CHEMICALS AND CANCER
What the Regulators Won’t Tell You About Carcinogenity Testing

A Report by PETA Europe • PETA.org.uk • PETA.de • PETA.nl • PETAFrance.com
“[C]ANCER RISK ASSESSMENT UNCERTAINTIES OF ANIMAL TESTS HAVE BEEN SWEPT UNDER THE RUG BY ADOPTING ASSUMPTIONS OF CORRESPONDING HUMAN VALIDITY THAT HAVE NO FOUNDATION IN FACT OR SCIENCE.”

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FOREWORD

Established in 1980, People for the Ethical Treatment of Animals (PETA) is the world’s largest animal rights organisation, with more than 1.1 million members and supporters who share the belief that animals are not ours to eat, wear, experiment on, use for entertainment or exploit in any way. For the past decade, PETA has been at the forefront of global efforts to modernise regulatory toxicology testing by documenting the scientific failings of conventional approaches and by financially supporting and promoting valid and humane alternatives that reduce or eliminate reliance on animal testing while better protecting public health and the environment. As a founding member of the International Council on Animal Protection at the OECD and at the ICH (ICAO and ICAP, respectively), PETA joins with animal protection organisations across Europe, North America and Asia to ensure that animals have an effective voice within the Organisation for Economic Cooperation and Development and the International Conference on Harmonisation as they establish internationally recognised guidelines and standards for the safety testing of chemicals and pharmaceuticals that affect the use of animals in laboratories the world over.

PETA, which has affiliates in France, Germany, the Netherlands and the United Kingdom, has also been an active participant in a number of important scientific and policy dialogues at the EU level. We have long advocated for more intelligent testing strategies for the safety assessment of pesticides, pharmaceuticals and the approximately 30,000 chemicals that will be covered under the forthcoming regulation on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). We successfully campaigned for the acceptance of non-animal methods for the detection of paralytic toxins in shellfish and are currently working to improve protection of animals through the forthcoming revision of the EU’s animal experimentation legislation (Directive 86/609/EEC) and the proposed Community Action Plan on the Protection and Welfare of Animals. This work is helping to ensure that new and revised EU legislation and Community strategies are not only consistent with EU member states’ longstanding commitment to the “3Rs” of reduction, refinement and replacement of animal use, but also based on sound science, which is a precondition of effective regulation.

INTRODUCTION

Since World War II, synthetic chemical pollutants have persisted and bioaccumulated in our bodies and in the environment on a global basis, threatening wildlife populations and presenting an ever-growing hazard to human health. Among the most significant of these health concerns is cancer, which alone was responsible for one-quarter of the more than 4.6 million deaths in the EU in 2000 (Cancer Research UK 2004). Relative to other diseases, the human and economic costs of cancer are very high (Knight and others 2006a) and can cost EU taxpayers more than €2 million per individual cancer death (Postle and others 2003).

Human exposure to carcinogens can come from a variety of sources, including the workplace, consumer goods and textiles, food additives and preservatives and medicinal products as well as from pesticide residues and other contaminants in food, water, air and soil. A World Bank study (Lvovsky 2001) estimated that in established market economies, pollution from agro-industrial chemicals and other sources may be responsible for up to 2.5 per cent of a country’s total disease burden (i.e., deaths and general ill health). In contrast, a European Commission (EC 2003b) staff working paper suggested that the burden of environmentally attributable disease could be as much as 150 per cent higher than the World Bank estimate.

Community legislation aimed at protecting the health and safety of workers includes Directive 90/394/EEC for the Protection of Workers from Occupational Exposure to Carcinogens, and Directive 98/24/EC for the Protection of Workers Health and Safety from Chemical Agents at Work. Despite these legislative measures, however, an estimated 32 million European workers are exposed each year to known or suspected carcinogens in the workplace, resulting in as many as 45,000 cancer deaths annually across EU Member States (EC 2004). Cancer deaths arising from exposure to occupational carcinogens are responsible not only for a vast amount of preventable human suffering, but also for annual societal costs of up to €70 billion (Postle and others 2003).

Chilling statistics such as these have led some stakeholder groups to conclude that what EU regulators lack is a sufficient quantity of information upon which to make public health and worker protection decisions. However, closer inspection reveals that testing for cancer hazard – or carcinogenicity – is a long-established legal requirement for regulated substances where human exposure is expected to be significant, repeated and/or long-term. For example, Directive 91/414/EEC requires long-term toxicity and carcinogenicity testing of pesticide “active ingredients”; Directive 93/41/EEC and Regulation 2309/936 impose similar requirements for the authorisation of pharmaceuticals; and Directive 76/769/EEC restricts the marketing and use in Europe of hundreds of known or suspected carcinogenic, mutagenic and reprotoxic (CMR) substances and preparations. In addition, the proposed REACH regulation lists carcinogenicity as a conditional testing requirement for chemicals produced or imported into the EU in volumes of 1,000 tonnes or more per year.
THE SCIENTIFIC CASE AGAINST RODENT CARCINOGENICITY STUDIES

OVERVIEW OF CARCINOGENICITY TESTING AND SCIENTIFIC VALIDATION

The current paradigm in regulatory toxicology has failed to prevent global contamination and environmental damage because it is based on test methods that have not been scientifically validated. Carcinogenicity studies are a case in point.

"The current 2-year rodent carcinogenicity study was never validated ... and there is little evidence supporting the repeatability and reproducibility of the current rodent carcinogenicity study."


The earliest recorded example of a laboratory carcinogenicity study dates back to 1915, when Japanese researchers Yamagiwa and Ichikawa induced skin tumours by painting rabbits' ears with coal tar for months in an attempt to "confirm" the correlation between soot and scrotal cancer in humans – an association which had already been documented clinically more than a century earlier by Sir Percivall Pott (1775). The decades that followed produced many similar examples; however, it was not until the 1960s and 70s that carcinogenicity testing became routine. The first standardised protocol for lifetime carcinogenicity studies in rats and mice was proposed by the US National Cancer Institute (Sontag and others 1976) and has since been refined by the US National Toxicology Program (US NTP) and adopted as internationally harmonised test guidelines of the Organisation for Economic Cooperation and Development (OECD 1981) and of the International Council on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH 1997).

A conventional rodent lifetime carcinogenicity study takes approximately five years to design, conduct and analyse and consumes at least 800 rats and mice at a cost of €1.5 million to €3 million per chemical tested (OECD 1981; NIEHS 1996). The study exposes groups of rats and mice of both genders to three different doses of a test chemical, while one or more "control" groups receive no chemical exposure; only recently has the EU discontinued its longstanding requirement of two control groups (Baldrick 2005). The chemically exposed animals receive daily doses of a test substance for their entire 18- to 24-month lifespan. A statistically and/or biologically significant increase in tumour rate relative to controls is taken as "evidence" of a chemical's carcinogenic potential. To date, more than 6,000 chronic/carcinogenicity studies have been reported in the peer-reviewed literature.
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In the absence of scientific validation, regulators are left with the proverbial "garbage in, garbage out" scenario, whereby data from non-validated test methods are always open for interpretation, and therefore manipulation, by a variety of vested interests.

**FAILURE 1: PERPETUALLY MISJUDGING HUMAN CANCER RISK**

"[R]egulators have chosen animal tests to forecast human cancer risks. To this end, animal data are filtered through a series of preconceived assumptions that are presumed to overcome a host of human/animal differences in biology, exposure and statistics – differences that in reality are insurmountable."

– Dr Gio Batta Gori, The Health Policy Center (2001)

When animal tests fail to accurately predict human results, they can either give a "false positive", in which they predict cancer risk where there is actually no risk to humans, or a "false negative", in which they do not detect an actual health risk to humans. The problems of false negatives – true human carcinogens that go undetected – is clearly of great concern from a public health perspective, as they allow for potentially widespread human exposure to dangerous chemicals. For example, critical public-health and worker-protection measures related to cigarette smoke, asbestos, benzene and other well-established human carcinogens were delayed for many years because of misplaced trust in animal tests, which for years could not replicate effects that had already been documented in humans (Laskin et al., 1994). In the absence of scientific validation, regulators are left with the proverbial "garbage in, garbage out" scenario, whereby data from non-validated test methods are always open for interpretation, and therefore manipulation, by a variety of vested interests.

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In order to objectively evaluate the relevance and reliability of rodent carcinogenicity studies, PETA scientists conducted an analysis of the largest publicly available database of standardised carcinogenicity studies, belonging to the US National Toxicology Program (US NTP). As of January 2006, this extensive database contained detailed reports and pathology data on 502 two-species rodent lifetime carcinogenesis studies of 476 unique chemicals – which comprise at least 10 million tissue sections from nearly 1,900 individual experiments that have been evaluated for carcinogenicity (Seidle 2006a).

Recognising that rats and mice are more biologically similar to one another than either species is tohumans, PETA set out first to determine quantitatively whether test results in one gender and species of rodents (e.g., male mice) could accurately predict the cancer risk for the other gender and species of rodents exposed to the same chemical. We found that across all 502 rat and mouse lifetime carcinogenicity studies in the US NTP database, results in one species and sex frequently underestimated cancer incidence in the other species and gender, with the average false negative rate being 27.5 per cent – but ranging as high as 40 per cent in one case. In light of these findings, it should come as little surprise that tests on rats and mice may be chemically related. Such dangerous comparisons are supported by the absence of quantitative data of the response is less than that required for clear evidence. Inadequate study: demonstrated by studies that because of major qualitative or quantitative limitations cannot be interpreted as valid for showing the absence or presence of carcinogenic activity.

Table 1: Chemicals recognised as definite or probable human carcinogens by international cancer authorities despite dubious or false negative results in conventional rodent carcinogenicity studies

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Asbestos (chrysotile and dimethyl hydrazine)</td>
<td>Cement</td>
<td>&lt;rats = inadequate study</td>
<td>rats = inadequate study</td>
<td>rats = no evidence</td>
</tr>
<tr>
<td>Asbestos (chrysotile)</td>
<td>Cement</td>
<td>&lt;rats = no evidence</td>
<td>rats = no evidence</td>
<td>mice = no study</td>
</tr>
<tr>
<td>Combination of aspirin, caffeine and phenacetin</td>
<td>Analgesic</td>
<td>&lt;rats = no evidence</td>
<td>rats = equivocal evidence</td>
<td>mice = no evidence</td>
</tr>
<tr>
<td>Dichlorvos as dosed feed</td>
<td>Insecticide</td>
<td>&lt;rats = no evidence</td>
<td>rats = no evidence</td>
<td>mice = no evidence</td>
</tr>
<tr>
<td>Lindane</td>
<td>Insecticide</td>
<td>&lt;rats = no evidence</td>
<td>rats = no evidence</td>
<td>mice = no evidence</td>
</tr>
<tr>
<td>Dichlorodiphenyl trichloroethane (DDE)</td>
<td></td>
<td>&lt;rats = no evidence</td>
<td>rats = no evidence</td>
<td>mice = no evidence</td>
</tr>
<tr>
<td>Hexachlorobenzene-p-dioxin as topical application</td>
<td>Combination byproduct and industrial contaminant</td>
<td>&lt;rats = no study</td>
<td>rats = no study</td>
<td>mice = no evidence</td>
</tr>
<tr>
<td>Selenium sulfide</td>
<td>Antidandruff shampoo</td>
<td>&lt;rats = no study</td>
<td>rats = no study</td>
<td>mice = no evidence</td>
</tr>
<tr>
<td>Bromochloromethane</td>
<td>Water disinfection byproduct</td>
<td>&lt;rats = no study</td>
<td>rats = no study</td>
<td>mice = no study</td>
</tr>
<tr>
<td>Gallium arsenide</td>
<td>Semiconductors</td>
<td>&lt;rats = no evidence</td>
<td>rats = clear evidence</td>
<td>mice = no evidence</td>
</tr>
<tr>
<td>Aerochlor 1254</td>
<td>Pesticide</td>
<td>&lt;rats = equivocal evidence</td>
<td>rats = equivocal evidence</td>
<td>mice = no evidence</td>
</tr>
<tr>
<td>p-Nitroflourene</td>
<td>Ingredient in dyes, pesticides and rubber chemicals</td>
<td>&lt;rats = equivocal evidence</td>
<td>rats = equivocal evidence</td>
<td>mice = no evidence</td>
</tr>
<tr>
<td>5-Azacytidine</td>
<td>Anti-cancer drug</td>
<td>&lt;rats = inadequate study</td>
<td>rats = inadequate study</td>
<td>mice = inadequate study</td>
</tr>
</tbody>
</table>

Definitions: 
- Inadequate study: demonstrated by studies that because of major qualitative or quantitative limitations cannot be interpreted as valid for showing the absence or presence of carcinogenic activity.
- Clear evidence: showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumours to progress to malignancy.

2US NTP 2006b.

In order to objectively evaluate the relevance and reliability of rodent carcinogenicity studies, PETA scientists conducted an analysis of the largest publicly available database of standardised carcinogenicity studies, belonging to the US National Toxicology Program (US NTP). As of January 2006, this extensive database contained detailed reports and pathology data on 502 two-species rodent lifetime carcinogenesis studies of 476 unique chemicals – which comprise at least 10 million tissue sections from nearly 1,900 individual experiments that have been evaluated for carcinogenicity (Seidle 2006a).
Turning to “false positive” results, perhaps the most infamous example of this problem is the artificial sweetener saccharin. In 1981, saccharin was given the dubious distinction of being listed among substances “reasonably anticipated to be a human carcinogen” because it caused bladder cancer in rats. The sugar industry capitalised on this finding, and for a time, in the US, packets and foods containing the sweetener were required to bear the warning: “Use of this product may be hazardous to your health. This product contains saccharin which has been determined to cause cancer in laboratory animals” (Vogt 1995). Two decades later, regulators were forced to admit that “observed bladder tumours in rats arose from a mechanism that is not relevant to humans”, which led to the de-listing of saccharin in 2000 (Schmidt 2006).

Saccharin’s regulatory history is a telling example of the problem with false positive results. For decades, scientists have criticised rodent carcinogenicity studies for implicating an implausibly large number of chemicals as carcinogenic. For example, the US NTP has estimated that about half the chemicals it has tested have produced evidence of cancer in rodents (Fung and others 1995; Haseman 1983). A review by academic cancer researchers (Kaid and Stone 1993) found that closer to two-thirds of 800 chemicals tested positive in rodent carcinogenicity studies, while other scientists have suggested that the false positive rate could be upwards of 90 per cent – meaning that rodent carcinogenicity studies are almost completely incapable of correctly identifying chemicals that truly do not pose a cancer risk to humans (Emmerv and others 1987). Overestimates of cancer risk can cost society billions in terms of loss of viable products in commerce, decreased international competitiveness, job loss, litigation and undue public anxiety.

### FAILURE 2: UNRELIABLE TEST RESULTS AND MEANINGLESS CLASSIFICATIONS

“The problem is we don’t know what the findings really mean.”
— Dr Robert Maronpot, US National Institute of Environmental Health Sciences (Brinkley 1993)

The OECD (2005) defines reliability as “the extent that a test method can be performed reproducibly within and between laboratories over time, when performed using the same protocol. It is assessed by calculating intra- and inter-laboratory reproducibility and intra-laboratory repeatability". Ideally, if a chemical is tested in several laboratories, each following the same protocol, variability in test results will be low and agreement between toxicity classifications will be high. However, a recent analysis by Austrian and German scientists of duplicate rodent carcinogenicity data showed that there was only 57 per cent agreement between results for 121 chemicals, each of which had been tested on two occasions (Gottman and others 2001). Concordance improved only marginally even when additional biological factors such as species, sex, strain and target organs were taken into consideration, which led the scientists to conclude, “These results indicate that rodent carcinogenicity assays are much less reproducible than previously expected”.

As previously discussed, a standard rodent carcinogenicity study consists of concurrent tests in four different “species/gender groups” – i.e., male and female rats and mice. Thus, PETA’s recent analysis of the US NTP’s extensive database of standardised rodent lifetime cancer studies (Bedliss 2006a) involved separate reviews of 1,872 individual species/gender group tests on 476 unique chemicals, each consuming approximately 215 animals and costing €400,000. Of these, a total of 243 individual species/gender group tests – or approximately one in every seven – were found to produce either “equivocal evidence” of cancer hazard or were written off altogether as being “inadequate studies” (Table 2). Either way, these studies contributed nothing of value to the understanding of whether or not the tested chemicals cause cancer in rodents, let alone in humans.

### Table 2 Animal lives and money wasted on rodent carcinogenicity studies by the US NTP that have produced equivocal or inadequate results

<table>
<thead>
<tr>
<th>Species</th>
<th>Equivalent or Inadequate Tests</th>
<th>Animal Lives (per species)</th>
<th>Monetary Cost (Euro 2000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>84</td>
<td>13,760</td>
<td>€25,000,000</td>
</tr>
<tr>
<td>Male</td>
<td>72</td>
<td>15,480</td>
<td>€28,000,000</td>
</tr>
<tr>
<td>Mouse</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>40</td>
<td>8,600</td>
<td>€15,000,000</td>
</tr>
<tr>
<td>Male</td>
<td>37</td>
<td>14,405</td>
<td>€28,000,000</td>
</tr>
<tr>
<td>Total</td>
<td>243</td>
<td>51,245 animals</td>
<td>€94.6 million</td>
</tr>
</tbody>
</table>

A variety of national and international agencies classify chemicals according to their perceived cancer risk to humans, including the World Health Organisation’s International Agency for Research on Cancer (IARC), through its monograph series; the US National Toxicology Program (US NTP), through its biennial Report on Carcinogens (ROC); and the US Environmental Protection Agency (US EPA), through its Integrated Risk Information System (IRIS) database. Although classification schemes vary somewhat among these agencies, there are essentially five broad categories in which a chemical may be placed:

- **Known human carcinogen**
- **Probable human carcinogen**
- **Possible human carcinogen**
- **Probably not carcinogenic to humans**
- **Unclassifiable as to human carcinogenicity**

In order for a chemical to be classified as a known human carcinogen, there must generally be “sufficient evidence of carcinogenicity in humans” (emphasis added) (IARC 2006a). In other words, animal data alone are never enough to classify, let alone regulate, a chemical as a human carcinogen. The most that can be said on the basis of animal test results is that a substance may be a probable human carcinogen – and even this classification generally requires there to be evidence of cancer risk in both rats and mice, as well as some level of evidence of cancer risk in humans (IARC 2006a). The lack of weight given to rodent carcinogenicity data is clearly illustrated in Figure 1, which contrasts the hundreds of chemicals tested by the US NTP and found to produce “positive” evidence of cancer in one or more rodent species/sex groups (light green bars) with the tiny proportion of these that have been classified as known (dark green bars) or even probable (white bars) human carcinogens.
While chemicals classified as known or probable human carcinogens are more likely to be subject to meaningful regulatory controls, PETA’s analysis determined that such classifications have only been assigned to a small fraction of the chemicals that yielded positive evidence of cancer risk in animal studies (Figure 2). For example, the US EPA has classified less than 15 per cent of the 476 US NTP cancer-tested chemicals in its IRIS database as to their cancer risk to humans, and IARC has classified less than 45 per cent in its monograph series (Figure 2). And of the US NTP-tested chemicals that these agencies have classified, most have simply been lumped into such uninformative and non-committal categories as possible human carcinogen, or unclassifiable as to human cancer risk (IARC 2006b; US EPA 2006; Knight and others 2006b). In fact, more than 82 per cent of all chemicals evaluated to date by IARC have been so classified (IARC 2006b). Such designations fail to address the central question of whether a substance does or does not cause cancer in humans and are therefore virtually meaningless from a public health perspective.
human carcinogens or simply unclassifiable. IARC, on the other hand, has written off fully 94.2 per cent of the 139 US NTP-tested chemicals it evaluated as unclassifiable or possible human carcinogens. Thus, even after hundreds of rodent carcinogenicity studies – each inflicting suffering and death upon more than 800 animals – regulators are still reluctant to commit to a meaningful classification of human cancer risk for most tested chemicals.

On the other hand, some of the chemicals that the US NTP has classified as known or probable human carcinogens do not produce strong evidence of cancer risk in rodent studies. For example, only three of the nine chemicals classified as known human carcinogens in the US NTP ROC caused cancer in both rats and mice. One known human carcinogen (an anaglypge mixture containing phenacetin) produced only inconclusive evidence of carcinogenicity in female rats and no evidence whatsoever of carcinogenicity in any of the other animals. Similarly, of the chemicals classified by the US NTP as probable human carcinogens, 18 (20 per cent) caused cancer in only one species tested, and four chemicals (including the pesticides DDT and lindane) produced no evidence of carcinogenicity in either rats or mice (US NTP 2006). These false negative animal test results could lead to dangerous human exposures if government regulators relied on them.

**FAILURE 3: IRRELEVANCE OF EXTRAPOLATION TO DIFFERENT SPECIES, STRAINS AND GENDERS**

*I believe it is irrational to use strains of rats and mice which are known to be subject to high spontaneous tumour rates, because to do so is to maximize the chances of confusion due to co-carcinogenic effects in such strains.*
– British toxicologist Dr F.J.C. Roe (1980)

*In the present state of the art, making quantitative assessments of human risk from animal experiments has little scientific merit.*
– Statisticians Drs David Freedman and Hans Zaisel (1988)

In addition to being reliable, a scientifically valid toxicity study must also be relevant, meaning that it accurately measures a particular biological effect in the species of ultimate interest – usually humans (OECD 2005). Genomic research has revealed that rats and mice diverged as separate species 18 to 24 million years ago, yet even so, are much more similar to one another than either is to humans, who diverged approximately 80 million years ago (Langley 2005). It should therefore come as little surprise that a number of tumour types and mechanisms of cancer induction in rodents have been determined by regulatory and other cancer authorities to be of little or no relevance to the human condition (Cohen 2002 and 2004; IARC 1995, 1999a and 2003). Gold and others (2002). For example:

- Binding of chemicals such as unleaded gasoline and d-limonene to the male rat-specific protein 2u-globulin results in its accumulation in renal tubular cells and concomitant cell death, compensatory cell proliferation and eventually kidney tumours. There is no functionally similar protein in humans and no evidence of a similar mechanism.
- High doses of sodium salts such as ascorbate and saccharin produce a calcium phosphate-containing urinary precipitate in rats, which results in urinary tract cell death,
- Elevated levels of thyroid-stimulating hormone (TSH) result in thyroid tumours in rats. Substances that interfere with interactions between the thyroid and pituitary glands lead to elevated levels of thyroid-stimulating hormone, increased cell proliferation and tumour growth. Rodents are much more susceptible to this carcinogenic mode of action than humans. Phenobarbitol is an example of a chemical that causes thyroid tumours in rats – but not humans – through the elevation of TSH levels.

Certain types of tumours have been identified in rodents for which there are no known human equivalents, such as spicilec mononuclear cell leukaemia in rats and the mouse submucosal mesenchymal lesion of the urinary bladder. In fact, there are entire organs in rodents that have no counterpart in human anatomy, such as the forestomach, Zymbal’s gland, and the Harderian gland. In addition, certain tumours of the hormonal and reproductive systems (particularly the thyroid, pituitary, adrenal cortex and medulla, parathyroid, pancreatic islets, gastrointestinal endocrine cells and reproductive organs), while common targets of cancer in rodents, are routinely dismissed as being of little or no relevance to humans (Cohen 2004). So lengthy is the list of irrelevant rodent tumours and mechanisms that IARC has published technical reports cautioning scientists and regulators not to rely on the results of rodent studies in which cancers are found in the thyroid, kidney or urinary bladder (IARC 1995), forestomach or gastric neuroendocrine tissues (IARC 1999a) or where the mechanism of action is associated with peroxisome proliferation (IARC 2003).

The interpretation of rodent carcinogenicity data is further complicated by the fact that the highly inbred strains most commonly used in these studies have very high “background” tumour rates even when they are not exposed to a test chemical (Haseman 2000). For example, the US NTP has reported that approximately 96 per cent of untreated control rats from the Fischer 344 strain may be expected to develop some type of spontaneous tumour, and 64 per cent of the males and 43 per cent of the females had at least one cancerous tumour (Haseman and others 1998). The same study similarly reported that more than two-thirds of untreated B6C3F1 mice developed some type of tumour and that 39 per cent of these mice had at least one cancerous tumour. Such high spontaneous-tumour rates create so much background “noise” that it can be nearly impossible to detect a small rise in chemically induced tumours.

Additionally, carcinogenicity studies using different rodent strains often produce conflicting results (Knight and others 2006c). For example, a chemical that is carcinogenic in Fischer 344 rats may be harmless to rats of the Sprague-Dawley strain or vice versa, which leads to...
debates over which strain (if either) is most relevant to humans (Fung and others 1983; Ettlin and Prentice 2002). Moreover, decades of inbreeding have resulted in unintended genetic changes over time in rodent strains commonly used in toxicology studies (Schwetz and Gaylor 1997). For example, the US NTP reports that Sprague-Dawley rats have increased in weight by up to 300 grams over several years, while Fischer 344 rats now weigh 25 per cent more than their predecessors. These increased body weights may result in “decreased lifespan and increased tumour incidences”, further complicating the interpretation of carcinogenicity study data (US NTP 1994). For example, a decade ago, the average two-year survival of untreated male Fischer 344 rats was 86 per cent; the survival rate has now fallen to less than 50 per cent (Haseman and others 1998).

The dubious relevance of extrapolating test results across species, strains and sexes has been recognised by the World Health Organisation (WHO) International Programme on Chemical Safety (IPCS) and the International Life Sciences Institute (ILSI), which have each developed their own “human relevance frameworks” for evaluating whether rodent carcinogenicity data have any bearing on real-world cancer risk (Meek and others 2003; Cohen and others 2004; Sonich-Mullin and others 2001). Various EU regulatory and advisory bodies have likewise issued cautionary statements regarding the interpretation of rodent carcinogenicity data for the purposes of human risk assessment.

---

**Figure 3**

Types of rodent tumours recognised as having little or no relevance to humans

<table>
<thead>
<tr>
<th>Organs Unique to Rodent Anatomy</th>
<th>Rodent Tumours With No Known Human Equivalent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1</strong> - Harderian Gland</td>
<td><strong>4</strong> - Splenic Mononuclear Cell Leukaemia in Rats</td>
</tr>
<tr>
<td><strong>2</strong> - Zymbal’s Gland</td>
<td><strong>5</strong> - Submucosal Mesenchymal Lesion of the Urinary Bladder in Mice</td>
</tr>
<tr>
<td><strong>3</strong> - Forestomach</td>
<td><strong>6</strong> - Buildup of α2u-globulin in the Kidneys of Male Rats</td>
</tr>
</tbody>
</table>

**Mechanisms of Cancer Causation Irrelevant to Humans**

<table>
<thead>
<tr>
<th>7 - Calcium Phosphate-Containing Urinary Calculi in Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>8</strong> - Peroxisome Proliferation in Rodent Livers</td>
</tr>
<tr>
<td><strong>9</strong> - Thyroid Follicular Cell Tumours in Rats</td>
</tr>
</tbody>
</table>

**Tumours of the Hormonal and Reproductive Systems to Which Rodents Are Much More Susceptible Than Humans**

<table>
<thead>
<tr>
<th><strong>10</strong> - Pituitary Gland</th>
<th><strong>11</strong> - Adrenal Gland</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>12</strong> - Luteinising Hormone-Induced Breast Tumours in Sprague-Dawley Rats</td>
<td></td>
</tr>
<tr>
<td><strong>13</strong> - Gastric Endocrine Cells</td>
<td></td>
</tr>
<tr>
<td><strong>14</strong> - Pancreatic Tumours Related to the Use of Corn Oil in Oral Force-Feeding Studies</td>
<td></td>
</tr>
<tr>
<td><strong>15</strong> - Reproductive Organ Tumours</td>
<td></td>
</tr>
</tbody>
</table>
CHEMICALS AND CANCER

REAL-WORLD HUMAN EXPOSURES

and pesticides sectors (Schwetz and Gaylor 1997; Gold and others 2002).

1985). Similar findings have been reported by regulatory scientists in the pharmaceuticals

thirds of the positive bioassays were positive

studies conducted by the US NTP arrived at much the same conclusion, reporting that “two-

as the chemicals being tested (Knight and others 2006c). A review of rodent carcinogenicity

very conditions of carcinogenicity studies may be as much responsible for causing cancer

Exposing cells to a nearly toxic dose of any chemical injures and kills some of them. The

life (Table 3; Seidle 2006a; ACSH 1997; Goodman 1994).

often many orders of magnitude above the exposure levels encountered by people in daily

MTD – defined as the highest-dose of a substance that will not shorten the animals’

may be given a nearly toxic dose of a test substance every day for their entire lives. For

animals in the highest dose group are given the so-called “maximum tolerated
dose” (MTD) – defined as the highest-dose of a substance that will not shorten the animals’

normal life span because of non-cancer-related toxic effects. Such experimental doses are

Exposing cells to a nearly toxic dose of any chemical injuries and kills some of them. The

natural response to cell injury and death is for the remaining cells to divide to replace those

cells that have been lost, and increased cell proliferation presents a risk for cancer. Thus, the

very conditions of carcinogenicity studies may be as much responsible for causing cancer

as the chemicals being tested (Knight and others 2006c). A review of rodent carcinogenicity

studies conducted by the US NTP arrived at much the same conclusion, reporting that “two-

thirds of the positive bioassays were positive only when the MTD was employed” (Haseman

1985). Similar findings have been reported by regulatory scientists in the pharmaceuticals

and pesticides sectors (Schwatz and Gaylour 1997; Gold and others 2002).

The MTD, by definition, “should be the highest dose that causes no more than a 10 per cent

weight decrement” (McConnell 1989). PETA examined study results of US NTP rodent

carcinogenicity studies for the 20 chemicals most recently tested and judged to produce
clear evidence of carcinogenic effects in both sexes of rats and mice to determine whether

weight loss ever exceeded the 10 per cent cut-off, which would mean that doses above the

MTD had been used. It is recognised that carcinogenic effects produced under such

conditions have little or no relevance for humans, who are typically exposed to much lower

doses (Haseman 1986). We determined that for the 20 most recently tested chemicals,
average decreases in body weight among animals in the high-dose group relative to

untreated controls did indeed exceed the US NTP’s 10 per cent cut-off, with chemical-
specific decreases in body weight as high as 45.8 per cent for female mice, 28.5 per cent

for male mice, 29 per cent for female rats, and 34.7 per cent for male rats (Seidle 2006a).

Rather than determining which chemicals in the environment pose real cancer risks to

humans, regulatory carcinogenicity studies simply show that virtually all chemicals cause

cancer in rodents at high enough doses (Gaylor 2005). This fact led the US NTP’s Board of

Scientific Counselors to conclude that “the implicit assumptions underlying extrapolation

from the MTD … do not appear to be valid. Therefore, both the criteria for selection of the

high dose used and the default criteria that are employed for extrapolation from high-dose
to low-dose must be reevaluated in a critical manner” (US NTP 1992). A similar conclusion

was reached by the UK Interdepartmental Group on Health Risks From Chemicals, which

stated that “extrapolation from high dose rodent data to humans is very uncertain. Thus

these models may give an impression of precision, which cannot be justified in the light of

the approximations and assumptions on which they are based. The UK does not therefore

support the use of such models for quantitative risk assessment of chemical carcinogens.

The reasons given in the [Committee on Carcinogens] guidelines in 1991 are still valid: the

methods are not validated; they are often based on incomplete or inappropriate data, and

derived more from mathematical assumptions than from knowledge of biological

mechanisms; and they demonstrate a disturbingly wide variation in the risk estimates,
depending on the model used)” (2002).

Table 3

Comparison animal/human doses for selected substances

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Daily Dose Fed to Rodents</th>
<th>Equivalent Human Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>1,000 mg/kg</td>
<td>70 times daily human dose for life</td>
</tr>
<tr>
<td>Agar</td>
<td>50,000 ppm</td>
<td>100 times daily human intake for life</td>
</tr>
<tr>
<td>Codeine</td>
<td>70 to 80 mg/kg</td>
<td>20 to 60 times the human dose, or 180 Tylenol 3 tablets per day for life</td>
</tr>
<tr>
<td>Locust bean gum</td>
<td>50,000 ppm</td>
<td>50 times the level found in most food products</td>
</tr>
<tr>
<td>Safrole</td>
<td>5,000 ppm in diet (0.3%)</td>
<td>613 12-oz. bottles of root beer daily</td>
</tr>
<tr>
<td>Cyclamates</td>
<td>2.18 grams/day (0.8%)</td>
<td>136 to 222 12-oz. bottles of soda daily for life</td>
</tr>
<tr>
<td>Aflatoxin</td>
<td>5,000 to 10,000 ppm in diet (0.5 to 1%)</td>
<td>12.7 tonnes of apples daily for 10 years</td>
</tr>
</tbody>
</table>
Table 4 Procedures to which all animals in a carcinogenicity study are routinely subject

<table>
<thead>
<tr>
<th>Procedure (OECD 1981)</th>
<th>Level of Distress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic (18- to 24-month) caging of 200 to 215 rats and mice:</td>
<td>Mild</td>
</tr>
<tr>
<td>- Numerous potential stressors are inextricably linked to the laboratory environment, including bright lights (which can damage rodents’ sensitive vision), loud and/or stressful noises (e.g., doors closing, cages being opened/moved, the constant “hum” of electronic equipment, ultrasound, etc.), strong odours (e.g., disinfectant cleaning products, etc.), which have been proved to cause physiological evidence of stress and distress in captive animals (CCAC 1993). These and other laboratory conditions have been found to elevate stress hormones, heart rate and blood pressure, depress immune function and induce sleep disorders and gastric ulcers in animals (Balcombe and others 1994).</td>
<td>Mild</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CHEMICALS AND CANCER

Administration procedure: While control animals are not given the substance under study, they may be administered a vehicle control (e.g., corn oil), or otherwise be subject to the same exposure regimen as animals in the test groups.

- **Dietary/drinking water:** PETA's analysis of more than 500 rodent carcinogenicity studies conducted by the US NTP revealed that exposure via food and/or drinking water was used in approximately 57 per cent of studies. Non-chemically exposed control animals would not be expected to experience any adverse effects in this scenario.

- **Oral gavage:** The second most common route of administration is via daily orogastric gavage (Figure 5), which was used in more than one-quarter (26 per cent) of studies. Non-chemically exposed control animals would not be expected to experience any adverse effects in this scenario.

- **Dermal application and intraperitoneal injection:** Less common exposure routes include topical application (4.2 per cent in US NTP studies) and intraperitoneal injection (2.2 per cent). Control animals subjected even to sham intraperitoneal injections could be expected to experience pain and distress, in addition to the physical stress of routine handling.

Many rodent strains are now recognised to be suffering the effects of decades of inbreeding, which may be extremely painful and distressing for animals who experience them. For example, the Fischer 344 strain of rats has inherent problems with debilitating seizures, which have been worsening over time, as well as the potentially lethal accumulation of lymph fluid in the throat (King-Herbert 2005). Likewise, the B6C3F1 strain of mice has experienced an inexplicable and scientifically worrisome weight gain over time and, along with it, an increased rate of spontaneous liver tumours (current upwards of 60 per cent) (King-Herbert 2005). Likewise, as previously discussed, highly inbred rodent strains commonly used in carcinogenicity studies have very high background tumour rates even when they are not dosed with chemicals (Haseman 2000). The US NTP has reported that approximately 96 per cent of untreated control rats from the Fischer 344 strain are now expected to develop some type of spontaneous tumour, and 64 per cent of the males and 43 per cent of the females had at least one cancerous tumour (Haseman and others 1998). The same study similarly reported that more than two-thirds of untreated B6C3F1 mice developed some type of...
is acceptable when the number of survivors of the lower doses or control group reaches 25 per cent". The fact that this situation is the rule, rather than the exception, flies in the face of the requirements of Directive 86/609.

### Methods of killing:

- **Intermediate dose:** 215 animals exposed to a daily dose of a test chemical which the OECD (1981) recommends "should be established in a mid-range between the high and low doses", or approximately one-half the MTD, according to the US NTP's protocol (BSC 1984).
- **Lowest dose:** 215 animals exposed to a daily dose of a test chemical which the OECD (1981) stipulates "should not interfere with normal growth, development, and longevity of the animal; and it must not otherwise cause any indication of toxicity. In general, this should not be lower than 10 per cent of the high dose". The US NTP protocol for rodent carcinogenicity studies recommends that the lowest dose be set at one-quarter the MTD (BSC 1984).

### Table 5 Additional procedures to which chemically exposed animals are routinely subject

<table>
<thead>
<tr>
<th>Procedure (OECD 1981)</th>
<th>Level of Distress</th>
</tr>
</thead>
</table>
| **High dose:** 215 animals administered a test substance at the MTD (or higher) every day for up to 2 years. While OECD guidelines (1981) specify that “[t]he highest dose level should be sufficiently high to elicit signs of minimal toxicity without substantially altering the normal life span due to effects other than tumours”, other OECD publications paint a more realistic picture of the adverse health effects that are likely to be experienced following daily dosing with chemicals at the MTD for most of the animals’ lives. Examples cited in the OECD Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation include lethargy, anaemia, diarrhoea, weight loss, fur loss, organ damage, unsteady gait, salivation, tremors, coma and even death (OECD 2000). Animals who survive until the end of a two-year study may be riddled with massive, debilitating tumours (Figure 4) and suffer other ill effects of cancer. However, as previously discussed, as many as 70 per cent of animals may not survive to the end of a two-year cancer study (Haseman and others 2003). Indeed, this problem is so common that internationally harmonised test guidelines (OECD 1981) specify that “termination of the study is acceptable when the number of survivors of the lower doses or control group reaches 25 per cent”. The fact that this situation is the rule, rather than the exception, flies in the face of the requirements of Directive 86/609.  
- **Intermediate dose:** 215 animals exposed to a daily dose of a test chemical which the OECD (1981) recommends “should be established in a mid-range between the high and low doses”, or approximately one-half the MTD, according to the US NTP’s protocol (BSC 1984).
- **Lowest dose:** 215 animals exposed to a daily dose of a test chemical which the OECD (1981) stipulates “should not interfere with normal growth, development, and longevity of the animal; and it must not otherwise cause any indication of toxicity. In general, this should not be lower than 10 per cent of the high dose”. The US NTP protocol for rodent carcinogenicity studies recommends that the lowest dose be set at one-quarter the MTD (BSC 1984). In our assessment, rats and mice assigned to the lowest dose and control groups in a chronic carcinogenicity study can reasonably be expected to experience modest to severe suffering and distress, depending upon the combination of husbandry conditions (including type of caging and provision of social, psychological and behavioural enrichment), the route of chemical administration, the inherent toxicity of the chemical being tested, the animals’ general health status (including the rate and severity of spontaneous tumorogenesis for a given species/strain) and the choice of euthanasia method. While we have considered that animals in the intermediate dose group most likely suffer to a moderate degree based solely on chemical administration at half the MTD, when the cumulative effects of chemical dosing are weighed alongside the other stresses and harms to which these animals are routinely subjected, we believe that overall classification of severe is most appropriate for animals in this group. Finally, there can be no question that animals in the high-dose group endure severe suffering and distress throughout the duration of a carcinogenicity study. | Severe | Severe | Moderate |
The weight of the evidence presented above calls into question the wisdom of continued reliance upon rodent carcinogenicity studies and, in particular, the continued requirement of such studies under REACH or similar regulations for pharmaceuticals and pesticides. This is especially true if EU policymakers hold out any hope of determining the cancer risk to humans of the more than 80,000 environmental chemicals that have not been specifically tested for carcinogenicity (Ward and others 2003) — a process which would require more than 7,000 years, 68 million animals and €127 billion at the current rate of progress using current methods (EC 2005; OECD 1981; NIEHS 1996). As Nobel laureate Dr Joshua Lederberg stated in 1981, “It is simply not possible with all the animals in the world to go through chemicals in the blind way we have at the present time, and reach credible conclusions about the hazards to human health”. Clearly, the time has come for a fundamental paradigm shift in the field of cancer hazard and risk assessment and for the rodent carcinogenicity study to be retired to the pages of history.

“We now have an opportunity to start with a clean slate and develop evidence-based tests that have true predictive value.”
— Dr Thomas Hartung, European Centre for the Validation of Alternative Methods (Abbott 2005)

There have been many proposals for refining carcinogenicity studies as a short-term animal reduction measure while better, more human-relevant test methods are developed. For example, Schach von Wittenau and Estes (1983) questioned the necessity of a study in a second species because of the redundancy of its results. The authors argued that since the classification of compounds depends upon the worst results in any species, it was not apparent why substances must be tested in both rats and mice when confirmatory or contradictory results have little impact. This logic has since been evaluated with respect to pharmaceuticals and marketing authorisations in Germany and The Netherlands. For example, van Oosterhout and colleagues (1997) sought to discover whether tumour findings in mice “ever caused the regulatory authorities to refuse registration, to restrict the proposed therapeutic indication of a pharmaceutical, or to apply a cautionary label. It was found that no tumor findings in mice alone ever led to such a regulatory action”. Similar findings have recently been reported by a panel of pesticide regulators and industry convened by the International Life Sciences Institute, which concluded that the “additional information provided by the mouse carcinogenicity study is of very limited additional value in risk assessment” (Doe and others 2006).

Other authorities have called for the acceptance of a “reduced protocol”, using one or the other gender of rats and mice (Lai and others 1994). For example, Huff and Haseman (1991) reported that a reduced protocol “using male rat and female mouse would have identified correctly 95 per cent of the positive or no evidence chemical carcinogenicity results obtained using the more extensive protocol”. Another approach accepted by pharmaceutical regulators at the ICH is to conduct a full two-year cancer study only in rats and to obtain second-species information from shorter-term studies using genetically engineered mice (Schwertz and Gaylor 1997).

Yet despite the potential for reduced animal use, the foregoing scenarios still fail to satisfactorily address the fundamental scientific and ethical limitations associated with animal-based carcinogenicity studies, as well as the inescapable fact that “[l]ong-term animal carcinogenicity tests are unable to keep up with the number of chemicals requiring testing” (Stiles 1986). Thus, the most promising solution must involve a move to purely non-animal test methods. Methodologies available at present include a multitude of cell-based in vitro test systems, computational tools such as (quantitative) structure activity relationship, or (QSAR), and expert system models and human population-based epidemiological studies.

**IN VITRO TESTS**

Since the induction of cancer has conventionally been divided into two categories according to their presumed mode(s) of action: genotoxic carcinogens (also known as “initiators”) and non-genotoxic carcinogens (also known as “promoters”), myriad in vitro tests for mutagenicity and other forms of genetic toxicity have been accepted internationally and in widespread regulatory use for decades (OECD 2003; Maurici and others 2005a; Table 6). Their very short timeframes (hours to days), large financial savings and tiny quantities of test chemical required all offer strong advantages over long-term carcinogenicity studies (Derenlanko and Hollinger 2002; Table 7). Despite these clear advantages, however, the acceptance of most in vitro genotoxicity tests also predates modern validation standards; thus, many of these tests suffer from the same scientific limitations as rodent carcinogenicity tests – including a high rate of false positive results.

**Table 6** In vitro tests currently accepted in the EU and internationally for mutagenicity/genetic toxicity testing

<table>
<thead>
<tr>
<th>EU Annex V</th>
<th>OECD TG</th>
<th>Endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.13-14 471</td>
<td>Gene mutation in bacteria</td>
<td></td>
</tr>
<tr>
<td>B.10 473</td>
<td>Chromosome aberration</td>
<td></td>
</tr>
<tr>
<td>B.17 476</td>
<td>Gene mutations</td>
<td></td>
</tr>
<tr>
<td>B.19 479</td>
<td>Mammalian DNA damage (sister chromatid exchange)</td>
<td></td>
</tr>
<tr>
<td>B.15 480</td>
<td>Gene mutation in yeast</td>
<td></td>
</tr>
<tr>
<td>B.16 481</td>
<td>Mitotic recombination in yeast</td>
<td></td>
</tr>
<tr>
<td>B.18 482</td>
<td>Mammalian DNA damage (unscheduled DNA synthesis)</td>
<td></td>
</tr>
<tr>
<td>B.21 —</td>
<td>In vitro mammalian cell transformation</td>
<td></td>
</tr>
</tbody>
</table>
Table 7 Comparative costs of animal-based vs. in vitro tests for genetic toxicity

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Typical Cost and Material Requirements</th>
<th>Animal Test</th>
<th>In Vitro Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosomal aberration</td>
<td>€23,900</td>
<td>€15,900</td>
<td></td>
</tr>
<tr>
<td>50–100 g</td>
<td></td>
<td>5 g</td>
<td></td>
</tr>
<tr>
<td>Solar chromatid exchange</td>
<td>€17,500</td>
<td>€8,800</td>
<td></td>
</tr>
<tr>
<td>25–50 g</td>
<td></td>
<td>5 g</td>
<td></td>
</tr>
<tr>
<td>Unscheduled DNA synthesis</td>
<td>€25,500</td>
<td>€8,800</td>
<td></td>
</tr>
<tr>
<td>25–50 g</td>
<td></td>
<td>5 g</td>
<td></td>
</tr>
</tbody>
</table>

Since there is currently no validated test – either in vitro or in vivo – that can provide information on all potential mechanisms of genotoxic action, it is necessary to utilise a battery of tests. A Commission Ad Hoc Advisory Group on Genotoxicity and Mutagenicity, with experts drawn from various EC services and stakeholder organisations, proposed a stepwise testing strategy for mutagenic/genotoxic potential (Maurit and others 2005a). Step 1 involves characterising a substance based on existing data and knowledge. Then, depending upon the level of existing information, a substance may proceed to Step 2, which consists of a battery of three in vitro tests: Salmonella reverse mutation (OECD 471), in vitro gene mutation in mammalian cells (OECD 478) and either in vitro chromosome aberration (OECD 473) or in vitro micronucleus (draft OECD 487). If any of these tests yield positive results, a substance would proceed to Step 3, where it would undergo further testing and assessment in order to minimise the number of false positive results from the previous tiers.

Kirkland and colleagues (2005, 2006) recently evaluated the ability of a battery of three in vitro genotoxicity tests (those included in Step 2, above) to correctly identify 553 chemicals classified as rodent carcinogens. In 93 per cent of cases, positive results were obtained in at least one of the in vitro tests, indicating a high sensitivity of the test battery. Only 9 per cent of the rodent carcinogens tested in all three in vitro tests gave consistently negative results, and most of these were either non-genotoxic carcinogens (e.g., liver enzyme inducers, peroxisome proliferators or hormonal carcinogens) or were otherwise considered to be of little or no relevance to humans. The authors further established that positive results in all three tests indicated more than three times more likely to be a rodent carcinogen than a non-carcinogen and, conversely, that negative results in all three tests indicated more than twice the likelihood of non-carcinogenicity than carcinogenicity.

Efforts are also underway to validate a new human cell-based in vitro test known as the GreenScreen HC (Gentronix.co.uk), which appears to possess the same degree of sensitivity as other in vitro genotoxicity tests without the problem of false positive results (Van Gompel and others 2005). In contrast to other genotoxicity tests, which can detect only one type of genetic alteration at a time, the GreenScreen HC has thus far been successful in detecting all classes of direct-acting genotoxic chemicals, as well as compounds that alter chromosome number or DNA replication and repair (Cahill and others 2004). Moreover, the GreenScreen HC system has been specifically developed for automated, high-throughput screening, making it more efficient and cost-effective than even other in vitro test methods. In vitro systems can also provide information regarding non-genotoxic modes of carcinogenic action. For example, cellular events associated with malignant transformations in the whole organism (e.g., changes in cell colony morphology and focus formation) can be detected through the use of cell transformation assays (Combos and others 1999).

Commonly used systems include the Balb/c 3T3 and C3H10T1/2 immortalised cell line and Syrian hamster embryo (SHE) primary cells (Maurit and others 2005b). In vitro cell transformation assays have been accepted in the EU for regulatory purposes since 1988 (Table 6) and, in contrast to other short-term in vitro assays, are capable of detecting both genotoxic and non-genotoxic carcinogens (Mauthe and others 2001). Pinta and colleagues (1977) documented a 91 per cent correlation between morphological transformation of SHE cells and the reported carcinogenic activity of a large number of chemicals. Similar findings have recently been reported in a draft OECD Detailed Review Paper on Cell Transformation Assays for Detection of Chemical Carcinogens (2006), which found the assays were capable of accurately identifying 80 per cent of carcinogenic substances, as well as 95 per cent of chemicals classified as probable human carcinogens. These findings confirm the important role for in vitro cell transformation assays as an alternative to rodent lifetime carcinogenicity studies under REACH and other EU regulatory programmes.
tests and predictions from validated (Q)SAR and expert system models.

4. Substantial, targeted funding should be made available to:
   - The European Centre for the Validation of Alternative Methods (ECVAM) to expedite the validation and enhancement of new and existing in vitro tests to detect both genotoxic and non-genotoxic carcinogens (e.g., the GreenScreen HC, cell transformation assays, etc.).
   - The European Chemicals Bureau (ECB) to expedite the validation of appropriate (Q)SAR and expert system models for carcinogen identification and classification.

5. Additional EU funding should also be made available for human epidemiological (population) studies for substances of high concern in order to confirm either the presence or the absence of a carcinogenic hazard to humans. Such information is vital not only for classification and labelling purposes, but also for ongoing efforts to validate new and revised in vitro, computational and other alternative methods.

SUMMARY AND RECOMMENDATIONS

"It should be apparent beyond doubt that presently no science is available for the translation of chronic animal test data into objective forecasts of human cancer risk. If that were possible, regulation would be a much easier task than the contentious babel it has come to be."

– Dr Gio Batta Gori, The Health Policy Center (2001)

"In the face of these shortcomings, many experts believe the scientific value of the 2-year bioassay is highly limited – barely worth the investments in personnel, animals, money, and time."


Rodent lifetime carcinogenicity studies take up to five years to produce results of dubious reliability and relevance to humans at a cost – in terms of finances, skilled personnel hours, and animal lives and suffering – that vastly exceeds their purported benefits. Quite simply, these animal tests have failed regulators and the public for too long and should be abandoned. The challenge presented by EU public health and environmental initiatives such as REACH and the revision of the pesticides and biocides directives is to develop testing strategies that are relevant to the needs of regulators and of public safety – strategies which employ 21st century (as opposed to 19th century) science and which can deal with the backlog of thousands of untested chemicals in a practical, humane and cost-effective manner. The use of existing data together with the non-animal test methods and computer modelling approaches outlined in this report can achieve these aims – if the political will is sufficient to make this vision a reality.

RECOMMENDATIONS

1. In the interests of public health and worker protection in the EU, the Commission, Parliament and member states must commit to a fundamental paradigm shift in the area of carcinogenicity testing and risk assessment.

2. Animal-based carcinogenicity studies should no longer be required or recommended for regulatory purposes, and reference to such testing should be removed from relevant EU legislation and Community strategies, including:
   - The proposed REACH chemicals regulation
   - The pesticides and biocides directives (91/414/EEC and 98/8/EC, respectively)
   - Pharmaceuticals regulations (Directive 93/41/EEC, Regulation 2309/936, European Medicines Agency guidance, etc.)
   - The 7th amendment of the cosmetics directive (76/768/EEC)

3. The aforementioned regulations should also be amended, as needed, in order to permit assessments of carcinogenic and other toxic hazards to be based on a weight-of-evidence evaluation of existing data, validated and/or accepted in vitro
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