Genetically Modified Laboratory Animals in the Name of the 3Rs?

Arianna Ferrari
Technical University of Darmstadt, Darmstadt, Germany

Summary
Although the introduction of GM animal models at first was viewed as a potent way to enhance experimental biology in the name of the 3Rs by its proponents, over the years, the number of animals used has greatly increased and concerns about the suffering of these animals have emerged in the debate. The purpose of this contribution is to show the need and the urgency for a systematic evaluation of genetically modified laboratory animals (GM animals) according to the 3Rs principle. This evaluation presents various difficulties due to the special features of the genetic modifications of animals, the variety of scientific purposes connected with the use of these animals, the lack of coherent statistical data about this use and the difficulties related to the welfare assessment of these animals. In this article I discuss the significance of the procedures involving GM animals for each of the 3R principles. On this basis, I offer an answer to the question of whether these procedures are compatible with the spirit of the 3Rs.

Zusammenfassung: Genetisch modifizierte Versuchstiere im Namen der 3R Prinzipien?
Obwohl die Einführung von GM Tiermodellen anfangs durch ihre Befürworter als ein bedeutender Weg zur Weiterentwicklung der experimentellen Biologie im Einklang mit den 3R Prinzipien angepriesen wurde, hat die Zahl der so genutzten Tiere in den letzten Jahren stark zugenommen, und Bedenken über das Leiden dieser Tiere wurden geäußert. Dieser Beitrag hat zum Ziel, die Notwendigkeit und die Dringlichkeit zur Durchführung einer systematischen Beurteilung der Nutzung genetisch modifizierter Versuchstiere (GM-Tiere) hinsichtlich der 3R Prinzipien darzustellen. Eine solche Beurteilung birgt vielfältige Schwierigkeiten durch die speziellen Eigenschaften, die die genetischen Veränderungen den Tieren verleihen, durch die verschiedenen wissenschaftlichen Fragestellungen, die für die GM-Tiere verwendet werden, durch den Mangel an kohärenten statistischen Daten zur Nutzung und durch die Schwierigkeiten hinsichtlich der Einschätzung des Leidens der Tiere. Im vorliegenden Aufsatz diskutiere ich für jedes der 3R Prinzipien die Bedeutung der Verfahren, für die GM-Tiere verwendet werden. Auf dieser Basis schlage ich eine Antwort auf die Frage vor, ob diese Verfahren mit dem Geiste der 3Rs kompatibel sind.

Keywords: 3R principle, ethics, genetically modified animals, transgenic animals, genetic engineering

1 Introduction

The genetic engineering of animals can be defined as the deliberate incorporation of exogenous DNA into an animal's genome with the goal of changing the characteristics of its phenotype (FELASA, 1992, revised 1995). The term "transgenic" was coined by Gordon and Ruddle (1981), who established the method of pronuclear[1] microinjection, applied first to mice and subsequently to many other animal species, such as rabbits, cows, pigs, birds and fishes[2]. The photo of the first transgenic animal – a mouse provided with a gene for rat growth hormone in its genome – next to a considerably smaller conventional mouse on the cover of *Nature* in December 1982 marked a sensational discovery in the history of experimental biology and very much impressed the public (Palmiter et al., 1982). Shortly thereafter, the other main technique for the genetic modification of animals was established, that of embryonic stem cell mediated gene transfer (Goessler et al., 1986), through which gene targeting[3] became possible. Another big success in the application of biotechnological procedures to animals was the cloning of the first mammal from an adult cell, the sheep Dolly, in 1996, through the method called somatic cell nuclear transfer[4] (Wilmut et al., 1997).

Since the beginning of the nineties, GM animals have been widely used in various fields of animal experimentation, such as in basic research – in particular for the biology of gene regulation – and as models for human diseases, toxicological and immunological reactions. Already in 1995 Wagner and his colleagues reported that since their first applications, GM procedures have served to link molecular biology with whole-animal physiology and that at the time they were writing, GM animal models were being used in almost every field of experimental research (Wagner et al., 1995). Furthermore, genetic engineering in biomedicine has made possible new uses of animals: xenotransplantation (in which organs, tissues and cells from GM animals aim to be used for transplants in human beings) and the so-called field of gene-pharming (in which farm animals

Received 13. 9. 2006; received in final form and accepted for publication 3. 11. 2006

are genetically modified so that they produce protein-based drugs in their body fluids).

Originally, its proponents had seen GM laboratory animals as the way to enhance experimental biology, because they expected that through them fewer animals would be needed for each experiment, thus obtaining more accurate results. Additionally, they had expected that it would be possible to substitute mice for monkeys or "simpler" or "lower" animals like fruit flies and earthworms for rodents (see for example Royal Society, 2001). For these reasons, GM-animals were also introduced as potent tools for the improvement of animal experimentation in the sense of the 3Rs. In some cases, the expression "alternatives" was even used for these animal models (Gordon, 1997).

Despite the optimism, as soon as the first reports about the effects of genetic modification on the welfare of the animals were published (Balls, 1995; Balls, 1999; Jenkins and Combes, 1999; Mepham, 1994; Mepham and Crilly, 1999; van der Meer et al., 1999; van der Meer, 2001; Salomon et al., 2001; ANZCCART, 2002; Delpire et al. 2000; see also Moore and Mepham, 1995), many authors began to focus their attention on the increase in the number of GM animals used, on the enormous number of animals needed in the creation and breeding of GM animals as well as on special concerns regarding the intensity and the length of suffering of these animals. Furthermore, concerns emerged in the ethical debate about the profound implications of interventions in the genome and of crossing species barriers (Rollin, 1986, 1995; Ferrari, 2005; Sandøe and Holtug, 1993; Sandøe et al., 1996; Verhoog, 1992; Vorstenbosch, 1993).

So far, in many countries specific guidelines about the use of GM animals in experiments do not yet exist. Pioneering exceptions are the guidelines formulated by the Canadian Council of Animal Care in 1997 (CAAC, 1997, see also Buy, 1997) and by the Animal Welfare Committee at the Monash University in Australia in 1999 (MUAWC, 1999, see also ANZCCART, 2001). In Europe, ECVAM organised a workshop on transgenic animals in 1997 (Mepham et al., 1998). However, it does not seem to have been widely considered by regulatory authorities in their decision-making process. Despite the current worldwide acceptance of the 3Rs as a legally and ethically important standard and despite the increasing prominence of GM animals in biological research, a systematic analysis of the 3Rs implications of the creation and use of these animals has been lacking for a long time in the discussion. Recent attempts focusing on the refinement and reduction of experiments and on the specific management of research with GM animals (Robins et al., 2003; Australian Government, 2005; GA mouse welfare assessment group, 2006) show the relevance of as well as an urgent need for strict regulations in this field, in order to ensure that every effort for the avoidance or minimisation of suffering of these animals is undertaken. However, these documents, trying to give suggestions for the avoidance of unnecessary creation of animals and infliction of pain, sometimes show an attitude of acceptance regarding the *status quo* of present research, which is clearly oriented toward an increase in the use of these animals. The current discussion still lacks an analysis of the deeper implications of the impact of the genetic engineering of laboratory animals on the reduction, refinement and replacement in the use of animals and an appraisal of the compatibility of these procedures with the general "spirit" of the 3Rs.

2 Reasons for the difficulties in the 3R evaluation of GM laboratory animals

The insufficiency in analysis of the implications of the use of GM animals for the 3Rs is a complex matter; three main reasons can be offered as explanation.

First of all, and banally, the 3Rs were formulated in a time when GM animals did not exist and were not even imaginable. From the general definition of the principles offered by Russell and Burch (1959/1992) it is not possible to derive immediate consequences for the introduction of genetic engineering, because this practice is new in experimental biology and poses unexpected challenges to the research praxis. Therefore, if on the one hand the significance of GM animals is to be interpreted on the light of the 3Rs, on the other hand also the 3Rs need to be reconsidered in the light of these technological changes to a certain extent (Vorstenbosch, 2005).

Secondly, great deficiencies exist in the collection and statistical evaluation of data relating to the general number of GM animals used in experiments as well as to the number of animals involved in the creation and breeding of GM lines. A separate classification for GM animals is not foreseen in the standard format of the Council of Europe for the collection of data on animal experimentation (Council of the European Union, 2005), with the result that in the statistics of only very few European countries GM animals are counted separately. Moreover, among the countries that separately classify GM animals, there are many discrepancies in the statistical methods used, with the result that the comparison turns out to be difficult in a number of cases. For example, the official German statistics provide the overall number of transgenic animals used in scientific procedures, but do not provide any data on the number of animals involved in the creation and breeding of GM lines[5]. On the other hand, Switzerland in its statistics also gives information on the number of animals involved in the

[1] The pronucleus is one of the two haploid nuclei of the gametes prior to the fusion in the fertilised egg.
[2] More precisely, the possibility of pronuclear microinjection in mouse embryos was first reported in 1980 by Gordon et al. (See Gordon et al., 1980). The term "transgene" was however introduced only thereafter.
[3] Gene targeting is the insertion of DNA into specific sites or genes within the genome of selected cells in order to alter gene expression.
[4] The Cloning of non-mammals was first accomplished in 1952. However major difficulties stood in the way of the cloning of mammals; and only in 1996 Campbell and Wilmut successfully obtained 2 sheep clones called Megan and Morag from embryonic cells grown in culture (Campbell et al., 1996). One year later the birth of the sheep Dolly was announced: it was the first mammal cloned from an adult cell.
[5] See the homepage of the German Ministry of Nutrition, Agriculture and Consumers' protection at http://www.bmbwk.gv.at/medienpool/13640/tvstatistik_2005.pdf. Also see the Report on animal welfare 2005 (Tierschutzbericht) at: http://www.bmelv.de/nn_753004/DE/07-SchutzderTiere/Tierschutz/Tierschutzberichte/Tierschutzbericht2005.html__nnn=true

creation of a GM model, but not on those animals needed for the breeding of a transgenic line[6] (Bundesamt für Veterinärwesen, 2004). On the contrary, in the UK statistics the number of animals involved in the creation of GM animals is counted separately from those involved in the breeding of a transgenic line (Home Office, 2002). Furthermore, as a unique feature, in the Netherlands' statistics, laboratory animals that are killed without experimental procedure are also counted as animal experiments, so that only here is it possible to count the GM animals killed but not used in the experiments (Voedsel en Waren Autoriteit, 2003)[7]. Therefore, in order to become able to really understand the significance of GM animals in experimental life sciences, the introduction of a precise differentiation of the animals used both in the creation and in the breeding of transgenic lines is urgently needed and it should be uniform for all countries.

Thirdly, the generation and use of GM animals has literally spread into a variety of contexts of experimentation. Consequently, in order to assess the 3Rs' implications for GM animals, a differentiated and punctual analysis is needed, i.e. a case-by-case-evaluation (Gruber, 1998). The current worldwide use of GM animals as disease models for a vast multitude of different human diseases provides an idea of the various scenarios of application of these models[8]. Furthermore, refinement measures to improve the welfare of a so-called GM model for Alzheimer, for example, can differ a lot from those necessary for GM pigs used in experimental xenotransplantation.

However, a more general analysis of the 3Rs' implications should not be deterred by the need for a case-by-case-evaluation. Nevertheless, it is possible to consider general characteristics related to the techniques of the genetic modification of animals, and to their effects on the creation, breeding, husbandry and use of these animals.

3 Relevant aspects of the 3Rs in the creation, breeding and genotyping of GM animals

Concerns arise from the fact that both the creation and the breeding of GM lines are very wasteful processes involving a large number of animals and from the negative impacts on the welfare of the animals involved.

One relevant difference to the conventional breeding of laboratory animals is the fact that in the creation of GM animals other animals are involved and suffer invasive procedures (Buehr et al., 2003). These animals are often called "waste animals" because they are only used for the creation of GM animals, no experiments are performed on them, and they are usually killed at the end of the procedure (see Salomon et al., 2001).

The first step in the creation of GM animals is obtaining the zygotes. In order to produce fertilised eggs, female mice are hormonally induced to superovulate[9] and mated with vasectomised[10] or sterile males. Superovulation can cause discomfort due to the stress from handling, the pain from injection, the potential for hormonal side effects and the potential stress and damage to young and/or small females during their subsequent mating (Salomon et al., 2001; Lane and Jennings, 2004). In addition, the process of embryo transfer, performed by surgery under general anaesthesia, is also painful and stressful for the females. The method used to vasectomise male mice to induce pseudopregnancy in female recipients can cause discomfort due to the anaesthesia, and also in the post-operative phase (Lane and Jennings, 2004; Hawkins et al., 2006). Recently it has been suggested to move from the ventral laparotomy to the incision of the scrotal sac in order to access the vas deferens. Thereby suffering could be reduced because the abdominal organs do not need to be manipulated (reducing the risk of infection) and because the scrotal incision does not affect the muscles that support the weight of the gut (Hawkins et al., 2006)[11].

Although GM animals can be generated using various methods[12], I will focus my attention on those two that are currently in main use and that are well documented: pronuclear microinjection and gene-targeting using embryonic stem cells. In the method of pronuclear microinjection, a transgene[13] is injected into the pronucleus of an embryo *in vitro* and then the embryo is transferred into a recipient mouse. The mechanism of integration of the transgene in the genome remains unknown and uncontrollable by this method, which results in a very low efficiency, both at the level of the survival rate of the microinjected embryos and of the expression of the transgene. In the mouse, 15-20% of the transferred embryos develop up until birth and of these only 4-5% carry the transgene (see for example van der Meer, 2001). The efficiency is even lower with farm animals (this is particularly important because microinjection is the principal method used on farm animals): 0.2% for pigs and 0.7% for sheep, goats and calves (Dove, 2000; van Reenen et al., 2001). Moreover, a significant proportion (about 30%) of the GM

[6] As a matter of fact, in accordance with the Swiss Animal protection Act, the breeding of a line (both transgenic and non-transgenic) does not have to be approved by the authorities, and animals resulting from such breeding do not have to be counted in the statistics.

[7] The number of GM animals killed without experimental procedure is registered in the Dutch national statistics but not in the European Statistics, because according to the European standard format for the collection of data the killing of animals on which no experimental procedure has been performed doesn't count as animal experiment. This generates confusion: The total number of animals used in experimental procedure in Netherlands for the year 2003 amounted to 620,875 according to the Dutch statistics and to 542,331 according to the European Statistics. See Tab. 7, Voedsel en Waren Autoriteit, 2003, p.27.

[8] In order to obtain an impression of the multitude of these models, it is sufficient to go on the website of the world's major laboratories specialized in the creation and breeding of GM laboratory animals – especially mice – such as the Charles River Laboratory (www.criver.com) and the Jackson Laboratory (www.jax.org).

[9] The superovulation consists of the administration of gonadothropins to female animals in order to increase the number of ovulated eggs.

[10] Vasectomised males are animals in which, through surgical interventions, the vas deferens has been removed (a sterilisation measure).

[11] The scrotal incision can present, however, some disadvantages: it can, for example, lead to swelling and pain, so that Hawkins et al. (2006) nevertheless recommend applying specific adequate medication.

[12] Other methods of creating GM animals include: sperm mediated DNA transfer, nuclear transfer cloning, integration of proviral DNA into germline cells, lentiviral vectors and transposons. Furthermore, the so called conditional transgenic technologies (such as the Cre/*loxP* and Flp/*frt* recombination systems) also exist which are applied under specific conditions.

[13] The term "transgene" refers to the integrated sequences of exogenous DNA.

pups, which are mosaic[14], are not able to transmit the mutation to the next generation, and only in the second generation (F2)[15] is it possible to obtain transgenic homozygous[16] animals (about 25% of the animals born are of this generation). These transgenic homozygous animals are essential for the establishment of a GM line, which is necessary in almost all research programmes[17]. De Cock Buning counts that the use of 300 to 4000 animals in a four-year PhD project is common (de Cock Buning, 2004, p. 462).

The method of gene targeting using embryonic stem cells (also called ES-cell mediated gene transfer)[18] enables the direct insertion of the transgene into the ES-cells, i.e. the homologous recombination[19], so that it presents a higher efficiency compared to the pronuclear microinjection. However, this method is currently only possible in the mouse. About 25% of the pups obtained are chimeric[20], i.e. they contain the transgene (van der Meer, 2001), but many of them are required in order to select a few founders to generate the correctly targeted mutation. Here again it is only possible to obtain homozygous animals for the targeted gene (25% of the entire number of animals) in the second generation (F2)[21].

Many animals are genetically modified to some extent both in the course of the creation and breeding of a GM line, but are nevertheless only "stages" necessary to reach the final goal of obtaining the "right" GM model, i.e. the one carrying the mutation and expressing it[22]. These animals, involved in the genetic modification but not usable in the experiment and/or for the breeding of a line, are normally killed. They represent the "forgotten parts" of animal experimentation, since they are not normally counted in the statistics (with the exception of the Statistics of the British Home Office).

Another technical step that is essential to the use of GM animals is the process of genotyping, i.e. of testing that the animal actually possesses the specified genetic modification. During this step some invasive procedures are also required, such as the identification of the newborn, tail biopsy and, in some cases, toe-clipping in rodents. In particular, the tail biopsy has the potential to cause acute and chronic pain at the time of, and following, the cutting. Due to the special adverse potential of identification methods in neonatal rodents it has been suggested to use a temporary identification initially and to postpone the permanent one until after the weaning phase (Australian Government, 2005, p.12), or to move from tail-clipping to ear notching (Hawkins et al., 2006).

To recapitulate, a great number of animals are involved in the process of obtaining a useful GM model, both at the stage of creation (as animals are involved in obtaining the zygotes, and many genetically modified embryos fail to develop), at the stage of breeding the genetic modification into the desired genetic background and at the stage of maintaining the GM line.

3 Relevant aspects of the 3Rs in the phenotyping, husbandry and use of GM animals

Phenotyping, or phenotype screening, can be defined as the analysis of visible or measurable characteristics of an animal that result from the genotype and its interaction with the environment (Brown and Murray, 2006). Although recent studies conclude that being genetically modified does not necessarily affect the welfare of an animal (GA mouse welfare assessment group, 2006; Wells et al., 2006; Buehr et al., 2003), other studies clearly show that the genetic modification is linked to a higher occurrence of pathologies. Van der Meer (2001), for example, affirms that the genetic modification influences both the survival rate of the pups as well as their body weight. Moreover, a study in Denmark indicates that welfare was affected in approximately 30% of GM strains (Thon et al., 2002; see also van der Meer, 1997 and Costa, 2000, 2001). In any case, all studies conclude that genetic modification has *the potential* to compromise animal welfare, so that careful monitoring is needed.

In particular, the random integration of the transgene (which occurs in all methods except that of gene targeting with ES cells) means a higher risk for the animal to present unintended alterations of its morphology, physiology and behaviour, which in many cases are not related to the experiment. Furthermore, unexpected effects of the genetic modification can al-

[14] A mosaic animal is an individual that presents cells of two or more genotypes. A mosaic animal differs from a chimeric animal – the one obtained by the method using ES-cells – because the cells of different genotypes emerge from the same zygote.

[15] The animals obtained by the recipient, into which the injected embryo (modified) has been transferred, belong to the generation 0 (F0). The one in which the transgene has been reported (about 25%) are then called founder transgenics and are hemizygote, i.e. they posses the exogenous DNA in only one copy in a diploid cell. The founder transgenes are then bred with wild-type animals to produce the next generation (F1). Homozygous transgenic animals can be obtained in the second generation (F2).

[16] Homozygous indicates the state of having two identical alleles of a particular gene.

[17] The establishment of a transgenic line is not required to obtain the so-called transient transgenics, in which the embryos derived by genetic engineering methods are collected from the recipient at a defined point of gestation and analysed for transgene expression. See Robinson et al. (2003).

[18] By this method, embryonic stem cells are first obtained, then they are modified *in vitro* (by adding modified, highly specific DNA sequences) and injected into the blastocysts. The modified blastocysts are then transferred into pseudopregnant recipients.

[19] The homologous recombination is the process by which one DNA segment can replace another DNA segment that has a similar sequence. Embryonic stem cells (ES cells) of the mouse are used both to eliminate gene expression (the so called "knock-out") and to introduce mutant alleles in a precise locus of the genome ("knock-in").

[20] A chimera is an animal that presents more than one genetically distinct population of cells derived from more than one zygote (that is from different embryonic sources).

[21] The founder chimeric mice, that belong to generation 0 (F0), are then mated with wild-type mice. Since most embryonic stem cells are derived from the male, chimeras are usually male. Mice of the first generation (F1) are then screened for the presence of the modified gene. Homozygous transgenic mice - about 25% - are then obtained in the second generation (F2).

[22] Although in common practice the terms "transgenic" and "GM" are used synonymously, there is an important difference between them: properly a transgenic animal is the only genetically engineered animal that shows a stable incorporation of the external DNA in its genome, i.e. one which can transmit the artificial modification to the next generation. The other modified animals are "merely" genetically modified animals. The use of these terms as synonyms is due, on the one hand, to the need for simplification and also because the gene construct inserted is defined as "transgene", and, on the other hand, to the fact that true transgenic animals (and not "simply" GM animals) are used as experimental models and are needed in order to establish a transgenic line. Nevertheless, an understanding of the details of the different procedures in the creation of GM animals is important in order to obtain an impression of the total amount of animals involved in these procedures.

so occur in the gene targeting with ES cells: Van der Meer (2001) has noted a higher mortality among the modified embryos and the death of many pups soon after birth as a result of this method.

In consequence, it can generally be said that GM animals have a higher risk of suffering and distress than conventional animals precisely because of the genetic modification.

In addition, it may well be that damaging mutations only arise in homozygous and not in heterozygous stages or even only in later generations. Therefore the overall unpredictability of the techniques makes it extremely difficult, or impossible, to reliably anticipate ill effects and to be able to prevent them by ameliorating external conditions.

Although consistent differences in the behaviour of GM or conventional animals have not been reported (except obviously for those models which are expressly genetically modified in order to show altered traits in their behaviour) (van Reenen and Blokhuis, 1993), the unpredictability of the phenotype can sometimes lead to each individual animal showing unique characteristics. Therefore the application of welfare measures in the breeding of these animals can require complicated steps. Moreover, in many cases phenotypic aspects that compromise the welfare of animals, but which do not have an impact on the disease process, oftentimes are not reported or are disregarded by researchers, which also circumvents the protection of the animals' welfare (Brown and Murray, 2006).

Furthermore, both the scarcity of inquiries about the effect of genetic modification on the welfare specific to each model, and the great variety of GM models created, represent hurdles for the application of refinement measures that are used in conventional animal research, such as enrichment or humane end-points. For example, after the first experiments and a careful welfare assessment it has been possible to observe that mouse models of cystic fibrosis lacking CFTR function need a liquid diet, for otherwise they die shortly after weaning due to intestinal obstruction (Grubb and Boucher, 1999). Some GM mice present facial or maxillary malformations and therefore also need a special diet (Robinson et al., 2003). It has to be questioned whether and in which consistency a careful monitoring of the welfare is thoroughly carried out in the everyday research practice.

In most cases, GM models present severe welfare problems, also because a large number of them are used to create disease models and models with harmful mutations (Falkner et al., 1997). However, it is not possible to make a general judgement of these models, since in many cases disease models do not mimic all the phenomena of the human pathology, and since each model presents unique characteristics that can be assessed accurately only as the model is generated and the phenotype analysed.

Moreover, in order both to prevent escape and to guarantee the maximum level of sterility, GM animals are very often bred in isolation and under pathogen-free conditions (mainly GM pigs for xenotransplantation and disease and toxicity models), which obviously can make it impossible to apply refinement measures at all (Robinson et al., 2003). In particular, due to the sterile conditions required, animal staff does not come into direct contact with the animals through handling very frequently, so that many abnormalities are not recognised during the earliest possible stages.

Last but not least, GM animals are often transferred between establishments, because many laboratories do not possess the necessary technology to create these animals, with the result that big centres worldwide have come to specialise in these techniques. GM animals are often very sensitive, so that not only their transport but also changes in their environment can affect their welfare. Recently, the GA mouse welfare assessment group (2006) has suggested creating a standard "mouse passport system", which includes the welfare profile for a particular mouse line and which contains an indication of the steps that should be taken to reduce adverse effects, in order to improve animal welfare aspects and the knowledge about that particular GM model (GA mouse welfare assessment group, 2006, p. 14 and Annex 5, p. 23-24).

4 Relevant aspects of the 3Rs in the cloning of animals via somatic cell nuclear transfer

Although cloning is not a form of genetic modification, in the sense that DNA has been added into the animal's genome from another strain or another species[23], the technique of cloning via somatic cell nuclear transfer is widely used in laboratory practices precisely in connection with GM procedures, because it permits targeted genetic changes in many species (it should be kept in mind that gene-targeting using embryonic stem cells is only possible in the mouse!). Furthermore, since obtaining the "right" GM animal model is such a difficult and long enterprise, once these models are created they are very often cloned, in order to become available for other experiments. Some authors even argue that in applied medicine there currently seems to be no alternative to cloning via somatic cell nuclear transfer (Revermann and Hennen, 2000, p. 86). The creation of GM animals with techniques including a cloning step raises additional welfare concerns in comparison to the microinjection technique. Therefore, an estimation of the implications of the creation of cloned animals (via somatic cell nuclear transfer) for the 3Rs should be seen as a necessary step in the 3R assessment of GM procedures.

Here again, the efficiency of this procedure is very low: the average proportion of transferred embryos that develop to birth is between 1 and 10% (National Academy of Science, 2002; Tian, 2003; Nationaler Ethikrat, 2004; 2006), depending on the species and on the stage of embryo[24] considered. For example, Clausen (2006) has calculated an average efficiency of 0.83% when performing nuclear transfer using adult cells (and an efficiency of 0.74% using embryonic or fetal cells) (see tables 1 and 2 in Clausen 2006, pp. 50 and 51)[25]. This low efficiency is due mostly to the high rate of foetal losses after embryo

[23] For example, the British Home Office has introduced a new term – *genetically altered* (GA) – which includes all GM animals, mutants (both natural and artificially induced), and animals resulting from nuclear transfer. See Lane and Jennings (2004).

[24] For example, the efficiency of the technique is higher when the embryos have developed into blastocysts. See Nationaler Ethikrat (2004).

[25] In order to clone Dolly, 277 fertilized eggs were used, collected from 40 different donors, with an overall efficiency quote of 0,36%. For further information, see National Academy of Sciences, 2002, tables on pp. 114-118, and Clausen (2006).

transfer and implantation (National Academy of Science, 2002).

It has been shown that pregnancies involving cloned foetuses present a higher rate of abnormalities, including abnormal placentation and an excessive fluid accumulation in the uterus often associated with foetus abnormality (hydroallantois) (Hill et al., 2001; Yong and Yuqiang, 1998; Shiga et al., 1999). Increased maternal morbidity and mortality have been seen most prominently in studies with cattle and sheep. Additionally, the National Academy of Science (2002) reports a personal communication from Tim King and Ian Wilmut who noted that "hydroallantois can affect up to 5% of established sheep pregnancies involving cloned offspring, although this condition is 'extremely rare' in normal pregnancies" (National Academy of Science, 2002, p. 42). In particular, the greater frequency of oversize foetuses seems to lead to obesity problems in later stages of life and causes discomfort for the surrogate females, which often need caesarean sections. Moreover, many cases have been reported showing a connection between animal cloning and a wide range of abnormalities, such as immune system deficiencies, respiratory problems and developmental defects in the cardiovascular and nervous systems (see van Wagtendonk-de Leeuw et al., 2000; National Academy of Science, 2002; Clausen, 2006).

Some authors try to deny the opinion that most cloned animals develop abnormally and are affected by many pathologies (Wilmut, 2002), such as for example Cibelli et al. (2002), pioneers in the field of cloning. However, when they discuss the possible application of this technique to human beings, apart from ethical concerns[26], they recommend not applying it until nuclear transfer is better understood, revealing that they judge the apparent "objective" question concerning the efficiency of the technique by two different standards when considering laboratory animals or human beings[27], respectively.

5 Towards a 3R assessment of GM animals: a contribution to reduction?

The increasing use of GM animals is considered to be a major cause for the persistent rise in the number of animal procedures in the European Union (with particular evidence for the UK and the Netherlands) and in Australia (and presumably worldwide) (Home Office, 2002; Australian Government, 2005; Council of European Union, 2005; Grindon and Boghal, 2005).

Hudson and Boghal (2004), explicitly indicating GM procedures to be the largest factor contributing to the continuous increase in the total number of laboratory animal procedures reported during the last couple of years, anticipate that there is a great probability that this trend will further increase, with the total number of laboratory animals used exceeding the levels of the mid 1970s within the next few years. It is remarkable that in 1995 in the UK there were 215,293 the procedures with GM animals, whereas in 2003 the number has reached 764,095 (Home Office, 2002). Mepham and Balls (2000) counted an increase of procedures on GM animals in the UK of approximately 900% from 1990 to 1998. In their study commissioned by the Dutch Foundation Sophia Vereeniging together with the Netherlands Centre for Alternatives in Animal Use (NCA), aiming at a monitoring of trends and developments in laboratory animal use, Boumans and Hendriksen (2006) find clear evidence that the increasing trend in animal use is due to animal experiments with GM animals. Both Sauer et al. (2005 and 2006) and Gärtner (2006) indicate that GM animals are the main cause for the increase in the number of laboratory animals used in Germany as well.

Furthermore, it is likely that this trend will be intensified by ongoing projects related to mapping the genome of various animals and to creating mouse models for the investigation of the functions of key genes involved in biological processes[28] such as for example in physiology (EUMORPHIA[29]) and for embryonic stem cell differentiation (FunGenES[30]). The International Mouse Mutagenesis Consortium has been established with the goals to produce a mouse model for at least one heritable mutation in every gene in the genome, to identify every gene that affects key traits of biomedical interest and to develop the infrastructure for distributing mutant cells and mice (Nadeau et al., 2001).

Although some authors argue that the number of animals needed to establish a line by conventional breeding is greater then the number of animals required for a transgenic line (Buehr et al, 2003, p. 324), from the statistical analysis on animal experimentation in the UK, it is possible to argue that the large number of "waste animals" involved in GM procedures exceeds the number of animals required for conventional breeding procedures. In the UK statistics it is reported that 96% of the total number of animals used in experimental procedures for breeding purposes are involved in the creation or maintenance of GM lines and, as a consequence, only 4% are needed in conventional breeding procedures (Home Office 2002, Tab. 3, p. 31-33 and Tab. 3.3, p. 37). In consequence, the Joint Group on Refinement has recently proposed using these animals also as source of tissue samples instead of simply killing them (Robinson et al., 2003, p.10).

The enormous number of "waste animals" alone should suffice to lead to the conclusion that intrinsically GM technologies cannot bring about a reduction of the total number of animals used for scientific purposes. In addition, the number of transgenic lines currently being produced outweighs the number of recombinant inbred lines being established (Buehr et al., 2003).

[26] The question of cloning in human beings (both for reproductive and for therapeutical purposes) is a very controversial and interesting issue but its discussion would exceed the topic of the present article. See for example Nationaler Ethikrat (2004) and Clausen (2006).

[27] This sort of double standard in judging the efficiency of the procedures is very common in the general discussion of human cloning.

[28] The European Union is a major sponsor of mouse functional genomics research: Since 2002, fifteen ongoing European collaborative projects received altogether €135 million from the EU's Fifth and Sixth Framework Programmes.

[29] The EUMORPHIA project is called "Understanding human disease through mouse genetics". See http://www.eumorphia.org/

[30] FunGenES is the project for the investigation of functional genomics in mouse embryonic stem cells. See http://www.fungenes.org

Even if it has become clear over the years that, in the case of GM animals, a reduction in the total number of animals involved cannot be obtained, some authors try to defend the contribution of these animals to reduction arguing that, due to the improved efficiency of GM models, more results (or a greater knowledge) are expected to be gained from one single model, in comparison to conventional models (Gordon, 1997). Moreover, although recognising that these procedures both lead to an increase in animal suffering as well as to the current and likely future increase in the number of procedures with animals performed, they argue that this kind of conflict is not at all typical for procedures with GM animals, but that it also arises due to procedures with inbred animals and mutants (Buehr et al., 2003).

From an epistemological point of view, the argument that more knowledge can be gained with a GM model in comparison to a conventional one is problematic. Even though it might be true that GM models have offered new research possibilities, which has effectively lead to an increase in the number of scientific questions that can be addressed, this was the case precisely because the questions at stake were thought and planned to be searchable only in *animal models*. Admitting that GM procedures are "inducing investigators to turn to animal research for the first time in order to obtain solutions to their problems" (Gordon, 1997, p.105), supporters of GM procedures also state that "if increased use of GM animals can answer more important questions, this need not to be viewed as a problem" (Buehr et al. 2003, p.333). The real question, however, regarding the contribution of GM animal procedures to the 3Rs seems to me to rely not on the intrinsic value of the rise in the number of investigable questions, but rather on the fact that the kinds of questions that become addressable through GM models are *only* addressable through animal models, so that they perpetuate the logic of animal experimentation.

The confusion about the topic at stake also leads to a misunderstanding of the issues connected with GM animals and reduction. When this topic is discussed, strategies to reduce the excessive number of unwanted GM animals and of animals involved in the creation and breeding of GM lines are often mentioned (Robinson et al., 2003; Nuffield Council of Bioethics, 2005), rather than discussing the general role played by GM procedures in reducing the overall number of research animals, which is precisely a negative one. In other words, when the problem of GM animals and reduction is discussed, ways to improve the production of GM animals are often mentioned, rather than questioning *whether* their use can contribute to an overall reduction in the number of animals used in the first place.

6 Towards a 3R assessment of GM animals: a way to refinement?

One argument that GM technologies are compatible with the 3Rs refers to the fact that these technologies permit the realisation of more efficient and precise models (Buehr et al., 2003).

For example, Wolf (1997) argues that GM disease models represent a form of refinement because through them it is possible to examine diseases at earlier stages, i.e. in stages where the animal does not show a great degree of suffering. This would be an example of genuine refinement. In this context, however, it must be kept in mind that in most cases the observation of the pathologic changes in the phenotype, i.e. of the outbreak of the disease, is a very important part of the research protocol, for example in the search for new drugs for the disease in question.

Moreover, in order to objectively assess the contribution of GM animals to refinement, it is important to consider the difficulties related to the welfare assessment of GM animals, which can present unique characteristics (van Reenen, 2001). It seems particularly difficult, for example, to determine humane endpoints for GM animals because of the unanticipated outcomes stemming from the genetic modification (Dennis, 2000; Toth, 2000). To overcome this problem, it would be necessary to observe several generations of the same GM model. For instance, for some time it has been observed that the p53-knockout mouse model develops tumours very early - differently from other non-genetically modified mouse strains – and also that the animals die after approximately the tenth month of life; thus it has been possible to establish a humane endpoint for this mouse model (Roths et al., 1999). However, this is a time-consuming and expensive practice, which unfortunately is not performed in every case.

Furthermore, the phenotypes of identical GM laboratory animals can vary depending on their physical, social and microbiological environment. Therefore careful monitoring of their welfare under each particular condition is required, also taking into account the fact that GM animals are often moved from one establishment to another, both nationally and internationally. For example, there have been observations of significant differences in behaviour between different laboratories (Crabbe et al., 1999).

Doubts about the GM animals' contribution to the refinement criterion come from special concerns about the welfare of these animals and consequently from the special requirements that are necessary in order to protect them, especially when they are used as disease models. The Austrian study on the 3Rs relevance of the introduction of genetic engineering into animal experimentation (Falkner et al., 1997) clearly reveals that the majority of animal experiments with GM animals have a negative impact on the welfare of the animals, particularly in the case of GM disease models[31]. The study by Sauer et al. (2005; 2006) – a very useful analysis of the current status of research with GM animals in Germany based upon a literature survey of 577 scientific publications – also provides evidence for a negative impact of GM procedures on the welfare of the animals. Moreover, the majority of toxicological GM models also do not seem to present a form of refinement for the welfare of the animals. On the con-

[31] The study of Falkner et al. (1997) represents the only one until now that systematically evaluates concrete experiments on GM animals on the basis of the 3Rs, providing a particularly useful case-by-case analysis on the basis of a detailed literature research.

trary, these models have been genetically modified to an extent that they are more sensitive to reactions, and in some cases they even carry more than one modification (famous examples are Big Blue and Muta Mouse); the purpose of which is to ensure that many different reactions can be tested on one single model (Falkner et al., 1997; Vogel, 2001).

Taking into consideration these problems leads to the question which sort of refinement can effectively be possible for the majority of experiments on GM models. It seems to me that most procedures with GM animal models can indeed lead to a refinement of the *scientific procedures*, yet this does not ever correspond with any refinement in the *treatment* of the animals, which is precisely the genuine sense of refinement promoted by Russell and Burch. This kind of amelioration of the experiments is not in the interests of the animal, but aims at a better constructed experiment, from which a greater knowledge is to be gained (regardless of the consequences for the animals). This misunderstanding is similar to the question of the GM animals' contribution to reduction.

In addition to these difficulties, one has to take into account that sometimes attempts to refine GM models and reduce their suffering conflict with attempts to reduce their number. As I stated before, sometimes pathological characteristics only occur in the homozygous transgenic form and not in the heterozygous one, so that in this case a refinement would be to maintain the GM model in the heterozygous form. However, more animals would be required for the breeding of a heterozygous transgenic line in comparison to the corresponding homozygous line, so that such a situation would lead to a clear conflict between attempts at reduction and at refinement (Australian Government, 2005).

Another example for such a dilemma arises due to the special features of GM phenotypes, which require careful analysis. Taking into account that GM animals can present unique characteristics, control groups are indispensable for welfare assessment. This means that extra animals presenting the same genetic modification are required as controls, and both groups should be housed in the same manner. This represents a challenge to the principle of reduction, however is necessary for refinement purposes. As a consequence, the Australian Animal Welfare Committee has tried to find a compromise between both aims, stressing the careful choice of control animals (Australian Government, 2005, p.8) and the Joint Group in Refinement has recently proposed using some of the so called "waste animals" as controls (Robinson et al., 2003, p.10). Such difficult situations are caused by the conflict between the principles of reduction and of refinement, which, although typical for the 3Rs in general (Anim.Al.See, 2004), is particularly acute in the case of GM technologies.

7 GM insentient animals as replacement?

The genetic modification of animals implies the use of animals; therefore, it is logically impossible for these techniques to serve as alternatives in the purest sense, that is, in the sense of replacement. Following the definition offered by Russell and Burch (1959/1992)[32], any attempts to substitute the use of "conscious living vertebrates" with "non sentient material" should be considered as a form of replacement. With the new tools offered by genetic modification, the idea of knocking-out sentience in animals has been formulated and it has been asked whether such animals could be seen as a form of replacement (Macer, 1989; Rollin, 1995; Reiss and Straughan, 1996; Appleby, 1999; de Cock Buning, 2004).

The idea of creating GM insentient animals leads to many different concerns, which are very complex and would therefore require a separate article to be discussed appropriately. However, since this possibility has been formulated in the course of the debate, particularly in relation to the topic of replacement of the use of animals for scientific purposes, I think that it should be mentioned and briefly discussed in this article.

When we consider the idea of insentient GM animals, the first question to be dealt with relates to its feasibility: Can it really be possible to knock out sentience in animals? In order to appropriately answer this question, it is not only necessary to have a broad and competent knowledge about GM technologies, but first and foremost, to know what sentience is, and where it is located at the genomic level.

In various dictionaries, "sentience" is defined as the general faculty through which the external world is apprehended, as a state of elementary and undifferentiated consciousness or the readiness to perceive sensations[33]. An ongoing and very interesting discussion on the philosophy of mind considers the question of if, and to what extent, animals have consciousness[34]. Sentience, on the other hand, is described as something more elementary than consciousness, such as the organism's capacity for embodied sensation. Precisely, this more basic characteristic of sentience – which has to be seen as a sort of vital experience – renders it a vague feature, constituting a problem for the planning of any experiment that aims at turning off sentience in animals.

In order to create GM insentient animals, it would indeed be necessary to identify the mechanisms underlying sentience. Does sentience depend on having certain cognitive capacities, such as consciousness, together with certain neurological traits (such as, for example, a neural cortex), or does sentience result from the complex combination of specific characteristics of an animal?

Broadly speaking, we face two options for defining the sentience of animals: a narrow one and a broader one. In accordance with the definition put forward by cognitive ethologists and philosophers of mind, the narrow view identifies sentience as *phenomenally conscious* sentience, which presupposes that the animal has passed some basic threshold of degrees of overall psychological, neurobiological,

[32] The term 'replacement technique' should be used "for any scientific method employing non-sentient material which may in the history of experimentation replace methods which use conscious living vertebrates" (Russell, Burch, 1959, p. 69).
[33] See "sentience" in the Oxford Dictionary on line (www.askoxford.com) and in the Wordnet Dictionary on line (www.wordnet-online.com).
[34] For this debate see for example Nagel (1974); Griffin (1992); Rollin (1989); Bekoff et al. (2002); Perler and Wild (2005).

and behavioural organisation into a centred form of sentience[35]. In the broader view, sentience is seen to be the expression of the complex biological and behavioural characteristics of an animal, a sort of "feeling of life" or "vital experience", independent of whether the animal has a subjective point of view or not. In the rest of this paragraph, I will try to show that working with either the narrow or the broader definition of sentience, the question of whether GM insentient animals can be seen as a form of replacement or not, is the wrong question, because there is something intrinsically wrong with the predicate "GM insentient animals". Furthermore, I will show that apart from these difficulties there are serious ethical concerns connected with these experiments.

In the narrow view of sentience, identified solely on the grounds of consciousness, only vertebrates are sentient/conscious animals, in the sense that they are able to have proper conscious sensations because they have of the relevant biological traits, such as nociceptive nerves and an organised nervous system[36], and possess certain psychological and behavioural characteristics[37]. Such conscious beings are also aware of their suffering and pain, which is precisely what raises ethical concerns about animal experimentation. If we follow the narrow view of sentience/consciousness, we have to consider the goal of creating GM insentient animals as a modification of vertebrate animals aiming at transforming them in some way into "painless" invertebrates. However, in order to accomplish this, we also have to admit that there are *gene* or *gene sequences* responsible for these phenomena, that is we need a *genetic conception of pain and suffering/phenomenal consciousness*. In other words, we have to be sure on the one hand, that by genetic modification we can turn off the part of the brain system that makes the difference between vertebrates and invertebrates, and that, on the other hand, we can maintain their bodily and biological characteristics of being vertebrates (otherwise it would suffice to merely use invertebrates).

Attempts to create somewhat less conscious animals are currently being made in neurological research, particularly in research dealing with the neural correlates of consciousness, for which certain GM mouse models called zombie-mice[38] are created (Koch, 2004a; 2004b; Moss, 2005). Unfortunately, the characteristics of these models have not yet been described, so that their detailed discussion is not yet possible[39]. However, the research of Koch and his team is based on a particular model for consciousness, which is very controversial in this debate, because it involves reducing consciousness to particular parts of the brain (Crick and Koch, 1997; Koch 2004a, 2004b).

If we want to speak of "insentient"/"non conscious" GM animals properly, we have to switch off not only their ability to feel physical pain, but also their ability to express their discomfort and stress, that is their emotional capacities. In fact, proposing GM insentient animals as a form of replacement, at least would require going beyond what it is being achieved by refinement measures aimed at reducing their suffering: we also have to modify the characteristics that determine their welfare. Investigations into animal welfare indeed confirm that there are other states apart from pain that negatively affect the welfare of an animal. It is the common and current opinion among ethologists that the welfare of animals is not merely reducible to units of pain, but rather consists of positive states and feelings, as well (Stafleu et al., 1996; Broom, 1998a; 1998b; 1999). Positive states and feelings are profoundly determined by the biological characteristics of each animal, and these characteristics are the product of the evolution and adaptation to particular environmental conditions. As a result, GM insentient animals would also have a different kind of self-perception, a different ability to cope with the external environment and other species-specific needs (i.e. the activities characteristic of its species) in comparison to non-modified animals of their species.

It seems to me that the narrow view of sentience/consciousness turns out not to be adequate as a standard for the creation of GM insentient animals as a form of replacement. Even if we pretend for a moment that we can genetically switch off the emotional capacities of animals, if we want to aim for insentient animals properly, we have to abandon this narrow view and to take the broader one, because the phenomenon of consciousness is closely interrelated with the other biological characteristics of animals, and to a certain extent forms them. If we take the broader view and identify sentience as "the general feeling of life" of the animal, we have to interfere with so many complex mechanisms when striving for insentient animals that the creature that would remain in the end would be something that could hardly be defined as an animal at all. In other words, we would have to make profound changes to the entire structure of the animal. Would such an enterprise really lead to useful

[35] In the philosophy of mind there is a further distinction between phenomenal consciousness and access consciousness, which is the state of being conscious of something in relation to abstract concepts. This notion was introduced by Block in 1995 to capture the sense in which mental representations may be poised for use in rational control of action or speech. Block believes that many animals possess access consciousness. His position is opposed to the one of Donald Davidson, who denies that animals can have intentional states. See for example: Block, N. (1995). On A Confusion About a Function of Consciousness?, *Behavioral and Brain Sciences* 18: 227-47; Davidson, D. (1975). Thought and talk, In S. Guttenplan (ed.), *Mind and Language*. Oxford: Oxford University Press.

[36] All vertebrates possess the areas of the brain to process nociceptive information, namely the medulla, thalamus and limbic system.

[37] The discussion on pain in animals is a very fascinating and complicated topic. For an interesting reconstruction of the difficulties connected to this problem from a philosophical and scientific point of view, see Galert (2005).

[38] Zombies are beings that have no conscious experiences. In the philosophy of mind, there is a large debate on the conceivability and possibility of zombies. See for example: Cottrell, A., (1999). Sniffing the Camembert: on the Conceivability of Zombies. *Journal of Consciousness Studies*, 6: 4-12; Dennet, D. C. (1995). The Unimagined Preposterousness of Zombies', *Journal of Consciousness Studies*, 2: 322-6. Furthermore, discussions are also ongoing about the implications of the possibility of zombies for the evolutionary theory. See also: Flanagan O., T. Polger (1995) 'Zombies and the Function of Consciousness', *Journal of Consciousness Studies*, 2: 313-321.

[39] In this context, it is interesting to note, that Koch and his team (2004a) openly point to the potential of their research for the cause of animal rights, while they admit that studying the evolutionary basis of consciousness could yield to new knowledge about animal capabilities, and that this could affect our attitude towards animals. However, this thought does not seem to let them understand that they are trying to "scientifically" assess the abilities of animals in order to demonstrate their right to be protected by performing very stressful and painful experiments on them.

models for experiments, which aim at investigating disease phenomena and toxicological reactions? As a matter of fact, one of the principal arguments that supports animal experimentation refers to the advantages of performing experiments *in vivo*, especially on vertebrates, exactly because of their complexity. The problems addressed here would require a more in-depth analysis, which cannot be discussed in this article.

What about the ethical acceptability of such procedures? Although the creation of GM insentient animals up until now is far from becoming *concrete* research projects, the idea of such models is connected to particular views about animals and to the possibilities offered by biotechnologies, which have ethical implications as well. The British Animal Procedures Committee (APC) has also briefly discussed this hypothesis in its Report on Biotechnology (2001), judging it as unacceptable (see Recommendation 4, p. 18)[40] (APC, 2001).

First of all, in the light of the hypothesis of this type of research, laboratory animals are seen as modifiable and determinable tools at the disposal of human beings for any need conceivable – in this case the desire to clear their bad conscience resulting from the performance of animal experiments. In consequence, the idea of GM insentient animals clearly supports an instrumental view of animals. As Vorstenbosch (2005) pointed out, this idea confronts us with the realisation that "it is not only the pains and pangs that occur in the animal as part of the experiment that worry us, but also that there is a living subject, with a life and standing of its own, that experiences these pains and pangs" (Vorstenbosch, 2005, p.340)[41].

Second, the creation of insentient animals, if it were possible, could paradoxically result in a very risky practice for the animals in terms of suffering and distress. To eliminate sentience, it is necessary to profoundly change the characteristics of the animals without exactly knowing the effects that this would have on these beings. Actually, we change what we understand as sentience, but we simply do not know if there are other ways of understanding sentience (at least with the current state of knowledge about pain, suffering and stress in animals).

Third, we also have to consider the necessary steps involved in the creation of such animals. In this article, I have shown that genetic modification is a wasteful process in terms of the number of animals involved in the procedures, and this has to be taken into account when assessing the ethical acceptability of attempts to create GM insentient models.

Fourth, the idea of GM insentient animals relies on an absolute faith in the power of biotechnology – genetic engineering can achieve anything by arbitrarily adding to or knocking out every characteristic of a living being – which is problematic both from an epistemological and ethical point of view.

And last but not least, supporting GM insentient models as a form of replacement could in practice serve to maintain the *status quo* of animal research, by forming new arguments to gain acceptance for the use of animals, which would take away precious resources from the research into alternatives.

For these reasons, I strongly propose refusing any attempts to create GM insentient animals.

8 Conclusion

Assessing the 3Rs' implications of GM procedures means evaluating whether the actual use of these procedures is compatible with the spirit of the 3Rs or not. But what exactly is the spirit of the 3Rs? In my introduction, I have stated that, notwithstanding recent attempts to stress the importance of reduction and refinement measures in procedures with GM animals (Australian Government 2005, GA mouse welfare assessment group 2006; Wells et al., 2006a and 2006b), these reports seem oriented at improving the situation of the animals within the existing scientific order and not at encouraging a further criticism of these practices as such. In his examination of the ethics of the 3Rs, Vorstenbosch (2005) emphasises that this tendency towards ameliorating the *status quo*, without deeply criticising it, results from a narrow interpretation of the 3Rs, which is, however, not the only possible one. We do need to remember that the 3Rs concept constitutes the basis for alternative methods (see Gruber and Spielmann, 1996) and that there are historical grounds for this[42]. In order to understand the spirit of the 3Rs, I think that we should reason from the perspective of implementing research based on alternative methods, i.e. to support every effort made in the direction of the alternatives, at all levels of reduction, of replacement and of refinement. If we want to speak of GM animals in the light of the 3Rs properly, we have to discuss which contribution these animals make to the 3Rs, and not just apply the 3Rs to current research which uses GM animals.

The current state of research shows that the creation and use of GM-animals have contributed to an increase in the total number of research animals, so that currently, and presumably in the near future, these animals will not make any contribution to a reduction of animal use in experimental procedures. The possibilities for the long term future remain unclear, but the expanding fields of genomic research and the fact that GM technologies intrinsically require a great number of waste animals do not leave any hopes for a reduction in the long term, either. Therefore, the use of GM animals acts contrary to the principle of reduction.

Alternatively, it could be argued that our society needs to reflect upon the choice between giving the priority to the principle of reduction – and thus to renounce or to restrict the use of GM tech-

[40] The Committee mentions the following arguments against creating insentient animals: the concern for the violation of the species-integrity and the repugnance to the very notion of producing creatures without a recognisable species-nature. See APC, 2001, pp. 17-18.

[41] In the current debate on animal ethics, an interesting main issue deals with the analysis of the relationship between pathocentric considerations based on the capability of experiencing negative states (such as suffering and pain) and of animal related and ethical considerations regarding the optimal conditions for living for animals, by also referring to notions such as naturalness, integrity and flourishing. See for example Rollin (1995), Dol et al. (1997).

[42] However, Russell and Burch's book did not have great success at first, and it remained a matter for scientists. Only in the 1970s did the topic of the 3Rs reach the larger public, particularly after the publication of David Smyth's book *Alternatives to animal experiments* in 1978, where the attempts offered by the 3Rs were incorporated into the definition of 'alternative' (Smyth, 1978).

nologies – or to the principle of refinement, that is to implement their use because these technologies serve to obtain better and more efficient models. However, my analysis has revealed, that the refinement in question is not a refinement in treating the animals more humanely in comparison to conventionally bred animals (in some cases even the opposite happens), but that it is a refinement of the scientific procedures, i.e. a refining of the animal model as such. Nevertheless, the question of obtaining better models (not addressing the scientific problem of whether they really are better) is not really an option in the sense of the 3Rs. According to the definition offered by Russell and Burch, refinement means "any decrease in the incidence or severity of inhumane procedures applied to those animals which still have to be used" (Russell, Burch, 1992, p.64). However, can GM models be viewed as animals that still have to be used in research? Obviously not, since they are *new* animals, created for addressing *new* questions.

Since the goals of any particular experimental research are closely interwoven with the scientific procedures chosen (Vorstenbosch 2005, p.343), GM models allow an increase in the number of questions that can be scientifically investigated only on animal models (more precisely on GM animal models). Therefore they are strongly oriented towards the use of animals and act contrary to the search for alternatives. In most cases (maybe with the exception of toxicology), the question of alternatives is not reducible to the problem of whether one single experiment on an animal can be replaced by an alternative method, but rather it involves reflections about the research strategies chosen in order to achieve specific goals. GM animals represent the consummation of laboratory animal research strategy, and therefore they cannot serve as a starting point for the application of the 3Rs. In other words, the problem is that in many cases this kind of research does not allow a one-to-one replacement of experiments with GM models by non-animal methods, because the kind of question formulated is thinkable only in such kinds of models. Paradoxically, this happens at a moment when the field of alternative methods is growing and flourishing, despite all difficulties. In a recent article, the scientific journalist Edgar Gärtner, analysing the German statistics on animal experimentation, points out that GM animal models are now essential and indispensable tools for the pharmaceutical industry and for basic research, with the consequence that, for a long time now in these fields, there has been no interest in developing alternative methods (Gärtner, 2006).

In summary, the fundamental orientation of research with GM laboratory animals towards supporting *animal* experimentation, together with the fact that, since the beginning, these methods have caused a huge increase in animal experiments and the use of laboratory animals, and in many cases are connected to higher risks of suffering and distress of the animals involved, render these technologies incompatible with a broad interpretation of the spirit of the 3Rs. Even the idea of creating GM insentient animals – an extremely complicated enterprise, which could only be achieved by applying a problematic view of sentience in animals and its biological foundation – as a form of replacement serves to perpetuate the view of the inevitability of animal experimentation.

I suggest that this incompatibility should be carefully taken into account in regulating the experimental practice using GM models, which is now very diffuse and destined to increase. In particular, it should be deliberated whether special restrictions on the creation of GM laboratory animals should be introduced, together with an increased financial support for alternative methods.

References

Anim.Al.See (2004). Final report and recommendations of a project funded by the European Commission. *Alternative methods in animal experimentation: evaluating scientific, ethical and social issues in the 3Rs context*, http://www.inemm.cnr.it/animalsee/Finalresult.html

ANZCCART (2001). Animal Welfare Committee, National Health, Medical Research Council. *The Welfare of transgenic Animals. Notes from workshops held in Sydney and Melbourne on 28, 30 November, 2001*, http://www.adelaide.edu.au/ANZCCART/publications/workshop_notes_11_2001.pdf

ANZCCART (2002).Animal Welfare Committee, National Health, Medical Research Council. Development and use of transgenic rodents in preclinical research – practical issues. *ANZCCART News 13*, 2.

APC, Animal Procedures Committee (2001). *Reports on Biotechnology June 2001,* http://www.apc.gov.uk/reference/biorec.pdf

Appleby, M. C. (1999). Tower of Babel: variation in ethical approaches, concepts of welfare and attitudes to genetic manipulation. *Animal Welfare 8*, 381-390.

Australian Government, Animal Welfare Committee (2005). *Guidelines for the creation, breeding, care and use of genetically modified and cloned animals for scientific purposes*. Draft consultation document 2005, http://www.nhmrc.gov.au/publications/_files/gmguidelines.pdf

Balls, M. (1999). Does the use of transgenic animals raise particular welfare and ethical concerns? *ATLA 27 (Suppl. 1)*, 811-813.

Balls, M., Goldberg, A., Fentem, J. H. et al. (1995). The Three Rs: The Way Forward. The Report and Recommendations of ECVAM Workshop 11. *ATLA 23*, 838-886.

Bekoff, M., Allen, C. and Burghardt, G. M. (eds.) (2002). *The Cognitive Animal*. Cambridge, MA: The MIT Press.

Block, N. (1995). On A Confusion About a Function of Consciousness? *Behavioral and Brain Sciences 18*, 227-47.

Boumans, V. and Hendriksen, C. (2006). The animal use barometer. Poster abstract *ALTEX 23*, 2/06.

Broom, D. M. (1998a). The effects of biotechnology on animal welfare. In A. Holland and A. Johnson (eds.), *Animal Biotechnology and Ethics* (69-82). London: Chapman and Hall.

Broom, D. M. (1998b). Welfare, Stress, and the Evolution of Feelings. *Advances in the Study of Behaviour, Vol. 27*, 371-403

Broom, D. M. (1999). Animal welfare: the concept and the issues. In F. L. Dolins (ed.), *Attitudes to Animals: Views in Animal Welfare* (129-142). Cambridge:

Cambridge University Press.

Brown, M. J. and Murray, K. A. (2006). Phenotyping of genetically engineered mice: humane, ethical, environmental, and husbandry issues. *ILAR Journal 47(2)*, 118-123.

Buehr, M., Hjorth, J. P. and Kornerup Hansen, A. (2003). Genetically Modified Laboratory Animals – What welfare problems do they face? *Journal of applied animal welfare science, 6(4)*, 319-338.

Bundesamt für Veterinärwesen (2004). *Statistik der Tierversuche in der Schweiz 2003*; http://www.bvet.admin.ch/tv-statistik/Jasta-03/D/Index.html

Buy, M. (1997). Transgenic animals, animal welfare and Ethics. Canadian Council of Animal Care (CCAC), *Resource Supplement*, Spring/Summer 1997, http://www.ccac.ca/en/Current/Current_Transgenic.htm#felasa

Campbell, K. H. S., McWhir, J., Ritchie, W. A. and Wilmut, A. (1996). Sheep cloned by nuclear transfer from a cultured cell line. *Nature 380 (6569)*, 64-66.

CCAC (1997). Canadian Council of Animal Care. *CCAC Guidelines on transgenic animal*, http://www.ccac.ca/english/gui_pol/gdlines/transgen/TRANSGE1.HTM

Cibelli, J. B., Campbell, K. H., Seidel, G. E. et al. (2002). The health profile of cloned animals. *Nature Biotechnology 20(1)*, 13-4.

Clausen, J. (2006). *Biotechnische Innovationen verantworten: Das Beispiel Klonen*. Darmstadt: Wissenschaftliche Buchgesellschaft.

Costa, P. (2000). Welfare aspects of the transgenesis. In M. Balls, A.-M. van Zeller and M. E. Halder (eds.) *Refinement and Replacement of Animal Experimentation* (1719-1723). Amsterdam: Elsevier Science.

Costa, P. (2001). La transgenesi animale tra speranze scientifiche e problematiche etiche. In A. Mannucci e M. Tallacchini (ed.), *Per un codice degli animali* (251-268). Milano: Giuffré Editore.

Cottrell, A. (1999). Sniffing the Camembert: on the Conceivability of Zombies. *Journal of Consciousness Studies 6*, 4-12.

Council of the European Union (2005). *Fourth report on the statistics on the number of animals used for experimental and other scientific purposes in the Member States of the European Union*. 5633/05 ENV 27 http://www.nca-nl.org/English/Docs/EU%20stats%202002.doc

Crabbe, J. C., Wahlstein, D. and Dudek, B. C. (1999). Genetics of mouse behavior: Interaction with laboratory environment. *Science 284*, 1670-1672.

Crick, F. and Koch, C. (1997). Consciousness and Neuroscience. *Cerebral Cortex*, http://www.paradise.caltech.edu/~cook/Workshop/Conscious/crick-koch-cc-97.html

Davidson, D. (1975). Thought and talk. In S. Guttenplan (ed.), *Mind and Language*. Oxford: Oxford University Press.

De Cock Buning, T. (2000). Genetic engineering: Is there a moral issue? In M. Balls, A. M. van Zeller and M. E. Halder (eds.), *Progress in the Reduction, Refinement and Replacement of Animal Experimentation* (1457-1464). Amsterdam, Lausanne, New York, Oxford, Shannon, Singapore, Tokyo: Elsevier.

De Cock Buning, T. (2004). Practical Difficulties in Balancing Harms and Benefits in the Modern Use of Laboratory Animals: Biotechnology. *ATLA, 32, Supplement 1*, 459-463.

Delpire, V., Mepham, T. B. and Balls, M. (2000). Proposal for a new ethical scheme addressing the use of laboratory animals for biomedical purposes. In M. Balls, A. M. van Zeller and M. E. Halder (eds.), *Progress in Reduction, Refinement and Replacement of Animal Experimentation* (841-849). Amsterdam: Elsevier.

Dennet, D. C. (1995). The Unimagined Preposterousness of Zombies. *Journal of Consciousness Studies 2*, 322-6.

Dennis, M. B. (2000). Humane endpoints for genetically Engineered Animals. *ILAR Journal*, 41(2).

Dol, M., Kasanmoentalib, S., Lijmbach, S. et al. (eds.) (1997). *Animal Consciousness and Animal Ethics: Perspectives from the Netherlands*. Assen, the Netherlands: van Gorcum.

Donnelly, S., McCarthy, C. R. and Singleton, R. Jr. (1994). The Brave New World of Animal Biotechnology. *Hastings Center Report 24 (1 Suppl)*, 1-31.

Dove, A. (2000). Milking the genome for profit. *Nature Biotechnology 18 (10)*, 1045-1048.

Falkner, E., Schöffl, H., Tritthart, H. A., Reinhardt, C. A. und Appl, H. (1997). *Tierversuche: Gentechnologie und Ersatz- und Ergänzungsmethoden – Endbericht zur Studie*. Wien: Rote Reihe des Bundesministerium für Gesundheit, Sport und Konsumentenschutz

FELASA (Federation of European Laboratory Animal Science Associations) September 1992, revised February 1995. *Transgenic Animals – Derivation, Welfare, Use and Protection.*

Ferrari, A. (2005). Zur Verantwortungsproblematik in Konfliktsituationen gentechnologischer Anwendungsbereiche. Albert Schweitzers Ethik und die genetische Modifikation von Tieren in der biomedizinischen Forschung. In G. Schüz (Hrsg.), *Leben nach Maß – zwischen Machbarkeit und Unantastbarkeit. Biotechnologie im Licht des Denkens von Albert Schweitzer* (257-280). Frankfurt am Main: Peter Lang Verlag.

Flanagan, O. and Polger, T. (1995). Zombies and the Function of Consciousness. *Journal of Consciousness Studies 2*, 313-321.

GA mouse welfare assessment working group (2006). *Assessing the welfare of genetically altered mice* http://www.nc3rs.org.uk/downloaddoc.asp?id=356

Galert, T. (2005). *Vom Schmerz der Tiere. Grundlagenprobleme der Erforschung tierischen Bewusstseins*. Paderborn: Mentis Verlag.

Gärtner, E. (2006). Tierversuche – wo sind sie ersetzbar? *Bild der Wissenschaft 2*, 36-45.

Gordon, J. W. (1997). Transgenic technology as an alternative to animal experimentation. In L. F. M. Van Zutphen and M. Balls (eds.), *Animal Alternatives, Welfare and Ethics* (95-112). Amsterdam: Elsevier Science B. V.

Gordon, J. W. and Ruddle, F. H. (1981). Integration and stable germ line transformation of genes injected into mouse pronuclei. *Science 214*, 1244-1246.

Gordon, J. W., Scangos, G. A., Plotkin, D. J. et al. (1980). Genetic transformation of mouse embryos by microinjection of purified DNA. *Protocols of the National Academy of Science USA. 77(12)*, 7380-4.

Gossler, A., Doetschmann, T., Korn, R. et al. (1986). Transgenesis by means of blastocyst-derived embryonic stem cell line. *Protocols of the National Academy of Science USA. 83*, 9065-9069.

Griffin, D. R. (1992). *Animal Minds*. Chicago: University of Chicago Press.

Grindon, C. and Bhogal, N. (2005). The Fourth EC Report on the Statistics of Laboratory Animal Use: Trends, Recommendations and Future Prospects. *ATLA, 33 (4)*, 417-426.

Grubb, B. R. and Boucher R. C. (1999). Pathophysiology of gene-targeted mouse models for cystic fibrosis. *Physiological Reviews, 79 (suppl.)*, 193-214.

Gruber, F. P. (1998). Transgenic Animals within the 3R concept. *ALTEX 15(1)*, 26-28.

Gruber, F. P. and Spielmann, H. (Hrsg.) (1996). *Alternativen zu Tierexperimenten. Wissenschaftliche Herausforderung und Perspektiven*. Berlin, Heidelberg, Oxford: Spektrum Akademischer Verlag.

Hawkins P. (2002). Recognizing and assessing pain, suffering and distress in laboratory animals: a survey of current practice in the UK with recommendations. *Laboratory Animals 36*, 378-395.

Hawkins, P.Felton, L. M., van Loo, P. et al. (2006). Report of the 2005 RSPCA/UFAW Rodent Welfare Group meeting. *Laboratory Animal, Vol. 35, N. 9*, 29-38.

Hill, J. R., Winger, Q. A., Burghardt, R. C. and Westhusin, M. E. (2001). Bovine nuclear transfer embryo development using cells derived form a cloned fetus. *Animal Reproduction Science 67 (1-2)*, 17-26.

Home Office (2003). Statistics of Scientific Procedures on Living Animals. Great Britain, 2002.

Hudson M. and Boghal N. (2004). Scientific Procedures on Living Animals in Great Britain in 2003: The Facts, Figures and Consequences. *ATLA 32*, 437-485.

Jenkins, E. S. and Combes, R. D. (1999). The welfare problems associated with using transgenic mice to bioassay for bovine spongiform encephalopathy. *Animal Welfare 8*, 421-431.

Koch, C (2004b). Consciousness is Localized and We can Discover Where it is. Intervista di Andrea Lavazza. *Networks, 3-4*, S. 71-76 http://lgxserve.ciseca.uniba.it/lei/ai/networks/04/koch_engl.pdf

Koch, C. (2004a). *The Quest for Consciousness. A neurobiological approach*. Englewood, Colorado: Roberts.

Lane, N. and Jennings, M. (2004). Supplementary Resources for lay members of Local Ethical Review Processes. Projects involving genetically modified animals. *RSPCA Research Animals Department*, 1st editon, July 2004.

Macer, D. (1989). Uncertainties about "painless" animals. *Bioethics Vol. 3, N.3*, 226-235.

Mepham, T. B. (1994). Transgenesis in Farm Animals: Ethical Implications for Public Policy. *Politics and Life Sciences 13 (2)*, 195-203.

Mepham, T. B. and Crilly, R. (1999). Bioethical Issues in the Generation and Use of Transgenic Farm Animals. *ATLA 27*, 847-855.

Mepham, T. B., Combes, R. D., Balls, M. et al. (1998). The Use of Transgenic Animals in the European Union – The Report and Recommandations. *ATLA 26*, 21-43.

Moore, C. J. and Mepham, B. T. (1995). Transgenesis and animal welfare. *ATLA 23*, 380-397.

Moss, H. (2005). *Christof Koch and the Quest for Consciousness. With Comments by Oliver Sacks*. New York Academy of Sciences. Posted Jul 29,2005 http://www.nyas.org/publications/readersReport.asp?articleID=27

MUAWC (1997). Monash University Animal Welfare Committee. *Policy on use of genetically modified animals* http://64.233.183.104/search?q=cache:DrvSDn98huYJ:www.monash.edu.au/research/ethics/animal/regguide/gmpol05.rtf+guidelines+transgenic+animal+monash&hl=de&gl=de&ct=clnk&cd=3&client=firefox-a

Nadeau J. H., Balling, R., Barsh, G. et al. (2001). International Mouse Mutagenesis Consortium. Sequence interpretation. Functional annotation of mouse genome sequences. *Science 291*, 361-379.

Nagel, T. (1974). What is it like to be a bat? *The Philosophical Review LXXXIII, 4* (October 1974), 435-50, http://members.aol.com/NeoNoetics/Nagel_Bat.html

National Academy of Science (2002). *Scientific and Medical Aspects of Human Reproductive Cloning*. The National Academy of Science.

Nationaler Ethikrat (2004). *Klonen zu Fortpflanzungszwecken und Klonen zu biomedizinischen Forschungszwecken. Stellungsnahme*. Berlin: Druckhaus Berlin-Mitte.

Nuffield council of bioethics (2005). The ethics of research involving animals. London: Nuffield Council of Bioethics. http://www.nuffield-bioethics.org/fileLibrary/pdf/RIA_Report_FINAL-opt.pdf

Palmiter, R. D., Brinster, R. L., Hammer, R. E. et al. (1982). Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. *Nature 300 (5893)*, 611-5.

Perler, D. und Wild, M. (2005). *Der Geist der Tiere*. Frankfurt am Main: Suhrkamp Verlag.

Reiss, M. J. and Straughan, R. (1996). *Improving Nature? The Science and Ethics of Genetic Engineering*. Cambridge:Cambridge University Press.

Revermann C., Hennen L. (2000), T. A. Projekt. "Klonen von Tieren". Endbericht. TAB, Büro für Technikfolgenabschätzung beim Deutschen Bundestag, TAB-Arbeitsbericht NR. 65.

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 Group on Refinement. *Laboratory Animals, 37 (Suppl. 1)*, 1-51.

Rollin, B. E. (1986). On Telos and Genetic manipulation. *Between the species 14/ 2/1986.*

Rollin, B. E. (1989). *The Unheeded Cry: Animal Consciousness, Animal Pain and Science*. New York: Oxford University Press.

Rollin, B. E. (1995). *The Frankenstein Syndrome. Ethical and Social Issues in the Genetic Engineering of Animals*. Cambridge: University Press, Cambridge.

Roths J. B., Foxworth, T. B., McArthur, M. J. et al. (1999). Spontaneous and engineered mutant mice as models for experimental and comparative pathology: History, comparison, and developmental technology. In: *Laboratory Animals Science 49*, 12-34.

Royal Society (2001). *The use of Genetically Modified Animals: Policy Document 5/01*. London: Royal Society.

Russell, W. M. S. and Burch, R. L. (1959). *The Principles of Humane Experimental Technique*. London: Methuen. Second edition UFAW, 1992.

Salomon, B., Appl, H., Schöffl, H. et al. (2001). *Erfassung und Bewertung des Leidens sowie der Belastung transgener Tiere im Tierversuch im Vergleich zu konventionellen Tierversuchen*. Wien: im Auftrag des Bundesministeriums für Bildung, Wissenschaft und Kultur.

Sandøe, P. and Holtug, N. (1993). Transgenic animals – which worries are ethically significant? *Livestock Production Science 36*, 113-116.

Sandøe, P., Forsman, B. and Hansen, A. K. (1996). Transgenic animals: the need for an ethical dialogue. *Scandinavian Journal of Laboratory Animal Science 1*, 3, 279-285.

Sauer, U. G., Kolar, R. and Rusche, B. (2005). Die Verwendung transgener Tiere in der biomedizinischen Forschung in Deutschland. Teil 1: Status Report 2001-2003. *ALTEX 22, 4*, 233-257.

Sauer, U. G., Kolar, R. and Rusche, B. (2006). Die Verwendung transgener Tiere in der biomedizinischen Forschung in Deutschland. Teil 2: Ethische Bewertung der Verwendung transgener Tiere in der biomedizinischen Forschung und Perspektiven für die Umstellung der Forschung auf tierversuchsfreie Verfahren. *ALTEX 23, 1*, 3-16.

Shiga, K., Fujita, T., Hirose, K. et al. (1999). Production of calves by transfer of nuclei from cultured somatic cells obtained from japanes black bulls. *Theriogenology, 52 (3)*, 527-535.

Smyth, D. H. (1978). Alternatives to Animal Experiments. London: Scholar Press Ltd.

Stafleu, F. R., Grommers, F. J. and Vorstenbosch, J. (1996). Animal welfare: evolution and erosion of a moral concept. *Animal Welfare 5*, 225-234.

Thon R., Lassen, J., Hansen, A. K. et al. (2002). Welfare evaluation of genetically modified mice? An inventory of reports to the Danish Animal Experiments Inspectorate. *Scandinavian Journal of Laboratory Animals Science 29*, 45-53.

Tian, X. C. (2003). Cloning animals by somatic cell nuclear transfer – biological factors. *Reproductive Biology and Endocrinology, 1*.

Toth, L. A. (2000). Defining the moribund condition as an experimental endpoint for animal research. *ILAR Journal 41*, 2.

Van de Meer, M. (2001). *Transgenesis and animal welfare. Implications of transgenic procedures for the welfare of the laboratory mouse*. Utrecht University: Department of Laboratory and Animal Science.

van de Meer, M., Baumans, V. and van Zutphen, L. F. M. (1997). Measuring welfare aspects of transgenic animals. *Animal Alternatives, Welfare and Ethics*, 229-233.

Van der Meer, M., Costa, P., Boumans, V. et al. (1999). Welfare assessment of transgenic animals: Behavioural Responses and morphological developments in new born mice. *ATLA 27 (Suppl. 1)*, 857-868.

Van Reenen, C. G., Blokhuis H. J. (1993). Investigating welfare of dairy calves involved in genetic modification: problems and perspectives. *Livestock Production Science 36*, 81-90.

Van Reenen, C. G., Meuwissen, T. H., Hopster, H. et al. (2001). Transgenesis may affect farm animal welfare: A case for systematic risk assessment. *Journal of Animal Science 79*, 1763-1779.

Verhoog, H. (1992). The Concept of Intrinsic Value and Transgenic Animals. *Journal of Agricultural and Environmental Ethics 5*, 147-160.

Voedsel en Waren Autoriteit (2003). *Zo doende 2003* http://www.vwa.nl/download/rapporten/Dierproeven/zo-doende_2003.pdf

Vogel, B. (1997). *Tappt die medizinische Forschung in die Mausefalle?* im Auftrag des WWF Schweiz und der Ärztinnen und Ärzte gegen Tierversuche, SAG (Schweizerische Arbeitsgruppe Gentechnologie). Freiburg: Zypresse Verlag.

Vogel, B. (2001). *OncoMouse™. Eine Recherche zur medizinischen und kommerziellen Bedeutung der Harvard-Krebsmäuse*. Greenpeace: Hamburg/Zürich; http://www.greenpeace.de/fileadmin/gpd/user_upload/themen/patente_auf_leben/OncoMouse.pdf

Vorstenbosch, J. (1993).The Concept of Integrity. Its Significance for the Ethical Discussion on Biotechnology and Animals. *Livestock Production Science 36*, 109-112.

Vorstenbosch, J. M. G. (2005), The ethics of the Three Rs principle: a reconsideration. In *Animal Welfare 2005, 14*, 339-345.

Wagner, J., Thiele, F. and Ganten, D. (1995). Transgenic animals as models for human disease. *Clinical and Experimental Hypertension 17(4)*, 593-605.

Wagtendonk-de Leeuw van, A. M., Mullaart, E., de Roos, A. P. et al. (2000). Effects of different reproduction techniques: AI MOET or IVP, on health and welfare of bovine offspring. *Theriogenology 15, 53(2)*, 575-97.

Wells, D. J., Playle, L. C., Enser, W. E. et al. (2006a). Assessing the welfare of genetically altered mice. Summary. *Laboratory Animals 40*, 111-114.

Wells, D. J., Playle, L. C., Enser, W. E. et al. (2006b). Assessing the welfare of genetically altered mice. Report at www.nc3rs.org.uk/GAmice or www.lal.org.uk/gaa.

Wilmut, I. (2002). Are there any normal cloned mammals? *Nature Medicine, Vol. 8, N. 3*, 215-216.

Wilmut, I., Schnieke, A. W., McWhir, J. et al. (1997). Viable offspring derived from fetal and adult mammalian cells. *Nature 385 (6619)*, 810-813.

Wolf E. (1997). Transgenic animals: an alternative? Proponent's statement. In: Van Zutphen L. F. M, Balls M. *Animal Alternatives, Welfare and Ethics* (271-275). Amsterdam: Elsevier.

Yong, Z. and Yuqiang, L. (1998). Nuclear-cytoplasmatic interaction and development of goat embryos reconstructed by nuclear transplantation : production of goats by serially cloning embryos. *Biology of Reproduction 58 (1)*, 226-9.

Correspondence to
Dr. Arianna Ferrari
Institute for Philosophy
Technical University of Darmstadt
Schloss
64283 Darmstadt
Germany
e-mail: ferrari@phil.tu-darmstadt.de