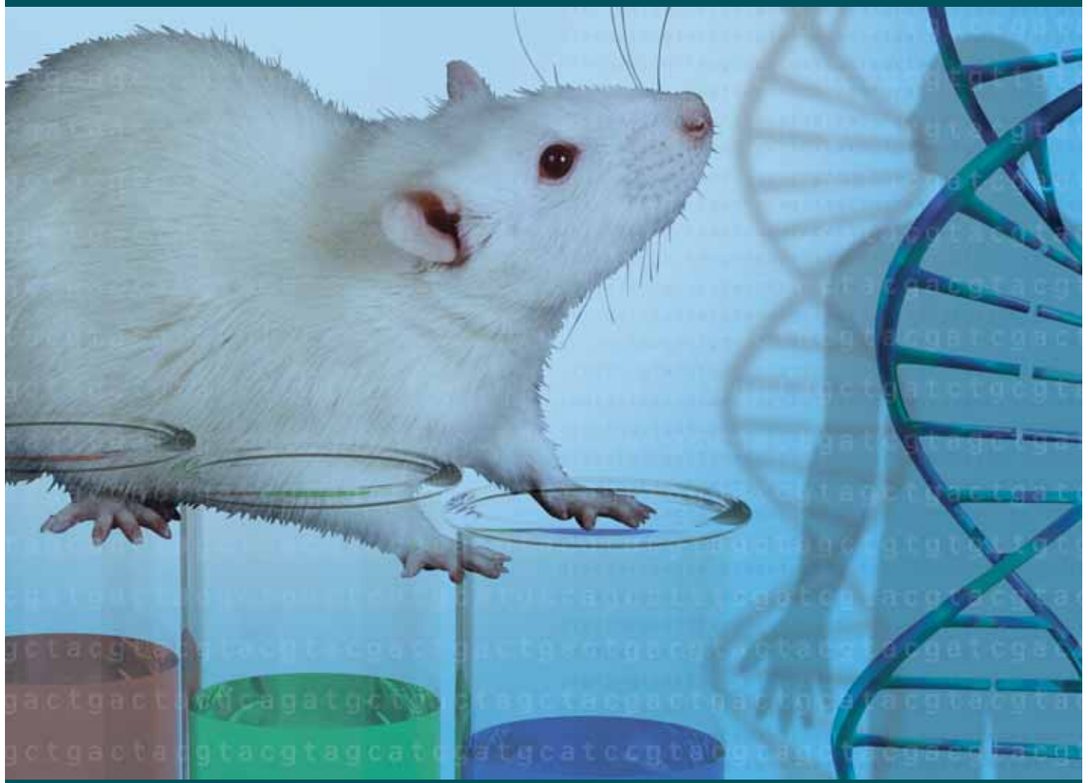
PETA

CHEMICALS AND CANCER

What the Regulators Won't Tell You About Carcinogenity Testing



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"[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."

– DR GIO BATTA GORI, THE HEALTH POLICY CENTER (2001)

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Published by: PETA Europe Limited
Date: September 2006
Author: Troy Seidle
Reviewers: Sadhana Dhruvakumar MPhil, Gill Langley PhD, Joseph Manuppello MS, Emily McIvor, Karen Porreca MA, Jessica Sandler MHS, Chad Sandusky PhD, Kristie Stoick MPH

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 (ICAPO and ICAPI, 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.

Clearly, requirements for testing and data submission are *not* what is lacking. Indeed, the EU's own studies reveal that only about 20 per cent of workplace cancer deaths are attributable to unknown chemical agents – meaning that the overwhelming majority of occupational cancer deaths result from substances that have already been identified as known or suspected human carcinogens (Knight and others 2006a). Therefore, what is truly lacking is not data quantity, but the *quality* and *utility* of test data as a basis for effective regulatory measures for cancer prevention.

> "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."
> – Dr Joshua Lederberg, Nobel laureate in medicine (1981)

This report provides an in-depth look at the conventional toxicological testing paradigm for the identification of human carcinogens; its history, scientific basis and limitations; and its implications from both public health and animal welfare perspectives and concludes with a series of recommended amendments to data and testing requirements in Community legislation, as well as priorities for future research and test method validation.

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."
> – Drs Joseph Contrera, Abigail Jacobs and Joseph DeGeorge, US Food and Drug Administration (1997)

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

scientific literature (Gold and others 2005), including tests of pharmaceuticals, pesticides, plastics, industrial chemicals and even natural plant extracts.

Official statistics published by the European Commission (EC 2003a) reveal that in 1999, at least 23,277 animals were used in carcinogenicity studies in EU member states. Within three years, this figure more than doubled – a 109.3 per cent increase (EC 2005) – and in all likelihood continues to rise. Yet despite decades of regulatory use, the scientific validity of rodent carcinogenicity studies has yet to be adequately evaluated according to modern standards. *Validation* refers to "the process by which the reliability and relevance of a procedure are established for a particular purpose" (OECD 2005):

- *Reliability* refers to "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".
- *Relevance* is a measure of a test method's accuracy in measuring the biological effect of interest in the species of ultimate concern.

Specific criteria for test method validation were first articulated in 1996 at an OECD Workshop on Harmonisation of Validation and Acceptance Criteria for Alternative Toxicological Test Methods in Solna, Sweden, and have since become *the* internationally accepted standard by which the scientific validity of a toxicity study is judged. The so-called "Solna criteria" form the basis of validation reviews by the European Centre for the Validation of Alternative Methods, or ECVAM (Hartung and others 2004), and the US Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM 2003) and have since been reaffirmed and expanded upon in the form of an OECD *Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment* (OECD 2005). This document was subsequently endorsed unanimously by the OECD's 30 member countries – including the EC and numerous EU member states.

In order for *in vitro* or other non-animal test methods to achieve regulatory acceptance, they must first undergo rigorous validation to verify their reliability and relevance according to the aforementioned criteria. In contrast, virtually none of the animal-based toxicity studies routinely required for the assessment of potential health and environmental hazards of chemical substances has been properly validated according to modern criteria. This glaring double standard is problematic for the following reasons:

- It puts public health and safety in jeopardy by basing critical decisions on the results of unproven – and potentially invalid – test methods.
- It undermines the strong political and social desire to see EU regulators and industry reduce their reliance on animal testing by giving preferential treatment to the very test methods that have been targeted for replacement.
- It reinforces the erroneous notion that the validity of prospective non-animal replacement methods can or should be judged against animal-based methods which themselves have never been properly validated.

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 problem 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

HUMAN CARCINOGENS UNDETECTED IN RODENT CANCER TESTS

Cigarette smoke – Despite ample human evidence of the link between smoking and cancer, the tobacco industry was successful in using the results of experiments – in which rodents and other animals were forced to inhale smoke but did not develop cancer – to delay health warnings about smoking for more than 20 years (Laskin and Sellakumar 1974).

Asbestos – Hundreds of animal tests of asbestos have been conducted, including more than 20 rodent cancer studies, yet the significance of the test results to humans has been debated and disputed for decades. A 1995 study by Rodelsperger and Woitowitz re-analysed rat and human data and concluded that humans are 300 times more susceptible than rats to lung cancer (mesothelioma) from inhaled asbestos fibers. This led the scientists to conclude that "inhalation studies in rats are not sufficiently sensitive for the detection of hazards and risks to humans exposed to man-made fibers".

Benzene – The causal link between benzene and human leukaemia was established as early as 1928, yet 14 subsequent animal studies failed to replicate benzene's cancer-causing effects (De Marini and others 1989). Only during the late 1980s were researchers ultimately able to induce cancer in animals by overdosing them with benzene, yet even this has not stopped researchers from continuing to use public funds to subject thousands of animals to lethal tests with this chemical, its derivatives and its byproducts.

and Sellakumar 1974; Rodelsperger and Woitowitz 1995; DeLore and Borgomono 1928; De Marini and others 1989). In addition, many of the known causes of human cancer are viruses, radiation and chemical mixtures that would not normally be tested using conventional rodent carcinogenicity studies, which have historically been used only to assess one chemical substance at a time (Ennever and Lave 2003).

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 to humans, 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 can also fail to identify cancer risks to humans. Table 1 presents more than a dozen examples of dubious and/or false negative results in US NTP carcinogenicity studies for substances classified as definite or probable human carcinogens by international cancer authorities.
A separate analysis by Dr David Salsburg (1983) of Pfizer pharmaceuticals calculated that the false negative rate for carcinogenicity studies could be as high as 63.2 per cent, based on his observation that of 19 chemicals classified as *known* human carcinogens by the International Agency for Research on Cancer (IARC), 12 showed no carcinogenic effects in rodent studies. The peer reviewed scientific literature is filled with similarly high estimates of false negative rates (e.g., Lave and others 1988; Johnson 2001). Such dangerous underestimates of cancer risk could expose millions of people to very real health risks and incur societal costs of hundreds of millions of dollars (Lave and others 1988).

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*

Chemical[1,2]	Major Use	Rodent Carcinogenicity Test Results
Asbestos (chrysotile and dimethyl hydrazine)	Cement	♂rats = inadequate study ♀rats = inadequate study ♂mice = no study ♀mice = no study
Asbestos (chrysotile)	Cement	♂rats = no evidence ♀rats = no evidence ♂mice = no study ♀mice = no study
Combination of aspirin, caffeine and phenacetin	Analgesic	♂rats = no evidence ♀rats = equivocal evidence ♂mice = no evidence ♀mice = no evidence
Dichlorvos as dosed feed	Insecticide	♂rats = no evidence ♀rats = no evidence ♂mice = no evidence ♀mice = no evidence
Lindane	Insecticide	♂rats = no evidence ♀rats = no evidence ♂mice = no evidence ♀mice = no evidence
Dichlorodiphenyl trichloroethane (DDT)	Insecticide	♂rats = no evidence ♀rats = no evidence ♂mice = no evidence ♀mice = no evidence
1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin as topical application	Combustion byproduct and industrial contaminant	♂rats = no study ♀rats = no study ♂mice = no evidence ♀mice = no evidence
Selenium sulfide	Antidandruff shampoo	♂rats = no study ♀rats = no study ♂mice = no evidence ♀mice = no evidence
Bromodichloromethane	Water disinfection byproduct	♂rats = no evidence ♀rats = no study ♂mice = no study ♀mice = no evidence
Gallium arsenide	Semiconductors	♂rats = no evidence ♀rats = clear evidence ♂mice = no evidence ♀mice = no evidence
Aroclor 1254	Pesticide	♂rats = equivocal evidence ♀rats = equivocal evidence ♂mice = no study ♀mice = no study
p-Nitrotoluene	Ingredient in dyes, pesticides and rubber chemicals	♂rats = equivocal evidence ♀rats = some evidence ♂mice = equivocal evidence ♀mice = no evidence
5-Azacytidine	Anti-cancer drug	♂rats = inadequate study ♀rats = inadequate study ♂mice = inadequate study ♀mice = clear evidence

Definitions:[2]
- **Inadequate study:** demonstrated by studies that because of major qualitative or quantitative limitations cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.
- **No evidence:** no chemical-related increases in malignant or benign neoplasms.
- **Equivocal evidence:** a marginal increase of neoplasms that may be chemically related.
- **Some evidence:** a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **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.

[1]IARC 2006b, US EPA 2006, US NTP 2006a.
[2]US NTP 2006b.

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 (Gold and Slone 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 (Ennever 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 (Seidle 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.

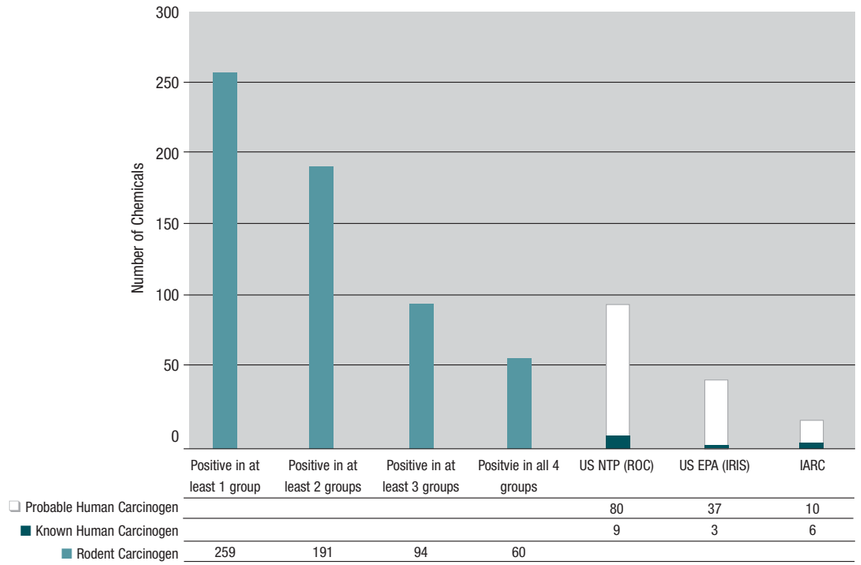
Table 2 *Animal lives and money wasted on rodent carcinogenicity studies by the US NTP that have produced equivocal or inadequate results*

Species		Equivocal or Inadequate Tests	Animal Lives (215/test)	Monetary Cost (€400,000/test)
Rat	Female	64	13,760	€25,000,000
	Male	72	15,480	€28,000,000
Mouse	Female	40	8,600	€15,600,000
	Male	67	14,405	€26,000,000
	Total	**243**	**51,245 animals**	**€94.6 million**

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

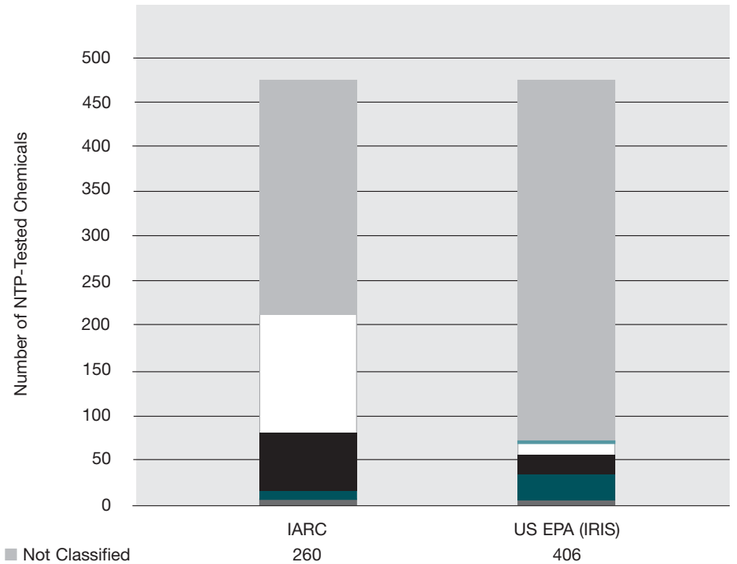
In order for a chemical to be classified as a *known* human carcinogen, there must generally be "sufficient evidence of carcinogenicity *in humans*" (*emphasis added*) (IARC 2006a). In other words, animal data alone are never enough to classify, let alone regulate, a chemical as a human carcinogen. The 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.



Positive Evidence of Cancer in Rodents *vs.* Agency Classifications

Figure 1 *Chemicals producing positive evidence of cancer in one or more rodent species/sex groups relative to the proportion of these same chemicals classified as known or probable human carcinogens by recognised cancer authorities*

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.



	IARC	US EPA (IRIS)
Not Classified	260	406
Probably Not	0	2
Unclassifiable	135	14
Possible	65	19
Probable	10	32
Known	6	3

Figure 2 *The vast majority of US NTP-tested chemicals have not even been classified in IARC monographs or the US EPA IRIS database as to their cancer risk to humans, and of those that have, most are deemed either unclassifiable or possible human carcinogens.*

Even when evidence of cancer is found in both rats and mice – which indicates that a chemical hazard is not unique or limited to a single species – cancer authorities may disregard these findings when making their determinations regarding human risk. For example, only 62 of the 114 US NTP-tested chemicals yielding this level of evidence (54 per cent) have been classified as *known* or *reasonably anticipated* human carcinogens in the US NTP's *Report on Carcinogens*. The remaining 46 per cent have simply not been classified by the US NTP. If studies producing evidence of cancer in two species are so freely dismissed, public health authorities will be even less confident in making meaningful classifications or regulatory decisions based on evidence from only one rodent species, which is all that is available for 383 (80.5 per cent) of the 476 US NTP-tested chemicals that PETA examined. Importantly, the majority of these 383 chemicals have not even been classified by IARC or the US EPA (59 per cent and 80.7 per cent, respectively). Only 54 have been classified in the US EPA's IRIS database, of which 32 (59.3 per cent) have been labelled either *possible*

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 reticent 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 analgesic 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 Zeisel (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 α2u-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, regeneration and, ultimately, tumourogenesis. There is no evidence that this mechanism operates in humans. The recognised irrelevance of this mechanism to humans led to the down-classification of saccharin by IARC and the delisting of the chemical from the US NTP's *Report on Carcinogens*.
- Peroxisome proliferation has been documented following exposure of rodents to a wide range of chemicals, including cholesterol-lowering drugs, herbicides and plasticisers. This leads to an increase in the density and activity of peroxisomes, which in turn results in the proliferation of hepatocytes, liver enlargement and tumour induction. Chemicals that are peroxisome proliferators in rats and mice have been demonstrated to have little or no effect on the human liver.
- 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.

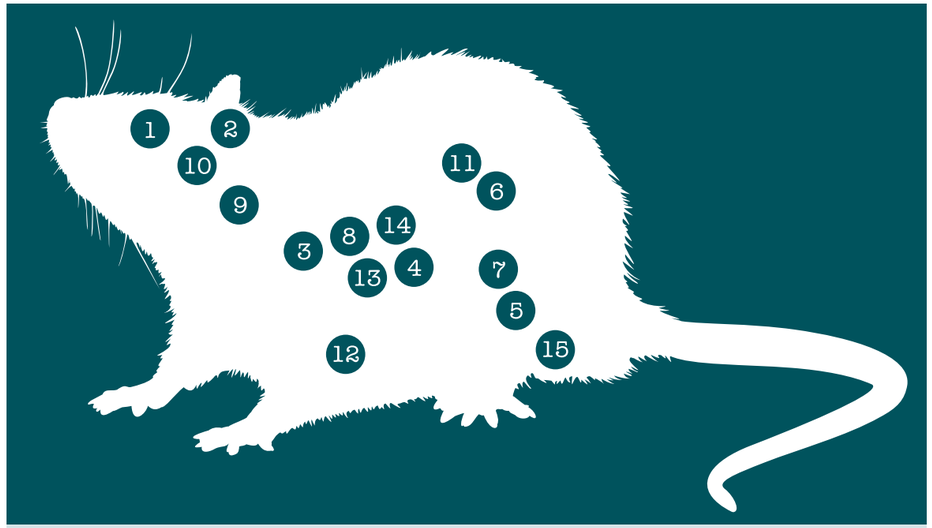
Certain types of tumours have been identified in rodents for which there are no known human equivalents, such as splenic mononuclear cell leukaemia in rats and the mouse submucosal mesenchymal lesion of the urinary bladder. In fact, there are entire organs in rodents that have no counterpart in human 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 1999b) 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 life-span 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 66 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.



Organs Unique to Rodent Anatomy
1 - Harderian Gland
2 - Zymbal's Gland
3 - Forestomach

Rodent Tumours With No Known Human Equivalent
4 - Splenic Mononuclear Cell Leukaemia in Rats
5 - Submucosal Mesenchymal Lesion of the Urinary Bladder in Mice

Mechanisms of Cancer Causation Irrelevant to Humans
6 - Buildup of α2u-globulin in the Kidneys of Male Rats
7 - Calcium Phosphate-Containing Urinary Calculi in Rats
8 - Peroxisome Proliferation in Rodent Livers
9 - Thyroid Follicular Cell Tumours in Rats

Tumours of the Hormonal and Reproductive Systems to Which Rodents Are Much More Susceptible Than Humans
10 - Pituitary Gland
11 - Adrenal Gland
12 - Luteinising Hormone-Induced Breast Tumours in Sprague-Dawley Rats
13 - Gastric Endocrine Cells
14 - Pancreatic Tumours Related to the Use of Corn Oil in Oral Force-Feeding Studies
15 - Reproductive Organ Tumours

Figure 3
Types of rodent tumours recognised as having little or no relevance to humans

FAILURE 4: IRRELEVANCE OF LABORATORY MEGA-DOSES TO REAL-WORLD HUMAN EXPOSURES

> "The increased incidence of tumors under the highest tolerated dose condition could be due to an indirect effect related to the high dose used, more than to the intrinsic carcinogenicity of the chemical under consideration."
> – Dr Harri Vainio and Julian Wilbour, International Agency for Research on Cancer (1992)

Rodent carcinogenicity studies are designed to maximise the likelihood of detecting a statistically significant increase in tumour incidence in chemically exposed, short-lived animals relative to untreated controls in order to apply observations taken from hundreds of animals in laboratories to millions of people throughout the world. In order to achieve this goal and cut through the "noise" created by rodents' high spontaneous-tumour rate, animals may be given a nearly toxic dose of a test substance every day for their entire lives. For example, 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 often many orders of magnitude above the exposure levels encountered by people in daily life (Table 3; Seidle 2006a; ACSH 1997; Goodman 1994).

Exposing cells to a nearly toxic dose of any chemical injures 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 (Schwetz and Gaylor 1997; Gold and others 2002).

> "There is more than a statistical difference in being hit by a speeding truck or by a falling feather, and a few mice exposed for life to the equivalent of 1,500 cans of diet soda daily could tell nothing of what may happen to one or to a million mice or humans at realistic doses."
> – Dr Gio Batta Gori, The Health Policy Center (2001)

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 1985). 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
Comparative animal/human doses for selected substances

Chemical	Daily Dose Fed to Rodents	Equivalent Human Intake
Penicillin	1,000 mg/kg	70 times daily human dose for life
Agar	50,000 ppm	100 times daily human intake for life
Codeine	70 to 80 mg/kg	20 to 80 times the human dose, or 180 Tylenol 3 tablets per day for life
Locust bean gum	50,000 ppm	50 times the level found in most food products
Safrole	5,000 ppm in diet (0.5%)	613 12-oz. bottles of root beer daily
Cyclamates	2.18 grams/day (5%)	138 to 522 12-oz. bottles of soda daily for life
Alar	5,000 to 10,000 ppm in diet (0.5 to 1%)	12.7 tonnes of apples daily for 10 years

FAILURE 5: ANIMAL SUFFERING

> "Although some claim that animals used in laboratory experiments are healthier now than in the past, the life of the animal who lives in a barren, sterile environment in which temperature, humidity and lighting are controlled and wall surfaces are disinfected, whose food and bedding are sterilized, and who is subjected to painful and debilitating experiments, is an unhealthy and abnormal one – and one which does not parallel the average human (or animal) condition."
> – Alix Fano, in Lethal Laws: Animal Testing, Human Health and Environmental Policy (1997)

Article 7.4 of Directive 86/609/EEC for the protection of animals used in experiments stipulates, "All experiments shall be designed to avoid distress and unnecessary pain and suffering to experimental animals". Yet while pain, suffering and distress may occur only as undesired consequences of an experiment for the purpose of basic research, toxicity studies are designed to produce clinical signs of toxicity and, by extension, some degree of animal pain and distress. In addition, animals used in regulatory toxicity studies are seldom, if ever, afforded any form of pain relief. This reality is clearly captured in international statistics, which reveal that regulatory toxicity studies can account for up to 82 per cent of the most invasive animal experiments – which, by definition, "cause pain near, at, or above the pain tolerance threshold of unanesthetized, conscious animals" (CCAC 1991).

Carcinogenicity studies are among the most objectionable toxicity tests from an animal welfare perspective, subjecting hundreds of rats and mice to a lifetime of misery and suffering for every chemical tested (Seidle 2006b). Tables 4 and 5 below summarise many of the routine procedures to which animals in control and treatment groups are routinely subjected according to internationally accepted OECD test guidelines for carcinogenicity testing (OECD 1981), together with a conservative estimation of the severity of each class of procedures as defined by the UK Home Office (2000).

Figure 4
Mouse overcome with massive debilitating tumours

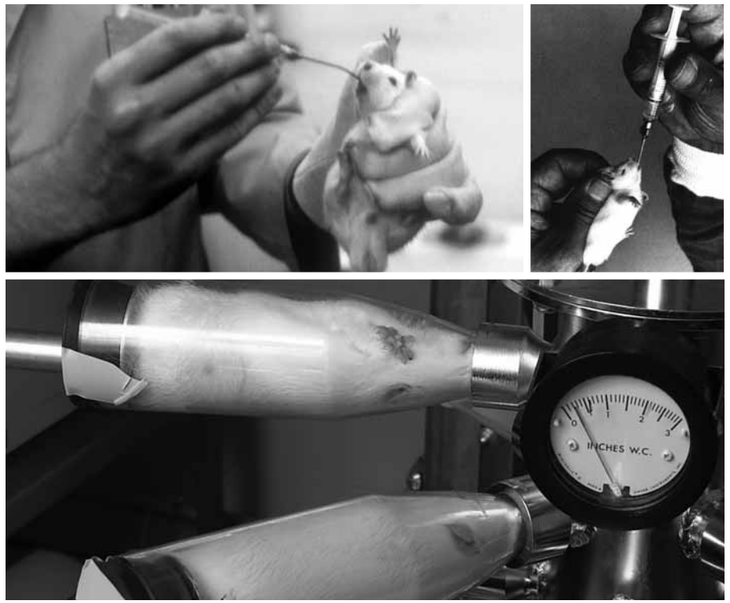


Figure 5
Oral gavage dosing of a mouse with a steel tube measuring the distance to the animal's stomach

Figure 6
Rats squeezed into inhalation tubes

Table 4 *Procedures to which all animals in a carcinogenicity study are routinely subject*

Procedure (OECD 1981)	Level of Distress
Chronic (18- to 24-month) caging of 200 to 215 rats and mice: • 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).	Mild
• In the best-case scenario, animals would be group-housed in solid-bottom polycarbonate cages, with suitable bedding and enrichment devices provided.	Mild

Procedure (OECD 1981)	Level of Distress
• It is still common practice in toxicity studies to house rodents in hanging wire-bottom cages, which can lead to foot ulcers, deformities, and other physical injuries, particularly among animals housed this way chronically, as is the case in carcinogenicity studies (Haseman and others 2003; Morton 2005; Weihe 1987).	Moderate
• It is also common to house animals individually (Haseman and others 1994), which deprives them of the comfort and stimulation that comes from social contact with others of their kind (APC 2003). Chronic boredom and frustration of animals' complex social and psychological needs has been clearly linked to a variety of anxiety-related behaviours, ranging from stereotypic biting of cage bars to self-mutilation (Patterson-Kane and others 1989). Such conditions fly in the face of the minimal animal care provisions of Directive 86/609.	Severe
Administration procedure: While control animals are not given the substance under study, they may be administered a vehicle control substance (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.	Mild
• **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 US NTP carcinogenicity studies (Seidle 2006a). Gavage administration "involves the physical stresses of handling and restraint, insertion of a rigid metal or flexible plastic tube … from mouth to stomach (with associated breathing interference), and stomach distention" where a chemical or vehicle is injected (Balcombe and others 2004). Complications associated with repeated gavaging can include puncturing of the throat and lungs, aspiration pneumonia and even death. Such severe damage is particularly common in chronic carcinogenicity studies. For example, one study reported that 32 per cent of female rats and 6.5 per cent of male rats had died within the first year of a cancer study as a result of suffocation related to repeated gavaging (Germann and others 1994). Even non-chemically exposed control animals can be expected to experience severe distress when routinely subjected to this violent procedure.	Severe

Procedure (OECD 1981)	Level of Distress
• **Inhalation:** Another common exposure scenario is forced inhalation, which was used in roughly 10 per cent of US NTP carcinogenicity studies. The OECD (1981) recommends either "daily exposure of 6 hours … for 5 days a week (intermittent exposure), or … 22-24 hours of exposure per day, 7 days a week (continuous exposure), with about an hour for feeding the animals and maintaining the chambers". Animals are either placed in cages that are sealed inside an inhalation chamber, or they are squeezed into restraint tubes (Figure 6), which are then connected to a tree-like inhalation device, which delivers vapours directly to the animals' respiratory systems. The latter scenario creates especially serious animal welfare concerns, resulting not only from the degree and duration of restraint, but also from the difficulty this creates in identifying clinical signs of pain and distress, such as convulsions and tremors (F. Cavender, personal communication, 24 Feb 2006). Even non-chemically exposed control animals can be expected to suffer considerable distress under such conditions.	Severe
• **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.	Severe
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 (currently 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	Severe

Procedure (OECD 1981)	Level of Distress
tumour and that 39 per cent of these mice had at least one cancerous tumour. Thus, even non-chemically exposed control animals are likely to suffer many of the same pathophysiological consequences associated with cancer.	
Methods of killing: At the end of a carcinogenicity study, animals who have survived the full 18 to 24 months are killed. Common methods of "euthanasia" in toxicity studies include CO_2 asphyxiation, cervical dislocation (neck-breaking), and decapitation (OECD 2000).	Severe

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

Procedure (OECD 1981)	Level of Distress
In addition to experiencing all of the same "costs" as described above, animals assigned to a treatment group are also administered daily doses of a test chemical for the majority of their lifespan. Thus, most if not all of these animals can be expected to spend most of their entire existence in some degree of sickness and distress.	Severe
• **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	Severe

Procedure (OECD 1981)	Level of Distress
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).	Moderate
• **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).	Moderate

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.

NON-ANIMAL APPROACHES TO CARCINOGEN IDENTIFICATION

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 with 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 (Schwetz 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" (Styles 1980). 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 (Q)SAR, and expert system models and human population-based epidemiological studies.

IN VITRO TESTS

Since the induction of cancer involves genetic alterations that can be induced either directly or indirectly, carcinogens have 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 (Derelanko 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*

EU Annex V	OECD TG	Endpoint
B.13-14	471	Gene mutation in bacteria
B.10	473	Chromosome aberration
B.17	476	Gene mutations
B.19	479	Mammalian DNA damage (sister chromatid exchange)
B.15	480	Gene mutation in yeast
B.16	481	Mitotic recombination in yeast
B.18	482	Mammalian DNA damage (unscheduled DNA synthesis)
B.21	—	*In vitro* mammalian cell transformation

Table 7 *Comparative costs of animal-based* vs. in vitro *tests for genetic toxicity*

Endpoint	Typical Cost and Material Requirements	
	Animal Test	*In Vitro* Test
Chromosomal aberration	€23,900	€15,900
	50–100 g	5 g
Sister chromatid exchange	€17,500	€6,400
	25–50 g	5 g
Unscheduled DNA synthesis	€25,500	€8,800
	25–50 g	5 g

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 (Maurici 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 476) 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 that a chemical was 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 rodent 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). And 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 (Combes and others 1999). Commonly used systems include the Balb/c 3T3 and C3H10T1/2 immortalised cell line and Syrian hamster embryo (SHE) primary cells (Maurici 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). Pienta 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 90 per cent of chemicals classified by IARC as *known* human carcinogens, 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.

COMPUTERISED STRUCTURE-ACTIVITY RELATIONSHIP AND EXPERT SYSTEM MODELS

Structure-activity relationships are computerised models used to predict toxic effects either quantitatively (QSAR) or qualitatively (SAR) based upon the presence of molecular substructures and/or other chemical properties that are known to be associated with a particular biological effect (e.g., carcinogenesis). Expert systems are another type of computer model that mimic the thinking and reasoning of human experts using knowledge-based rules for chemical classes to predict a toxic effect of concern (Cronin and others 2003). For example, the Danish EPA (2001) has examined approximately 47,000 chemicals with the aid of (Q)SARs, reporting that many computer models "are now so reliable that they are able to predict whether a given substance has one or more of the properties selected with an accuracy of approximately 70–85%".

A large number of (Q)SAR models and expert systems exist for the prediction of carcinogenicity. For example, computational toxicologists with the US Food and Drug Administration (Matthews and Contrera 1998) have described the beta-test evaluation of a QSAR expert system that demonstrated 97 per cent sensitivity for carcinogens (i.e., very few false negative predictions) and 98 per cent specificity for non-carcinogens (i.e., even fewer false positive predictions). Another cancer expert system is OncoLogic (LogiChem Inc.), which is widely used in the US EPA's new chemicals programme to evaluate the cancer-causing potential of a wide range of substances, including organic chemicals, polymers, fibres and a variety of metal-based compounds (Woo and others 1995). In addition to their ever-increasing predictive accuracy, (Q)SAR and expert system analyses also have the very strong advantages of being relatively inexpensive and virtually instantaneous.

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."
> – Charles W. Schmidt, MPH (2002)

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* 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.

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PETA Europe, Ltd.
P.O. Box 36668
London, SE1 1WA
020 7357 9229
PETA.org.uk

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