



Session 3.4 Policy implementation

Three Years of Animal Welfare in the German Constitution – the Balance from an Animal Welfare Perspective

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Summary

The inclusion of animal welfare into the German Constitution in 2002 gained worldwide attention. Animal welfare organisations had been striving to reach this goal for more than a decade, and they had good reasons to do so: in several court cases the regulations of the German Animal Welfare Act had been overruled by basic rights laid down in the Constitution, such as the freedom of research and the freedom of education.

To review the situation, case studies focussing on court cases and assessments by members of ethics committees are used to demonstrate what consequences the change of the German Constitution has brought about.

Keywords: animal protection, animal welfare legislation, ethics committees

Introduction

Before the year 2002, the appropriate regulation of animal experimentation in Germany was severely compromised by the fact that the German Constitution did not include animal welfare as a specific objective, whereas freedom of (*inter alia*) arts, religion, education and research were, and still are, guaranteed as basic rights (Caspar und Schröter, 2003).

For that reason, High Court Decisions directly interfered with regulations laid down by the German Animal Welfare Act (Bundestag, 1998), as well as EU Council Directive 86/609 (Anon., 1986a), and the Council of Europe Convention ETS 123 for the Protection of Animals Used for Experimental and other Scientific Purposes (Anon., 1986b) in those cases where there was a conflict between animal welfare and constitutional rights.

Court cases before 2002

Freedom of education overrules the Animal Welfare Act

In 1993 a professor at the University of Giessen filed a lawsuit against the local competent authority's decision to not grant licenses for experiments on live rats for educational purposes (demonstration of glucose uptake) that he had applied for. In its decision on this case, the competent Supreme Administrative Court (*Verwaltungsgerichtshof*) of the City of Kassel came to the conclusion that the experiments were legal, even though it had been demonstrated that an alternative (a film showing the experiment) existed. It stated that animal welfare, based on ethical grounds, did not have constitutional status and therefore could not compromise the university teacher's basic right to freedom of education.¹

German Federal Constitutional Court restricts ethical examination of animal research

In 1994, a professor at the University of Berlin filed a lawsuit against the local competent authority's decision to no longer

¹ Verwaltungsgerichtshof (VGH) Hessen, Kassel, Urteil vom 29.12.1993, AZ 11 TH 2796/93. NJW 1994, 1608 ff.



grant licenses to continue experiments on newborn non-human primates that he had applied for. The Federal Administrative Court (*Bundesverwaltungsgericht*) of Berlin regarded the authority's decision as a restriction to the basic right of freedom of research and called upon the Federal Constitutional Court (*Bundesverfassungsgericht*, the highest court in Germany) to solve this conflict between provisions of the German Animal Welfare Act and the German Constitution. The Federal Constitutional Court, surprisingly, concluded that no conflict existed. In its opinion, the Animal Welfare Act had to be interpreted in a way that it would conform to the Constitution. Such an interpretation would suggest that the authority was not entitled to make its own conclusions on the ethical justifiability of applications for animal experiments. Instead, it had to accept the applicant's reasoning, as long as this was coherent, and as long as no formal reasons stood against it.²

This decision had concrete consequences for the practice of licensing animal experimentation in Germany. For instance, after that decision the local competent authority of Berlin was officially advised to restrict its examination of applications for animal experiments to formal criteria.³

Not only to experts in the field such a restriction appeared to be in clear contradiction to basic provisions of the German Animal Welfare Act, and to Council Directive 86/609/EEC for the Protection of Animals Used for Experimental and other Scientific Purposes that Germany had to implement and enforce as a member of the European Union. In its Article 12, the Directive explicitly states "the authority shall take appropriate judicial or administrative action if it is not satisfied that the experiment is of sufficient importance for meeting the essential needs of man or animal". This provision clearly implies that the authority is expected to make its own assessment of the ethical justifiability of a proposed animal experiment.

The debate on the amendment of the Constitution in Germany

These court cases, and other court decisions that compromised animal welfare provisions, also because of the fact that they conflicted with other basic rights that were guaranteed by the German Constitution (such as the "art" of publicly killing a fish in a kitchen mixer), raised great concern not only in the German animal welfare community, but also in politicians and lawyers. They ignited a public debate that was to last for a decade. In this debate, the German Animal Welfare Federation and other animal welfare organisations lobbied and campaigned hard for insertion of a reference to animal protection in the German Constitution, so that it could be balanced against the established constitutional rights. The argument behind these activities was clear: as long as animal welfare provisions could be overruled because of the Constitution, the German Animal Welfare Act, often claimed to

be one of the best legislations for animal protection in the world, would be nothing but a toothless tiger (Hobe, 1998).

On the other hand, the scientific community, in Germany and also abroad, drew up a horror scenario, prophesying that any reference to animal welfare in the Constitution would hamper scientific progress in Germany, and lead to emigration of researchers and scientific institutions, see e.g. a *Nature Neuroscience* editorial (Anon., 2002): "The likely result will be great damage to German biomedical research." These extreme reactions of researchers in Germany were not always understood well by scientists outside of Germany, as the German legal system was unique worldwide anyway, see e.g. an *ALTEX* editorial (Balls, 1999): "How can any groups in our societies expect to be allowed freedom to do what they want, unless that freedom is expressed in an acceptable ethical framework?"

The amendment of the German Constitution – wording and implications

In the year 2002 the German Constitution was amended. The amendment concerned Article 20a, which before 2002 read: "The state takes responsibility for protecting the natural foundations of life in the interests of future generations." The legal interpretation of this article had been that "life" was to mean "human life", therefore animals were not addressed in this article before 2002. The amendment of Article 20a basically consisted of the addition of the words "and animals" to the clause, which now reads:

"Mindful also of its responsibility toward future generations, the state shall protect the natural bases of life *and animals* by legislation and, in accordance with law and justice, by executive and judicial action, all within the framework of the constitutional order."

The legal interpretations of this amendment generally concluded that there were three main aspects regarding its implications (see for example Caspar und Schröter, 2003):

- Animal welfare had become a "state goal" – a matter that requires consideration when the government formulates new legislation, or existing law is interpreted by authorities and courts.
- The amendment does not grant individual rights to animals.
- Instead, it provides a legal basis for weighing animal protection measures against human interests in matters such as research and teaching.

The reality today: Court cases after 2002

To analyse whether these interpretations are reflected in legal practice, it is worthwhile to look at a court case from the year 2003, when the University of Marburg filed a lawsuit against the local competent authority's decision to not grant licenses for experiments on rats in the context of research on drug-induced pathophysiology of weight regulation.

The Administrative Court of the City of Giessen rejected the University's lawsuit and based its decision on the amended

² Bundesverfassungsgericht (BVerfG), Urteil vom 20.06.1994, AZ 1 Bv12/94. *Natur und Recht* 1995, Heft 3, 135-137.

³ Verwaltungsgericht (VG) Berlin, Urteil vom 07.12.1994, AZ VG 1 A 232/92.



Constitution: After a reference to animal welfare had been inserted into the Constitution, the local authorities had the right and the duty to perform their own ethical evaluation. The court also made it clear that the authorities had the duty to reject applications if provisions of the Animal Welfare Act (they referred to indispensability, ethical justification) were not met.⁴ The University of Marburg then appealed to a higher court to revoke that decision. That court, the Supreme Administrative Court (*Verwaltungsgerichtshof*) of the City of Kassel rejected the appeal in 2004. It based its decision on the fact that, in its opinion, the applicant was unable to demonstrate the indispensability of the proposed experiment.⁵

The Court also directly referred to the new legal situation after the change of the Constitution (see above).

The reality today: A survey among members of ethics committees

The Animal Welfare Academy intended to analyse the reality of regulation of animal experiments after the Constitution's amendment. That reality should be reflected in the work of the licensing authorities and ethics committees. Therefore it is presently undertaking a survey among licensing authorities and members of ethics committees on selected aspects of the workings of the ethical evaluation process. This is being done in co-operation with a Ph.D. study at the University of Tuebingen. The Animal Welfare Academy had already undertaken surveys among members of ethics committees in Germany in the years 1989 and 1995 (Rusche, 1997; Gruber and Kolar, 1997).

The new survey will be based on questionnaires that will be sent to all local competent authorities for factual information, and to members of ethics committees for their individual assessment of the situation. Already, a pilot study has been completed: telephone interviews with animal welfare members of five (out of 33 in total) different ethics committees across the country have been carried out on both aspects (tab. 1).

⁴ Verwaltungsgericht (VG) Gießen, Urteil vom 13.08.2003, AZ 10 E 1409/03.

⁵ Verwaltungsgerichtshof (VGH) Hessen, Kassel, Urteil vom 16.06.2004, AZ 11 ZU 3040/03.

Summary of results from telephone interviews with members of ethics committees

- The local competent authorities have not taken any substantial measures in reaction to the change in the Constitution.
- The ethics committees' work has not changed.
- At least some applications (still) lack an appropriate ethical justification.
- In a substantial number of cases, the task to evaluate whether an experiment is at all justifiable for the proposed research goal is (still) not performed.

If the results of the telephone interviews are confirmed in the overall survey, the conclusion must be that implementation of the state goal "animal welfare" is evidently unsatisfactory.

Discussion

From the analysis of court decisions before and after the year 2002, when the change in the German Constitution took place, there is an indication that the change has had a significant impact on the way issues of licensing of animal experiments are handled by courts in Germany. Their decisions now follow the amended Constitution's principle that constitutional rights must be balanced against animal welfare requirements, and that freedom of research can no longer be regarded a right that outweighs any regulations in the field of animal welfare.

However, what remains unchanged is the fact that researchers (or institutions) can still go to court to question rejections of applications by the licensing authority. On the other hand, there is no legal provision in place to allow questioning of the approval of an animal experiment by the authority. A real balance would require such a provision - for ethics committees, animal welfare organisations and others (see also Caspar und Schröter, 2003).

The number of telephone interviews with members of ethics committees carried out in the pilot study does not allow for definite postulations, however, regarding the questions selected for this publication, the answers received give clear indications.

From these interviews it became evident that the practice of licensing of animal experiments does not appear to have changed to a satisfactory extent. There are at least some local authorities that never reject an application. From previous experiences the conclusion can be drawn that this is at least partly

Tab. 1: Telephone interviews with animal welfare members of five (out of 33 in total) different ethics committees across Germany

Questions	Answers	
	yes	no
Has your competent authority informed your ethics committees members that a new legal situation exists after the change in Article 20a of the German Constitution?	2	3
Have you observed any changes in the evaluation of applications for animal's experiments after the adoption of animal welfare into the German Constitution in August 2002?	0	5
Have you observed any changes in the work of the competent authority after inclusion of animal welfare into the German Constitution?	0	5
Do you receive applications that contain standard arguments to answer questions regarding the ethical justifiability of the proposal?	4	1
According to your opinion, do the present circumstances allow for sound ethical evaluation?	2	3

due to the fact that the composition of ethics committees does not provide for a balance between interests in using animals and interests in protecting them. In ethics committees in Germany only one third of the members represent animal welfare whereas two thirds normally come from the scientific community.

Independent of these analyses, the fact has to be mentioned that an ethical evaluation of animal experiments carried out for regulatory purposes is still not foreseen in Germany. Such experiments only need to be reported to the authorities, and are never seen by ethics committees. A true balancing of costs for animals and benefits for humans would need to include an examination of the need for substances/products (Kolar, 2000).

Another worrying problem when talking about balancing animal welfare against human benefit is the fact that there is still no defined minimum standard for the benefit. This is to say that no research goal is insignificant enough, or, in the case of toxicological testing, no product is unessential enough to set up a legal requirement against animal experiments for that purpose. However, there are some exemptions to this. For example, in the European Union, legal measures have been taken to exclude animal testing of cosmetic products and ingredients (Anon., 2003; Ruhdel, 2004). The German Animal Welfare Act also prohibits animal experiments carried out for the development or testing of weapons, ammunition, tobacco products, and detergents. There are historical reasons why these product categories have gained specific attention, but from an ethical point of view there are other product categories that would require equal consideration. Similarly categories of academic research aims have to be examined regarding their capacity to justify animal experiments.

Like no minimum standard exists for the benefit, there is no absolute (defined) limit to (the suffering in) animal experiments, i.e. the cost. No procedure is painful or unbearable enough to be excluded by legislation. There are voluntary restrictive policies, such as in Switzerland, where the Swiss Academy of Sciences has set up guidelines that include a renunciation of extremely painful animal experiments, independent of the importance of the gain of knowledge they would promise (1995). However, from an animal welfare point of view, there is a need for legal provision in this context.

There are many other issues in animal research that need to be considered and regulated appropriately, if the state-goal animal welfare is to be taken seriously by decision-makers in Germany. As a matter of priority, however, the existing regulations of the German Animal Welfare Act need to be enforced. This seems to require immediate action by the licensing authorities and ethics committees, in particular with regard to the need to give more weight to the protection of animals when there are doubts about the indispensability of the proposed research. If at this level no significant shift of the cost-benefit balance towards animal protection can be observed, other levels of governmental administration must address this issue.

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Strategies to Reduce Animal Testing in US EPA's HPV Programme

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Summary

The High Production Volume (HPV) programme was launched by the US Environmental Protection Agency (EPA) in 1998. To reduce animals killed, the animal welfare community negotiated basic principles set forth in a letter from EPA to HPV participants, and reiterated in the Federal Register (2000). After five years reviewing more than 370 test plans, the outcome of this effort is disappointing. However, successful strategies were developed by these reviewers and in collaboration with conscientious companies to reduce testing and still meet the Screening Information Data Set (SIDS) requirements. These strategies are explored as they might apply to future testing programmes.

Keywords: high production volume (HPV), three Rs, chemical testing, toxicity testing, animal testing

Introduction and historical overview

The EPA's HPV programme has been in progress for five years, and was envisioned to involve commitments for 2,200 chemicals by 400 companies. Submitters were to assess existing hazard data and "data gaps", and propose a plan to fill these perceived gaps. Originally designated as a "voluntary" programme, since its inception hundreds of test plans have been submitted, many of which propose animal tests to complete the SIDS base set of Tier I data requirements. The HPV programme was modeled after a similar programme administered by the OECD, i.e., the Task Force on Existing Chemicals. In the U.S. programme, the SIDS is considered a minimum for hazard evaluation and hazard is stressed over considerations of potential exposure. While each of the 3Rs (replacement, reduction, refinement) is available to HPV participants, they have frequently been ignored and/or followed to varying degrees. This current paper provides an update to a previous presentation at the Fourth World Congress on Alternatives and Animal use in the Life Sciences, in which the US HPV programme was critiqued shortly after its implementation (Nicholson et al., 2004).

Screening information data set (SIDS)

The Tier I SIDS data requirements which use animals are provided in table 1, along with the corresponding OECD Test Guideline number and the number of animals used for each test.

The numbers of animals used per test can vary depending on exact study design, but the total number killed for a complete data set ranges from 750-870 mice, rats, and fish. Although an exact number is not possible to calculate, we have estimated that since the inception of the HPV programme, upwards of 150,000 animals have already been killed (through April 2005).

Animal welfare guidance and principles

When animal protection organisations became aware of the proposed programme, they maintained that the programme objectives, primarily the protection of human health, would not be met and that the cost in animal lives would be exorbitant. Through the White House, they also negotiated with stakeholders (EPA/Environmental Defense/American Chemistry Council) to put minimal animal welfare principles in place. The result was

Tab. 1: Screening Information Data Set (SIDS)

Test	OECD TG	# ANIMALS
Acute oral toxicity	425	3 - 10
Repeat Dose (28-day and 90-day) toxicity	407, 408	40 - 65
Combined reproductive/developmental toxicity	421	675
Combined repeated dose/reproductive/developmental toxicity	422	675
Acute toxicity to fish	203	40 - 120



the “October 1999” agreement letter, sent by EPA to programme participants (Wayland, 1999). These principles were subsequently published in the *Federal Register* (2000). The main tenets of these principles encouraged the following:

- The use of *in vitro* genetic toxicity testing rather than *in vivo* (unless impossible).
- Maximising the use of existing data.
- The use of weight-of-evidence and avoid “checklist toxicology.”
- The use of Structure Activity Relationships (SAR) to form chemical categories.
- No terrestrial testing (e.g., birds, etc).
- No new dermal testing (generally).
- No sub-chronic or reproductive toxicity testing on closed system intermediates.
- Special considerations for chemicals which have been previously determined to be GRAS (Generally Recognised As Safe).
- The use of validated non-animal tests as they become available and the delay of certain testing until some non-animal methods were in place.

The goal of these animal welfare principles was to minimise animal use, while still meeting the stated hazard identification goals of the programme. Our aim was to assist companies in avoiding check-the-box toxicology to fulfill the basic SIDS data set. If the recommended generalised principles of the October 1999 letter were indeed followed, the result would be a reduction in the numbers of animals killed under the HPV programme. Each of the 3R principles (replacement, reduction and refinement) was available to HPV participants. Reductions in the numbers of animals could be accomplished, for example, by using categories of chemicals to maximise existing data or by using established OECD combined protocols such as the OECD TG 422, a combined repeat-dose, reproductive, and developmental toxicity screen, instead of three separate tests to fulfill the endpoints. Refinements to tests involving animals included the use of OECD TG 425 and cytotoxicity tests instead of the traditional LD₅₀. Finally, replacing animals completely was possible in some cases, such as the use of *in vitro* genetic toxicity tests rather than *in vivo*. The principle of “thoughtful toxicology,” outlined in the October 1999 letter, provided an overarching opportunity for companies to carefully analyse existing data and decide whether additional animal tests would provide information that would be useful or relevant and to avoid such testing where it would not.

What went wrong with implementation of the HPV programme?

Once initiated, it became clear that the sponsors of HPV test plans often failed to follow even the minimal guidance offered above. The guidelines were not enforceable, and there is still no mechanism in 2005 to ensure that animal welfare guidelines are followed. In many cases, companies duplicated testing unnecessarily by conducting animal tests that had already been conducted but were conducted prior to implementation of Good Laboratory Practices (GLP), or by failing to coordinate efforts

with other companies sponsoring similar chemicals. In other instances, companies did not use existing published data, individually or in conjunction with other data (in a weight-of-evidence approach), to avoid new animal testing. Often times, sponsors would fail to show relevance, such as proposing acute fish toxicity tests on water-insoluble chemicals. In many cases, when it was clear that a test was not needed for HPV, the study was proposed in “anticipation” of future data requirements, primarily Registration, Evaluation and Authorisation of Chemicals (REACH). Some companies refused to use combined protocols, sometimes doubling the number of animals killed under their test plans. Even in obvious replacement opportunities, such as the use of *in vitro* genotoxicity tests, there was an inconsistent application of the principle. In its responses to test plan proposals, EPA itself frequently failed to follow or encourage sponsors to follow basic animal welfare guidance.

HPV since 2000

Scientists at PCRM and PETA have reviewed approximately 376 test plans through August 2005, representing both individual chemicals and small to large groups of chemicals. According to our figures, a full 50% of the test plans proposed from 2000 through 2002 called for animal testing, another 50% of the test plans submitted in 2003, 45% of the test plans in 2004, and 33% of the test plans submitted through May 2005 proposed new animal testing.

These test plans account for more than 150,000 animals used to date. Importantly, these figures do not include animal tests requested by EPA above and beyond those proposed in the original test plans. It is noteworthy that after more than five years and 150,000 animals killed, no additional protections have been implemented to protect human health or the environment as a result of the HPV programme. Importantly, hazard data being generated offer little in the way of assessing human risk in that exposure characterisations are discouraged and to some degree specifically excluded from the programme. Thus, there is no context to assess the large amount of hazard information being generated by the sponsors.

Additional strategies to reduce animal use in the HPV programme

In the process of reviewing hundreds of test plans, additional strategies have been developed to supplement those envisioned in the original October 1999 letter. Some of these are extensions of the original recommendations, e.g., “common sense” toxicology, identification of duplicative testing and/or overlooked data, promoting stronger weight-of-evidence approaches, etc. In addition, the wise use of resources has been stressed (a full SIDS data set may cost up to \$400,000 USD) as well as encouraging companies to resist regulatory pressures when testing does not make sense. In addition to the existing guidelines, and based on an extensive review of HPV test plans, additional animal welfare principles are described below.



- **Rapid Hydrolysis of Parent Chemical.** The parent chemical need not be tested in animals if it hydrolyses to well-characterised products in an aqueous environment at low pH. A bench study at stomach pH may be used to determine rate of hydrolysis and hydrolytic products. Existing data on the hydrolysis products may then be used to meet SIDS endpoints without additional testing.
- **Acidic/Corrosive/Irritating Materials.** These are usually strong acids; they may be completely ionised in aqueous environments and are expected to cause localised, corrosive effects in the GI tract. Results from animal tests will be confounded by the corrosivity of the chemical and mammalian testing would not yield meaningful results. Animal tests using such material are particularly painful.
- **Highly Reactive Materials.** These chemicals are highly reactive to air and/or water as demonstrated by physical/chemical data. Mammalian and ecotoxicity testing with these types of chemicals is not feasible.
- **Gases.** Primary concerns with these chemicals are flammability, explosivity at test levels, and/or insolubility in water. Many are asphyxiants, some are minimally toxic and rapidly excreted, so additional testing may not be feasible or will not yield meaningful results.
- **Complex Mixtures.** The product is a mixture from different manufacturing processes and/or waste streams. Additional testing with a variable mixture may not provide useful information and existing data on major constituents may be sufficient to fill SIDS endpoints.
- **Weight-of-Evidence.** Additional testing for reproductive toxicity can be eliminated if histopathology data on reproductive organs from a 90-day subchronic study are available, in combination with a negative developmental study. This guidance is provided in the Manual for Investigation of HPV Chemicals OECD Secretariat (SIDS Manual, 2004). In some cases, traditional reproduction/developmental studies are not required if existing data from other studies, such as 2-year cancer bioassays, have evaluated reproductive and developmental parameters. A separate developmental study is not required if data exists from one- or two-generation reproduction studies.

These additional strategies have been employed successfully in the US HPV programme, and have resulted in saving thousands of animals.

Implementation and implications

In order to implement these strategies, much time is spent by PCRM and PETA reviewing each test plan, conducting internet data searches, submitting detailed comments during the public comment period, and finally, contacting individual companies to discuss opportunities to eliminate or at least reduce animal testing. We encourage companies to submit revised test plans, and we offer support to those that have already used creative and well developed strategies that reduce testing (sometimes in the form of letters to the EPA). Continued review and comment will hopefully result in future opportunities to reduce animal testing further, both in the impending REACH programme and the recently announced "Extended HPV programme," planned for a January 2006 initiation and running through 2010.

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LD₅₀ Testing of Botulinum Toxin for Use as a Cosmetic

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Summary

In 2003, the Fund for the Replacement of Animals in Medical Experiments (FRAME) revealed that the potency of botulinum toxin for use as a popular wrinkle treatment is assessed by using the LD₅₀ Test. The endpoint in this mouse-based testing is death through suffocation. In 2004, The Humane Society of the United States (HSUS) sought to work with Allergan, the US-based manufacturer of Botox® Cosmetic, and the U.S. Food and Drug Administration (FDA), on ways to refine, reduce, and replace this LD₅₀ testing. This article summarises The HSUS's campaign in the United States and provides an update on FRAME's continuing efforts in Europe.

Keywords: Allergan, LD₅₀, Botox, botulinum toxin, Dysport, FDA, potency

Introduction

Botulinum toxin, produced by various bacteria, including *Clostridium botulinum*, is one of the most powerful biological toxins known, which blocks transmission of nerve impulses to muscles. Eight subspecies of the bacterium produce seven distinct types of toxin (types A-G), which act through different mechanisms. Food-borne botulinum toxin causes Botulism.

Several companies use botulinum toxin as the active ingredient in therapeutic products for treatment of conditions such as cervical dystonia, strabismus, blepharospasm, and hyperhidrosis. For example, Ipsen Limited UK markets a botulinum toxin Type A product, Dysport®, while the Allergan Corporation, based in the United States, markets two botulinum toxin Type A products: Botox®, for therapeutic applications, and Botox® Cosmetic, the popular wrinkle treatment – a cosmetic application.

Botulinum toxin is produced for commercial application in fermentation batches seeded with the bacteria. The standard method for assessing the potency of botulinum toxin batches is a mouse LD₅₀ Test (Bottrill, 2003). In this procedure, mice are sorted into dose groups, given a single injection of toxin, and monitored over 3-4 days. Death is the endpoint, which results from suffocation through paralysis of the diaphragm musculature. Although the precise details are not available, over 100 mice are used per test, and the mouse testing is carried out up to three times prior to batch release. Calculations from the test data yield an LD₅₀ value (the dose which would kill half the number of animals in a test group), which is then standardised as one “mouse unit”.

The LD₅₀ testing of botulinum toxin products runs counter to three trends in the application of the Three Rs of replacement, reduction, and refinement, and in animal welfare generally. First, the use of the LD₅₀ test is being phased out worldwide. This was symbolised most dramatically in the field of industrial chemicals, when, in 2002, the Organization for Economic Cooperation and Development deleted the LD₅₀ Test (its Test Guideline 401) from its Health Effects Test Guidelines (OECD, 2002). Second,

the use of death as an endpoint is the *bête noire* of the growing field of humane endpoints (Olfert et al., 1998; OECD, 2000; ILAR, 2000). The third trend, applicable to LD₅₀ testing of Botox Cosmetic, is the phasing out of animal testing of products with a cosmetic use. For example, in 2004, the European Union banned all forms of animal testing of cosmetic products (Europa, 2003).

In 2003, the Fund for the Replacement of Animals in Medical Experiments (FRAME) drew attention to the issue of LD₅₀ testing of botulinum toxin products with the publication of an exposé entitled “Growing Old Disgracefully ...” (Bottrill, 2003; Balls, 2003). The Humane Society of the United States (HSUS) then took up the challenge in the USA. FRAME focuses its efforts on the European scene, and The HSUS on the US scene, but we are pleased to have this opportunity to show that we work together, and to provide brief updates on earlier assessments of alternatives to the mouse LD₅₀ testing of botulinum products (Bottrill, 2003; Balls, 2003) and on the FRAME campaign, as well as a summary of the HSUS campaign.

Background

An update on alternatives for the potency assessment of botulinum toxin products

Table 1 summarises some of the potential alternatives to the mouse LD₅₀ Test for assessing the potency of botulinum toxin products. Much of this information is taken from Bottrill's 2003 review. Potential refinements include mouse-based methods that assess local paralysis either *in vivo* or *ex vivo*, in contrast to systemic paralysis *in vivo*, as in the LD₅₀ assay. Potential replacements target the specific molecules involved in nerve transmission that are disrupted by the various types of botulinum. For example, the SNAP-25/endopeptidase assay (Ekong et al., 1997) assesses *in vitro* the extent to which botulinum toxin Type A disrupts the activity of synaptosomal-associated protein of molecular mass 25kDa (SNAP-25), a molecule with a critical role in transmitting nerve signals.



In their recent monograph on testing botulinum toxin, the influential European Pharmacopeia (Anon., 2005) recognised the potential of these methods to substitute for the mouse LD₅₀ test, stating that: “After validation with respect to the LD₅₀ assay (reference method), the product may also be assayed by other methods that are preferable in terms of animal welfare”, including the *in vitro* endopeptidase assay, the *ex vivo* assay using the mouse phrenic nerve diaphragm, and the “mouse bioassay using paralysis as the endpoint.”

The European Pharmacopeia monograph was published prior to the publication of the Endopep-MS assay *in vitro* method (tab. 1), which has the potential advantage of permitting assessments of the potencies of all of the types of botulinum toxin.

An update on the FRAME campaign

FRAME is concerned that Ipsen Limited UK continues to use the mouse LD₅₀ test to measure the potency of Dysport, and urges the Home Office (the British Government department responsible for the control of animal experimentation) to do more to bring about an end to the animal testing of botulinum toxin products for clinical and/or cosmetic use, and considers that the Government should close the loophole which permits botulinum toxin destined to be used for cosmetic purposes to be tested in animals, despite the ban on testing cosmetic products in the UK. The claim is that botulinum toxin is only officially licensed (and therefore tested) for clinical use, and its use for cosmetic purposes involves a private contract between a physician and a patient, at their own risk.

FRAME applauds the efforts of the UK national control agency, the National Institute for Biological Standards and Control (NIBSC), to develop and use refinement and replacement alternatives. The NIBSC uses *in vitro* methods on a routine basis, and only uses a non-lethal *in vivo* test when, rarely, the results of an *in vitro* test are inconclusive or close to pass/fail specifications.

FRAME is also encouraged by the effort being put by the NIBSC into the development of methods which could totally obviate the need for animal testing (eg, Ekong et al., 1997), and also that Ipsen Limited UK are working with the NIBSC and others to develop suitable batch release tests.

Meanwhile, at the European level, the European Centre for the Validation of Alternative Methods (ECVAM) and the European Directorate for the Quality of Medicines (EDQM) are working together and with others to review what progress is being made in applying the Three Rs to botulinum toxin testing and to assist in moving forward.

Summary of HSUS campaign

The HSUS campaign focuses exclusively on the testing of Botox Cosmetic by its manufacturer, Allergan, Inc., based in California. Botox Cosmetic wrinkle treatment is the most common cosmetic procedure in United States, with 2.8 million treatments carried out in 2004 (Allergan, 2005; ASAPS, 2005), accounting for 40% of net Botox sales or \$295M. The HSUS regards the LD₅₀ testing of products for cosmetic use as unacceptable, and seeks to hold Allergan accountable.

The strategy was to first seek to work *with* Allergan, and only if that approach failed, would The HSUS seek to pressure the company from the outside. Three things were sought from Allergan:

1. public disclosure of the details of its current potency testing of Botox Cosmetic;
2. public disclosure of the details of its current efforts to develop alternatives to the mouse LD₅₀ testing of Botox Cosmetic; and
3. adoption of a well-funded and publicly available plan to rapidly end the LD₅₀ testing of Botox Cosmetic.

For several months, beginning in January 2004, The HSUS engaged in cordial, but largely fruitless, communication with Allergan. The company communicated with The HSUS only through its legal staff. At The HSUS's request, the company met with Alan Goldberg, Director of the Johns Hopkins Center for Alternatives to Animal Testing (CAAT), to discuss potential CAAT/Allergan collaboration on alternatives, but the company never followed up this suggestion.

Allergan did confirm that the company uses the LD₅₀ Test to assess the potency of Botox Cosmetic, and claimed to have an active alternatives program to replace this testing. However, the company provided few details either of its current testing practices or of its alternatives efforts. In its defense, Allergan noted that Botox Cosmetic and its sister product, Botox, share the same active ingredient, botulinum toxin Type A, so LD₅₀ testing for the two products is inextricably linked and testing for cosmetic purposes cannot be cleanly separated from testing for therapeutic purposes. Allergan also noted the international regulations calling for the LD₅₀ testing of botulinum toxin products.

The HSUS took note of these claims, but concluded that they collectively failed to justify the company's secrecy concerning its testing and alternatives practices. In The HSUS's view, any company that is making \$300M a year on the backs of suffocating animals deserves to be held publicly accountable for working towards an urgent solution, especially in the context of a cosmetic application.

Tab. 1: Promising alternative methods to the mouse LD₅₀ test for assessing the potency of botulinum toxin products

Name of Test	System	Endpoint	Duration	Reference
Mouse hind-limb assay	<i>in vivo</i>	local paralysis	2 days	Pearce et al., 1995
Abdominal ptosis assay	<i>in vivo</i>	local paralysis	< 1 day	Takahashi et al., 1990
Mouse phrenic nerve-hemidiaphragm system	<i>ex vivo</i>	muscle contraction	< 1 day	Bigalke et al., 2001
SNAP- 25/Endopeptidase assay	<i>in vitro</i>	molecular disruption of nerve transmission	< 1 day	Ekong et al., 1997
Endopep-MS	<i>in vitro</i>	molecular disruption of nerve transmission	< 1 day	Boyer et al., 2005



Consequently, The HSUS decided to implement the second, conditional part of its strategy towards Allergan, namely, applying public pressure to the company. Beginning in October 2004, The HSUS began issuing calls to its members and constituents to urge the company to work with The HSUS on rapidly replacing LD₅₀ testing for Botox Cosmetic. The calls were issued through the organisation's electronic and hard-copy publications. In response to one appeal, thousands of e-mails to Allergan compelled the company to shut down an e-mail account.

Since Allergan refused to work with The HSUS or to disclose information about its testing and alternatives practices, The HSUS turned to the U.S. Food and Drug Administration (FDA), which had approved Botox Cosmetic and Botox. The FDA regulates these products as pharmaceuticals, and now oversees their manufacture and sale. The HSUS was specifically interested in information about the potency testing currently required or encouraged for these products. It was hoped that the agency could help answer several key questions, including the following.

1. What are the current testing practices?
2. Does the FDA require or encourage these practices?
3. How have these practices changed over the years?
4. What is the FDA itself doing to promote LD₅₀ alternatives?

In 2004, The HSUS filed two Freedom of Information Act requests with the FDA, in order to obtain the sought-after information, but the agency was largely unresponsive. Consequently, the HSUS initiated legal action in 2005 to obtain the requested documents. This legal action is still pending.

Discussion and conclusions

FRAME, The HSUS, and similar organisations engage in advocacy of the Three Rs, because they want to accelerate the pace of progress in the development, validation, and implementation of methods to replace, reduce and refine animal experimentation and testing. In the case of the potency testing of botulinum toxin products, it is clear that some progress on alternative methods had been made prior to the launch of the FRAME and HSUS campaigns. However, it is clear that an unknown, but undoubtedly large number of mice were being used, and are still being used, in painful and lethal procedures for the testing of products destined for use for cosmetic, as well as for clinical, purposes. We are prepared to give Allergan, Ipsen Limited UK, and other manufacturers of botulinum toxin products the benefit of the doubt, by accepting that they are seeking to contribute to progress in the right direction.

Given this concession, some might conclude that our advocacy efforts are misplaced. We would disagree. As outlined above, the LD₅₀ testing of botulinum toxin products in general, and of products for cosmetic use in particular, runs counter to three trends: the phasing out of the LD₅₀ test, of the use of death as an endpoint, and of any animal testing of products with a cosmetic purpose. Consequently, the continuation of such testing is particularly out of step with the times, and is therefore particularly in need of scrutiny and action. Instead of assurances that

progress is being made, what is needed is a demonstration of goodwill and verifiable action on the part of the manufacturers and the agencies responsible for the registration and use of pharmaceutical and cosmetics products and for the regulation of laboratory animal experimentation. The technical challenges to developing a non-animal alternative for botulinum toxin product testing are formidable, and are best met with collaborative efforts open to scrutiny and to constructive criticism, not with alleged programs happening behind closed doors. We note for the record that none of the published studies of alternatives to LD₅₀ testing of botulinum toxin products, of which we are aware, were conducted by scientists working for the manufacturers of these products.

One of the factors that has worked against the FRAME and HSUS campaigns is the limited media interest that these efforts have generated. We suspect that this stems, in part, from limited public (and media) sympathy for mice. This is unfortunate, given that the capacity of mice to suffer is similar to that in most other animals used in laboratories. We suspect that the complexity of the relevant issues also limits the media appeal of our campaigns, including the technical nature of the non-animal alternatives and the dual use of botulinum toxin production batches for both therapeutic and cosmetic purposes. Our campaigns are also hampered by the lack of publicly available details about current testing practices and alternatives efforts.

FRAME is encouraged by the attention now being paid by the Home Office to the questions we have raised, and by the work being conducted by the NIBSC, as well as by the attention now being paid to the botulinum toxin testing issue by ECVAM and the EDQM. However, having legitimately raised an important issue of great concern in relation to both the severity of animal procedures and the need for an active commitment to finding relevant and reliable replacement alternatives, FRAME will expect progress to be made and to be kept fully informed about it.

The HSUS anticipates that its legal action against the FDA will yield critical information about the botulinum toxin testing, including the numbers of animals used per test and the number of tests conducted prior to release of a given batch of product. If Allergan continues to spurn legitimate demands for information and for co-operation, The HSUS will seek to increase the public pressure on the company, in a manner consistent with the successful campaign strategy of that late American activist, Henry Spira (Singer, 1998).

Meanwhile, both FRAME and The HSUS are willing to work collaboratively with the relevant authorities, and with institutions such as ECVAM, the EDQM, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), and the NIBSC, to accelerate the pace of progress, in the confident belief that we all share the same interest in making available products which are made as safe as possible for human use, but by using modern methods and progressively reducing reliance on the traditional application of painful test procedures to laboratory animals.

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