Researched and written by **Dr Adrian Stallwood**

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SCIENCE CORRUPTED

Revealed: the nightmare world of GM mice

This report charts, for the first time, the true scale and nature of the GM mouse revolution. The appalling animal suffering and immense squandering of scientific resources are at last laid bare.

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See also our *Science Corrupted* short film, which includes footage of some of the experiments described in this report: <http://www.animalaid.org.uk/GMmice

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'… this peculiar science continues to lead to ineffective drugs, disastrous clinical trials, and the dashing of the elevated hopes of hundreds of thousands of patients and their carers…'

EXECUTIVE SUMMARY

The number of genetically modified (GM) mice bred for, and used and killed in, animal experiments has reached staggering proportions. The numbers continue to increase both in the UK and globally.

Every year, millions of these sensitive and vulnerable animals suffer a chain of misery. It begins with the invasive procedures needed to create new genetic lines, and carries on with the harmful effects of genetic alteration, colony breeding, experimentation and traumatic death. The scale of suffering involved is incomparably greater than any other area of laboratory activity using animals. Furthermore, using GM mice to mimic human disease is not delivering meaningful healthcare advances. This peculiar science continues to lead to ineffective drugs, disastrous clinical trials, and the dashing of the elevated hopes of hundreds of thousands of patients and their carers.

Science Corrupted draws together a mass of expert testimony, research articles and critical commentary, and describes how:

- Mice are intelligent and complex small mammals, who feel pain in a comparable way to people. Their rich emotional lives encompass excitement, pleasure in social contact, and empathy for their fellows, as well as fear and despair. The laboratory cages in which they live and die are alien and hostile environments, replete with multiple stressors.

- The use of mice as 'tools' in animal experiments has long-standing historical roots. The first steps towards organised 'mouse laboratories' were taken in the US at the start of the last century. Mice were popularised as experimental subjects because they were easy to breed and house on a large scale, not because of genetic similarities to humans.

- The last decade has witnessed a further dramatic increase in the use of GM mice. Several hugely expensive international consortia have created thousands of new lines, with researchers attempting to produce 'mouse models' of almost every human ailment, including Alzheimer's, cancer, heart disease, lung disease and obesity. In the UK in 2011, more than 1.85 million procedures were started on mice whose genetic status had been altered (GM mice and those with a harmful mutation that was often induced through the use of poisons). Universities now perform more animal experiments than all other sectors combined.

- The two principal categories of GM mice in use today are 'transgenic' and 'knockouts'. Transgenic animals have been altered to carry a foreign gene from another organism within their natural genome. Knockout animals have certain genes prevented from working. In addition, highly speculative random mutations have been induced in millions of mice through chemical poisoning.

- The two most widely used methods of creating new lines of GM mice are pronuclear microinjection and gene targeting in embryonic stem (ES) cells. These techniques involve several invasive and painful procedures, including major surgery, castration and ear or tail mutilation. In addition, because the procedures are inherently inefficient, they entail the deaths of hundreds of animals to produce only one 'founder'.

- Most GM and mutation procedures are crude and unpredictable, and cause a high attrition rate. Many mice die from severe 'side effects'. Mice have been created who bleed to death *in utero*. Many pups who survive to birth suffer conditions such as water on the brain, cleft palate, or other severe facial deformities, meaning they must be killed or they will starve. Others die from asphyxia due to undeveloped lungs or obstructed airways; or they have exposed internal organs; or they fatally dehydrate due to undeveloped skin. Some obese mice are so heavy that they fall over on their backs and cannot right themselves. Other mice have been inadvertently produced with missing limbs or missing the front of their heads.

EXECUTIVE SUMMARY

- The subsequent breeding of an established colony of GM mice, to satisfy the need for experimental subjects, is not a simple or painless process. It involves the manipulation of the reproductive cycles, behaviour, living conditions and health status of millions of animals. Mass killing regimes are necessary to ensure colonies remain 'productive' – across the UK every year, millions of 'excess' or 'spent' mice have their necks crudely broken or are gassed with carbon dioxide.

- Mice are often bred to suffer 'harmful phenotypes' – painful or distressing alterations to their physical or mental condition. Mice have been engineered to develop lethal heart failure, and suffer severe swelling and breathlessness before they die. Many mice endure cancers. GM epilepsy mice die, by ten weeks of age, of constant seizures, malnutrition or dehydration. Mice used to model neurological diseases are tremulous and lose control of their bodily movements. Mice have been created so mentally disturbed that they chew through their own skin and wound themselves in the face, or are so anxious that they constantly try to hide.

- In many cases, the GM mice are subjected to further surgery, poisoning, unnatural diets, trauma or psychological distress. Examples include mice given strokes via wires inserted into blood vessels in their brains, mice forced to inhale cigarette smoke, mice whose hearts are more likely to burst after a surgically induced heart attack, and mice who die from incessant seizures induced by injecting acid into their abdomens. Psychiatric experiments include placing mice in deep, enclosed cylinders filled with tepid water, and waiting until they stop swimming – having despaired of ever escaping from drowning. Other tests include water deprivation and electric shocks, separating mothers from their pups to make them squeal in distress, the insertion of rectal probes, exposure to predators, and destruction of their sense of smell with surgery, thereby inducing chronic fear and withdrawal.

- Mouse units typically hold very large numbers of animals, making it more likely that mice will suffer through neglect or incompetence. Examples that have come to light as a result of covert investigations, or published in Home Office reports, include mice being drowned, killed through overheating, starved to death, left to die in scanners, and used for stitching practice.

- There are many important scientific reasons why GM mouse models represent a poor approach to human medicine. They include fundamental interspecies differences, the complexity of genetic machinery, a reliance on misleading and inaccurate models of disease, and an emphasis on curiosity-based basic research.

- GM mouse models have a very poor track record in actually helping the sick. Evidence is presented of their systematic failure in cancer and Alzheimer's, cardiovascular and respiratory diseases – all major areas of human suffering and mortality. It can, therefore, be confidently stated that GM mouse experiments have diverted funds from more promising and humane methods of investigating and ameliorating the impact of human illness.

Science Corrupted, accordingly, concludes that the genetic modification of mice is proving to be a hugely expensive, cruel and tragically wasteful enterprise. Human patients as well as animals suffer the consequences.

The widely reported Home Office annual statistics represent merely a fraction of the true scale of mouse suffering in pursuit of this vainglorious enterprise. The millions of mice who are violently killed as 'surplus' are not even accorded the dignity of official recognition. And, in the future, neither will millions more with supposedly 'non-harmful' genetic alterations. It is as if they had never existed. *Science Corrupted* is the first step in a campaign to make their immense suffering visible and, ultimately, to prevent it.

INTRODUCTION

The desire to purge human beings of weakness and disease has led, over the centuries, to transformative public health initiatives and medical breakthroughs – but also to some extreme and desperate measures. Examples range from the medieval surgeons and their use of red-hot irons and spells, to the eugenicists of the early 20th century, who believed that the human gene pool could be purified by preventing the unfit and feeble-minded from reproducing.

The ambition persists to our own day, as does the age-old conceit that the crude excesses of the past can never be repeated. History, it is supposed, has made us too wise, while the immensely powerful tools at our disposal, we tell ourselves, can be deployed only with societal approval.

At the heart of the modern disease-purging project is DNA – the molecule containing the instructions used for the development and functioning of all known living organisms. Specialists in the field can read and manipulate DNA to the extent that life itself can be reconfigured. They tell us that they can produce bespoke 'mouse models' to act as accurate surrogates for afflicted human beings. Cures for cancer, Alzheimer's, Parkinson's, heart disease, stroke, epilepsy and other conditions are now said to be within reach.

But, as this uniquely revealing, fully referenced report sets out, the GM mouse revolution is not about to take us to the Promised Land. This is because the project rests on the faulty scientific premise that these animals can act as reliable human surrogates when they can not. There is also the deeply troubling treatment of the 'disease models' themselves. The scale and severity of the suffering being inflicted on the mice is genuinely nightmarish. Drawing from the researchers' own published papers, we describe animals who are genetically programmed to die from continuous seizures, or from massive internal bleeding due to the rupture of a major artery. Others can't help but excessively groom themselves until they rip their own skin, or they repeatedly bite and pull the skin of cage mates. For many mice, the torment caused by genetic programming is just a start. Next come the experiments themselves and, in this regard, GM researchers are nothing if not supremely innovative.

GM female mice are poisoned with salt to induce stroke, and suffer a range of disabilities before dying from brain haemorrhage. For depression studies, mice are dropped into a beaker of cool water and monitored for several minutes as they move from frantic attempts to save themselves from drowning, to despair, defeat and immobility. Epilepsy 'models' have seizures triggered by being rapidly and repeatedly tossed in the air. The same effect is achieved in other mice by injecting acid into their abdomens. Some of the injected animals die from uninterrupted fitting. Other genetically manipulated animals must endure water deprivation, exposure to predators, rectal probes and electric shocks.

Does it matter? Do mice deserve consideration, given that they are mere rodents? And a second point: if what is described delivers major health benefits for human medicine, doesn't that trump any mice suffering? The answer to the first point is found at the opening of our report. Mice might be small but their capacity to feel pain is as fully developed as any other mammal's – humans included.

The answer to the second point is embodied in this report's main title: *Science Corrupted*. The deliberate use of such terrible and macabre cruelty and on such a massive scale – in Britain alone millions of GM mice are bred, disposed of and experimented on every year – is inherently corrupting of the culture that sanctions it.

But as indicated above, even if the GM enterprise were to be judged strictly on the grounds of expediency, it would still fail the test. An objective reading of the scientific literature shows that the GM mouse revolution is not delivering. Nor can it do so. Mice might share around 80 per cent of genes with human beings but the way those genes function and are regulated is self-evidently very different. And adding or subtracting a gene or two (the main alterations done to GM mice) does not take the practitioners to where they want to go. As important as genes might be, they represent a tiny fraction (about 2 per cent) of a person's DNA that – in sum total – governs his or her development and functioning. Researchers used to regard the DNA that isn't arranged into gene sequences as 'junk'. Now, it is widely acknowledged by geneticists that DNA's regulatory and 'switching' functions are fundamental... though still essentially mysterious.

There are other reasons why GM mice do not function as reliable human disease surrogates. One is that researchers cannot reproduce in these animals disease states sufficiently similar to those experienced by people. Another is that drugs and other treatments tried out on the GM mice cannot later be relied upon to function in the same way in human patients.

Little wonder that a long list of candidate drugs that produced positive results in GM mice went on to prove useless or harmful when tried out on patients with conditions such as Alzheimer's, cancer, Parkinson's and heart disease.

A seminal figure in the development of the mouse disease model industry was Clarence Cook Little (1888-1971). Little was an American genetics and cancer specialist who, during his early career, reared and sold thousands of inbred mice for cancer research. In 1929, he founded Jackson Laboratory in Maine, which, by 1944, and under his continuing direction,

INTRODUCTION

was reported to be shipping out 9,000 mice a week to other laboratories. Today, Jackson employs 1,400 staff and offers, to research labs around the globe, 5,000 strains of mostly GM mice.

Another of Little's notable interests* was eugenics. In 1929 (the same year he founded Jackson Laboratories) he was appointed president of the American Eugenics Society (AES). During his tenure, the AES advocated sterilisation laws, the segregation of the 'feebleminded', race separation statutes, larger families for the middle and upper classes, and birth control programmes targeting the poor and unfit.

By invoking Little, this report does not argue that the GM mouse revolution heralds a new era of state-enforced eugenics. For one thing, the modern project to eradicate structural weaknesses in the human genome is in many ways self willed on the part of eager consumers. But while the grave excesses of the 1920s and '30s are not about to be replicated, we can see worrying echoes of that earlier time by way of the frenzied ambition accompanying the GM mouse project; the towering, boastful rhetoric; and the cruel insensitivity with which the objectives are being pursued. And just as early 20th Century eugenics had leading cultural, intellectual, political and industrial forces ranged behind it, so too does the GM mouse project.

Little and his contemporaries developed different strains of mice – for instance, animals prone to cancer and other diseases – by mating mice who were genetically closely related, or by opportunistically breeding from animals manifesting a desired weakness or malformation.

In 1974, three years after Little's death, came the first transgenic animals – created by the insertion of a gene from another organism. Then came the creation of the first 'knockout' mouse, whereby instead of adding a foreign gene, one of the mouse's own genes was 'knocked out' or 'silenced'.

The publication of the entire mouse genome in draft form in 2002 was to unleash a massive international collaboration to knock out each of the mouse's 20,000 genes, one at a time, to see what would result. Millions of mice have already been killed in the project.

Most GM mice are still created through the transgenic or knockout methods. But there is a third means: mutagenesis. The huge, multi-centre mutagenesis programmes that began in the 1990s are further evidence of the desensitisation that some lab researchers undergo – a process that allows them to use these animals as though they are unfeeling *materials*, rather than sentient beings.

Under the mutagenesis programmes, millions of male mice around the world were systematically poisoned by having DNA-damaging chemicals injected into their abdomens. The chemicals caused genetic damage to the mice's sperm, which meant that when they were subsequently mated, their offspring were born malformed, though in ways that could not be predicted.

Most of the damaged progeny were of no interest to the researchers as future 'disease models'. But some were selected for colony breeding. The rate of 'wastage' can be gauged by the fact that one UK centre screened 26,000 mice and recovered 500 usable 'mutants'. The 'failures' were killed with gas or had their necks broken.

Whatever the GM method – transgenic, knockout or mutagenesis – the vast majority of progeny are killed. This is because they are born either with unintended malformations (such as limb deformities, emaciation, water on the brain or a swollen heart) or because the judgement is made that the planned deformity is not, after all, useful. Or they might be killed because they are surplus to requirements. Many of the junked mice don't even receive basic bureaucratic recognition by being reported in government statistics.

When we began researching this report, we were convinced, based on our existing knowledge, that we would be disturbed by the findings. What we have uncovered has been even more profoundly distressing and shocking.

The world of GM mouse production and experimentation, it turns out, is a nightmarish realm of barely restrained cruelty, in which the normal moral considerations that give rise to compassion and empathy seem to have been discounted. Until now, the public discourse around the subject has reflected the narrative voiced by the practitioners. Their message has been that the GM mouse revolution is morally benign and immensely beneficial medically. This report tells the real story.

The logic of what we reveal is simple: the manufacture and use of GM mice does not merit the public's support.

Andrew Tyler, Director Animal Aid

See also our *Science Corrupted* short film, which includes footage of some of the experiments described in this report: <http://www.animalaid.org.uk/GMmice

* Between 1954 and 1969, Little held a senior scientific post with what came to be called the Council for Tobacco Research – a leading voice of the tobacco industry. Perversely for a man who had devoted so many years to cancer research (using mice as the principal research tool), he declared that 'smoking does not cause lung cancer and is at most a minor contributing factor'. In 1969, five years after the US Surgeon's landmark report setting out the health damage caused by smoking, Little insisted: '… there is no demonstrated causal relationship between smoking or any disease'.

‘… a perception that mice are “primitive” is still the undercurrent in many defences of their experimental use…’

SECTION ONE Mice Matter

> Snazzy was a cute fuzzy little guy with a pink nose and grey fur. For the first two months he was very shy and just tried to get back in his cage, but soon enough we could watch a two-hour movie with him sleeping in our laps. He loved it when we cuddled him, and when we woke him up, he used to lick our fingers and groom them with his paws.

> I had two rescue mice called George and Dylan. They were the sweetest little things. They would hang out on my shoulder/head or, if I was wearing a hoodie, they would hang out in the hood. They each had their own personalities. George was bigger and a bit bossy whereas Dylan was quite shy and timid. It was heartbreaking when they were gone.

> I worked for an old lady of 89 who had a friend of a similar age. When the friend came up to stay with my old lady, she’d tell her neighbour to pop in every day and leave six peanuts on a saucer in her larder. The neighbour said she would oblige but asked her why. “Well, it’s for the field mouse that pops in every day. He has lovely manners as he leaves the shucks [shells] on the side of the saucer and doesn’t touch anything else in there.” Of course the neighbour didn’t believe her, but after a couple of days she could see the old lady was telling the truth. A well mannered field mouse did pop in, eat his nuts, and neatly piled his shucks on the side.

> One of the first mice I got was Daisy. Her companion sadly died and, for a couple of weeks, Daisy only had me for companionship and we bonded then. She was a lovely natured brown and white girl and when I introduced some baby mice to her, she took to them straight away, grooming them and making sure they knew who was boss. Daisy loved being out the cage and would often be found scattering dirt from the plant in the lounge. Even if it was put up high, she would reach it and there would be dirt thrown everywhere!

> I had a little mouse who always hated going back in his cage (I no longer keep pets in cages) and when I would put him back in he would be visibly angry... One day I put my finger through the bars to stroke him and he bit my finger hard and then turned his back on me just out of reach...

> A few weeks after Florence came to live with me, I noticed a small lump on her abdomen... it turned out that she had something similar to a hernia, so she was fixed pretty quickly, stitched up and left to recover. Florence hated me handling her, and when I returned to pick her up at the vets, you should have seen the reaction I got. She peeked her head from under her little blanket when she heard my voice and then got very excited. That evening, she was first out the cage for a play and she spent a lot of time “popcorning” (mice do funny little jumps when they are happy or excited). She even came over to me and kept jumping on and off me. Florence lived a long life, despite various illnesses. She still hated being held, right until the end.

> One time, [my mouse] Madeleine got really sick and was close to dying, so she had a visit to the vet and had to have Baytril [an antibiotic] given to her. The other mice mostly ignored her, except for Anastasia, who snuggled with her and groomed her. Anastasia was the runt who was usually picked on by Madeleine. When Madeleine got better, she stopped picking on Ana, so maybe she appreciated the comfort and love she was given by her.

SECTION ONE Mice Matter

The stories overleaf illustrate the intelligent, complex and sensitive nature of mice. A perception that mice are somewhat 'primitive' is still the undercurrent in many defences of their experimental use. However, first hand accounts, rigorous observational studies and, sadly, cruel research, affirm that mice are highly developed, responsive creatures – making their widespread institutionalised abuse even more disturbing.

The inbred and genetically modified strains of mice used in laboratories are mostly descended from *Mus Musculus*, or house mouse. Animals in laboratories are no less perceptive or vulnerable than their wild-living counterparts. The sterile environment in which they live and die is completely hostile to them, and is fraught with stressors even before the trauma of experimentation.

Wild mice are by nature highly exploratory, and extremely active from dusk to dawn. They are omnivores, and can range widely over large territories in search of food (a mouse may visit up to 30 sites, and consume 200 small feeds in a single night). Their home base can be a simple tunnel with a nest, or a complex network of burrowed chambers. Mice are a prey species, and are highly motivated to stay close to safe cover, disliking barren open spaces. They find human contact very stressful unless they are properly habituated, and are especially upset by being caught or handled. In the laboratory setting, they are traditionally picked up by their tails, which unsurprisingly induces severe anxiety.[1]

They are gregarious animals and form complex social networks, communicating by touch, smell and sound. They use an exquisitely detailed system of scent-marking to identify territories and as a means of communication. Mice also have excellent and sensitive hearing, with a broad frequency range including ultrasound. Their sociability means that any periods of isolation are damaging for their welfare. Companionless mice suffer anxiety, boredom and physical illness.[2]

The courtship, mating and pup-rearing behaviours of mice are intricate and fascinating. Male mice use ultrasonic vocalisations, which have been characterised as 'love songs', to court available females.[3] In turn, the objects of their attention clean themselves vigorously all over to demonstrate their interest. Mouse pups are born deaf, blind, and hairless, and require significant nursing if they are to survive. Maternal behaviours, such as nest building, gathering pups together and keeping them warm, are therefore crucial. The smell of the pups activates this nourishing care from their mothers, who in turn employ a range of senses including scents in their milk and urine to identify their litters as unique. Interference with these olfactory cues – such as adding unwanted scents from handling, or removing them through cage changes – can disrupt this vital nurturing.

Mice are intelligent creatures with a highly evolved, sophisticated mammalian nervous system. They feel pain in the same way (physiologically speaking) as humans. Ironically, animal experimenters have felt it necessary to 'prove' what sensitive and ethical observations had already demonstrated – that mice have rich emotional lives, and experience fear, despair, excitement and pleasure in social contact.

Someone who has shared her home with numerous mice over the years put the case in simpler terms: 'They may be different to us in size and the way they look, but they have very similar personalities to humans. Some are shy, some are confident and adventurous, sometimes they are greedy (and prone to weight gain!) and some of them are friendly, while others are bullies. They are entertaining little creatures and misjudged by people, who generally think they are dirty and worthless.'

In a number of experiments, researchers have 'rigorously demonstrated in a scientific context' that mice empathise with each other, and are conscious of pain and suffering in their fellows. In one 2006 experiment, a Canadian team injected acid into the paws of adult mice.[4] They found that 'a mouse injected with acid writhed more violently if his or her partner had also been injected and was writhing in pain'. In another experiment, mice emitted distress calls as they were shocked by noise or electrocuted. Other mice learned to associate their cries with something unpleasant.[5]

Prey animals such as mice have an inherent tendency to hide signs of pain or distress. This makes them wholly unsuited to the mass-production, time-constrained laboratories that this report will describe. They can become unwell and deteriorate quickly, with often only subtle signs of suffering.

Mouse being used in brain damage experiment

'… [mice] continue to be treated as little more than biological tools, advertised in trade catalogues and shipped around the world…'

Double helix – the structure of DNA

SECTION TWO A Brief History of the Genetic Manipulation of Mice

Despite the evidence highlighted in Section One, the suffering of mice has historically been given short shrift in the drive to popularise them as research 'tools'. From the outset, their use as experimental subjects has been interwoven with wholesale human interference in their genetic make-up and life cycles. Therefore, before presenting a focused narrative of mice in laboratories, this report sets out some basic genetic terminology.

The language of genetics and animal genetic modification

DNA (deoxyribonucleic acid) is the biochemical alphabet in which genes are written. Genes are discrete units of DNA, and essentially act as blueprints for the creation of living organisms. A genome is an organism's entire genetic complement, with the term 'genotype' often used synonymously. An animal's phenotype is, essentially, what it looks like and what it does. Its phenotype encompasses development, physical make-up and behavior, and is the result not only of an organism's genes but also of environmental factors.

The process whereby genes serve as templates for the manufacture of cellular proteins is called gene expression. Genes are classified as 'structural' or 'regulatory,' depending on their function. Structural genes code for proteins that comprise cells and organs, and for enzymes that carry out chemical reactions vital for life. Regulatory genes control the expression of structural and other regulatory genes, increasing and decreasing their levels of activity.

Gene expression is a highly complex and intricate process, with even minor faults having potentially far-reaching effects. The protein products of genes do not act in isolation; there is an almost infinitely complex array of interactions between them and other cellular components that can alter their functions drastically. Furthermore, many proteins interact with other regulatory genes. One gene can give rise to many different proteins, which in turn have varied functions and interactions.

Genetically modified (GM) animals have had their DNA artificially manipulated in some way. The term includes cloned animals, who are virtually identical and produced from the same DNA blueprint. The two principal categories of GM animals are transgenic and 'knockouts', whose production and use constitute a large and rapidly expanding sector of contemporary biomedical research. The former are animals who have been altered to carry a 'foreign' gene from another organism ('transgene') within their natural genome. Knockout animals are used, at least in theory, to study a gene's function by halting its expression, and observing the effects of its absence.

The term 'mutant' is often used loosely to refer to all kinds of GM animals, but is only used here to indicate animals with genes that have been changed in structure, as opposed to deletions or insertions. These alterations – mutations – can occur naturally or be induced by poisons in a laboratory. In UK government statistics, there is a distinction between GM animals and those with naturally occurring but harmful mutations.

As pointed out in a previous Animal Aid report,[6] the complexity of genetic processes means that 'the generation of genetically modified animals, by their very nature, is a highly complicated, difficult, imprecise, inefficient and crude method (in terms of results) of determining or altering the function of a gene'.

The history of mice in laboratories

The first steps towards organised 'mouse laboratories' were taken in the US around the start of the last century. The systematic inbreeding (mating of closely related animals) of many generations of mice was carried out by two key

figures. Then, as now, mice offered advantages to these early experimenters: they are relatively cheap to house and feed en masse; they are small, easy to capture and handle; they are docile; they have good-sized litters; and they can be readily shipped from breeding facilities to research locations.

Mice were emphatically not chosen as experimental subjects due to their genetic similarity to humans – the nature of the respective genomes was, anyway, totally unknown at this time. They were selected because they were convenient to use.

Miss Abbie Lathrop, a retired schoolteacher from Massachusetts, initially began breeding mice for pets, but became a systematic animal experimenter when laboratories began purchasing her animals.[7] She noticed that her mouse inbreeding programme was leading to the emergence of 'skin lesions', which were diagnosed as cancer by the academic institutions. In collaboration with the University of Pennsylvania, she performed a series of breeding experiments on mice with breast cancer. A description of her set-up evokes a modern-day puppy farm: 'From around 1910 until her death in 1918, Miss Lathrop's barn and sheds contained more than 11,000 mice, several hundred guinea pigs, rabbits and rats, and occasional ferrets and canaries. The mice were housed in light-tight wooden boxes, filled with straw, and were fed on a diet of crackers and oats.' A local newspaper report in 1913 shows how little ethical consideration was afforded these animals:

'In one of the cages of Miss Lathrop's mouse barn may be seen a lively little fellow with a lump upon his shoulder as big as a hickory nut. His days are numbered, for the cancerous tumor will strike a vital spot before very long and, with the delicacy characteristic of creatures low on the scale of life, he will probably succumb.'[8]

A contemporary of Lathrop, Clarence Cook Little, also embarked on a mass mouse inbreeding programme, driven by his interest in genetics.[9] He was also the President of the American Eugenics Society, part of a movement which advocated selective breeding and the forced sterilisation of human 'defectives'.[10] Little went on to found the Jackson Laboratory, which today is one of the largest suppliers of GM mice in the world, and believed that breeding mice could give insights into the 'making of men'. Over three years from 1909, he reared more than 10,000 mice, systematically killing the weaker animals. The aforementioned academic interest in mouse cancers gave Little a burgeoning market for his inbred victims – he 'offered a cheap supply of tiny patients to try things out on'.[11]

Decades of mouse breeding programmes followed, with 'curing cancer' often declared as the driving force. Highly inbred strains yielded naturally occurring mutants prone to

Abbie Lathrop, early mouse breeder for labs

Clarence Cook Little (centre), large scale mouse supplier and noted eugenicist

many kinds of disease, including malignancy. Severe combined immune deficiency (SCID) mice, for example, are luckless mutants who suffer from almost complete immune system failure. Cancer researchers have for years exploited their defencelessness, and grafted human tumours into their bodies in the hope of developing new drugs for people.

The arrival of genetic modification

However, even before the SCID mouse appeared, it was obvious that occasional treatments that worked on mutant or inbred mice very rarely translated to people, and researchers were casting around for better animal 'tools'. In 1974, the German biologist Jaenish created the first transgenic animal. He did this by attaching foreign DNA to a virus, and taking advantage of the virus's ability to penetrate through to the cell nucleus of mouse embryos. Techniques that ensured transmission of genetic modifications to the offspring of the altered mice followed in the early 1980s, and the details are set out in the next section of this report. It was now possible to create lines of transgenic mice in the laboratory.

Although the breeding terms 'line' and 'strain' are often used interchangeably in scientific literature, this report uses strain to refer to an inbred non-GM population, and line for a GM population.

The first 'oncomice', genetically modified to develop cancer, followed in the mid-1980s. Controversially, one research team patented their 'transgenic non-human animal', and licensed the patent to the Dupont Corporation.[12] By 1989, a British team had used mouse embryonic stem cells to develop knockout mice.

Genome sequencing

Academic interest in genetics reached a new intensity with the decision to sequence the human genome in the early 1990s. A draft 90 per cent complete sequence was published by The International Human Genome Sequencing Consortium in 2001, and the full sequence in April 2003. Declarations were made in reverent terms of the almost miraculous power of this information. Francis Collins, the then Director of the National Human Genome Research Institute in the US, enthused: 'It's a transformative textbook of medicine, with insights that will give health care providers immense new powers to treat, prevent and cure disease.'[13] By 1991, the project had cost 2.7 billion US dollars,[14] one third of which was provided by the UK Wellcome Trust.[15]

In 1999, with the human genome sequencing project well underway, three major genetic research centres launched the Mouse Genome Sequencing Consortium. The group quickly expanded, and a draft sequence of one widely-used strain (C57BL/6J) was published in 2002. Seventeen more strains had been sequenced by 2011. Researchers are now inundated with a mass of genetic information, the implications of which remain far from clear.

The mouse mutagenesis project

Running alongside human genome work (and pre-dating the systematic mouse 'knockout' studies described later) are ongoing experiments to warp the genetic make-up of mice, and, basically, see what happens. In these 'mutagenesis' programmes, chemicals that are powerful DNA-damaging agents are injected into the abdomens of male mice. This causes genetic damage to the victims' sperm, as well as to other cells in their bodies, leading to cancer. Subsequent matings produce offspring with a large variety of genetic alterations. Any mice with 'interesting' phenotypes are then selected for further experiments, in an attempt to identify which of their thousands of genes have been mutated, and whether these changes are responsible for their afflictions.

This blunderbuss technique has formed the basis of several large-scale research programmes, involving the systematic

poisoning of millions of mice. The most commonly used chemical is ENU (N-ethyl-N-nitrosourea), which has a marked ability to damage DNA. Hundreds of mutant mouse lines have been created, after experimenters have eliminated the physically deformed or behaviourally damaged results of their work. One UK centre alone, the Mammalian Genetics Unit at Harwell (see page 16), had already 'generated and screened more than 26,000 mice, and recovered some 500 new mouse mutants', by the turn of the millennium.[16]

Mass mutagenesis projects such as these, in common with the more focused GM techniques described below, entail large scale deliberate killing and unintended deaths. In fact, almost all progeny are killed immediately, as they offer nothing 'fresh' to study. Of the one to two per cent who are regarded as of interest, only a quarter will possess a new mutation. The rest are discarded. Other mutations will have already killed progeny animals during prenatal or postnatal development. Finally, any breeding programme entails the mass killing of surplus mice (see page 16).

A GM mouse for every ill

The account above shows that mice in laboratories have been subjected to a commodification process for more than a century. They continue to be treated as little more than biological tools, advertised in trade catalogues and shipped around the world. This process dramatically accelerated in the new millennium, with the advent of international and expensive 'consortia' tasked with creating thousands of new GM lines. In parallel, a mass of laboratories, both publicly and privately funded, have been busy creating genetically engineered 'mouse models' of almost every human ailment, from baldness and the common cold, to Alzheimer's and cancer.

The mice are used for a vast array of purposes, ranging from basic research (see page 28) to testing candidate drugs.

Somewhat predictably, even before the C57BL/6J genome was published, mouse genetics laboratories were discussing plans to knock out every gene in the mouse genome – some 20,000 of them. The International Mouse Knockout Consortium began this enormous task in 2006, using mouse embryonic stem cells. By the end of 2012, and more than a hundred million dollars later, the project was nearing completion. More than 2,100 new lines had been created.[17] Millions of mice have been used and killed worldwide in the service of this objective.

One researcher from the Wellcome Trust commented breathlessly in 2002: 'The avalanche of genome sequence will be followed by an explosion of mutant mice, requiring new mouse facilities to house and phenotypically evaluate this global genetic resource.'[18]

It is accurate to observe that the experimenters do not know the consequences of their genetic alterations in mice, let alone whether they have any relevance to human medical progress. The same researcher points out that 'a knockout phenotype often shamelessly displays our collective ignorance about gene function'.[19] Another, a US geneticist, commented in 2011 that 'knocking out the mice is simple relative to the huge task of finding out what all those genes do'.[20] Hence the 'need' for another hugely expensive multi-centre project – the International Mouse Phenotyping Consortium – formed in 2011, which aims initially to characterise 5,000 knockout mice in five years. The longer-term goal, to be completed by 2021, is the generation and phenotyping of a line for every gene that mice possess. This is projected to cost around six hundred million dollars.[21]

'Mouse clinics' have sprung up around the world, clamouring for funds to undertake these analyses. The phenomenal expenditure of resources devoted to knockout mice is probably at least equalled by the sum spent on creating and experimenting on their transgenic kindred.

It is hard to find a current figure for the number of GM lines now created. A 2004 *Nature* article, entitled 'Geneticists prepare for deluge of mutant mice', predicted that 300,000 new lines of mice could be created over the following two decades.[22] The Jackson Laboratory alone offers more than 5,000 'genetically defined' lines. This report now examines the current extent of GM mouse use in the UK, firstly by looking at the trend since the 1970s.

'… the university sector has been carrying out progressively more animal experiments since the late 1980s…'

SECTION THREE
The Use of Genetically Modified Mice in the UK

The number of experiments using animals hit a UK high in the early 1970s, when more than 5.5 million regulated procedures took place. (A procedure is defined as an action 'likely to cause pain, suffering, distress or lasting harm'.) For most of that decade, the figure exceeded five million procedures annually, after which time a generally steady decline began until the late 1990s. Since then, the decreasing trend has reversed and, in 2011, more than 3.79 million experiments were started on animals.[23] This is the highest figure since the introduction of the Animals (Scientific Procedures) Act in 1986.

Most of the 25-year rise has been due to the increasing use of GM animals, the vast majority of whom are mice. It was in 2009 that experiments on genetically altered animals first exceeded those on normal animals.

The number of procedures performed on mice, in general, has also increased dramatically. They are up from 1.45 million in 1995 to nearly 2.68 million in 2011 – with 70 per cent of that 2011 total accounted for by procedures on mice who have been genetically altered.

GM mice are currently used far more than mice with harmful mutations, and almost twice as often as normal mice. In 2011, they were used in 1.5 million procedures. While 'breeding' as a procedure accounted for two-thirds of the 1.5 million total, that still left more than 0.5 million other kinds of experiments – the vast majority of which came under the heading of 'basic research' (see Section Five).

It is important to stress at this point the difference between 'basic' and 'applied' research. The first is curiosity-driven. Applied research, by contrast, focuses on practical matters such as the discovery and validation of medical treatments. Only a tiny proportion of the procedures using GM mice (two per cent in 2011) involve applied rather than basic research.

The main users and breeders of GM mice

In the UK in 2011, 1.47 million procedures performed on GM mice used animals sourced from within the same establishment. Another 47,000 procedures used GM mice transported from another designated UK establishment. A further 3,000 procedures used mice shipped from the European Union, and 9,000 others used mice transported from even further afield.

Thus, nearly 60,000 GM mice were transported out of the establishments in which they are bred – a noteworthy figure, given that transportation is so stressful for animals.

The university sector has been carrying out progressively more animal experiments since the late 1980s. It is now responsible for more than all other sectors combined – just over 50 per cent in 2011. Although the Home Office does not provide specific details on university animal experiments, it states that 'the difference in trends between the commercial sector and the university sector is likely to reflect the increase in fundamental research using GM animals within universities'.[24] Understanding the primacy of academia in mouse experimentation is a fundamental part of interpreting this contemporary trend.

It is likely that universities that have not custom-bred their own GM mice, will have established close links with the UK's two major commercial breeders – Harlan Ltd and Charles River Ltd.

Charles River was founded in 1947 and, although its headquarters are in the US, it has more than 60 'facilities' in 16 countries. The company owns a large site in Margate, Kent, which breeds and supplies mice, rats, gerbils, guinea pigs, hamsters, rabbits and chickens for animal experiments. In 2011, its revenue was 1.14 billion dollars. In the same year, Charles River signed an agreement to market and distribute GM animals developed by the drug company Pfizer.[25]

Harlan, founded in 1931, is another large multinational based in the US. It has four UK sites, and in addition to GM animals, supplies marmosets, beagles, cats, rabbits, guinea pigs, rats, mice, gerbils and hamsters for laboratory research. It also carries out contract research, which involves animal poisoning studies (toxicology). According to *The Guardian*, the company's site in Blackthorn, Oxfordshire houses 52,000 rats and mice 'destined for use in medical experiments', with 6,000 being shipped out each week. Customers 'include pharmaceuticals such as GlaxoSmithKline and academic centres such as University College London and King's College London'.[26]

'… [the process] involves the wholesale manipulation of the reproductive cycles, behaviour, living conditions and health status of millions of animals…'

SECTION FOUR The Creation and Colony-breeding of GM Mice

It is plain from the figures in Section Three that the creation and use of GM mice is something of a contemporary juggernaut, with few researchers, so far, inclined to consider applying the brakes. In response to concerns about the resultant mass animal suffering, several expert bodies have produced guidelines and recommendations. They make clear that GM programmes cause pain, suffering and distress, and are thus legitimate areas of public interest and, often, unease.

Newborn mice

The creation of GM mice

Before examining how mice suffer at all stages of these programmes, it is necessary to set out in simple terms what is involved. The first stage in both knockout or transgenic research is the often speculative creation of novel animals, with a genetic profile that has never before existed. This is done through the insertion of a modified section of DNA into a mouse genome. In the case of knockouts, the inserted material is a copy of the target gene with a segment deleted or otherwise altered so as to disable its function. These founder mice are then bred on, to confirm that genetic alterations are reliably transmitted to their offspring. If this check confirms the existence of a new and stable GM line of mice, the offspring can then be kept and bred en masse in laboratories around the world, and serve as experimental subjects.

This initial creation stage involves several highly invasive procedures. It also entails the deaths of hundreds of animals to produce only one 'founder'. Across the UK every year, millions of animals are killed and often, literally, binned like so much rubbish (see page 17). The scale of the slaughter has been reported to leave some of the animal technicians responsible 'physically and emotionally exhausted'.[27]

The two most widely used methods of creating GM mice are pronuclear microinjection and gene targeting in embryonic stem cells (ES) . (Other techniques, such as the use of viruses, are not commonly employed, and will not be featured in this report.) Both techniques are inherently inefficient, and notable for the sheer waste of life they inevitably cause.

Pronuclear microinjection

In this technique, young female mice are injected with powerful hormones to make them superovulate (produce an unnaturally large quantity of eggs), and are then allowed to mate. On the morning after the introduction of a male, the mated females are killed. A section of their reproductive organs is removed, and 'embryo clumps' harvested. At this time, the genetic material from the egg and sperm is still separately visible within these embryos, in the form of two structures called pronuclei. Foreign genetic material, called a construct or transgene, is then injected into one of the pronuclei using a microscope and a tiny needle.

A number of these injected embryos are then transplanted into 'pseudopregnant' female mice, who act as surrogate mothers. The state of pseudopregnancy is established by treating the females with hormones and mating them with sterile (usually vasectomised) male mice. These processes trick their bodies into 'thinking' they are pregnant, and prepare their wombs for the implantation of the GM embryos. Large numbers of female mice are bred for this purpose.

Roughly three weeks afterwards comes the birth of any surviving pups. Most of the embryos will have already perished *in utero*, either due to the presence of lethal genetic changes, or simply due to the sheer violence of the process. Typically, between 20 and 30 per cent will develop to term.

Of the mice who do survive to birth, a minority will be successfully genetically modified – a typical figure is 10 to 20 per cent. This is because the injected transgene does not integrate reliably into the fertilised embryos: there may be some offspring with multiple copies, and some with none. All the mouse pups must, therefore, be genetically analysed, and have to endure the cutting off of a piece of their tail or ears to provide a tissue sample. The animals with the transgene inserted to the researchers' satisfaction are kept alive for breeding; those who do not are destroyed as 'failures'. Overall, around 95 per cent of the original cohort of mouse embryos will have perished.[28]

Gene targeting in embryonic stem cells

This method uses mouse embryonic stem cells (ES), which have the potential to develop into any kind of specialised tissue, but not to form an entire animal. To obtain these cells, female mice are again allowed to mate and become pregnant. Very shortly afterwards, they are killed and their embryos harvested, from which the stem cells are isolated and incubated *in vitro*, prior to the introduction of the transgene.

The next stage of the process requires the killing of yet more newly pregnant mice, to obtain embryos only a few days old, known as blastocysts. The engineered ES cells are injected into these hollow spheres of around a hundred cells, and the resulting cell mixture surgically implanted into pseudopregnant surrogate mouse mothers. Between 15 and 25 per cent of the manipulated embryos survive to birth.

Around half of the survivors will be chimeras. They are so called because their bodies are composed of some cells derived from the transgenic ES cells, and others derived from the blastocyst. Often, the original cells are obtained from animals with different coat colours as well as different genetic backgrounds. It is then possible to tell immediately which animals are transgenic chimeras simply by observing their colouring. Any non-chimeric animals are killed.

The process, however, is still not finished. Only a proportion of the mice have the ES cells in their reproductive system; only they will be able to pass the modification on to their offspring. Additional breeding is now undertaken to obtain mice with such reproductive characteristics, with yet more animals killed along the way. Overall, the technique is often no more efficient than microinjection in terms of the high numbers of wasted animal lives.[29]

Building and maintaining GM colonies

The development of a colony of GM animals involves the breeding of GM founder animals with genetically normal or 'wild type' mice. Once a GM line has been established with the above techniques, breeding programmes are set up to maintain the resultant colonies, and satisfy the need for experimental subjects. This is not a simple or painless process. It involves the wholesale manipulation of the reproductive cycles, behaviour, living conditions and health status of millions of animals. These breeding programmes are in many ways the laboratory equivalent of factory farming, with the same inherent problems of animal neglect, mass suffering, and a casual attitude to piles of dead bodies.

Most GM mouse breeding involves the transmission of novel genetic alterations down the generations. However, many founder animals have the transgene integrated only at one site on one chromosome. When these mice are bred with wild type (non GM) animals, 50 per cent of the offspring will also be wild-type and are usually killed. Subsequently, regular killing of 'failed' animals is essential to maintain the productivity of colonies.

An insight into the methodology of laboratory mouse breeding is provided by a resource manual from the Jackson Laboratory.[30] Mice in laboratories become sexually mature between five and eight weeks of age, although females of some strains can conceive when they are as young as 23 days. Mice are usually mated when they are six to eight weeks old. Their gestation period is around three weeks, and so a generation of mice can therefore be produced in 12 weeks. Litter sizes vary from two or three pups, to 12 or more in prolific strains. Typically, the mice breed for seven to eight months, producing four or more litters.

In order to induce numerous females to synchronise their reproductive cycles and produce pups of the same age, they are literally crammed in as densely as legally permitted. They are made ready to be impregnated via exposure to male hormones or litter shavings from a male cage. Females, thus stimulated, are then added to the cage of a 'stud' male who has been housed on his own for up to two weeks.

The Jackson Laboratory recommends getting the most out of especially fertile partners before killing them: 'If a pair is breeding well beyond its expected reproductive life span, retain it until the female is not pregnant within 60 days of her previous litter's birth.'[31] Usually, however, breeders should be 'replaced before their reproductive performance declines', which entails a regular weekly or monthly kill.

'…it is well recognised that mice, like other prey species, do not display overt signs of their pain – indeed they are adept at concealing their suffering…'

SECTION FIVE
The Suffering of GM Mice

The mouse victims of genetic modification programmes suffer in a whole host of ways. A chain of misery is formed by the procedures needed to create new lines, and by the harmful effects of genetic alteration, through to subsequent colony breeding, and finally experimentation and death. In addition, the mice endure a range of more generic stresses caused by the unnatural laboratory environment and, often, by transport both within and between establishments. These are described at the end of this section, together with examples of animal cruelty resulting from neglect, incompetence and even deception.

Mouse undergoing surgery to induce a stroke, via a wire inserted into blood vesssels in his brain

A Joint Working Group report from 2003, led by the RSPCA, and which continues to be influential, states that 'from an animal welfare standpoint, the use of GM mice is of serious concern because of the numbers of animals involved, the surgery and other invasive procedures used, and the deleterious effects that genetic modification can have on animal welfare'.[32] Despite this observation, many of the procedures involved in the production and maintenance of GM animals are still officially classified as 'mild' in severity.

It is well recognised that mice, like other prey species, do not display overt signs of their pain – indeed, they are adept at concealing their suffering. Recognition of their distress is therefore difficult, a situation which is compounded by their normal quietness during daytime working hours. The sheer numbers of GM mice housed in some establishments means welfare is inevitably compromised. A 2002 RSPCA survey of establishments conducting animal experiments described a facility in which technicians were responsible for checking 500 cages of four to five mice every day – equivalent to around ten seconds for each mouse even if a full six hours were spent on the task.[33] This overcrowding is not a problem only of the past, as recent undercover investigations have revealed.

Suffering during the creation of GM mice

It is worth considering in more detail what mice endure during the procedures they are put through in order to start the GM process. The variety of surgical procedures necessary to generate new transgenic or knockout mice are undoubtedly stressful and painful. Although major surgery is usually performed under general anaesthesia, this is in itself unpleasant. In addition, the above-mentioned RSPCA survey discovered an 'element of sizeism or speciesism in practice, as rodents sometimes received less consideration than larger animals'.[34] For example, one academic establishment gave no painkiller to mice before or after embryo transfer. Another gave post-operative pain relief only to larger animals.

- The hormone (gonadotrophin) injections, necessary to stimulate the overproduction of eggs, are administered deep into the abdominal cavity. The Working Group report referred to above cautions that 'intraperitoneal injections can be difficult to perform in mice because of their small size, and care should be taken... to avoid puncturing the abdominal viscera'.[35]

- The transfer of embryos into female mice involves major abdominal surgery, with significant post-operative pain.

- As only pseudopregnant females are initially required, founder male mice have to undergo vasectomy if they are not already sterile. In 2005, the most common vasectomy procedure involved a large abdominal incision, and 'substantial manipulation of the abdominal contents'.[36] This can lead to catastrophic postsurgical infection (especially as the procedure is not always performed

aseptically), carries a greater likelihood of wound breakdown, and mandates pain relief for possibly more than 24 hours. There is now a refinement to this procedure – a scrotal incision akin to that performed in humans. It is not clear how widely this refinement is implemented.

- Every individual animal from a GM breeding colony must have his or her genetic make-up analysed – a process called genotyping. This requires a tissue biopsy. A still widespread practice is to cut off the tip of the mouse's tail with a scalpel or scissors. Studies have demonstrated clearly how painful this is – the last 5mm of tail (a length commonly removed) contains tendons, a generous nerve supply, and spinal bones. Tail tipping causes both acute and chronic pain that can persist for many months in some strains.[37] If too much tail is taken due to poor technique or carelessness, the mice can also suffer from balance or gait problems. Although ear biopsy was acknowledged to be a scientifically superior technique in 2005, the Home Office is still prepared to license tail tipping 'on a case by case basis'. A coalition of animal experimenters has lobbied the Home Office to exempt genotyping procedures from new UK welfare legislation.[38]

- As well as tissue sampling for genotyping, many GM or potentially GM mice must also endure further distress due to invasive identification methods. Although observable features like coat colour can be used, this does not apply in many cases. Ear mutilation, via notching or the punching out of multiple holes, is commonly used instead. The procedure is performed using an ear punch or fine-tipped straight scissors, and can tear the ear tissue widely if performed carelessly. Nonetheless, these practices are exempt from UK legislative control and will remain so under the new EU Directive, as it is claimed that they cause 'only momentary pain or distress'.

Suffering during the breeding of GM mice colonies

The mice suffer many stresses, and sometimes physical injuries, due to the wholly artificial breeding regimes used in the creation of GM lines. In order to maximise the production of an unnaturally large quantity of eggs, three to five-week old prepubescent female mice are traditionally mated with older and larger 'stud' males, then killed. These vulnerable animals can be injured during the mating process. It is, therefore, recommended by the Working Group that 'to avoid harming females, over-sized or over-aggressive stud males must not be used'.[39] However, this recommendation has no legal force.

The stud males, used for both the creation and maintenance of colonies, are also regarded as just another commodity. If they do not function satisfactorily, they are summarily killed. They have to be 'housed singly to avoid fighting or injury'.[40] The RSPCA states that housing social animals without companions 'will seriously limit the animals' ability to express their natural behaviour and will have a big impact on their welfare'.[41]

A 2006 National Anti-Vivisection Society (NAVS) undercover investigation revealed widespread flouting of welfare recommendations at the Mammalian Genetics Unit, Harwell.[42] One of the organisation's investigators was able to obtain employment at this research centre. Run by the Medical Research Council, it is an 'international centre for mouse genetics', and can house 65,000 mice. The NAVS investigator reported that 'problems of over breeding and an inability to manage colonies, coupled with staff inadequacies, meant keeping control of the colonies was almost impossible'. Among the examples recorded were: signs of copulation being missed, resulting in unplanned litters; a male mouse 'mated' by his father; and female mice aged three and a half weeks becoming distressed after being put to mate with older males about three times their size.

The NAVS investigator attempted to postpone the deaths of some smaller pups by leaving them with their mothers a little longer, but was reprimanded and told to accept that *'some will die'.*

The killing of millions of 'surplus' animals during breeding programmes

The GM mouse industry is responsible for the creation and destruction of living creatures on a profligate scale. But under UK law, the killing of an animal by a permitted ('Schedule 1') method is not a 'procedure' and does not appear in the Home Office statistics. Overbreeding of rodents for use in laboratories is routine, as there is no official sanction for unnecessary mass killing. The unrecorded death toll runs into millions of animals.

The killing methods permitted for rodents, found in both old and new UK laws governing animal experiments, include death by inhalation of carbon dioxide, neck dislocation, and a direct blow to their heads against a work surface. Undercover investigations have repeatedly revealed the truth about the lives and deaths of these 'excess' or 'non-suitable' mice, who are not even accorded statistical recognition.

Charing Cross and Westminster Medical School (CXWMS) remains a tragic example of both overbreeding and violent killing, as revealed by NAVS. Although its investigation was carried out in 1994, there is no reason to believe such carnage does not continue. The NAVS report, *Access Denied*,[43] highlights how gassing of rodents was a particular problem at CXWMS. Because of faulty equipment or because of the quantities of animals being killed at the same time, animals often did not die quickly. Live unconscious animals were inadvertently thrown into the rubbish bags along with the dead bodies of others after inadequate gassing.

Its investigator commented: 'Picking up the bodies out of the gas chamber, it is obvious that it is a stressful way to die. The animals' bodies are wedged in corners, desperately trying to escape. Their eyes are almost always open. I've never seen a rat or mouse body from a gas chamber with closed eyes. It can be seen as the gas starves their lungs that they become panicked, and scurry to and fro, climbing over their fellows in a desperate attempt to escape. The positions I find them in (often huddled together as they clamour for an exit that isn't there, or trying to burrow through the corner of the steel cage) is the one they collapse in. Their lungs still pump and their eyes stare until they die.'

At CXWMS, records showed that, of 52,435 rodents bred, 'just' 15,198 (that is less than 3 out of 10) were used in experiments. 3,889 died before they were weaned, and 33,348 (68 per cent of all born) were killed, usually gassed, simply because they were surplus to requirements.

'Harmful phenotypes' – programmed to suffer extreme torments

An animal's phenotype, as previously noted, is a composite of his or her bodily form, physical functioning, and behaviour. The Joint Working Group report referenced above describes harmful phenotypes that can result from genetic modification. These can involve 'morphological, physiological, biochemical and/or behavioural abnormalities that compromise animal welfare by causing, or predisposing, mice to pain, suffering, distress or lasting harm. Animal welfare can be affected even if the phenotypic effects of the genetic modification are subtle.' In other words, GM mice may be deformed, their organ systems rendered dysfunctional, or their minds and emotions damaged. Mice with harmful phenotypes are without doubt bred to suffer – it is literally 'in their genes'. It is important to realise that these harms are present in the mice even before they are subjected to the traumatic experiments set out in this report.

To date, almost every area of human dissatisfaction or disease, from the trivial to the terminal, has been 'modelled' in GM mice. It is unsurprising, therefore, that there are thousands of examples of clearly detrimental genetic alterations to mice. Estimates vary as to the percentage of GM mice with a harmful phenotype (as opposed to a genetic alteration that is not officially recognised as causing harm or suffering). One researcher's 'personally communicated' low guess was that ten per cent of all GM mice suffer in this way.[44] However, a more systematic review reported that 21 per cent of lines experience minor discomfort, 15 per cent severe discomfort and 30 per cent suffer increases in mortality and susceptibility to disease.[45] There are major difficulties inherent in deciding formally whether or not a phenotype is harmful, a topic to which this report returns in the concluding section.

Until recently, the rat was a preferred 'model' for studying cardiovascular disease, chiefly due to a high blood pressure strain that has been subjected to innumerable experiments. However, transgenic mice are now widely used to model not just hypertension, but also heart failure, heart attacks, heart muscle disease, atherosclerosis (narrowing of the arteries), high blood cholesterol, strokes and obesity.

- **Heart Disease:** end-stage congestive cardiac failure in mice causes severe breathlessness and swelling, with fluid collecting in their lungs and abdomens. The 'muscle LIM protein knockout mouse', and the TNF-alpha overexpressing mouse, are destined to develop severe and ultimately lethal heart failure.[46] The Col1a1 knockout mouse developed by British Heart Foundation researchers is liable to die suddenly from massive internal bleeding due to aortic rupture (bursting of a major artery).[47]

- **Obesity,** despite its obvious dietary and social origins, is a favourite condition for researchers to study. The Mc4r knockout mouse overeats, and becomes massively obese and unwell, a condition totally unknown in her wild cousins.[48]

- **Cancer research** remains a focus of GM mouse programmes. The obvious clinical irrelevance of grafting human tumours into mice with a deliberately disabled immune system (see Animal Aid's *Victims of Charity* report, (page 7) has spurred the development of thousands of GM cancer 'models' – with consequent large-scale animal suffering. However, a 2010 document, produced largely by cancer research and drug industry interests, entitled *Guidelines for the welfare and use of animals in cancer research*[49] gives far more information on using animals than on their welfare. The only detailed references to

A fur-removing cream is applied to a mouse prior to surgery to induce a heart attack

animal suffering describe 'rare' symptoms of illness so severe that the victims need to be immediately killed. Tellingly, there is no guidance on objective assessments of animal pain or distress throughout the experiments. The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) is only now explicitly addressing the issue of pain monitoring in experimental animal cancer. It acknowledges that their tumours are 'assumed to cause pain and/or distress'.[50]

- Recent decades have seen the development of many GM models of **respiratory disease**. An emphysema experiment describes how one mouse became severely unwell at eight weeks of age and had to be killed.[51] Before death, she was allowed to suffer a week of wasting away, lethargy and turning blue. At post-mortem, her lungs were severely diseased with complete loss of normal anatomy. Other mice apparently 'appeared outwardly healthy' despite having severe lung disease, which again indicates the difficulty of spotting 'harmful phenotypes'. Transgenic mice, produced to study lung cancer, develop so many malignant lesions that they die of respiratory failure.[52]

- Making animals distressed is often the explicit purpose of **neurobehavioural and psychiatric research**. This experimental field has been notable over decades for its intentional pursuit of animal misery, and for the irrelevance of this enterprise to the human condition. Some of the torments inflicted on GM mice are due to the 'tests' they are forced to go through, most especially to mimic depression (see below). Others have suffering 'built in', like many Obsessive-Compulsive Disorder (OCD) 'models'. Hoxb8lox mutants, for example, excessively groom themselves and each other, resulting in hair removal and skin lesions.[53] Serotonin receptor knockout mice 'compulsively chew non-nutritive substances'.[54] Sapap3-mutant mice 'display excessive and self-injurious behaviors, including self-inflicted facial lesions' and increased anxiety.[55] D1CT-7 transgenic mice repeatedly bite and pull the skin of cage mates during grooming, and display 'abnormal digging, climbing, and tic-like behaviors'.[56] 'Severe anxiety' mice constantly try to hide when placed in mazes or test boxes.[57]

- **Epilepsy:** the medical charity Epilepsy Research UK has funded a researcher to use knockout mice who suffer fits from three weeks post-natally.[58] They are destined to die from continuous seizures in early adulthood. Other widely used epilepsy mice also begin to fit at a similar age, with 'head nodding, rearing up on the hind limbs, repetitive forelimb clonus [muscle spasms] and occasional loss of upright posture with generalized repetitive clonus of all limbs'.[59] They then lose condition, fail to thrive, and die by ten weeks of age, either of constant seizures, malnutrition or dehydration.

- **Alzheimer's** and other neurodegenerative diseases have been widely modelled in GM mice. According to the Nuffield Council on Bioethics, the animals 'may show a variety of neurological impairments including, for example, tremors and ataxia (loss of full control of bodily movements). The diseases may also affect a mouse's ability to interact with other animals, and to carry out behaviours such as play, running and climbing.'[60]

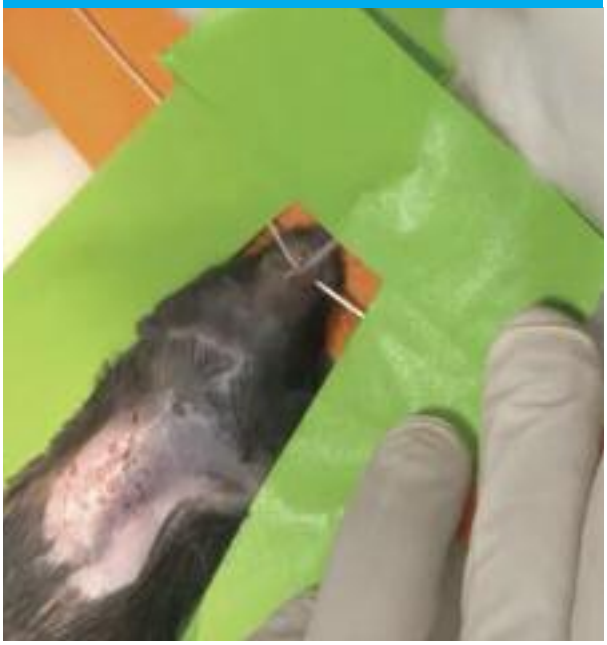
Other examples of harmful and cruel GM traits were noted by the NAVS investigator at MRC Harwell – 'severe limb deformities; fused lung lobes; Huntington's Disease mice with hard lumps in their abdomens, strong tremors, immobility, priapism [painful swelling of the penis] and weight loss of up to 30 per cent; cataracts and other eye problems; mutants with extremely short faces and upturned noses caused by abnormal bone growth; self harm, such as animals chewing through their own skin; and congestive heart failure which caused one mouse to swell to about three times normal size'.[61]

Distressing and lethal 'side effects' of genetic manipulation

Many animal victims of genetic manipulation suffer unforeseen and unpredictable 'side effects', in addition to the

intended suffering from their designer diseases. The reasons lie in the inherent crudeness of the techniques, and a lack of knowledge of the complex interplay of genetic regulatory processes. Disabling a gene's function – as in the creation of knockout 'models' – may have wide-ranging and unexpected consequences. Alternatively, when transgenes are introduced into a foreign 'host', they may take effect in the wrong tissue, switch on at the wrong time, or be uncontrolled in their effects and inflict damage on non-target organs or tissues. Transgene insertion can disrupt crucial host genes, rendering them useless, as well as critical 'control regions' of DNA that switch genes on and off. The 2003 Working Group report states that the effects of transgenesis are 'variable, unpredictable, and influenced by many factors'.[62]

Mouse about to suffer a surgically induced heart attack, via the tying off of coronary arteries



The unintended effects can begin in the womb with phenotypes that are so-called 'embryonic lethal'. Examples include mice that bleed to death *in utero*, or GM mice used to model Down's syndrome who develop fatal cardiovascular malformations during gestation.[63] Pups who survive to birth can suffer severe developmental abnormalities, such as hydrocephalus (water on the brain) and cleft palate.[64] These conditions often mean affected mice will be killed, or will starve to death if they cannot suckle. Other GM pups die from asphyxia due to undeveloped lungs or obstructed airways, have exposed internal organs (which can lead to them being eaten alive by their mothers), or suffer fatal dehydration due to water loss through undeveloped skin.[65] Some GM lines have bad teeth, no teeth, or facial deformities that make them unable to eat grain pellets.[66] They require special foods, such as ground or dampened grain. Obese mice can be so heavy that they cannot lift themselves up to where food hoppers are normally placed, or fall over on their backs and cannot right themselves. Other mice have been produced with unintended missing limbs, shortened jaws, or missing the front of their heads. Not surprisingly, the DNA poison ENU used in mutagenesis programmes is also a carcinogen. Many treated mice therefore succumb to different types of cancer, which halves their life span and causes significant suffering.[67]

Sometimes, the vital bonds between dams and pups are adversely affected. Genetic alterations can destroy mammary gland function, even making the mother's milk toxic or lethal to her pups.[68] Females of some GM lines cannot nurse or are poor mothers, and some males are aggressive and attack their mates and offspring.[69]

Even if not deliberately 'programmed-in', genetic tampering can cause increased anxiety and frustration, especially if the mice are more motivated to perform natural activities that are prevented in laboratories.[70] This can lead to the emergence of psychological distress syndromes such as purposeless and repetitive movements (stereotypies, see page 23).

Many GM lines have diminished fertility, which makes the maintenance of colonies 'challenging'. Some mice, for example, develop unintended leukaemia, and must be 'replaced' when they are about six months old. Others stop breeding early because they have a high frequency of ovarian cysts and tumours. Certain females develop diabetes when they are 12 weeks old, but 'their reproductive lives can be extended with foot pad injections of Freund's Adjuvant' (an excruciatingly painful injection of an emulsified microbiological solution).[71] Yet other mice suffer tremors and seizures by nine to 11 weeks of age, with the males having a breeding lifespan of only three to four weeks.

Cystic fibrosis (CF) mouse models, while exhibiting elements of the human disease, do so in an extreme and unrepresentative manner that is scientifically highly problematic. Sixty per cent of certain lines of CF mutant mice die after weaning, with those who survive suffering bowel narrowing and obstructions, and painful peritonitis.[72] Only 40 per cent of other, transgenic CF mice make it even that far. The majority die from suffocation due to a blocked airway.[73]

Some GM mice have reduced resistance to infection, which makes them highly vulnerable to lethal diseases. The lung pathogen *Pneumocystis carinii*, for example, hardly affects most mice, but can cause pneumonia and death in immuno-compromised GM animals.[74] ENU-'treated' mice are particularly susceptible to infection, as a consequence of damage to stem cells, caused by the poison, that are important for their immune systems.[75] The consequent need to house them individually is a great upset to these social animals.

GM mice as experimental subjects – suffering and traumatic death

Sometimes, the purpose of a research project is to see what sort of mice result from a gene knockout or insertion. Often, however, the production and breeding of GM mice are only preludes to their use in subsequent experiments. In many cases, the mice are programmed to develop certain diseases, but only if subjected to further surgery, poisoning, unnatural diets, trauma or psychological distress. Many of the experimental procedures they are put through can be categorised as basic research, with no direct relevance to the discovery of medical treatments. Examples include the following:


Mouse being given a fluid injection after heart attack surgery

- Transgenic mice used by Alzheimer's Society researchers (see below) were given strokes via the insertion of tiny wires into blood vessels in their brains. Other mice had coils inserted into their carotid arteries to permanently deprive their brains of oxygen.[76]

- The Alzheimer's Society has also funded experiments in which transgenic mice were subjected to swimming tests in a Morris Water Maze. This forces mice (innately strictly terrestrial animals) to swim in a tank of water until they locate a surface platform on which to rest. The platform is subsequently hidden, and mice must remember its location, at the same time as trying to escape through frantic swimming. Two 'neuroscientist members' of the lobby group Understanding Animal Research claimed in 2011 that the water in the tanks is not cold, and that the procedure is not cruel. A group of Finnish researchers, who say they have tested about 3,000 transgenic mice in this way, state clearly that animals are exposed to cold water ('to ensure sufficient motivation to escape') and that 'young healthy mice can become severely hypothermic during the task'. This is due to the mice's small body size and thin layer of subcutaneous fat. 'Alzheimer's model' transgenic mice are more vulnerable to hypothermia because of their smaller body weights. As to the cruelty, the Finnish group are categorical that the test involves 'unavoidable stress'.[77]

- Both the British Heart Foundation and the British Lung Foundation have funded recent studies forcing knockout mice to inhale cigarette smoke and have their lungs repeatedly 'washed out' to detect inflammation.[78] Mice were put into a plexiglass chamber, and smoke from Marlboro cigarettes was repeatedly pumped in. The experimenters even went as far as injecting 'Cigarette Smoke Extract' into the abdomens of some mice in order to induce peritonitis. One US researcher cynically described these victims as 'Marlboro Mice'.[79]

- For cardiovascular experiments, mice have been developed who are more likely to suffer cardiac rupture – a burst heart – after a surgically induced heart attack. In one experiment, mice who did not develop this outcome went on to suffer severe shortness of breath and die within a week.[80] The British Heart Foundation has recently funded similar studies.[81] In stroke research, GM female mice were poisoned with salt or other chemicals to raise their blood pressure. Experimenters then waited until the mice developed signs of a stroke – extended limbs, circling behaviour, or other disabilities – before killing them. All the

Mouse subjected to 'behavioural despair' test. He will struggle for several minutes to escape from drowning and then give up.

poisoned mice died of brain haemorrhages within ten weeks.[82]

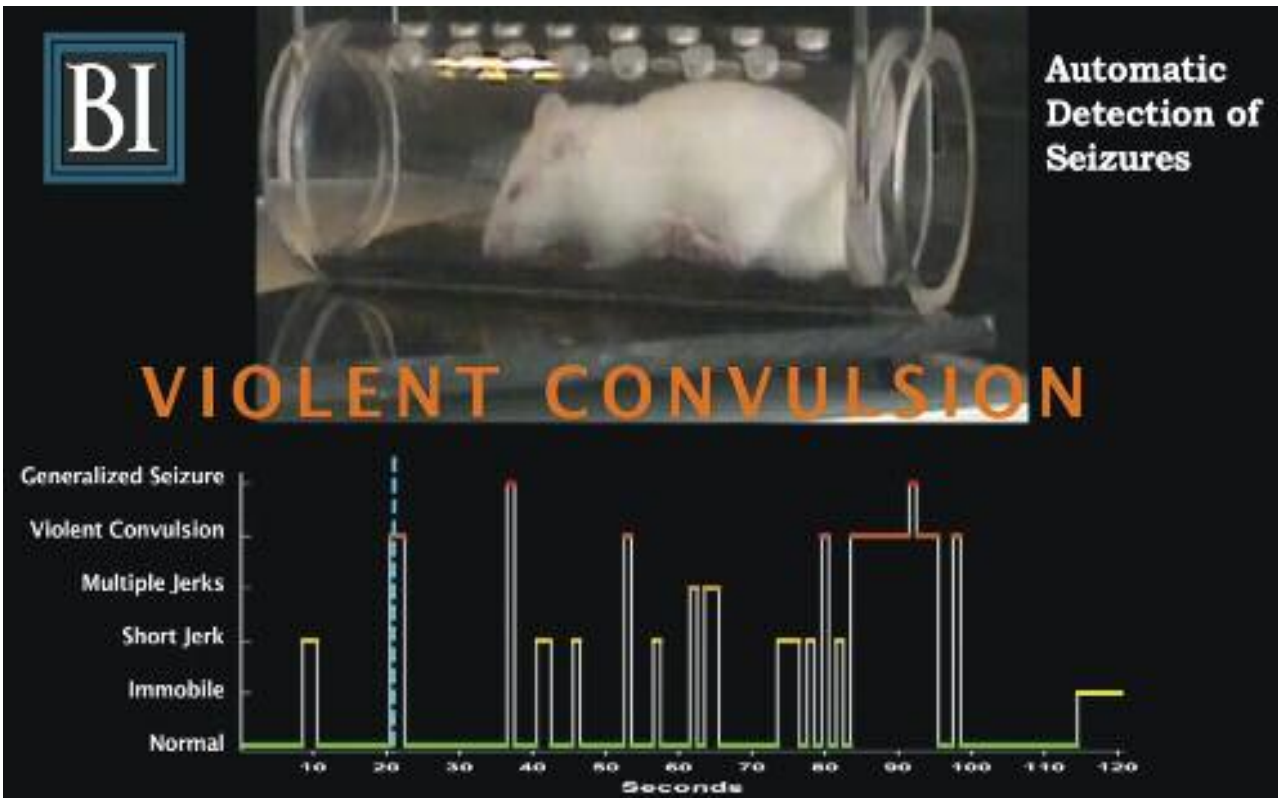
- The 'tests' inflicted on mice by the psychiatric establishment, apart from being outlandish parodies of human distress, are defiantly cruel. The following descriptions are from a 2005 review paper:[83]

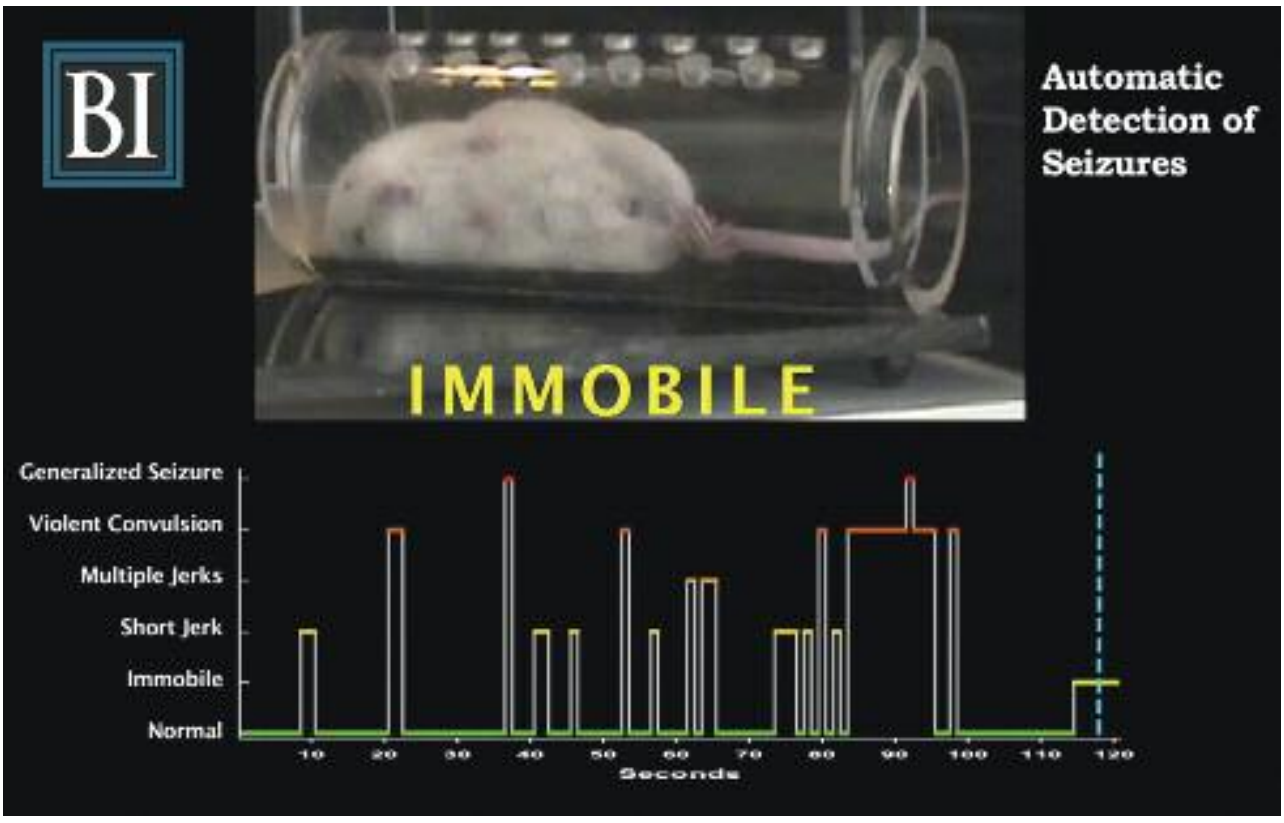
'Many models and tests for assessing depression-related behaviour in rodents involve exposure to stressful situations. Of these experimental procedures, the forced swim test (FST) – also known as Porsolt's test, a behavioural despair test – is probably the most widely and frequently used. The FST is based on the observation that rodents placed in an enclosed cylinder filled with tepid water will initially engage in vigorous escape-orientated movements, but then within minutes will exhibit increasing bouts of immobility. A related but not synonymous task is the tail suspension test (TST), in which mice hung upside-down by their tails also exhibit passive immobility after minutes of futile struggling.'

'Another model based on exposure to repeated but unpredictable stressors is the chronic mild stress (CMS) model... this model involves repeated exposure to relatively moderate stressors, such as wet bedding, constant lighting and food deprivation. The CMS procedure induces various long-term behavioural, neurochemical, neuroimmune and neuroendocrine alterations that resemble those observed in depressed patients.'

Other torments inflicted on mice include water deprivation and electric shocks, separating mothers from their pups to make them squeal in distress, the insertion of rectal probes, exposure to predators and destruction of their sense of smell with a crude surgical procedure. The latter makes them chronically scared, withdrawn and mentally unwell.[84]

- Some GM epilepsy mice have seizures set off by 'rhythmic gentle tossing' – being bounced in the air at a rate of 256 cycles per minute.[85] In an experiment partly funded by Epilepsy Research Foundation UK, indwelling electrodes were implanted into the brains of both GM and wild type mice to monitor their brain waves. Severe seizures were later induced via injections of an acid into their abdomens. The animals were allowed to fit for an hour before receiving an injection to halt their misery. Some of the GM mice died from status epilepticus – uninterrupted fitting.[86] There are many references to GM mice being killed by chemically induced seizures in the reports of the animal experimenters.





Device for measuring extent of seizures in GM mice

The 'background' suffering of mice in laboratories and during transportation

It would be an omission not to describe briefly the general suffering of mice – whether or not they are genetically modified – in laboratories.

Mice are highly social and inquisitive creatures, with a wide repertoire of natural behaviours that are stifled in a laboratory environment. They are also scared by being moved around, even within an establishment, let alone over long distances. There is a wealth of evidence that testifies to the welfare burdens imposed by such ordeals.

A current overview of such evidence is provided by Knight.[87] He points out that 'to standardise experimental conditions, and to facilitate access for experimental procedures and cage-cleaning, laboratory animals are typically kept in small cages, with a minimum of environmental enrichment materials'. Knight goes on to describe a lack of natural lighting (which can rapidly cause eye degeneration and cataracts in mice)[88] and noisy laboratory conditions that can be the equivalent of the din generated by an underground train.

Cage living does not permit mice to interact socially in natural ways. Young mice in laboratories are, typically, separated from their mothers when they are just three weeks old; in the wild, they would not leave their birth territories until they were twice that age.[89] Solitary housing remains commonplace, especially when animals are the subjects of experiments. Furthermore, as an outbreak of infection in a laboratory could necessitate the killing of entire colonies, procedures to isolate 'risk' animals are often required. The housing conditions provided for such mice amount to extreme levels of sensory deprivation, with many systems even preventing the transfer of sound and odour cues between cages. Mice housed like this are therefore utterly bereft of social stimulation.

Many mice in laboratories experience long-term psychological damage, as indicated by the emergence of stereotypies or 'barbering'. A stereotypy is an apparently functionless, repetitive behaviour, such as circling, route-tracing, back-flipping or bar biting. Stereotypies are estimated to afflict some 50 per cent of all laboratory-confined mice.[90] Whisker or fur-plucking is known as 'barbering', and is associated with both genetic factors and boredom. Importantly, such adverse effects are often not prevented even with the provision of the limited enrichment that is standard in most laboratories. A 2010 review of laboratory rodent welfare makes the point clearly: 'The current trend toward environmental enrichment is positive, but an enriched cage is still fundamentally impoverished... Enriched caged animals cannot exercise control over where they go. They cannot forage or burrow. They cannot explore or escape aversive noises, odors or (sometimes) lights.'[91]

A large number of GM mice are transferred between establishments (see Section Two). Animals ordinarily find being 'freighted' a distressing experience, but the distress can be heightened for some GM mice, due to the abnormal nature of their phenotypes.[92] Even short journeys can upset such animals and disrupt their physiology. A 1995 study that monitored mouse behaviour, as well as blood hormones, found that animals had not fully acclimatised a full four days after transport from one room to another.[93] Although there are codes of conduct governing animal transport, it is inevitable that blunders will occur. A 2003 newsletter for animal experimenters admits candidly:

'Though it's not done intentionally, there are many examples of animals being poorly treated while under the control of carriers. Instances of animals sitting for hours on runways in airplane cargo holds; animals being lost or misrouted; animals exposed to temperature extremes; or careless, unnecessary accidents, still occur.'[94]

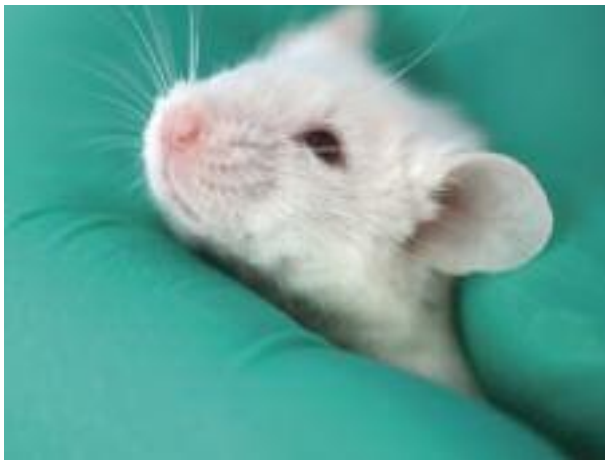
Lastly, even so-called 'routine' procedures exert a stressful toll on mice. Knight describes how handling (mice are traditionally caught and picked up by the tail), force-feeding and taking blood samples cause fear in common laboratory species, including mice. Animals have been documented, in many instances, as having suffered 'rapid, pronounced and statistically significant' distortions in their physiology and biochemistry – in short, a marked stress response.

Officially reported 'infringements' and covert investigations

The fact that transgenic mouse units typically hold large numbers of breeding animals magnifies the ubiquitous stressors set out above. It also makes it more likely that mice will suffer the kind of casual neglect and indifference uncovered by NAVS at Charing Cross Medical School and MRC Harwell. Poignant accounts of mouse suffering were recorded by the investigator at the former establishment:

- 'While doing cage cleaning I found a cage with three blotchy mice, all females, 30 days old. One was dead, the other two were ill, lethargic and shivering violently. K said it was probably due to a blocked nozzle on the drinking bottle.'

SECTION FIVE The Suffering of GM Mice



- 'While cleaning out today, I found an M9 breeder severely emaciated. The bones felt very clear through the skin. She was 91 days old. Eventually she was gassed because she seemed beyond hope. Her problem was actually one of grossly overgrown teeth preventing her from eating.'

- 'I didn't find many dead today. There was one in a cage of two (non-breeders), which looked as though it had been there for ages. It was hard, but not with rigor mortis – it was hard because it had dried out.'

- 'I took some stills of a T/O mouse due to be culled. He had severe injuries to his tail and body around the hind legs. A patch by the upper part of one of his rear legs was bleeding and stripped of fur, and his tail was a mass of scabs and dried blood. The digits on the front paws were indistinguishable, all I could see were blood clots.'

NAVS concluded that, within such regimes, 'ailments are only spotted if they become extremely visible or the animal is found dead. Treatment is rare amongst rodents because they are a cheap, disposable laboratory commodity.'[95] Its more recent (2006) Harwell investigation found examples of water leaking into mouse cages, resulting in severe cold, discomfort and even death. One mouse, with her feet and face bright pink, appeared to have hypothermia and so was killed.

These are instances of abuse that were exposed by covert investigations. However, annual Home Office reports published by the Animals Scientific Procedures Inspectorate (ASPI) verify that 'infringements' of licence conditions frequently involve mice, and at least some experimenters are capable of severe neglect.[96] The frequency of such occurrences is impossible to measure, due to pervasive, legally sanctioned secrecy and lack of public scrutiny.

Some recent examples of mouse suffering and death, reported by the ASPI, include:

- A licensee undertook to investigate problems being encountered with wound closure in an embryo transfer programme. Instead of seeking advice from care staff, the individual tried to resolve the problem by practising different closure techniques.

- Two mice were left over a weekend in an imaging chamber where they were discovered three days later. One was dead.

- 24 genetically altered mice died, and a further 14 had to be killed, as a result of a control unit failure, which resulted in raised room temperature. The alarms had been switched off, and the failure of the steam valve to close was attributed to faulty maintenance.

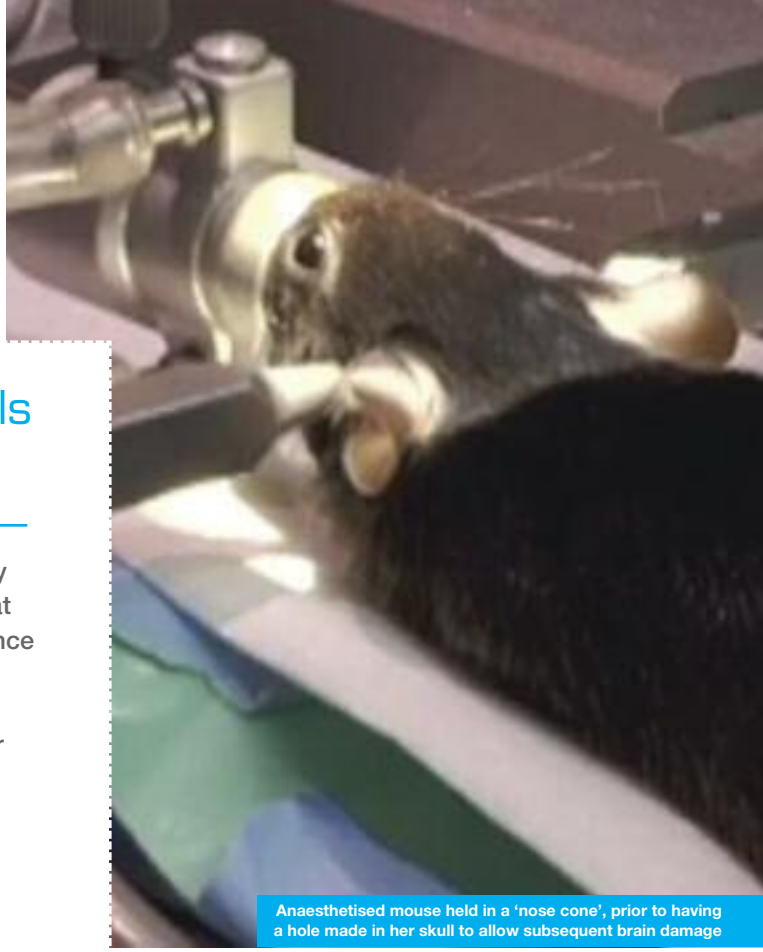
- 25 mice were starved to death, and another died from overgrown teeth.

- 208 mice were drowned when cages were flooded by a drinking water system, on two separate occasions.

- When a project licence was almost expired, experimenters illegally transferred mice to another licence. They mislabelled cage cards in an attempted cover-up, and failed to check on the mice to the extent that a number died from infection.

- A number of mice were found dead, and others had to be killed because the suffering they were enduring exceeded the severity limit stipulated on the project licence. Subsequent investigation by the Home Office found that the experimenter had caused the deaths of other mice 'because of a failure to take proper responsibility for the animals' care'.

'… the simple observation that humans are very unlike mice clearly shows that genes work in different ways in different species…'



Anaesthetised mouse held in a 'nose cone', prior to having a hole made in her skull to allow subsequent brain damage

SECTION SIX GM Mouse Models and their Medical Failure

'In our opinion, the current extent to which GA [Genetically Altered] mice are used cannot be justified on the basis that they are vital for the development of human medicines, since few human medicines have so far been developed which were largely or exclusively based on the use of GA mouse models. This unsatisfactory situation is despite nearly four decades of studies in GA mice – the first knockout, transgenic and trisomy [extra chromosome] mice were all produced in the 1980s. Indeed, the inability of many GA mouse models to recapitulate all the features of a human disease has often resulted in several mouse models being created, for studies on different aspects of the disease in question. Together with problems of differences in the genetic backgrounds of the mice used in these mutagenesis studies, this has confused the interpretation of the information provided, and has potentially slowed, rather than expedited, the development of new medical treatments.'

The above quote is from a paper published in 2006 by two scientists working for the UK Fund for the Replacement of Animals in Medical Research.[97] It encapsulates much of the discussion that follows, and demonstrates that objections to GM mouse experiments encompass human suffering as well as that of animals.

The myth that the gross and varied miseries detailed earlier are an essential pre-requisite to the alleviation of human suffering is, nonetheless, widely promulgated by the animal research industry and government. For instance, the lobby group, Understanding Animal Research claimed in 2011 that 'genetic modifications can produce better and more predictive animal models for human disease'.[98] By this, it presumably means 'better than the poorly predictive animal models that preceded the non-GM ones'. The sobering reality, however, is that 'genetically based' medicine, and even gene-based preventative strategies, are failing to live up to the starry-eyed promises initially made on their behalf.

One of the confounding factors is that individual mutations in the human genome seem to account for only a small part of disease risk. Even where a strong genetic component is suspected, widespread diseases are often linked to many rare variants, rather than a few common ones. In 2010, Harold Varmus, now director of the US National Cancer Institute, said '… genomics is a way to do science, not medicine'.[99] Even Lord Professor Robert Winston, a staunch supporter of animal experiments, declared in June 2012 that the 'hype' about the sequencing of the human genome is 'complete balls'. Winston went on to say that genetic studies 'certainly don't apply to all cancers, or even most cancers. The genome is also not really applying to heart disease, which will affect one third of us. And it frankly has been a real disappointment in the brain.'[100]

These opinions must surely apply as much to GM animal research as they do to human genetic studies, if not more so – given that mice are obviously much further removed from the origins of human illness. It is not surprising, therefore, that using genetically altered mice to mimic human disease is simply not delivering. *The mouse model industry, to stand any chance of success in helping the sick, needs three key assumptions to be true. Briefly, these assumptions are: that mice are similar to humans in the way their genes work, that genetic changes in mice can produce diseases similar to human diseases, and that treatments that work in these mice will work in people. There is now compelling evidence that all these assumptions are seriously flawed.*

The analysis below examines some general reasons why GM animal models represent a poor approach to human medicine. There follows a disease-specific look at how these experiments betray patients suffering from a multitude of conditions. The text builds on evidence presented in Animal Aid's *Victims of Charity* report (2011).

GM Mice – key reasons why their use does not aid human medicine

There are many reasons why the results of genetic experiments on mice do not 'translate' to humans. Some of the most important are set out briefly below.

1. Fundamental interspecies differences

Regardless of any genetic correspondences, humans are not giant mice. Mice have fundamentally different physiology and anatomy, especially with regard to their cardiovascular and respiratory systems (see page 30). There are profound differences between human and mouse immune systems, in terms of development, activation, and response to challenges.[101] Mice do not naturally develop many neurological conditions like Alzheimer's or Parkinson's Disease. High incidences of some rare human tumours occur spontaneously in certain mouse strains, whereas common human tumours hardly ever develop in rodents.[102]

2. Differences in disease status

The mice used in laboratories, whether GM or not, are very different from human populations. Strains are highly inbred and genetically homogenous. In contrast, genetic variability is a given in humans, and such variability plays a part in the risk, development and progression of disease. Additionally, the GM animals used in experiments are not suffering from the long-term, multi-faceted and interacting damage of chronic illnesses (such as diabetes and hypertension) found in human patients.

3. Differences in environment

The preceding sections of this report have shown just how stressful laboratory life is for mice. Force-feeding, multiple painful injections, surgical procedures, and restraint are all liable to provoke stress-related hormone responses. These repeated events predispose animals to poor immunity and cancerous changes. Simply handling mice has been shown to unpredictably increase the spread and growth of their cancers.[103]

4. Evolutionary biology and complexity theory

During the evolution of species, the more successful individuals are those who fit best into their environment, and are best equipped to deal with the challenges it poses. The evolution of mice took a very different turn from that of humans around 70 million years ago, with the former evolving strategies to feed efficiently, hide and reproduce in abundance. Primates took at that time a unique evolutionary path, with increased brain size and resultant cognitive advancement.

Such differences in evolutionary history have led to significant differences in the way the genetic machinery is configured. Altering a gene in an animal is not a straightforward matter with easily discernable consequences. The biochemical 'machinery' responsible for these processes differs between species, with similar genes performing different functions. Minimal changes in DNA sequence can lead to profound differences in biochemistry and physiology. These differences occur predominantly due to interspecies variation in regulatory genes and DNA regions.

Extrapolating the results of genetic alterations between different species is therefore rich with the possibility for huge error. In attempts to lend credibility to their work, animal experimenters cite the fact that 80 per cent of human genes have a mouse counterpart. The simple observation that humans are very unlike mice clearly shows that genes work in different ways in different species. An apparently closely related set of genes produces a vastly different animal.

Some of the explanation for this divergence is only now being understood, through the study of the human genome. In August 2012, a number of papers were published in leading scientific journals concerning so-called 'junk DNA'. It has been recognised for some time that around 98 per cent of the DNA in the human genome does not code for proteins. It is not, therefore, organised into gene sequences. This non-gene material was misleadingly labelled 'junk' in the 1970s, although ongoing work since that time has demonstrated that this material plays a critical role in regulating how genes are expressed. Genes are regulated in various ways, through the action of various interconnected 'switches'.

An extraordinary complexity has now been established, and the junk DNA concept is largely redundant. A UK researcher claimed in September 2012 that 'nearly all the genome is in play for doing something, or if you change it maybe it would have an effect somewhere'.[104] Such complexity partly explains why the results of genetic modification are not even predictable between different strains of mice, let alone between different species.

Bailey, in the Animal Aid report *Man or Mouse*, explains how this complexity can confound attempts to model human diseases with GM animals:

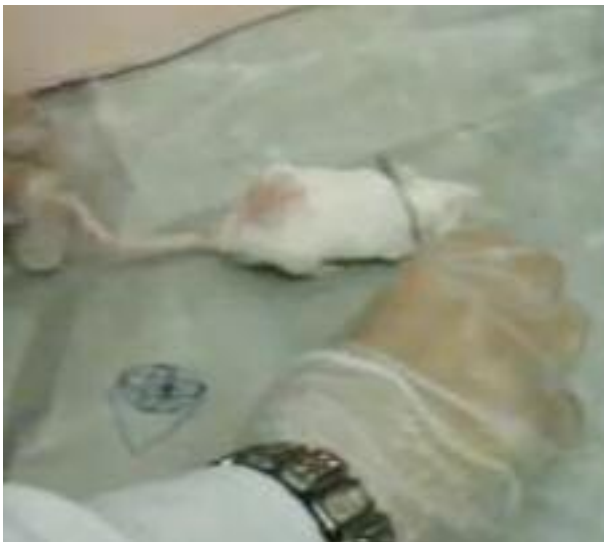
'No matter how similar our structural genes may be, if they are regulated differently, we're looking at a whole new scenario. One simple analogy of this is to imagine two huge, complex and almost identical church organs side-by-side.

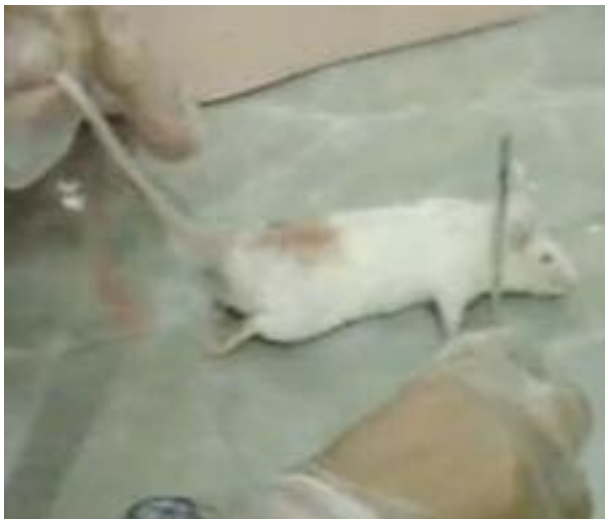
The hundreds of stops either side of the keyboards are the regulatory genes and regions of our DNA, able to exert subtle changes in the sound of the instruments individually but also able to act in countless combinations together to alter the sounds drastically. Even if the same music is played on both organs, the sound will be entirely different unless the stops (i.e. the regulatory genes and regions) are in identical positions. Change the order and timing with which the keys are operated, and the end products are completely unrecognisable from one another.'[105]

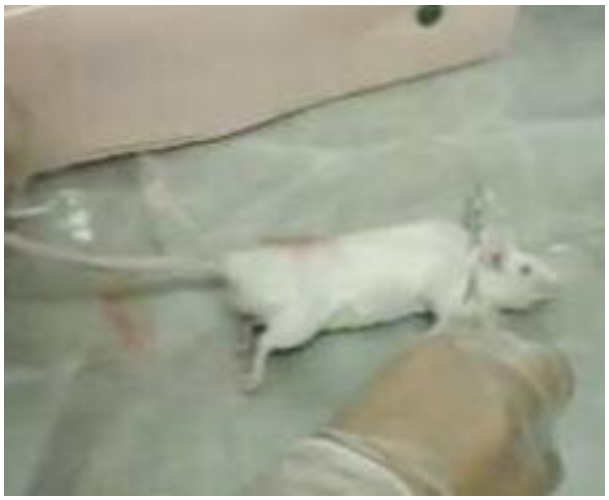
It follows that altering the genetic make-up of any organism, including mice, may lead to completely unforeseen consequences. In some cases, these may be dramatic and fatal. In others, the results may be too subtle to be picked up by crude laboratory animal studies, but still be of vital importance. This comes back to evolutionary biology. A US geneticist commented with regard to mouse genome experiments: 'Survival in the laboratory for a generation or two is not the same as successful competition in the wild for millions of years... Darwinian selection is a tougher test.'[106] In other words, mice have regions of so-called junk DNA that are in fact essential but poorly understood. The same goes for humans, and these black holes of knowledge are not trivial issues.

'Personalised medicine' is nowadays a much used term. It is based on the concept that different people with the same disease may respond differently to drug treatments. What works in one patient population may not work in another. It is becoming clear that most human diseases with a major genetic component are influenced by many gene products. Only in exceptional cases do researchers understand all the pathways that are involved. Nevertheless, most GM experiments to date have relied on simple, often single, gene deletions or insertions. This is un-illuminating reductionist science, and a poor way to make advances in human personalised medicine. A group of specialists on this issue commented in 2012:

'[I]n any given complex system, small changes in initial conditions can result in dramatically different outcomes. Despite human variability and intraspecies variation in other species, nonhuman species are still the primary model for ascertaining data for humans. We call this practice into question and conclude that human-based research should be the primary means for obtaining data about human diseases and responses to drugs.'[107]







Mouse being killed by having her neck broken

5. Misleading and inaccurate models of disease

In many cases, experimenters are trying to mimic in GM mice what they do not understand in humans. A classic – and tragic – example of this back-to-front process is epitomised by decades of Alzheimer's research (see below). There is still a poor understanding of the biological causes of the disease in people. However, this did not prevent researchers assuming that certain findings in Alzheimer's sufferers were critical, and then modelling them with GM mice. The mice were then used to develop many treatments for the disease, which all failed dismally. Similarly misleading animal models have been developed in other disease areas, including cancer and heart disease.

6. Curiosity-driven research with no benefits

Much animal experimentation is purely speculative, with the goal being 'the advancement of knowledge'. Unlike applied or 'translational' research, there is no legal requirement under the licence conditions for there to be any relevance to alleviating human or animal suffering or disease. Experiments of this nature very seldom lead to medical benefits. It has been shown that only around 0.004 per cent of the publications in high ranking journals result in a new class of drugs.[108] It should be noted that GM mice are the most frequently used animal for basic research.

Pro-animal experiments lobby groups constantly emphasise the value of mice to human medicine. However, in the UK in 2011, nearly three times as many procedures (more than 864,000) were performed on mice for basic research, as compared with applied medicine or dentistry studies.[109] Of those procedures, nearly 475,000 used GM mice. The comparable figure for applied research was around 31,500 GM mouse procedures. Fifteen times more procedures were therefore performed on GM mice in curiosity-driven experiments.

One example is a ridiculous 2010 experiment funded by Cancer Research UK, in which researchers deleted a gene that they considered 'essential for life', and 'remarkably' found that the mice survived with 'unexpected and extraordinary phenotypes'.[110] Even in these days of supposed 'reduction and refinement', it is still considered acceptable to produce phenotypes expected to be lethal. Even more scandalously, researchers have admitted contriving connections to human disease in order to boost a grant or paper. They have also confessed to using 'creative ways of selling their research as potentially having a rapid clinical application'.[111] It is likely, therefore, that the number of genuinely 'applied' GM procedures was even lower than suggested by the official statistics.

The failure to translate – mouse experiments that fail patients

It should not be necessary to state that patients view any benefits from research in terms of treatment success or otherwise. People suffering from painful, disabling or potentially lethal conditions are not as a whole interested in whether researchers have added to their databanks of knowledge. If such knowledge is not clinically relevant – or if it is misleading – then the only concrete beneficiaries are those whose core objective is to conduct animal experiments. The analysis that follows draws on commentaries from many different researchers and reviewers. It shows that GM mouse models, overall, have a very poor track record with regard to actually helping the sick.

Alzheimer's Disease

Perhaps the starkest example of the failure of the GM mouse project is research into Alzheimer's Disease. Enormous sums of both public and private cash have been spent 'modelling' what historically have been presumed to be key features causing the disease, namely amyloid plaques and tau protein tangles. The results have been a slew of ineffective drugs, disastrous clinical trials, and the dashing of the elevated hopes of hundreds of thousands of patients and their carers.

The disease that researchers produce in mice via genetic alterations is emphatically not the same as the human Alzheimer's, and is widely pilloried in the scientific literature as 'Mouseheimers' in recognition of this fact. A year on from a 2011 analysis[112] of the dire track record of GM mouse experiments, the situation has deteriorated further. According to a 2012 article in *New Scientist*, researchers are now looking for 'a new direction':

'The awful truth is sinking in: getting rid of the most obvious hallmarks of Alzheimer's Disease, the sticky plaques that clog up people's brains, isn't working. In August 2012, the two largest trials of treatments to attack plaques failed. In fact, between 1998 and 2011, 101 experimental treatments for Alzheimer's were scrapped, with only three drugs making it to market.'[113]

The animal researchers are not blind to the problems with their GM models. Their journals teem with critical commentary, of which the following is just a sample:

'Amyloid-beta accumulating in the brains of APP transgenic mice is neither physically, chemically or functionally equivalent to that characteristic of human AD. The APP transgenic mice


Mouse undergoing brain injury experiment

represent a reductionist approach to AD modeling in which massive overexpression of a single gene profoundly alters mouse physiology and behavior... interspecies differences in amyloid-beta toxicity might explain why recent therapeutic approaches work so consistently and dramatically in the mouse model but not nearly as effectively in AD patients'.[114]

'Primates and mice diverged about 85 million years ago and consequently exhibit vast differences over a wide range of fundamental attributes, including life span, and an age-dependent repression of broad-spectrum neuronal genes, a feature of humans and Rhesus macaques that is not replicated in mice, suggesting that the assumption of evolutionarily conserved biochemical equivalence between human and mouse aging is erroneous.'[115]

'A possible failure of a drug in clinical settings is often interpreted as the failure of the basic hypothesis on which the target for the drug was selected, rather than the failure of the animal models in which the drug was active. Several essential neurochemical differences between, for example, rodents and men might hinder a successful clinical development of a candidate drug; for example, (i) the different pharmacology of the same drug for rodent versus human target subtypes; (ii) the different wiring of specific neurotransmitter circuits in rodent versus human brain; and (iii) the difference in drug metabolism which makes it difficult to simulate the human drug exposure'.[116]

However, despite these extensive biological and evolutionary differences, all the failed Alzheimer's drugs were deemed powerfully effective in so-called 'validated' animal models, not purely in terms of amyloid removal but also with regard to cognitive improvements. The drug-treated mice performed better in facile tests supposedly relevant to human disease, such as swimming in tanks or navigating mazes. It appears that GM 'Mouseheimers' (rather like GM mouse cancer) can be treated successfully over and over again. This is cold comfort to those seeking meaningful medical progress.

Professor Lawrence Hansen, a distinguished specialist in geriatric neuropathology, has recently spoken out on this issue: 'Setting aside the ethical dimensions (which we should never do) of inflicting pain and suffering on any animal, even mice and rats, the amoral scientific problem with using rodents as models for neurodegenerative diseases is that rodents do not naturally develop Alzheimer's Disease or Parkinson's Disease. The only way to get what looks even a little like AD or PD pathology in rats and mice is to make them transgenic – that is, to insert human disease-causing genes into the rodents. This does create a Frankenstein-like mutant model with some expression of AD or PD pathology, which can be manipulated to make it go away. But reversing artificially induced AD or PD changes in animals that never naturally develop them, is a far cry from curing the human diseases. The "cures" that work in the rodents have never worked when applied to humans... The species differences that have evolved over millions of years make animal models largely useless, except for the purposes of enhancing scientific careers and attracting lots of grant money.'[117]

A mouse falls off a rotating drum in 'performance' test.

Cardiovascular disease

The picture with regard to the most common cause of death in the UK, cardiovascular disease, is not much better. The familiar tendency of researchers to praise GM mouse models for basic research discoveries, whilst lamenting their poor translation to the clinic, is much in evidence. In terms of new treatments for patients, the situation has not altered significantly from that in 2007, when the following was written by two US researchers:

'However, despite these impressive advances [in basic research] using the mouse models and the many conferences trumpeting their imminent translation into clinical practice, these studies generally have not yet resulted in significant changes in clinical practice.'[118]

The explanations for this lack of medical progress are by now familiar ones: 'transgenesis can often lead to developmentally inappropriate expression or to very high expression levels of a protein that is normally present in very low amounts, resulting in side reactions and artifactual physiological responses [i.e. that would not naturally occur] that are fundamentally misleading... The devil lies in the details, and the details have been and are often overlooked in the first rush to study all of the fascinating phenotypes.'[119]

In other words, the researchers are so dazzled by their GM toys that they are neglecting the fundamentals. In fact, profound differences exist between mice and humans, even before any genetic modifications. Mouse heart muscle is biochemically different from that of humans, and mice have different cardiovascular physiology. A mouse heart beats about 600 times per minute, compared with the human average of 72. The effect of genetic changes can be to amplify such differences, or create new ones. The over-expression of various genes can lead to 'highly artificial physiologic conditions that may not provide clinically relevant information'.[120] A team of French researchers commented in 2007 that the GM mouse 'may not be the most relevant model for directly extrapolating human clinical disease, especially because of the high heart rate, low cardiac mass and differences in the expression and distribution of gap junctions and ionic channels [structures concerned with the electrical activity of heart muscle]'.[121]

Two US heart researchers pointed out in 2004 that 'a number of nonsurgical mouse models of heart failure utilize a genetic "lesion" [transgene or gene-targeting event] without a natural analogue in typical human disease... Moreover, the mouse and human differ for a number of more obvious reasons such

as overall size, lifespan, circulatory physiology, and pharmacological response heterogeneity'.[122] Nonetheless, they still felt able to praise the GM mouse as a useful model for basic research and for 'identifying novel therapeutic strategies given the ease and relative speed of performing genetic manipulations'.

This primacy given to adventurism and curiosity, at the expense of good medicine, has led to wasted resources and failed treatments. Clinical trials in the decade up to 2010 have produced no 'novel therapeutics' for acute heart failure which could demonstrate 'a consistent benefit on in-hospital and/or post-discharge survival or in readmissions compared to placebo or conventional therapies. Moreover, the only two approved drugs for the treatment of AHFS [acute heart failure syndrome] have had serious safety concerns.'[123]

Many experimental chronic heart failure treatments, successful in GM mice, do not work in humans. For example, mice can be engineered to overproduce a chemical suspected to worsen heart failure (TNF-alpha), and suffer enlarged, baggy hearts and premature death. Some of them showed improvements in their cardiac function when treated with a drug that blocked TNF-alpha receptors. A human drug trial using the same substance failed. In other heart-failure mice, antioxidants were shown to improve cardiac function, but 'to date no antioxidant strategy has translated to a therapeutic in the heart failure clinic'.[124]

Especially worrying for cardiac patients is the fact that some drugs that affect the cardiac rhythm, developed and safety tested using GM mice, could prove fatal when trialled in people. In 2011, a researcher from Washington University who specialised in heart physiology showed that a drug target that looked promising in knockout mice would not work in humans. Two drugs that had been studied in mice in 2010 were tested on human hearts in 2011. One was found to work on completely different areas of the heart in the two species, and the other would have caused fatal heart rhythm disturbances in people.[125]

The researcher was quoted as saying 'the difference in gene expression between the mouse and the human is very very large... You can mutate in mice the gene thought to cause heart failure in humans and you don't get the same disease, because the mouse is so different... So, unfortunately, even with the help of transgenic mice, very few results made it from the animal model to the clinic.' Rather than relying on this failed 'model', he has established connections with local institutions that supply his laboratory with human hearts (either removed with consent during transplants or otherwise ethically donated) to avoid the expense of failed clinical trials or putting patients at risk.

Atherosclerosis (hardening and narrowing of the arteries) is the most common killer in the developed world, and is responsible for the majority of heart attacks. Mice engineered to have high blood fats – hyperlipidemia – are today the most widely used models of human atherosclerosis. However, these GM animals are acknowledged by researchers to be poor surrogates for a complex disease. A group of US researchers stated in 2011:

'The accelerated atherogenesis in mice contrived to have hypercholesterolaemia requires cholesterol levels that far exceed those commonly encountered in the clinic, and does not reflect the chronic nature or complexity of the human disease... too often, the pharmaceutical or biotechnology sector adopts or abandons targets or strategies on the basis of uncritical acceptance of the results of animal studies. The recognition of animal preparations as "models" of human disease requires considerable scepticism. For example, atherosclerotic lesions in the commonly used genetically modified mice seldom develop plaque disruption with thrombosis – a mechanism that commonly complicates the human disease. Mouse studies generally focus on the aorta and proximal great vessels, whereas the most important clinical consequences of atherosclerosis in humans arise from lesions in the coronary, carotid and cerebral arteries'.[126]

Most patients with atherosclerosis are not treated before symptoms develop, usually due to plaque disruption (the fatty material clogging a vessel splits open, with consequent clot formation and blockage). The disease processes identified in GM mice may not even be important in the clinic, and the ones that are important are not present in the mice.

Cancer

Mice are by far the major animal victims of cancer research. Up until relatively recently, the standard animal model for both basic and applied cancer research was the mouse xenograft. In this approach, cells from human tumours are transplanted into mice, usually under the skin – a thoroughly artificial representation of the human disease. Furthermore, while the resultant cancer is sourced from human tissue, it is growing in a surrogate host. Mouse xenografts have been described as 'far from an ideal model for many reasons, including their notorious lack of predictive value for human response'.[127] GM mice have, therefore, been increasingly promoted as

superior successors to such poorly performing disease parodies.

A key deficiency of most mouse models to date, including GM ones, is the failure to induce metastasis – the spreading of cancer cells from the original site of the disease to other parts of the body. It is this progression that typically causes the most serious complications for human patients.

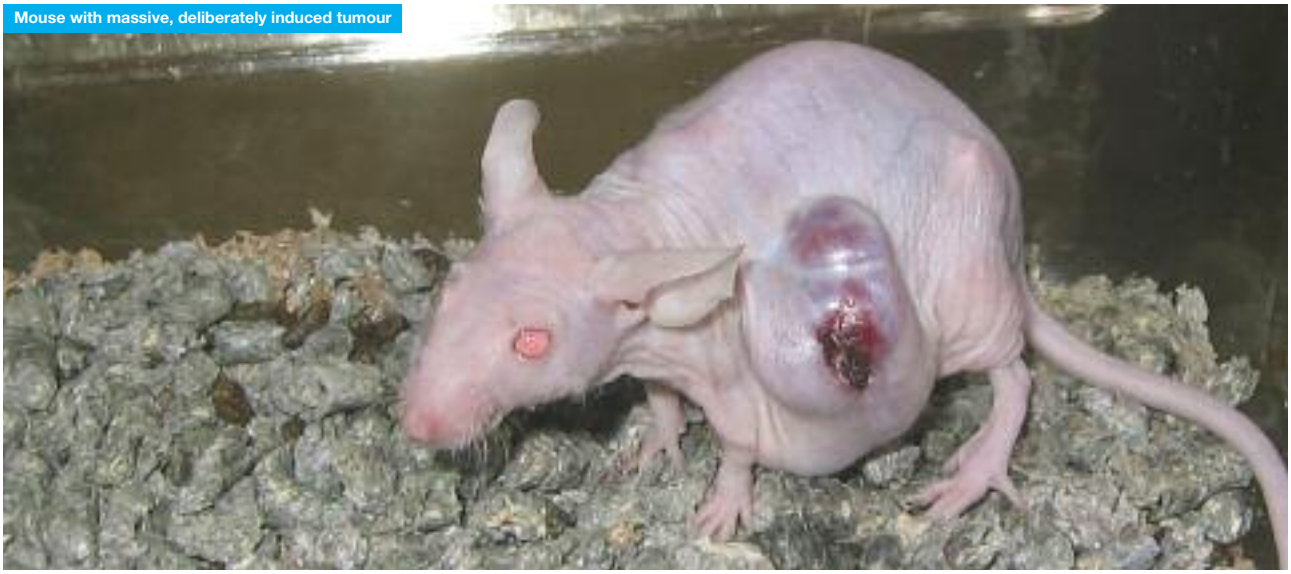
Whilst it is true that mouse genetic research was involved in the development of some contemporary cancer drugs, this does not mean that such work was essential. While some discoveries in mice will inevitably tally with those in people, this is to be expected given the sheer volume of animal experimentation. The few examples of correlation are much better described as luck than science. A 2011 editorial published in *Nature Reviews Clinical Oncology* bemoaned the extremely high attrition rates for cancer drugs: 'Only 5% of agents that have anticancer activity in preclinical development are licensed after demonstrating sufficient efficacy in phase III testing [large scale human clinical trials]... To compound this issue, many new cancer agents are being withdrawn, suspended or discontinued.'[128] Another 2011 *Nature Medicine* article quotes a US cancer researcher: 'We've been banging our heads against this cure thing for three, four decades now and really made almost zero progress.'[129]

The failure rate of cancer drugs in large-scale human trials is higher than in any other therapeutic area. A 2010 review in *Nature Biotechnology*[130] points out how 'in non-small cell lung cancer, for example, with the exception of a bevacizumab (Avastin) trial, every one of over a dozen phase 3 trials combining a "targeted" biologic agent with standard chemotherapy used for first-line treatment has failed to provide an overall survival benefit'. The review states that one source of this 'dismal' performance is 'preclinical studies with animal models and, in particular, with genetically engineered mouse models'. The authors point out that these models are partially to blame for 'exposing thousands of people to ineffective therapies' and contributing substantially to the high cost of most newly approved anti-cancer drugs. (Each failure has been estimated to cost on average $1.7 billion.)

The account above illustrates the case that mice are poor models of human cancer. There are fundamental differences between species, with the life spans of laboratory mice 'simply not long enough for the full chain of events that includes tumor initiation, interplay between the tumor and the environment, initial response and ultimate resistance to therapy, and development of long-term side effects'.[131] Most human malignancies are far more common in older people, due to multiple 'hits' on their DNA by cancer-causing agents. In addition, the immune response to candidate therapies is different in mice and humans, and the small size of mice means that injections of test substances into their tumours may affect relatively larger proportions of host organs. Even the needle wound can affect how the cancer subsequently behaves.[132]

GM mouse cancer models have not been shown to be superior to mouse xenografts. So far, they have proved eminently suitable for cruel tinkering, but far less useful in

Mouse with massive, deliberately induced tumour



bringing cures or relief to patients. A 2012 review of GM mice by leading US cancer researchers points out that 'despite much enthusiasm generated during the early years of the new millennium, their use in drug discovery and development has remained limited'.[133]

The problems with GM mice mean that this situation is unlikely to change for the following reasons:

- Animal models are not good at replicating advanced cancer, particularly metastasis. This often makes them clinically unhelpful, and in some cases essentially valueless in treatment development. For example, the clinical problems associated with prostate cancer are largely restricted to its dissemination throughout the body.

- Transgenic mice develop cancer through the expression of 'foreign' inserted genes, which means 'disease evolution is unlikely to be similar to that of their human counterpart'.[134] The introduced gene can also employ artificial gene promoters (DNA segments that regulate how genes work), which themselves influence how the resultant cancers originate, progress and spread.

- Evolutionary biology has ensured that the process of cancer development is very different between humans and mice. It has been shown that mouse cells require far fewer genetic alterations to transform into cancer, and that tumour progression is far simpler than the comparable processes in humans. The evidence is that 'most of the anticancer protective mechanisms that are present in human cells must have been developed, or at least perfected, during the time since our evolutionary lineage diverged from that of rodents'.[135]

- Perhaps the most fundamental problem lies with over-simplistic models, which involve turning off biological pathways (or key chemical reactions). In fact, cancers are usually caused by multiple mutations in co-existent cells, and are critically dependent on a highly individualised cellular environment. Many researchers are now coming to terms with the fact that human cancers are far more complex in their behaviour and genetic make-up than was previously thought. In 2010, US scientists discovered a staggering 1,700 gene mutations in the cancer genomes of just 50 breast cancer patients. Most were unique to individual patients' tumours, and only three occurred in 10 per cent or more of the cancers studied.[136]

More and more discoveries like this are being made. A 2012 review by Cancer Research UK scientists commented that 'the sequencing of increasingly larger numbers of cancer genomes has revealed extraordinary complexity, including the presence of thousands of genetic alterations and considerable genetic heterogeneity [differences], not only between different tumours but also within an individual cancer'.[137]

Such complexity is not reproducible in animals, despite researchers' attempts to 'humanise' them with even multiple genetic alterations. An American cancer biologist commented in 2008 with reference to GM mice: 'If one wants to know whether a patient's tumor will respond to a specific therapeutic regime, one must examine the response of that human tumor, not a mouse tumor, to the therapy.'[138]

Given the poor record of animal research to bring forward cancer treatments, researchers have been busy reframing the markers for success. Progress is now often measured by 'insights into cancer biology', or to show 'proof of concept' in animal models. However, the scientific literature is inundated with such insights or proofs, with little to show for the information overload.

Overall, the story of animal cancer research illustrates a tendency – depressingly common amongst experimenters – to champion the merits of the 'animal model' in use at the time, only to disparage it when a 'better' version or approach is developed:

'The use of genetically engineered cancer-prone mice as relevant surrogates for patients during the development of pertinent clinical applications is an unproven expectation that awaits direct demonstration. Despite the generally disappointing findings using tumor xenografts and certain early transgenic cancer models to predict therapeutic efficacy in patients, the dramatic progress of mouse models in recent years engenders optimism that the newest generation of mouse models will provide a higher standard of predictive utility in the process of drug development.'[139]

If history is to act as a guide, then such optimism about animal models is misplaced. However, the drive to do basic research is deeply embedded amongst cancer researchers. As one noted, 'The rodent biology will be internally consistent, informative, and fascinating in its own right – and yet may not mimic the biology of human patients.'[140] Cancer sufferers could be forgiven for being less fascinated by failure.

Respiratory disease

The use of GM mice, true to form, is leading to treatment failures for patients with respiratory illness. Chronic Obstructive Pulmonary Disease (COPD, also known as emphysema) is a serious and disabling lung condition usually caused by smoking. None of the current COPD therapies reduce disease progression or significantly prevent exacerbations. TNF-alpha is an inflammatory substance that is increased in the sputum of COPD patients. In an attempt to discover disease-modifying drugs, knockout mice were engineered to be less sensitive to TNF-alpha. They were then forced to inhale cigarette smoke long-term. Compared with their wild-type counterparts, they developed less of the sort of lung damage commonly found in COPD patients. This led researchers to conclude that inhibiting TNF-alpha production could work in COPD. However, the drugs designed to do so were ineffective in patients. This led a drug company-funded researcher to conclude: 'The failure of anti-TNF in patients with COPD also questions the value of animal models in predicting useful therapies, since inhibiting TNF-alpha has a marked inhibitory effect on experimental emphysema and inflammation induced by cigarette smoke.'[141]

A recent article on GM mouse models of COPD in the *European Respiratory Journal* suggests why such failures occur.[142] Even though mouse lungs bear a superficial resemblance to those of humans, they crucially lack respiratory bronchioles. These are the tiny airways where the form of emphysema associated with smoking begins, and where inflammation is concentrated.

Neither are GM models helping patients with asthma. An NC3Rs-led review from 2011 points out how asthma is an area of unmet medical need, and that 'few new drugs have made it to the clinic during the past 50 years, with many that perform well in preclinical animal models of asthma failing in humans owing to lack of safety and efficacy'.[143] The authors point out that GM mice have been used extensively in asthma research, with questionable usefulness for 'studying a disease that is associated with several molecular and cellular pathways that function synergistically or independently of each other... it is still too early to assess whether they [GM models] are useful predictors of efficacy in humans, although initial results suggest the data should be treated with some caution'.

The need for wider public scrutiny

The above account represents only an overview of the ways in which GM mouse models have hindered and misled medical progress. Several major disease areas have been examined in this report but, in many more, there exist similar doubts about the ability of these models to deliver useful therapies to the bedside and clinic. Patients should be braced for more disappointments, given the continued faith being placed in GM animal experiments. The criticism that their proponents have served to divert funds from more promising and more humane methods by which to investigate and ameliorate human diseases is not trivial. Nor are the examples of cruelty meted out to the victims of this expanding enterprise. These issues merit far wider public scrutiny and engagement, and it is this topic to which this report now turns by way of conclusion.

Researchers time how long a mouse takes to stand up, having just suffered severe brain damage. Many mice die outright from these procedures.

'… the lobbyists went even further in their desire to hide GM breeding programmes from the public, proposing their complete removal from Home Office returns…'

SECTION SEVEN The Industry Campaign to 'Disappear' Millions of GM Mice

This report has shown that the suffering endured by millions of mice, before, during and after they are genetically altered, is the largest single area of laboratory animal cruelty in the UK today. Whilst many other species are still forced to endure torments at the hands of experimenters, public opprobrium directed at experiments on cats, dogs and monkeys has undoubtedly led to a reduction in their use in laboratories.

The same cannot yet be said for the humble *Mus Musculus*. The breeding of GM mice now accounts for the largest proportion of the rising number of procedures carried out in the UK. However, this (by now familiar) annual increase is not greeted warmly in many quarters. In response, animal experimentation lobby groups want GM breeding programmes expunged from the official figures. The 2012 revision of the UK's 1986 animal protection laws has given them a way to do just that.

The push for deregulation

A powerful coalition of vested interests claimed in 2011 that 'the breeding of established colonies of GM animals (beyond two generations) with "non-harmful phenotypes" should be treated as any other breeding colony and discharged from the controls of the new Act' (see below).[144] This would mean that even the moderate legal protection currently afforded these animals would disappear. The coalition went even further in its desire to hide GM breeding programmes from the public, proposing their complete removal from Home Office returns.

GM animals with 'non-harmful phenotypes' have always, in theory, been 'dischargeable' from Home Office scrutiny on a case-by-case basis. Under the 1986 Act, animal lines had to be 'demonstrably not predisposed to pain, suffering, distress or lasting harm'. This required evidence of at least two generations of the animals 'living a normal lifespan and displaying no welfare problems attributable to their phenotype'. According to the government's Animal Protection Committee, the lack of an acceptable agreed welfare screening protocol was one reason why GM animals were not being released from the modest legal safeguards afforded by the 1986 Act.[145]

However, this situation could be about to change dramatically. The new EU Directive has now been 'transposed' into UK law, thereby amending and renewing the UK Animals (Scientific Procedures) Act 1986. An endorsed consensus document on Genetically Altered (GA) animals was agreed in March 2012 at an EU meeting in Brussels. It made explicit that 'the use of animals for the maintenance of colonies of genetically altered established lines without a likely harmful phenotype is not considered a procedure and thus does not require a project authorisation'.[146] A new GA rodent welfare assessment will form a key part in deciding whether or not animals are suffering harm. The assessment lists numerous distressing outcomes of genetic alteration including skeletal deformities, a hunched posture and reluctance to move, hyperactivity, increased respiratory rate, seizures and death.

The Home Office has indicated that all animals exhibiting any such symptoms will fall within the legal safeguards. But, time and resource pressure, and the desire of establishments to discharge as many animals as possible from legal controls, may act to fatally undermine the entire process.

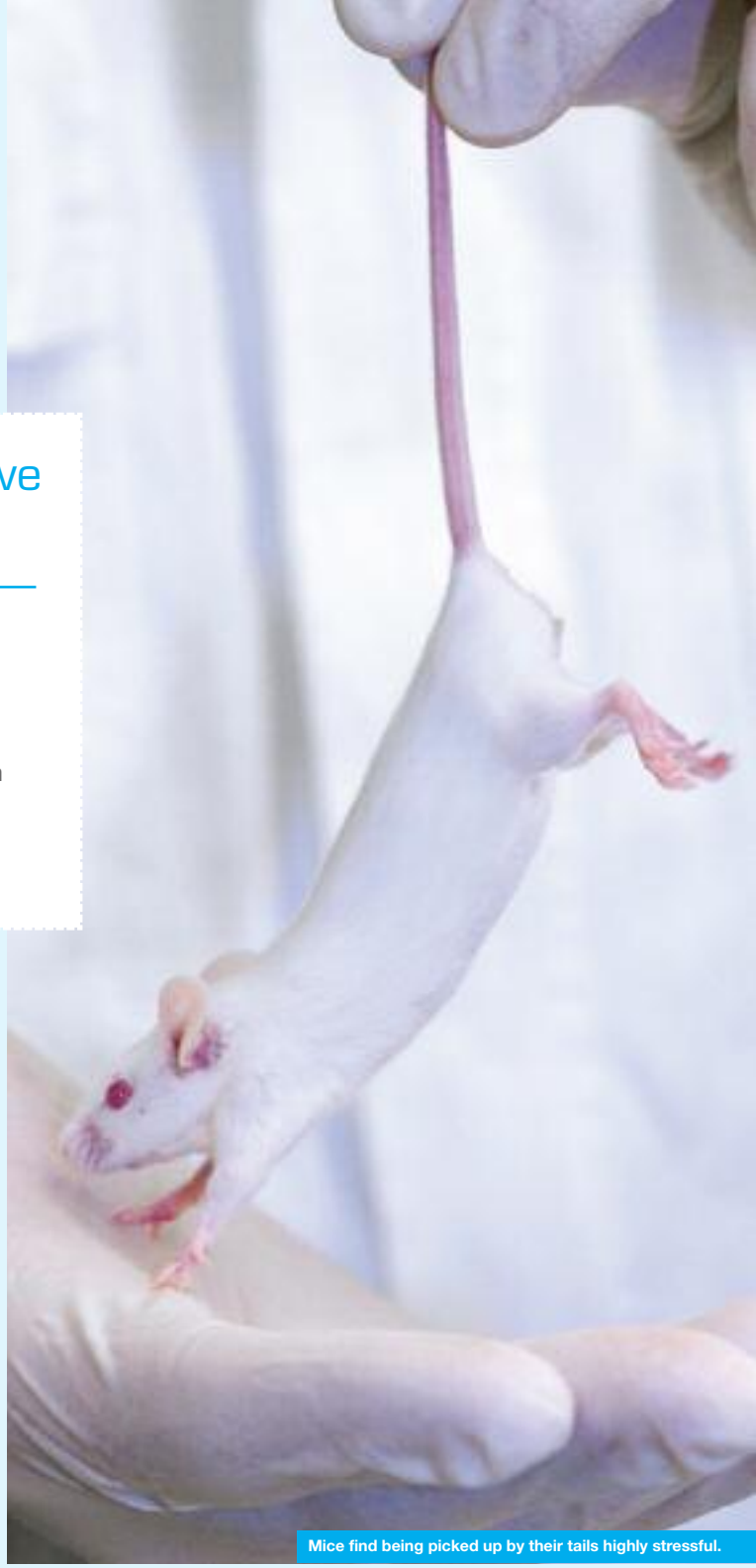
It is because a proper phenotypic assessment is costly and time-consuming, that a widely cited Danish study from 2003 reported: '...it is likely that potential welfare problems in phenotypically uncharacterised strains remain undetected'.[147]

Although both old and new UK legislation mandates two generations of mice for observation, this is likely to miss even major health problems. It is well known that the effects of genetic modification can be very subtle and difficult to recognise. In addition, a study into the genetic modification of livestock concluded that 'accurate assessment of the consequences of transgene expression is impossible without multigenerational studies'.[148] The first two generations of transgenic pigs modified with a gene for growth hormone had no ill effects. However, the third generation suffered kidney disease, enlarged hearts, arthritis, gastric ulcers and infertility.

'… their full capacity to experience pain and mental anguish is acknowledged by all who have contact with them, from laboratory technicians to animal campaigners…'

CONCLUSION A Cruel, Expensive and Wasteful Enterprise

For far too long, mice have been almost the acceptable victims of animal experiments. For far too long, the public has been lulled into a feeling that mice do not really matter, that their suffering is less relevant or more tolerable than that of larger mammals. For far too long, an almost feverish urge to mutate and create novel mice has been held aloft as clever science, as cutting edge technology, and as medically laudable.



Mice find being picked up by their tails highly stressful.

These ideas are not supported by reality. Mice are highly developed, sophisticated small mammals. Their full capacity to experience pain and mental anguish is acknowledged by all who have contact with them, from laboratory technicians to animal campaigners.

The genetic modification of mice is, on the available evidence, proving to be a hugely expensive, painfully cruel and tragically wasteful enterprise. Genetic tampering with a species markedly different from humans, in often highly speculative and sometimes totally random research, would seem doomed from the start to produce little of medical benefit. But, as the history of mice in laboratories illustrates so well, considerations such as convenience and cost, rather than a wisely planned blueprint for better healthcare, have led to this contemporary debacle.

A late 2012 survey of public opinion demonstrated that support for animal experiments is now on the wane in the UK.[149] In response, 41 UK institutions signed a highly publicised declaration to be more open about their animal research. Given the multiple and decisive legal impediments to obtaining information in this area, it is hard to view such initiatives as anything more than a public relations exercise. The new drive towards 'openness' will doubtless present a sanitised picture of mice in laboratories, with brain damage, smoke chambers, gassing and seizures all firmly off limits.

Statistical trickery and tokenistic transparency are givens in the emerging battle for the mice. But perhaps the most frequent technique of victim denial is the 'sizeism' identified by the RSPCA. An article in the *British Medical Journal* in October 2012 describes how David Willetts, the Minister for Universities and Science, claimed that a concerned public was not aware that 'things had moved on'.[150] In support of this supposed progress, the Minister was quoted as saying 'the vast majority of what we are talking about is mice and fish'. It is clear that both government and the bioscience lobby wish to redefine the discourse, and win back lost support for animal experimentation. Much will depend on whether critics of animal suffering can mobilise a vocal constituency to speak up for GM mice.

REFERENCES

1 RSPCA (2011). *Mice: Good Practice for Housing and Care: Supplementary Resources for Members of Local Ethical Review Processes*. RSPCA Research Animals Department

2 Balcombe JP (2006). Laboratory environments and rodents' behavioural needs: a review. *Lab Anim*. 40(3):217-35

3 Viegas J (2010). Male mice sing ultrasonic love songs. *Discovery News* [online], 5 Mar. Available from http://news.discovery.com/animals/mouse-courtship-songs-ultrasonic.html [Accessed November 2012]

4 Langford D J, Crager S E, Shehzad Z, et al (2006). Social Modulation of Pain as Evidence for Empathy in Mice. *Science*. 312:1860-1861

5 Chen Q, Panksepp J B, Lahvis G P (2009). Empathy Is Moderated by Genetic Background in Mice. *PLOS ONE* [online]. Available from http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0004387 [Accessed November 2012]

6 Bailey J (2005). *Man or Mouse – Genetically Modified Animals in Medical Research*. Animal Aid

7 Morse H C (1978). *Origins of Inbred Mice*. Academic Press. Available from http://www.informatics.jax.org/morsebook/chapters/morse1.shtml [Accessed November 2012]

8 *Springfield Sunday Republican* (1913), 5 Oct, p12

9 Wolf G (2010). How the Inbred Lab Mouse Helps Reprogram the Human Genome, *Wired* [online], 22 Feb. Available from http://www.wired.com/magazine/2010/02/ff_lab_mouse/all/ [Accessed November 2012]

10 Stern A M (2005). Sterilized in the Name of Public Health. *Am J Public Health*. 95(7): 1128–1138

11 Wolf G (2010). How the Inbred Lab Mouse Helps Reprogram the Human Genome, *Wired* [online], 22 Feb. Available from http://www.wired.com/magazine/2010/02/ff_lab_mouse/all/ [Accessed November 2012]

12 Hanahan D, Wagner E F, Palmiter R D (2007). The origins of oncomice: a history of the first transgenic mice genetically engineered to develop cancer. *Genes Dev*. 21: 2258-2270

13 National Human Genome Research Institution (2012). *An Overview of the Human Genome Project* [online]. Available from http://www.genome.gov/12011238 [Accessed November 2012]

14 National Human Genome Research Institution (2010). *The Human Genome Project Completion: Frequently Asked Questions* [online]. Available from http://www.genome.gov/11006943 [Accessed November 2012]

15 Wellcome Trust Sanger Institute (2011). *Celebrating a 'decade of discovery' since the Human Genome Project* [online]. Available from http://www.sanger.ac.uk/about/press/2010/100624-hgp.html [Accessed November 2012]

16 Nolan P M, Peters J, Strivens M et al (2000). A systematic, genome-wide, phenotype-driven mutagenesis programme for gene function studies in the mouse. *Nat Genet*. 25(4):440-3

17 International Mouse Knockout Consortium (2012), *IKMC Gene Progress Summary* [online]. Available from http://www.knockoutmouse.org/ [Accessed November 2012]

18 Bradley A (2002). Mining the mouse genome. *Nature*. 420:512-514

19 Ibid

20 Dolgin E (2011). Mouse library set to be knockout. *Nature* [online]. 15 Jun. Available from http://www.nature.com/news/2011/110615/full/474262a.html [Accessed November 2012]

21 International Mouse Phenotyping Consortium (2010). *Concise Business Plan* [online]. Available from http://commonfund.nih.gov/pdf/IMPC_Business_Plan.PDF [Accessed November 2012]

22 Abbott A (2004). Geneticists prepare for deluge of mutant mice. *Nature*. 423:541

23 Home Office (2012). *Statistics of Scientific Procedures on Living Animals Great Britain 2011*. London: The Stationery Office

24 Ibid

25 Charles River (2011). *Charles River Announces Agreement to Supply Pfizer's Genetically Modified Research Models* [online]. Available from http://www.criver.com/en-us/newsevents/pressreleases/pages/pfizer.aspx [Accessed November 2012]

26 McKie R (2012). Animal activists' terror tactics drive staff out of laboratories. *The Observer* [online], 29 July. Available from http://www.guardian.co.uk/science/2012/jul/29/animal-activists-terror-tactics-harlan-laboratories-staff [Accessed November 2012]

27 Coghlan A (1999). Hidden Sacrifice. *New Scientist*.162(2):4

28 Wight D C, Wagner T E (1994). Transgenic mice: a decade of progress in technology and research. *Mutation Research*. 307:429-440

29 Lane N, Jennings M (2004). *Supplementary Resources for Lay Members of Local Ethical Review Processes: Projects Involving Genetically Modified Animals*. RSPCA Research Animals Department

30 The Jackson Laboratory (2009). *Breeding Strategies for Maintaining Colonies of Laboratory Mice: A Jackson Laboratory Resource Manual* [online]. Available from http://knockout.cwru.edu/info/breeding_strategies_manual.pdf [Accessed November 2012]

31 Ibid

32 Robinson V, Morton D B, Anderson D et al (2003). Refinement and reduction in production of genetically modified mice. Sixth report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. *Laboratory Animals* 37(Suppl 1):1–51

33 Hawkins P (2002). *Recognising and assessing pain, suffering and distress in laboratory animals: A survey of current practice in the UK with recommendations*. RSPCA Research Animals Department.

34 Ibid

35 Robinson V, Morton D B, Anderson D et al (2003). Refinement and reduction in production of genetically modified mice. Sixth report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. *Laboratory Animals*. 37(Suppl 1): 1–51

36 Hawkins P, Felton L M, van Loo P et al (2006). Report of the 2005 RSPCA/UFAW Rodent Welfare Group meeting. *Lab Anima*l 35(9):29

37 Ibid

38 UK Bioscience Sector Coalition (2011). *UK Bioscience Sector Coalition response to the Home Office Consultation on the Transposition of European Directive 2010/63/EU* [online]. Available from http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC008195 [Accessed November 2012]

39 Robinson V, Morton D B, Anderson D et al (2003). Refinement and reduction in production of genetically modified mice. Sixth report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. *Laboratory Animals* 37(Suppl 1): 1–51

40 Ibid

41 Lane N, Jennings M (2004). *Supplementary Resources for Lay Members of Local Ethical Review Processes: Projects Involving Genetically Modified Animals*. RSPCA Research Animals Department

42 Animal Defenders International (2012) *Inside a GM animal factory* [online]. Available from http://www.ad-international.org/publications/go.php?id=2751 [Accessed November 2012]

43 National Anti-Vivisection Society (1996). *Access Denied: A report on animal experiments in two British laboratories: Charing Cross & Westminster Medical School, London, W6 and The Institute of Neurology, London, WC1*. Available from http://www.navs.org.uk/about_vivisection/27/42/336/

44 Robinson V, Morton D B, Anderson D et al (2003). Refinement and reduction in production of genetically modified mice. Sixth report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. *Laboratory Animals*. 37(Suppl 1): 1–51

45 Thon R, Lassen J, Hansen A K et al (2002). Welfare evaluation of genetically modified mice. *Scandinavian Journal for Laboratory Animal Science*. 29, 45-53

46 Patten R D, Hall-Porter M R (2009). Small Animal Models of Heart Failure: Development of Novel Therapies, Past and Present. *Circ Heart Fail*. 2:138-144

47 Smith L B, Hadoke P W F, Dyer E et al (2011). Haploinsufficiency of the murine *Col3a1* locus causes aortic dissection: a novel model of the vascular type of Ehlers–Danlos syndrome. Cardi*ovasc Res*. 90(1):182-190

48 Butler A A, Cone R D (2002). The melanocortin receptors: Lessons from knockout models. *Neuropeptides*. 36(2-3):77-84

49 Workman P, Aboagye E O, Balkwill F et al (2010). Guidelines for the welfare and use of animals in cancer research. *British Journal of Cancer*. 102:1555–1577

50 Roughan J V (2010). Assessing the welfare of mice used in cancer research. *The National Centre for the Replacement, Refinement and Reduction of Animals in Research* [online]. Available from http://www.nc3rs.org.uk/researchportfolio/showcatportfolio.asp?id=187 [Accessed November 2012]

51 Hoyle G W, Li J, Finkelstein J B et al. (1999). Emphysematous Lesions, Inflammation, and Fibrosis in the Lungs of Transgenic Mice Over expressing Platelet-Derived Growth Factor. *American Journal of Pathology*. 154 (6):1763-1775

52 Zhou Y, Rideout W M, Zi T et al (2010). Chimeric mouse tumor models reveal differences in pathway activation between ERBB family- and KRAS-dependent lung adenocarcinomas. *Nature Biotechnology*. 28(1):71-79

53 Smolinsky A N, Bergner C L, Hart P C et al (2010). The Utility of Genetically Modified Animals in Modeling OCD-Spectrum Disorders. In: Kalueff A V, Bergner C L, eds. *Transgenic and Mutant Tools to Model Brain Disorders (Neuromethods)* 1st ed. US: Humana Press, 139-149

54 Ibid

55 Ibid

56 Ibid

57 Egan R J, Bergner C L, Hart P C et al (2010). Genetic Animal Models of Anxiety. In: Kalueff A V, Bergner C L, eds. *Transgenic and Mutant Tools to Model Brain Disorders (Neuromethods)* 1st ed. US: Humana Press,179-189

58 Errington A C, Gibson K M, Crunelli V et al (2011). Aberrant GABAA Receptor-Mediated Inhibition in Cortico-Thalamic Networks of Succinic Semialdehyde Dehydrogenase Deficient Mice. *PLOS ONE*. 6(4): e19021. doi:10.1371/journal.pone.0019021

59 Zeng L H, Rensing N R, Zhang B et al (2011). Tsc2 gene inactivation causes a more severe epilepsy phenotype than Tsc1 inactivation in a mouse model of Tuberous Sclerosis Complex. Hum. Mol. Genet. 20 (3):445-454

60 Nuffield Council on Bioethics (2005). *The ethics of research using animals*. Available from http://www.nuffieldbioethics.org/animal-research

61 Animal Defenders International (2012). *Inside a GM animal factory* [online]. Available from http://www.ad-international.org/publications/go.php?id=2751 [Accessed November 2012]

62 Robinson V, Morton D B, Anderson D et al (2003). Refinement and reduction in production of genetically modified mice. Sixth report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. *Laboratory Animals*. 37(Suppl 1): 1–51

63 Yutzey K E, Robbins J (2007). Principles of Genetic Murine Models for Cardiac Disease. *Circulation*.115:792-799

64 Vogel P, Read R W, Hansen G M et al (2012). Congenital Hydrocephalus in Genetically Engineered Mice. *Veterinary Pathology*. 49(1):166-181

65 Turgeon B, Meloche S (2009). Interpreting Neonatal Lethal Phenotypes in Mouse Mutants: Insights Into Gene Function and Human Diseases. *Physiol Rev*. 89: 1–26

66 Robinson V, Morton D B, Anderson D et al (2003). Refinement and reduction in production of genetically modified mice. Sixth report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. *Laboratory Animals*. 37(Suppl 1): 1–51

67 Justice M J, Carpenter D A, Favor J et al (2000). Effects of ENU dosage on mouse strains. *Mammalian Genome*. 11:484–488

68 Litscher E S, Liu C, Echelard Y et al (1999). Zona pellucida glycoprotein mZP3 produced in milk of transgenic mice is active as a sperm receptor, but can be lethal to newborns. *Transgenic Research*. 8: 361–369

69 Nelson R J, Chiavegatto S (2000). Aggression in Knockout Mice. *ILAR Journal*. 41(3) [online] Available from http://dels-old.nas.edu/ilar_n/ilarjournal/41_3/Aggression.shtml [Accessed November 2012]

70 GA mouse welfare assessment working group (2006). Assessing the welfare of genetically altered mice [online]. Available from http://www.nc3rs.org.uk/downloaddoc.asp?id=356&page=231&skin=0 [Accessed November 2012]

71 The Jackson Laboratory (2009). *Breeding Strategies for Maintaining Colonies of Laboratory Mice: A Jackson Laboratory Resource Manual* [online]. Available from http://knockout.cwru.edu/info/breeding_strategies_manual.pdf [Accessed November 2012]

72 The Jackson Laboratory (2012). *Jaxmice Database* [online]. Available from http://jaxmice.jax.org/strain/002515.html [Accessed November 2012]

73 The Jackson Laboratory (2012). *Jaxmice Database* [online]. Available from http://jaxmice.jax.org/strain/006176.html [Accessed November 2012]

74 Robinson V, Morton D B, Anderson D et al (2003). Refinement and reduction in production of genetically modified mice. Sixth report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. *Laboratory Animals* 37(Suppl 1):1–51

75 Justice M J, Carpenter D A, Favor J et al (2000). Effects of ENU dosage on mouse strains. *Mammalian Genome*. 11:484–488

76 Scullion G A, Coltman R, Fowler J, et al (2010). The influence of chronic cerebral hypoperfusion on Alzheimer-like pathology in 3XTg mice. *Alzheimer's & Dementia: The Journal of the Alzheimer's Association*. 6(4,Suppl):S414

77 Tanila H (2012). Wading pools, fading memories – place navigation in transgenic mouse models of Alzheimer's disease. *Frontiers in Aging Neuroscience*. 4:1-7

78 Paul-Clark M J, McMaster S K, Sorrentino R et al (2009). Toll-like Receptor 2 Is Essential for the Sensing of Oxidants during Inflammation. *Am J Respir Crit Care*.179:299–306

79 Shapiro S D (2000). Animal Models for Chronic Obstructive Pulmonary Disease: Age of Klotho and Marlboro Mice. *Am J Respir Cell Mol. Biol*. 22(1): 4-7

80 Schellings M W M, Vanhoutte D, Swinnen M et al (2008). Absence of SPARC results in increased cardiac rupture and dysfunction after acute myocardial infarction. *JEM*. 206(1):113-123

81 Lim S Y, Hausenloy D J, Arjun S et al (2011). Mitochondrial cyclophilin-D as a potential therapeutic target for post-myocardial infarction heart failure. *J Cell Mol Med*. 15(11): 2443–2451

82 Iida S, Baumbach G L, Lavoie J L et al (2005). Spontaneous Stroke in a Genetic Model of Hypertension in Mice. *Stroke*.36:1253-1258

83 Cryan J F, Holmes A (2005). The ascent of mouse: advances in modelling human depression and anxiety. *Nature Reviews Drug Discovery*.4:775-790

84 Ibid

85 Frankel W N, Taylor B A, Noebelst J L et al (1994). Genetic Epilepsy Model Derived From Common Inbred Mouse Strains. *Genetics*. 138:481-489

86 Huang Z, Walker M C, Shah M M (2009). Loss of Dendritic HCN1 Subunits Enhances Cortical Excitability and Epileptogenesis. *The Journal of Neuroscience*. 29(35):10979 –10988

87 Knight A (2011). *The Costs and Benefits of Animal Experiments*. Basingstoke, UK: Palgrave Macmillan

88 Balcombe J (2010). Laboratory-rodent Welfare: Thinking Outside the Cage. *Journal of Applied Animal Welfare Science*. 13(1)

89 Ibid

90 Balcombe JP (2006). Laboratory environments and rodents' behavioural needs: a review. *Lab Anim*. 40(3):217-35

91 Balcombe J (2010). Laboratory-rodent Welfare: Thinking Outside the Cage. *Journal of Applied Animal Welfare Science*. 13(1)

92 Robinson V, Morton D B, Anderson D et al (2003). Refinement and reduction in production of genetically modified mice. Sixth report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. *Laboratory Animals*. 37(Suppl 1): 1–51

93 Swallow J, Anderson D, Buckwell A C et al (2005). Guidance on the transport of laboratory animals: Report of the Transport Working Group established by the Laboratory Animal Science Association (LASA). *Laboratory Animals.* 39, 1–39.

94 Association for Assessment and Accreditation of Laboratory Animal Care International (2003). *AAALAC International Connection*.

95 National Anti-Vivisection Society (1996). *Access Denied: A report on animal experiments in two British laboratories: Charing Cross & Westminster Medical School, London, W6 and The Institute of Neurology, London, WC1*. Available from http://www.navs.org.uk/about_vivisection/27/42/336/

96 Home Office (2010-2012). *Animals in Science Regulation Unit, Annual Reports*, 2009-2011. London: The Home Office.

97 Bhogal N, Combes R (2006). The Relevance of Genetically Altered Mouse Models of Human Disease. *ATLA* 34: 429–454

98 Understanding Animal Research (2011). *Animal research in the UK: the numbers in perspective* [online]. Available from http://www.understandinganimalresearch.org.uk/assets/document/AC13C7F5-B48C-B33C-668872770F913A2A/2011%20Briefing%20on%20numbers%20of%20animals2.pdf [Accessed November 2012]

99 Wade N (2010). A decade later, genetic map yields few new cures. *The New York Times* [online], 12 Jun. Available from http://www.nytimes.com/2010/06/13/health/research/13genome.html?pagewanted=all [Accessed November 2012]

100 Winston R, as quoted in Whipple T (2012). Gene 'revolution' has stalled, says Winston. *The Times*, 9 Jun, p3

101 Mestas J, Hughes C C W (2004). Of Mice and Not Men: Differences between Mouse and Human Immunology. *J Immunol*. 172:2731-2738

102 Knight A (2011). *The Costs and Benefits of Animal Experiments*. Basingstoke, UK: Palgrave Macmillan

103 Ibid

104 Geddes L (2012). Unfathomable. *New Scientist.* 215 (2881):40-43

105 Bailey J (2005). *Man or Mouse – Genetically Modified Animals in Medical Research*. Animal Aid

106 Khamsi R (2004). Mice do fine without 'junk DNA'. *Nature News*. [online], 20 Oct. Available from http://www.nature.com/news/2004/041018/full/news041018-7.html [Accessed November 2012]

107 Greek R, Menache A, Rice M J (2012). Animal models in an age of personalized medicine. *Personalized Medicine* 9(1), 47–64

108 Crowley, W F Jr (2003). Translation of basic research into useful treatments: how often does it occur? *Am J Med*. 114(6):503-5

109 Home Office (2012). *Statistics of Scientific Procedures on Living Animals Great Britain 2011*. London: The Stationery Office

110 Finn R D, McLaughlin L A, Hughes C et al (2011). Cytochrome b5 null mouse: a new model for studying inherited skin disorders and the role of unsaturated fatty acids in normal homeostasis. *Transgenic Res*. 20:491–502

111 Greek R (2012). *Trouble in Basic Research Land*. Part II [online]. Available from http://www.opposingviews.com/i/society/animal-rights/trouble-basic-research-land-part-ii [Accessed November 2012]

112 Stallwood A, Menache A (2011). *Victims Of Charity. A report on the cruel and scientifically invalid experiments funded by medical research charities*. Animal Aid

113 Hamzelou J (2012). Why Alzheimer's hits older brains. *New Scientist*. 215 (2884):6-7

114 Kokjohn T A, Roher A E (2009). Amyloid precursor protein transgenic mouse models and Alzheimer's disease: Understanding the paradigms, limitations and contributions. *Alzheimers Dement*. 5(4):340–347.

115 Ibid

116 Van Dam D, De Deyn P P (2011). Animal models in the drug discovery pipeline for Alzheimer's disease. *British Journal of Pharmacology*. 164 1285–1300

117 Antidote Europe (2012). *Professor Larry Hansen on ethical dilemmas* [online]. Available from http://antidote-europe.org/interviews/professor-larry-hansen-on-ethical-dilemmas [Accessed November 2012]

118 Yutzey K E, Robbins J (2007). Principles of Genetic Murine Models for Cardiac Disease. *Circulation*.115:792-799

119 Ibid

120 Ramachandran A, Jha S, Lefer D J (2008). Pathophysiology of Myocardial Reperfusion Injury: The Role of Genetically Engineered Mouse Models. *Vet Pathol*. 45: 698

121 Thireau J, Zhang B L, Poisson D et al (2008). Heart rate variability in mice: a theoretical and practical guide. *Experimental Physiology*. 93: 83-94

122 Dorn G W, Molkentin J D (2004). Manipulating Cardiac Contractility in Heart Failure: Data From Mice and Men. *Circulation*.109: 150-158

123 Tamargo J, Amorós I, Barana A et al (2010). New Investigational Drugs for the Management of Acute Heart Failure Syndromes. *Current Medicinal Chemistry*. 17(1):1-28

124 Sawyer D B (2011) Oxidative stress in heart failure: what are we missing? *Am J Med Sci*. 342(2):120-4.

125 Lutz D (2011). New study calls into question reliance on animal models in cardiovascular research [online]. Available from http://news.wustl.edu/news/pages/22540.aspx [Accessed November 2012]

126 Libby P, Ridker P M, Hansson G K (2011). Progress and challenges in translating the biology of atherosclerosis. *Nature*. 473:317-325

127 Ebner R, Strovel J W, Horrigan S K et al (2011). Mighty, but how useful? The emerging role of genetically engineered mice in cancer drug discovery and development. In: Green J E, Ried T, eds. *Genetically Engineered Mice for Cancer Research*. 1st ed. New York: Springer, 591-618

128 Hutchinson L, Kirk R (2011). High drug attrition rates – where are we going wrong? *Nature Reviews Clinical Oncology*. 8:189 -190

129 Drake N (2011). Forty years on from Nixon's war, cancer research 'evolves'. *Nature Medicine*. 17:57

130 Francia G, Kerbel R S (2010). Raising the bar for cancer therapy models. *Nature Biotechnology*. 28:561–562

131 Lampson L A (2012). Brain Tumor Models to Predict Clinical Outcome: Like the Phoenix? In: Martinez-Murillo, R, Martinez A, eds. *Animal Models of Brain Tumors (Neuromethods)*, 1st ed. US:Humana Press, 3-20

132 Ibid

133 Ebner R, Strovel J W, Horrigan S K et al (2011). Mighty, but how useful? The emerging role of genetically engineered mice in cancer drug discovery and development. In: Green J E, Ried T, eds. *Genetically Engineered Mice for Cancer Research*. 1st ed. New York: Springer, 591-618

134 Abate-Shen C, Brown P H, Colburn N H et al (2008). The Untapped Potential of Genetically Engineered Mouse Models in Chemoprevention Research: Opportunities and Challenges. *Cancer Prev Res*.1:161-166

135 Rangarajan A, Weinberg R A (2003). Comparative biology of mouse versus human cells: modelling human cancer in mice. *Nature Reviews Cancer*. 3: 952-959

136 Wadman M (2011). Fifty genome sequences reveal breast cancer's complexity, *Naturenews* [online], 2 April. Available from http://www.nature.com/news/2011/110402/full/news.2011.203.html [Accessed November 2012]

137 Hoelder S, Clarke P A, Workman P (2012). Discovery of small molecule cancer drugs: Successes, challenges and opportunities. *Mol Oncol*. 6(2):155–176

138 Richmond A, Su Y (2008). Mouse xenograft models vs GEM models for human cancer therapeutics. *Disease Models & Mechanisms*. 1: 78-82

139 Olive K P, Tuveson D A (2006). The use of targeted mouse models for preclinical testing of novel cancer therapeutics. *Clin Cancer Res*. 12(18):5277-87

140 Lampson L A (2012). Brain Tumor Models to Predict Clinical Outcome: Like the Phoenix? In: Martinez-Murillo, R, Martinez A, eds. *Animal Models of Brain Tumors (Neuromethods)*, 1st ed. US:Humana Press, 3-20

141 Barnes P J (2007). Unexpected Failure of Anti–Tumor Necrosis Factor Therapy in Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care*.175: 866-867

142 Shapiro S D (2007). Transgenic and gene-targeted mice as models for chronic obstructive pulmonary disease. *Eur Respir J*. 29:375–378

143 Holmes A M, Solari R, Holgate S T (2011). Animal models of asthma: value, limitations and opportunities for alternative approaches. *Drug Discovery Today*.15 (15/16):659-670

144 UK Bioscience Sector Coalition (2011). *UK Bioscience Sector Coalition response to the Home Office Consultation on the Transposition of European Directive 2010/63/EU* [online]. Available from http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC008195 [Accessed November 2012]

145 Animal Procedures Committee (2011). *Consultation on options for the transposition of European Directive 2010/63/EU on the protection of animals used for scientific procedures: Response from the Animal Procedures Committee* [online]. Available from http://www.homeoffice.gov.uk/publications/agencies-public-bodies/apc/Animal-procedures?view=Binary [Accessed November 2012]

146 National Competent Authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes (2012). *Working document on genetically altered animals*. [online]. Available from http://ec.europa.eu/environment/chemicals/lab_animals/pdf/Endorsed_consensus_doc.pdf [Accessed November 2012]

147 Jegstrup I, Thon R, Hansen A K et al (2003). Characterization of transgenic mice – a comparison of protocols for welfare evaluation and phenotype characterization of mice with a suggestion on a future certificate of instruction. *Laboratory Animals*. 37:1–9

148 Pursel V G, Pinkert C A, Miller K F et al (1989). Genetic engineering of livestock. *Science*. 244 (4910): 1281-1288

149 Ipsos MORI (2012). Views on the use of animals in Scientific Research. Available from http://www.ipsos-mori.com/research publications/publications/1512/Views-on-the-use-of-animals-in-scientific-research.aspx [Accessed November 2012]

150 Willetts D, as quoted in Kmietowicz Z (2012). Researchers promise to be more open about use of animals in their work. *British Medical Journal*. 345:3

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