cell culture assays are also under development to detect chemicals toxic to male cells in the testis and work on these should be accelerated.

There are *ex vivo* tests for estrogenic effects on fish⁸⁰. These measure levels of a protein, produced in the liver, which is a precursor to yolk protein. A rise in this

protein, called vitellogenin, indicates that a chemical has estrogenic activity in fish. At the moment the test relies on fresh liver cells from fish. Even though several chemicals can be tested on cells from one liver, this is not ideal because fish have to be killed for the purpose. There are fish liver cell cultures that survive in the test tube for long periods, and research should be undertaken to see if these may form the basis of a completely non-animal test. *In vitro* vitellogenin assays would also be applicable to other egg-laying animals, such as turtles.

Endocrine disrupting chemicals could affect embryonic development or male fertility, and may cause genetic damage. Chemicals can be screened for these properties in the test tube. A method based on embryonic stem cells has already been validated for detecting chemicals that cause embryotoxicity and is now being further improved^{81,82}. Chemical toxicity to sperm (e.g. structure and movement) can be demonstrated in the test tube⁸³. Finally, there is a range of established non-animal tests already in use to detect genetic damage, such as the Ames test, mammalian cell mutation tests and chromosome aberration assays.

9.2 From cells to whole organisms

Identifying chemicals that have hormone-related activities at the molecular and cell levels is the crucial step in identifying chemical hazards. But to understand the risk

chemicals may actually pose to humans and wildlife requires these results to be interpreted at the level of the whole person or animal. However, this need not, as is often argued, require cruel tests on living animals.

If a chemical cannot get into the bloodstream it cannot have toxic effects on the whole organism. Simple tests using gut cells and skin fragments in the test tube, plus computer modelling, can predict this, and cell-based models of the blood-brain barrier are being developed.

If a chemical is absorbed into the bloodstream it can have toxic effects anywhere in the body. Then it becomes necessary to predict which organs it might affect, how long it remains in the body and whether it is converted by metabolism into other more, or less, toxic forms.

The rate at which a chemical is metabolised, and the substances it is changed into, are very important parameters in determining its toxicity. These properties can be studied in the test tube using human liver cells or enzyme mixtures. Methods for assessing metabolism *in vitro* are undergoing validation⁸⁴, although additional research is required to ascertain their suitability for use in *in vitro* ED assays.

Mathematical models are also being developed which, on the basis of test-tube data on human enzyme systems, can predict the rate of clearance of a drug from the bloodstream. For example, a computer model called Simcyp has already produced results more predictive of human drug clearance





than scaling up from animal test data⁸⁵. The model could be adapted to produce similar data for chemicals.

The distribution of the chemical and its duration of effect in the body can be assessed by another computer approach known as physiologically-based pharmacokinetic (PBPK) models. These computer models have been used as decision-support tools for some time by the pharmaceutical industry and in occupational toxicology. They are being further developed for risk assessment purposes⁸⁶, and could be used in conjunction with other non-animal techniques for hazard and risk assessment.

PBPK models require information about the chemical – gained from its molecular properties, its solubility in fats and water and similar data, which can be obtained by non-animal methods. The models are also programmed with standard physiological information (for the species of interest, human or otherwise) including blood flow and respiration rates, enzyme activities and volumes of different tissues in the body.

The outcome is a set of computer predictions about the activity of the chemical within the body, the relationship between exposure to the chemical and its concentration in different tissues, and where toxic effects might be expected.

Being based on information specific to the chemical and to the species of interest (usually human), PBPK systems model the mechanisms underlying chemical effects as well as ‘real life’ exposure scenarios. They can also be used to predict the effects of a

chemical on different sub-populations, such as pregnant women, infants and children⁸⁷. Although the models are already in use, a dedicated, international effort to develop and perfect PBPK models for these applications is required.

There are also ethical opportunities to study the absorption, distribution, metabolism and excretion of chemicals at harmless ‘microdoses’ in human volunteers. These studies are being used in drug development⁸⁸ and also have applications in chemical testing. Incredibly sensitive analytical techniques, such as accelerator mass spectroscopy (AMS), offer the potential to provide gold-standard human data, without risk to volunteers. European funding to study and standardise such methods as AMS is urgently required.

10.0 Attitudes to non-animal tests

The non-animal methods described above are either under development or are already being validated for their relevance and reliability to detect EDs. In this respect, most are as advanced as the proposed animal testing methods, which are also being assessed for their validity (albeit not rigorously enough; see section 8.2).

Because new methods are having to be developed from scratch or modified, the ED situation offers a superb opportunity to develop a programme of testing that is ethical, practical and scientifically sound. Non-animal tests can be applied now in a

weight-of-evidence approach using expert judgement and informed by the precautionary principle. This will permit the identification of key suspect chemicals, for banning or tighter regulation in the immediate future. Additional non-animal methods are being developed and validated to allow more accurate and precise results to be generated, and to extend testing to protect the health of more wildlife species. The expenditure of millions of pounds and person-hours developing tests that cause animals to suffer and die en masse is unethical, especially when it is feasible and realistic to create an entirely non-animal programme.

One problem is the excessive conservatism of scientists and regulatory agencies. For several decades, and despite its obvious limitations, animal testing has been the cornerstone of efforts to regulate chemicals. Most scientists feel comfortable with the notion that tests using other mammals provide a level of reassurance simply because we too are mammals. However, scientific analysis is actually stripping away the basis of this comfort zone, as more and more studies undermine the value of animal tests for human health effects.

A second barrier is that toxicologists and regulators, by and large, are not sufficiently concerned about the pain and distress of laboratory animals, whether they are fish, rats, frogs or monkeys. However, the public – in whose name the testing is conducted – is very concerned indeed, as evidenced by numerous public opinion polls⁸⁹. One of the

most recent, conducted by TNS Media in Britain in August 2003, revealed that 87% of those polled were opposed to the use of millions of animals in chemical tests as planned by the European Commission⁹⁰.

The British House of Lords Select Committee on the European Union recommended recently that *“... the very success of any chemicals strategy depends on the public being reassured that a serious effort is being made to develop alternatives to animal testing”*.⁹¹

There is general agreement that non-animal methods have an essential role to play in testing for EDs. But, despite the process of validation that the tests will undergo, most toxicologists and regulators mistrust these novel methods and want to limit them to first-stage or screening assays only. This means that they intend to confirm the results by using hundreds of thousands of animals. Japan and the USA have made it clear that this is their stance; other countries, such as Denmark, are taking a more rational approach and have confidence in the new techniques.

As a testing paradigm, hoping to confirm results from human-based non-animal tests by means of animal assays, is doomed to failure. If human cell tests indicate that a chemical is an ED and tests on rats are contradictory, regulators will call for more and yet more animal testing to try and clarify the situation. In the meantime, potentially dangerous chemicals remain in wide use and continue to contaminate the soil, water and air.

11.0 Conclusions

It is certain that some wildlife species are already threatened by endocrine disrupter chemicals, and it is very likely that human health is also being damaged. Definitive information is needed, but in the meantime the precautionary principle must be invoked and suspected EDs should be withdrawn. Immediate action is required to draw up and implement an effective and rapid testing programme based on non-animal techniques.

Thus, a range of complementary approaches should be deployed. High-quality studies of reproductive disorders in people are urgently needed, using standard diagnostic methods and reporting requirements. This will clarify the types of health effects caused by EDs, and inform the kinds of laboratory tests that need to be developed.

Information is also required about the exposures of people and wildlife species to a range of potential EDs. This requires routine, real-time monitoring using sensitive analytical techniques that can detect low levels of chemicals. Detecting chemical build-up in humans and in the environment will identify emerging toxins and pollutants. A precautionary policy requiring evidence of safety to permit the marketing of chemicals is essential, as it places greater emphasis on health and environmental protection than does the present regulatory system.

Valid non-animal tests should be pressed into service without delay. Additionally, a

well-funded, high-priority programme of non-animal test development and validation is needed to ensure that EDs can be effectively and rapidly identified. Resources currently going into animal tests should be redirected into non-animal methods for humane, scientific and practical reasons.

A non-animal testing programme will enable chemicals with endocrine disrupting properties to be rapidly screened; priority chemicals identified; and hazards and risks predicted. Computerised QSAR methods can provide very rapid results extremely cost-effectively. In vitro tests based on cells and molecules from the target species (whether human or wildlife) will eliminate problems of extrapolating between species as well as being able to detect low-dose effects and chemical cocktails.

International experience has shown that all programmes based on animal tests attempting to assess thousands of chemicals are running years or decades behind schedule. The risk evaluation aspect of these programmes is delayed by conflicting data due to the limitations of the animal methods. A testing system using only non-animal methods would deal more rapidly with the chemical backlog. Avoiding the suffering and death of hundreds of thousands of animals is ethically sound and supported by the public. A weight-of-evidence approach and expert judgement, set in a precautionary framework, will support a regulatory approach that better protects our health and that of wild animals and the environment.



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 - Of 19 known human carcinogens, rodent tests identified only 37 per cent (Salsburg, D (1983). *Fund. & Appl. Toxicol.* 3:63-67);
 - They produce too many false positive results e.g. 19 of 20 substances accepted as safe in humans had been carcinogenic in rodents (Ennever, FK et al (1987). *Mutagenesis* 2:73-78);
 - 46 per cent of substances were found to have been carcinogenic in rats but not in mice, and vice versa (Di Carlo, FJ (1984). *Drug Metabol. Rev.* 15:409-413);
 - With 121 chemicals that had been tested twice in rodent assays, the results only agreed 57 per cent of the time (Gottmann, E et al (2001). *Data quality in predictive toxicology: reproducibility of rodent carcinogenicity. Environ. Health Perspect.* 109:509-514).
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both cases, the use of established cell lines kept alive in culture (in vitro) avoids the acquisition of fresh cells. One test mentioned in this section investigates potential estrogenic effects in fish and currently uses fresh fish tissue (ex vivo).

From an ethical standpoint the top priority must be to end experiments on living animals (in vivo). In the future, it should be possible to move beyond that aim and also replace the use of fresh cells from animals.

It should also be noted that no test described here is based on any use or expected use of human embryonic stem cells. Adult human stem cells are becoming increasingly available for testing purposes and, as they can be obtained without harm to donors, are both scientifically and ethically acceptable.

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