Three Rs Potential in the Development and Quality Control of Pharmaceuticals

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Content

Summary

The intention of a pharmaceutical company is to develop new, efficient products quick and with a minimum of costs. Compared to in vitro methods, animal experiments in general consume much more time and resources (costs as well as time to the market) than in vitro methods. Therefore, the use of whole animal models depends primarily on the judgement of their efficacy in the screening process, but the willingness to incorporate in vitro methods in general is high and is furthered by new developments such as high-throughput screening. Nevertheless, in vitro tests might be politically promoted by increasing their costs (quality controls, requested housing conditions) and duration (time to start of an experiment, sequential performance).

Which models are favoured by industry to include them in a screening process: They have to be based on our most recent understanding of the respective disease, well characterised to allow interpretation of results and require only limited development time. All these aspects argue in favour of collaboration between industry and academia, where our understanding of pathophysiology is generated and mechanism based models are developed and characterised. However, technology transfer towards industry represents a bottle-neck for industrial use of these new in vitro models. New platforms to promote this transfer should be developed in order to bring together developer and user of novel in vitro systems and promote demonstration projects. Financing of such collaborations is not the key problem (the development of a single drug makes up to 500 million $) but the dilemma of publication of results: The development advantage compared to competitors depends on the exclusive use of novel models. The protection of intellectual property rights and the public interest in spreading alternatives to animal experiments must be balanced, e.g. by delayed but indispensable publication or advantages for companies employing alternatives in the regulatory approval process for a new drug.

Quality control of therapeutic drugs (except hormones and blood products) represents a minor field of animal consumption with the exception of pyrogenicity testing despite considerable progress due to the introduction of the Limulus assay which represents the most successful in vitro alternative in use so far. However, some limitations of this in vitro test might be overcome in the near future by the currently validated human whole blood assay. During the last few years considerable progress has been made in the replacement (and deletion) of animal tests required for the potency and safety testing of hormones. This has been made possible by biotechnical production methods, by better-defined products, and because physico-chemical methods can be used for the potency testing of these products. In general, the better defined a drug is, the easier chemical, physical or in vitro techniques can be used for batch control. Control au-thorities should therefore urge the use of highly standardised components.

Zusammenfassung: Das 3R Potenzial bei der Entwicklung und Qualitätskontrolle von Arzneimitteln

Das primäre Ziel der pharmazeutischen Industrie ist es, neue und effiziente Medikamente schnell und kostengünstig zu entwickeln. Im Vergleich zu in vitro Methoden verbrauchen Tierversuche im Allgemeinen wesentlich mehr Zeit und Ressourcen (sowohl im Sinne von Kosten als auch von "time-to-market"). Deshalb hängt das Ausmaß der Verwendung von Tierversuchen im Wesentlichen von der Bewertung ihrer Effizienz im Screening-Prozess ab. Die Bereitschaft, in vitro Methoden im Screening einzusetzen, ist generell sehr hoch und wird durch aktuelle Entwicklungen wie das High-Throughput-Screening

noch verstärkt. Trotzdem ist es vorstellbar, Ersatzmethoden dadurch zu begünstigen, dass Tierversuche durch Auflagen (Qualitätsanforderungen, vorgeschriebene Haltungsbedingungen) und Steigerung des Zeitaufwandes (Verzögerungen bis zum Start der Versuche und vorgeschriebene sequentielle Testprozeduren) erschwert werden.

Welche Modelle werden in der industriellen Pharmakaentwicklung favorisiert? Sie sollen auf dem aktuellen Verständnis der jeweiligen Krankheit basieren, sollen gut charakterisiert sein, um die Interpretation der Befunde zu ermöglichen, und sollten im Industrielabor möglichst wenig zusätzliche Entwicklungsarbeit beanspruchen. All diese Aspekte sprechen für eine enge Zusammenarbeit der industriellen mit der Grundlagenforschung, wo das jeweils aktuelle Verständnis der Pathophysiologie geprägt wird und mechanistische Modelle entwickelt und charakterisiert werden. Leider stellt der Technologie-Transfer in die Industrie heute noch oft einen Flaschenhals für die Verwendung neuer in vitro Methoden dar. Neue Plattformen, die diesen Transfer erleichtern, könnten den Kontakt von Entwicklern und Anwendern von Ersatzmethoden herstellen und Pilotprojekte fördern. Die finanzielle Förderung stellt hier nicht einmal das Schlüsselproblem dar (die Entwicklung eines einzigen Medikamentes kann bis zu 500 Millionen Euro ausmachen), aber sehr wohl die Frage der Publikation von Resultaten: Der Entwicklungsvorsprung gegenüber Konkurrenzfirmen hängt oft an der exklusiven Verwendung neuer Modelle. Es muß deshalb ein Ausgleich zwischen dem Schutz und der Verwertung der eigenen Ergebnisse und dem öffentlichen Interesse, Ersatzmethoden zu verbreiten, gefunden werden. Dies könnte z.B. mit einer verzögerten aber verpflichtenden Publikation bezüglich Ersatzmethoden erreicht werden oder durch eine Kompensation durch Begünstigung derjenigen im Zulassungsverfahren, die Ersatzmethoden einsetzen.

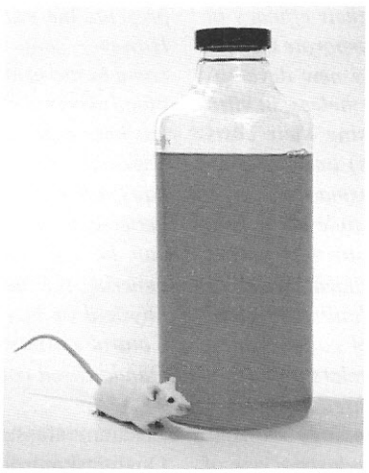
Die Qualitätskontrolle von Medikamenten (außer von Hormonen und Blutprodukten) stellt nur einen kleinen Teil des Tierverbrauchs dar mit der Ausnahme der Pyrogentestung. Letzteres gilt trotz des erheblichen Fortschritts durch die Einführung des Limulus-Testes, der die bisher wohl erfolgreichste Ersatzmethode zum Tierversuch überhaupt darstellt. Einige der Limitationen dieser Ersatzmethode könnten in Kürze durch den derzeit in der Validierung befindlichen Vollbluttest überwunden werden. In den letzten Jahren wurden sehr viele Fortschritte beim Ersatz und der Streichung von Tierversuchen bei der Potency- und Safety-Testung von Hormonen erzielt. Dies wurde möglich durch biotechnologische Produktionsverfahren, besser definierte Produkte und die Einführung von physiko/chemischen Nachweisverfahren insbesondere für die Potency-Testung. Ganz generell kann desto eher für die Chargenkontrolle eines Medikamentes ein in vitro Verfahren oder ein chemisch/physikalisches Meßprinzip genutzt werden, je besser das Medikament definiert und standardisiert ist. Die Zulassungsbehörden sollten deshalb mit Nachdruck auf die Standardisierung der verwendeten Komponenten hinwirken.

Keywords: pharmacology, toxicology, animal, test, alternative methods, in vitro, replacement

1 Introduction

Pharmaceutical industry represents a major consumer of animals. The situation differs for classical drugs and vaccines (blood products playing a role in between these two categories): While animals are used predominantly for drug development, in case of vaccines animals are mostly used for quality control. This implies that the use of animals is regulated in case of vaccines by pharmacopoeia and requires formal replacement while for drug development the use of animals depends on the indivdual development strategy of a company (except toxicology issues not handled here). Therefore, both areas complement each other and might serve to characterise the overall situation in pharmaceutical industry.

Batch quality control of therapeutic drugs (except hormones and a few blood products) represents a minor field of animal use with the exception of pyrogenicity testing. Despite considerable progress due to the introduction of the Limulus assay which represents the most successful *in vitro* alternative in use so far, in Europe about 200.000 rabbits per year are required for pyrogenicity testing. However, some limitations of the Limulus test might be overcome in the near future by the novel *in vitro* pyrogen tests being currently validated. In general, the better defined a drug is, the easier chemical, physical or *in vitro* techniques can be used for batch quality control. Competent authorities should therefore urge the use of highly standardised components.



2 Do we need animal research in the development and quality control of pharmaceuticals at all?

This question can be broken down in the two separate questions, whether animal research is required for development and whether it is required for quality control. The latter is much easier to discuss.

2.1 Do we need animals for quality control of pharmaceuticals?

These quality controls are mostly regulated by pharmacopoeias and therefore can be easily surveyed but are difficult to replace since a formal replacement for each and any given test is required. The better we know what the quality of a pharmaceutical is based on, the easier we can develop specific measures of these qualities to avoid animal use. To date, analytical chemistry allows to control any synthetic

compound with regard to purity and this can be complemented by *in vitro* tests, when a measure of specific biological activity of the ingredient is required.

The situation is different, when complex mixtures of components are used as drugs, which holds true for about 50% of the drugs on the market, which are produced from life material. Although, mak-ing up much less share of the market of drugs sold, these drugs are difficult to control. A special problem is given in case of herbal medicinal products: These preparations can include up to 40.000 individual components, the relative composition varying dramatically depending on the batch and its storage. Influences such as herb harvest (place of grow, climate, weather, time of harvest, duration of harvest, contamination with other plants), processing (storage, transport conditions, duration of handling, fermentation) and storage until use (storage conditions within the company, transport, pharmacy and at the end-user's place) will dramatically effect the composition of a herbal medicinal product. These issues have been overlooked by purpose for several decades, the main reasons being

- the impossibility to control all these factors
- the disbelief of many physicians and regulators in the strength of effect and side-effect of these agents (which does not hold true, as seen easily considering only digitalis, which is clinically highly effective and its toxicity is a major obstacle for broader use, or the couple of hundred thousand patients suffering from chronic kidney damage due to herbal teas)
- the traditional use of these drugs which raises the myth of safety; if we consider that for a new drug often a single death per hundred thousands treated suffices to recall the product from the market, it becomes evident that nobody did similar surveys for these herbal drugs
- the fact that herbal medicinal products are produced by small and medium enterprises which can not afford such safety controls
- the positive public opinion towards these "alternative" treatment options which hampers restrictive regulation

As exemplified here for herbal medicinal products, the indispensable safety of the patient is hardly controlled for these complex products. A similar situation is given in case of biologicals, i.e. drugs derived from biological materials such as animal organs or human blood. Any batch of a biological represents an individual. To illustrate this, factor VIII preparations used for hemophiliacs usually contain less than 1% of the compound but a broad and varying mixture of the couple of hundred other serum components.

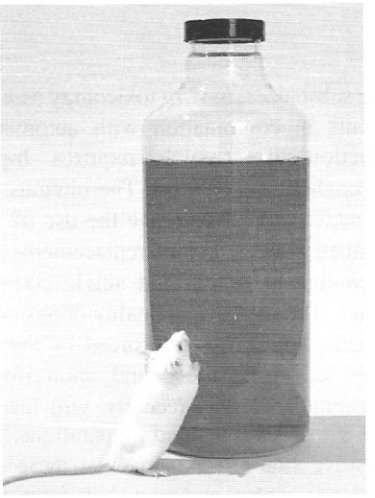
The challenge of quality control of these drugs has not yet been met, which might even call for new animal tests, since these are the most integrative safety controls (i.e. they integrate a broad panel of reactions to the unknown components).

Drugs can not be used without safety controls. The better standardised the drug and the better the risk factors are characterised, the less animals will be required to control these agents.

2.2 Do we need animals for the development of pharmaceuticals?

Some people even ask more strictly: Do we need development of more pharmaceuticals? Yes, we do. Those who dare to pose the question have most probably never felt the loss of a beloved person because there was no cure or felt like a physician who has nothing to offer for somebody who is seeking his help as a last hope. Many challenges of disease have not yet been met such as cancer, many infections and arteriosclerosis. The doubling of mean lifetime during the last century is impressive, but only to maintain this, we need the continuous development of new drugs: The most dramatic gain in lifetime resulted from the reduction of infections. Both the occurrence of resistance to antibiotics and the emergence of new pathogens such as HIV demand the development of new drugs. Thus, there is need for the development of new drugs, but does this require animal research?

There is little doubt, that toxicology of a drug has to be assessed before it is given to volunteers or patients. These issues are discussed elsewhere, but a few remarks: Similar to the question of quality control, the more we know about the mechanisms of toxicity, the easier we can model and study this *in vitro*. Such tests can serve as filters to reduce the number of animals finally exposed to drug candi-



dates. Some aspects of toxicity are, however, too complex to break them down even into a battery of *in vitro* tests, e.g. chronic effects, effects on behaviour, impairments of host defence.

However, do we need animals for the development of drugs? The answer is: Often but not necessarily (Hartung, 1998). Some agents are strictly species-specific, for example recombinant human proteins, and can not be tested in animals (except often in primates), or the infectious pathogen to fight is restricted to man (e.g. pox, measles, polio). These few cases show, that we can proceed to clinical trials without animal use. However, in general many diseases can to date only be modelled *in vivo*. Thus, as long as developments shall be pursued and not hampered by legislation, only a step by step replacement and reduction of animal experiments can be achieved. However, when screening for new active agents animal experiments are not indispensable, showing that this field has enormous potentials for reduction. Putative measures to promote this change are discussed below.

We need drug development, but we do not necessarily need animal experiments for drug development. However, the shift from *in vivo* to *in vitro* techniques takes time and requires the development and acceptance of alternatives.

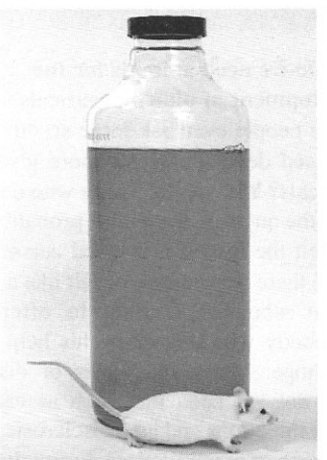
3 Three Rs potential in the batch control of pharmaceuticals

As ruled out earlier, most requirements for batch control result from ill-defined or variable compositions of drugs. It will be necessary to promote alternatives in this field, e.g. analytical fingerprinting of

the substances, *in vitro* toxicology assessments in combination with automated fractionation (which requires high-throughput-toxicology). The obvious alternative is to discourage the use of ill-defined drugs. Obvious replacements are recombinant or purified single components. The increase in quality control demands, which was reinforced by the issues of viral safety and spongiform encephalitis (BSE) recently, will favour the use of such defined preparations, the positive side-effect being that these are easier to control and on a short-term will require less or no animal testing.

There are two quality controls which are required for a broad variety of products, i.e. sterility testing and pyrogenicity testing. While sterility testing does in general not require animal experiments (even viral contaminations can be detected most sensitively by polymerase chain reaction PCR, however, tests for BSE might call for animal tests which are more sensitive than current *in vitro* tests but the long duration of several months hampers routine use), pyrogenicity testing still represents a major consumer of animals, namely rabbits: The use of rabbits in Europe for this purpose has been estimated to be around 200.000 animals per year (i.e. a considerable portion of all animal use and especially of large animal use) although 80% of pyrogenicity test is now carried out *in vitro* employing the Limulus assay (Flint, 1994). The use of the Limulus assay on the one hand represents a historic breakthrough in the replacement of animal experiments, on the other hand the safety concerns have never really been ruled out: To mention only a few aspects, the Limulus assay is restricted to the detection of endotoxin from Gram-negative bacteria while failing to detect pyrogens from fungi or Gram-positive bacteria, it does not reflect the varying potency (a difference of factor 10.000 for individual endotoxins) in mammals and it is disturbed by many endotoxin-binding components such as serum proteins.

In Europe, in recent years, a number of alternative cellular assays have been developed which aim to exploit the human primary fever reaction in order to replace the animal test (rabbit pyrogen test) and offer testing in the relevant species (man). All of these test systems are based upon the response of human leukocytes (principally monocytes), which release inflammatory mediators (endogenous pyrogens) in response to pyrogenic contamination (exogenous pyrogens). However, the cell-based *in vitro* assay systems differ with regard to the cells employed (isolated primary blood leukocytes (Poole, 1988a & 1988b) or whole blood (Hartung, 1995 & 1996; Fennrich, 1998, 1999a & 1999b; Pool, 1998; Jahnke 2000) or immortal monocytic cell lines (Taktak, 1991; Werner-Felmayer, 1995; Eperon, 1996 & 1997; Peterbauer, 2000), the mediator determined (interleukin-1, interleukin-6, tumor necrosis factor, neopterin, or NO) and the precise set-up of the test. These new developments towards an *in vitro* pyrogen assay based on the human fever reaction are summarised elsewhere (Bonenberger, 2000; Hartung, 2001).



Current status: In 2000, the ECVAM workshop on Novel Pyrogen Tests Based on the Human Fever Reaction was held in Konstanz (Hartung, 2001). The objectives of the workshop were: a) to identify the need for new pyrogen tests; b) to review the current status of the development of new pyrogen tests; c) to evaluate the capabilities of the new pyrogen tests and to give recommendations for further development; d) to identify regulatory requirements and to give recommendations for promotion to regulatory acceptance. In the same year, the EU funded project 'Human(e) Pyrogen Test' (QLRT-1999-00811) started which aims to select and to validate the most appropriate *in vitro* method(s) for pyrogen testing. The importance of novel pyrogen tests has been recognised by European Pharmacopoeia by establishing a new Group of Experts on Alternative Pyrogen Testing, which first met in January 2001.

Pyrogenicity testing represents a prominent example, how animal experiments in quality control can and have to be replaced: The basis of test development should be to identify what we have to measure (e.g. what are pyrogenic agents) and the mechanisms of their pathophysiology (e.g. fever induction); then tests can be developed which detect such compounds based on mechanistic understanding, avoiding false-positive or – even worse for safety tests – false-negative results.

As already mentioned in the introduction, there are only a few monographs in European Pharmacopoeia (*Ph. Eur.*), which stipulate *in vivo* tests for the quality control of pharmaceuticals. Most of the animals are needed for the batch testing of hormones. In the last ten years, remarkable progress was achieved with regard to the use of animals for the quality control of hormones and related products (van Noordwijk, 1996; Charton, 1999a). This is due to the initiative and willingness of *Ph. Eur.* to replace *in vivo* potency test with physio-chemical methods (e.g. insulin, oxytocin, calcitonin) and to take into consideration the recommendations of the ECVAM workshop on "Safety and Efficacy Testing of Hormones and Related Products" (Garthoff, 1995). Participants in the workshop questioned the relevance of the abnormal toxicity test (ATT), the testing of urokinase for vasoactive substances, the testing of heparin and other products for depressor substances, which led to inquiries pub-lished in Pharmeuropa (Council of Europe, 1996a, b, c) and subsequently to the omission of these tests for batch quality control (Tab. 1).

The shift in the production from human or animal tissue-derived hormones to chemical synthesis has made it possible that a number of new monographs never included an *in vivo* potency test since validated physicochemical method had already been in place. However, there are still a few monographs on hormones extracted from human and animal tissues, which require animals for potency testing. According to Charton (1999a), the monograph on corticotropin and glucagons are of minor importance, since only a few batches of corticotropin are produced and the use of extracted gluca-

Tab.1: Replacement of animal experiments by *in vitro* methods in quality control of biologicals and in toxicology

Animal In Vitro Method	Experiment	Replacement
QUALITY CONTROL OF BIOLOGICALS		
Pyrogenicity testing	*rabbit*	almost complete replacement* *Limulus-(LAL)-test & human blood cells*
Vitamin and Hormone determination *(Oxytocin, Sexual hormones)*	**Bioassays in** *Chicken, rat & mice*	complete replacement* *HPLC, cell lines with specific receptors*
Insulin determination	Convulsion test & blood glucose test *Mice & rabbit*	complete replacement* *HPLC*
TOXICOLOGICAL TESTING		
Eye irritation	Draize test on *Rabbit eye*	partial replacement for strong irritants and corrosive agents* *Hen egg test (HET-CAM test)*
Skin irritation	corrosive effect on *rabbit* skin	complete replacement for strongly corrosive and acidic agents *Artificial human skin cultures*
Phototoxicity of chemicals	UVA irridation Rabbit & mice	complete replacement* Photocytotoxicity on fibroblasts
Delayed neurotoxicity of organophosphates	Neurotoxicity of organophosphates in *chicken*	partial replacement posisible* *NTE-esterase determination in neuroblastoma cells*

*The in-vitro-test is internationally approved for risk assesment (OECD, ICH, pharmakopoiea).
Table modified from H. Spielmann "In vitro Methoden" in H. Marquardt und S.G. Schäfer "Toxikologie", 2nd edition, BI Wissenschaftsverlag, Mannheim, in press.

gons may be decreased due to the introduction of rDNA glucagons. The monograph on rDNA glucagons will include an HPLC assay.

3.1 Testing of Gonadotropins

Large numbers of animals are still needed for the quality control of gonadotropins. The numbers of animals stipulated by the monographs are exceeded by far in practice, for example, instead of 30 rats per batch 50 rats are used in the case of gonadotropin for veterinary use and up to 150 rats for gonadotropin chor. or urofollitropin (van Noordwijk, 1997). Due to the heterogeneity of gonadotropins extracted from human or animal tissue replacement of the *in vivo* test is not very likely but that these products will be totally replaced with better characterised recombinant hormones (Charton, 1999).

Such products are already on the market e.g. follicle stimulating hormone (FSH) and luteinising hormone (LH), however, they are tested according to the old gonatropin monographs. Thus, one of the main European manufacturers states that 60.000 rats/year are needed for the production and quality control of FSH (personal communication).

Mulders et al. (1997) report the development of a physicochemical method (isoelectric focusing, IEF) for the potency testing of recombinant FSH [rFSH]. The method was successfully transferred to NIBSC (UK), and, in early 1999, an ECVAM-funded study on prevalidation of physicochemical methods (IEF, capillary zone electrophoresis) for the potency testing of rFSH started at NIBSC, which will be finalised in 2001.

3.2 Testing of rDNA products

Depending on the production method rDNA products may be tested without the use of animals. The potency of products as erythropoeitin, which derive from mammalian cells or yeast, is, however, still tested in animals since erythropoeitin (EPO) is a complex glycoprotein (Charton, 1999). The potency testing of EPO can be carried out either with normocythemic or polycythemic mice. Both tests are equally valid. However, in the interests of animal welfare, the normocythemic test should be preferred. With respect to the replacement of the *in vivo* test, it is hoped that the successful development of a physicochemical method for the potency testing of rFSH, will have a positive spin-off to other therapeutical glycoproteins such as erythropoeitin and LH.

3.3 New products

The introduction of new animal tests in the pharmacopoeias should be discouraged. Therefore, regulatory acceptance of a new drug should depend at least on a proof that batch quality control can not be carried out *in vitro*. An ethical review board at these regulatory bodies should be installed to control this.

The balance of consumer safety and animal use can only be overcome by well-defined products and the identification of risk factors/mechanisms for patients by basic research. In some areas, the introduction of new animal tests should be prevented by stimulating the development of *in vitro* test ahead of time.

4 Three Rs potential in the development of pharmaceuticals

As explained above, the use of animals is not an absolute prerequisite for drug development but it often represents to date the quickest, most valid and within industry/regulatory bodies/scientific community most broadly accepted approach. In order to reduce animal use, these driving forces have to be considered.

4.1 Time consumption

The intention of a pharmaceutical company is to develop new, efficient products quick and with a minimum of costs. For those less familiar with this process, a few numbers: To bring a single product to the market, about 10.000 substances have to be screened (this number is nowadays dramatically increased to several hundred thousands by high-throughput screening, i.e. robotted testing in substance libraries)

to identify about 100 substances which go into animal experiments; from these about 10 will make it to testing in men, i.e. first volunteers and later patients. The mean time to market of a single product through this process lasts more than 12 years and the development of a single drug costs 200 to 500 million Euro.

Compared to *in vitro* methods, animal experiments in general consume much more time and resources (costs as well as time to the market) than *in vitro* methods. Therefore, the use of whole animal models depends primarily on the judgement of their efficacy in the screening process; however, the general willingness to incorporate *in vitro* methods is high and is promoted by new developments such as high-throughput screening, functional genomics and proteomics. Nevertheless, *in vitro* tests might be politically promoted by increasing the costs (quality controls, requested housing conditions) and duration (time to start off an experiment, sequential performance) of *in vivo* tests. Possible measures:

- request for larger cages with enriched environment
- installation of an animal health officer in each enterprise consuming a threshold number of animals per year
- installation of an internal ethical review board for animal experiments in industry (which overcomes the problem of secrecy of development), whose decisions have to be included into the documents for registration/approval of a new drug; this will on the one hand delay the commencement of the experiment and in-between-reviews might urge a sequential performance.
- increase the cost of laboratory animals by fees for the maintenance of ethical review boards, inspections and quality controls (e.g. genotyping, microbiology, mandatory banking of cryopreserved embryos in public strain banks).

Increasing the costs of animals and their handling as well as the delay and paper work required makes animals a resource to spare. Simple mechanisms of the market will then favour the use of alternatives.

4.2 Validity

Which models are favoured by industry to include them in a screening process? They have to be based on the most recent understanding of the respective disease, be well-characterised to allow interpretation of results and require only limited development time. All these aspects argue in favour of collaboration between industry and academia, where the understanding of pathophysiology is generated and mechanism-based models are developed and characterised. However, technology transfer towards industry represents a bottleneck for industrial use of these new *in vitro* models. New platforms to promote this transfer should be developed in order to bring together developer and user of novel *in vitro* systems and promote demonstration projects. Financing of such collaborations is not the key problem (the development of a single drug makes up to 500 million Euro) but the dilemma of publication of results: The development advantage compared to competitors depends on the exclusive use of novel models. The protection of intellectual property rights and the public interest in spreading alternatives to animal experiments must be balanced, e.g. by delayed but indispensable publication or advantages for companies employing alternatives in the regulatory approval process for a new drug.

The validation process which has been developed for alternatives to animal experiments during the last decade represents the most highly sophisticated quality assurance process in the life sciences, which is only paralleled in evidence-based medicine. Often these large-scale comparisons of alternatives with well-established animal models (which consumed millions of Euro) had to face a major problem: The limited validity of the animal model used as a gold standard. No animal model has been validated and standardised with similar efforts as some of the new *in vitro* alternatives. This observation itself should be stressed much more to increase awareness of industry, regulatory bodies and scientific community. Surveys of data challenging the validity of animal models should be stimulated by grants and awards. A public resource, either in a Medline-listed journal, a book series or a publically accessible Internet database summarising the validity of individual animal models would strengthen the impact of such surveys.

On a long-term, the level of standardisation, quality assurance and validation achieved for *in vitro* tests will favour their use. Taking into account that *in vitro* tests can be much more easily be validated in collaborative studies, the *in vitro* alternatives have an evolutionary advantage. This standard can not be met by *in vivo* models, since ethical aspects and costs inferred limit the efforts for statistical evaluation to validate the model. Simultaneously, all efforts to develop an arsenal of biometrical analysis of models and their validity will further promote the *in vitro* alternative in comparison to the animal model. Furthermore, the opportunity to make use of human materials further increases the objective and face-validity of these models.

Industry will use *in vitro* tests, if they trust in the individual model. Quality control in the development of the model, adequate evaluation and proper description in journals are major selection criteria. A major step towards a prominent quality standard are ongoing efforts to establish a guideline for Good Cell Culture Practice for especially academic research (Gstraunthaler, 1999; Hartung, 2000; Hartung, 2001), where the demands of Good Laboratory Practice e.g. on documentation can not be met. GCCP aims to define minimal requirements for quality standards in cell and tissue culture. The GCCP initiative shall establish principles for standardization, rationalisation, and international harmonisation of cell and tissue culture laboratory practices. Therefore, in analogy to Good Laboratory Practice (GLP), a Good Cell Culture Practice (GCCP) was initiated at the 3rd World Congress on Alternatives and Animal Use in the Life Sciences, Bologna, 29. August - 2. September 1999. This "Bologna Statement on Good Cell Culture Practice" was presented, discussed, and refined in a Workshop, and a final version was approved at the closing ceremony of the Congress by the scientific audience.

Based on the Bologna Statement, an ECVAM Task Force on GCCP was initiated, that is chaired by Dr. Thomas Hartung and Dr. Sandra Ceocke, in which experts in the field should elaborate minimal requirements for quality standards in cell culture. It is the intention of the GCCP Guidelines to encourage consensus among all concerned with the use of *in vitro* systems, in order to establish

and maintain best laboratory practices, to promote effective quality control systems, to facilitate education and training, to support journal editors, and to help any authorities who need to interpret and apply conclusions based on *in vitro* data.

The validation of *in vitro* models serves more than the final proof of the suitability of an individual test, but it stresses the quality control in the *in vitro* field in general. This objective advantage of *in vitro* tests should be stressed stronger in public awareness. Development of quality standards, biometrical tools and databases on both the validation of *in vitro* tests and the lack of validity of animal models serve this purpose.

4.3 Acceptance

Acceptance, in the broad sense of belief in the validity, is often more a psychological than an evidence-based process. A positive point of view towards alternatives can result from data, and matters discussed above to improve the awareness of these; in parallel, a general impression ("gut feeling") adds to these judgements which is based on experiences and impressions shared in peer groups. The latter is usually dominated by the academic community and transmitted via publications, presentations and education programmes.

The increasing number of journals, conferences, grant offers and scientific awards have promoted alternative methods very successfully. However, the opportunities of these measures seem to be somewhat saturated. Except very few underdeveloped fields, e.g. biometry and quality control (see above) and perhaps surgery, long-term/chronic toxicity, medical device testing and ecotoxicology, the progress made is impressive, steadily reducing the number of animals employed. Pursuing the existing programmes will gradually diminish animal numbers further. In some areas, e.g. immunotoxicology, infectious diseases and allergy, we have to prevent the introduction of new animal models by the prospective development of alternatives. Here, the efforts of funding institutions should be intensified.

While the scientific development of alternatives was successfully initiated, these developments are hardly reflected by integration into education programmes. Alternatives to animal use is not yet part of the respective study programmes and graduate courses. A first step towards a lobby and representation of the subject here, would be the installation of professorships in alternatives to animal experiments. By networking of these with ex-isting institutions, education programmes and integral parts in exams can be introduced. New opportunities such as teaching via the Internet ("virtual high-school") can make this available also at places who do not host such departments themselves. Students who have been taught the opportunities and advantages of alternative methods are the most effective messengers. On a mid-term they will be in positions who have to decide what is valid, what is acceptable and what is used.

A scientific community furthering alternatives has formed during the last decade, which is increasingly organised in societies such as MEGAT and InViTox. These developments have strengthened the development of alternatives. However, here people meet which are convinced anyway. In the future, it will be necessary to carry the message into the classical societies, e.g. by joined conferences/symposia, sharing of newsletters/society journals and installing of study groups on alternative methods in e.g. pharmacological, toxicological, immunological and medical societies. Sponsor programmes for such meetings could tremendously speed up this process. The scientific approach in animal protection in the life sciences has moved the field of alternatives from the image of a sect to an accepted pressure group within science. Now alternatives have to become an integral part of established areas. In a natural way, the exchange of generations of scientists will allow this fluently and the earlier the next generation is familiar with alternatives the better.

Public acceptance of alternatives has most development potential by formal implementation in education programmes. Both the installation of professorships for alternative methods and of initiatives with and within the respective societies in the life sciences will further this aim.

5 Catalogue of measures suggested

5.1 Batch control

- Replace the animal experiments listed in pharmacopoeias

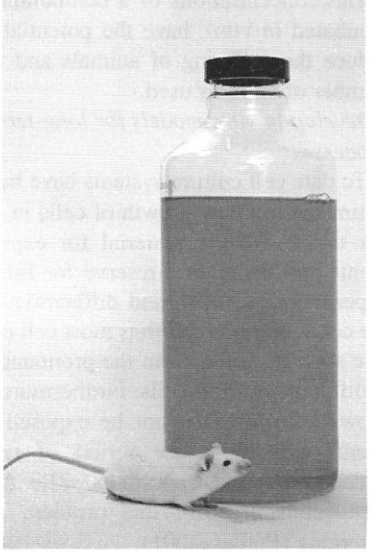
Beside the rabbit pyrogen test, few tests are currently listed in the pharmacopoeia to control batches of drugs. The most prominent examples are in the field of hormones. State-of-the-art analytical procedures in combination with bioassays should allow to replace this in the near future. Pharmacopoeia should be encouraged to call for alternative monographs and producers of these products should be made responsible of developing suggestions.

- Avoid the installation of new animal tests in pharmacopoeias

It should be the responsibility of a pharmaceutical company to develop and suggest *in vitro* methods for batch control suitable for later implementation in pharmacopoeia. In case no such tests can be suggested, an independent evaluation shall be initiated with the consequence of delay for registration and approval.

- Increase the degree of standardisation of drugs

Ill-defined and variable preparations for clinical use have to be replaced step by step. Poor definition of composition and active principles is a contradiction to any proof of efficacy, which will not be valid for the next batch of the drug. It makes no sense to compensate the costs either by public health systems or insurances for drugs lacking proof of efficacy except in controlled studies.



5.2 Research and Development (R&D) of drugs

- Broaden the spectrum of in vitro tests supported as a development of alternative methods

Screening for new drugs is a sequential process. The later *in vivo* testing is initiated the less animals are involved. Thus, the availability of *in vitro* test serving as filters is crucial to reduce the number of compounds tested *in vivo*. Furthermore, the later within the development process the less likely are severe side-effects, i.e. harm for the animal, of the drug itself and the testing of components with little potential for subsequent development. Therefore, in R&D not only full replacements of animal experiments but also *in vitro* tests to reduce the number of candidate substances serve the overall goal of reducing animal consumption.

In some areas, up to now few alternative models are available, especially in the area of chronic and degenerative diseases and those involving complex mechanisms of the immune system (infection, allergy, auto-immunity). These areas will need the continuos development of perhaps a battery of models which break down the complex pathogenesis in aspects which can be modelled *in vitro*.

The opportunity to develop *ex vivo* models is not yet fully exploited. These models, where either drug treatment is carried out *in vivo* and isolated cells and organs are challenged or a disease process is induced and a number of agents/concentrations of a compound is incubated *in vitro*, have the potential to reduce the suffering of animals and the number of animals used.

- Develop in vitro models for long-term processes

To date cell culture systems have been optimised to allow growth of cells in order to expand the material for experiments and maintain a reserve for future experiments. Growth and differentiation are contradictions and thus most cell culture systems suffer from the pronounced dedifferentiation of cells. Furthermore, a growing culture can not be exposed to agents over a longer period of time under controlled conditions. The first approaches to solve this problem are emerging (Pfaller, 2001).

- Hamper the broad use of animals making it expensive and more importantly time-consuming

Animals must be a valuable resource. Animal welfare regulations which foresee quality improvements and controls as well as require more and better trained personnel will make it less attractive to use animal experiments. Within the expensive R&D of drugs, any delay due to the need of justification of experiments and its review is even more effective to discourage the use of *in vivo* models or to postpone it to late stages of development.

- Demonstrate the validity of in vitro models and the lack of validity of existing in vivo models

Making data on validation of *in vitro* models and discrepancies of animal models available in a comprehensive easily accessible data-base will favour the use of *in vitro* tests, which have the inherent advantage that they can be evaluated with relatively high numbers of replicates.

- Increase the quality of in vitro work especially in academia

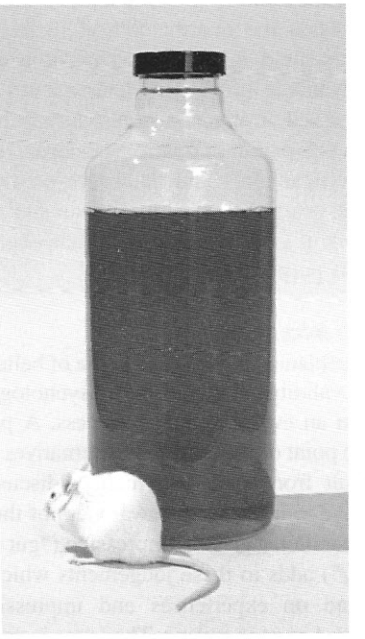
In vitro tests are prone to artefacts due to ample influence factors difficult to control. The quality of performance and documentation as well as the overall quality control of the results is crucial for the reliability of results. The current efforts of an ECVAM taskforce towards a GCCP guideline might represent an important step if these minimum requirements become mandatory for *in vitro* work to be published in leading journals.

- Promote technology transfer

One of the key problems for the use of *in vitro* test systems is their transfer to industrial routine use. Databases as provided by ZEBET or ECVAM serve to identify interesting approaches. Nevertheless, there is still a tremendous gap towards use, since the models often are no longer in use in the developing group. The re-installation and prevalidation of such models represents an interesting field for contract research. Funding institutions should be encouraged to sponsor such efforts.

- Make alternatives to animal experiments part of the scientific education in the life sciences

On a long-term the most important measure: Awareness of the opportunities and the concepts will minimise animal use. As described above, professorships for alternatives to animal experiments might represent an important signal and the start of a lobby. The close collaboration of animal welfare activists with the respective scientific societies will ultimately lead to the implementation in curricula and exams.



6 Final thoughts

Industry is not "the opponent" of alternatives to animal experiments. In order to survive on the market, industry has to be flexible and progressive. Companies depend on public opinion and thus do care about animal welfare debates. The objective advantages of *in vitro* methods, especially with regard to throughput and automated performance, have in the past resulted in an enormous promotion of *in vitro* technologies. The quality and validity of *in vitro* work and the general acceptance within the scientific community will determine how quickly the number of animal experiments in the development and batch control of pharmaceuticals decreases.

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