PERSPECTIVES
INTERNATIONAL MICROBIOLOGY (2005) 8:59-62
www.im.microbios.org

Joan-Albert Vericat

Neuropharma, Madrid, Spain

REACH in industrial platforms. The role of microbiologists

Address for correspondence:
Neuropharma S.A.
Av. de la Industria, 52
28760 Tres Cantos (Madrid), Spain
E-mail: jvericat@neuropharma.es

Introduction

One may wonder what a biologist working in preclinical development in a pharmaceutical industry—which does not develop antimicrobials—can offer to the readers of a microbiology journal. This is what I also wondered when I started writing this article for International Microbiology. Although I received a Ph.D. in microbiology twenty years ago, currently, the only close relationship my work has with microbiology is through the use of bacterial assays for genotoxicity testing in the different phases of drug development (lead optimization and regulatory preclinical development). These tests, including the SOS chromotest, the Umu test (both carried out in *Escherichia coli*), and any of the different versions of the Ames test (all of them using different strains of *Salmonella typhimurium*), are well known to microbiologists and other scientists involved in assessments of genotoxic potential. As a final user of these models, I do not think that I could offer interesting findings, other than rates of positives/negatives or correlations with other assays for genotoxicity or with carcinogenicity. In fact, these models are so widely used that I do not consider them to be an important issue in the field of microbiology. These assays, however, and especially the Ames test, are of great relevance to one of the most controversial issues in Europe, the new chemicals policy of the European Union.

European Commission proposal for REACH (Registration, Evaluation and Authorisation of Chemicals)

On October 29, 2003, the European Commission adopted a proposal for a new EU regulatory framework for chemicals, following The White Paper issued on February 27, 2001, on a strategy for a future chemicals policy. Under the proposed new system, called REACH (Registration, Evaluation and Authorisation of Chemicals [1]), enterprises that manufacture or import more than one tonne of a chemical substance per year would be required to register these activities in a central database. The aims of the proposed new regulation are to improve protection of human health and the environment while maintaining competitiveness and enhancing the innovative capability of the EU chemicals industry. Furthermore, REACH would give greater responsibility to industry to manage the risks from chemicals and to provide safety information on the substances. This information would be passed down the chain of production. Although some discrepancies occur, it can be said that REACH represents some form of consensus, as parties potentially affected by the new regulation were consulted previously. This has allowed the Commission to propose a streamlined, cost-effective system, and the proposal is now under examination by the European Parliament and the EU's Council of Ministers for adoption under the so-called co-decision procedure. However, the most difficult aspect of REACH is predicting its acceptance, since, according to the EU, REACH will increase competitiveness, whilst the different industrial associations (CEFIC-European Chemical Industry Council, EMCEF-European Mine, Chemical and Energy Workers Federation, and ECEG, European Chemical Employers Council) maintain that competitiveness will be reduced [www.cefic.be/Files /NewsReleases/Press%20 release_021203.pdf]. In particular, SMEs (Small or Medium-Sized Enterprises) are concerned about the possible economic consequences that REACH may have on their operations, and the potential difficulties associated with the increase in costs.

The new regulation specified by REACH includes both new and existing chemicals. "Existing chemicals" refers to those marketed before September 1981, which represent

more than 100,000 compounds (96% of the total), whereas "new chemicals" are those marketed after September 1981, translating into 3600 notifications (a 2003 figure). Although "new chemicals" have been annotated with respect to an adequate testing strategy following the different international guidelines, for "existing chemicals" the available information is rather limited. In fact, it is difficult for citizens to even know which chemicals are "new" and which are "existing", neither do they know whether data on the safety of "existing chemicals" are available.

The cost of safety

The cost of applying REACH to existing chemicals would be, according to DG Enterprises, around 2.3 billion Euros, as reported by Dr. Egbert Holthuis [2,3]. Other evaluations result in much higher figures, with costs as high as 9000 million Euros and, until 2048, potentially involving the use of around 13 million vertebrates (worst-case scenario) [4]. More recent evaluations result in slightly lower figures. Note that, in 1999, around 10 million animals per year were used to cover the testing needs in the 15 member states comprising the EU at that time (Table 1). The testing schedule depends on the number of tonnes of each chemical produced per year: For chemicals produced in amounts of 1000 tonnes and more per year, safety data (genetic toxicology. Animal toxicology, ecotoxicology, etc.) should be available in 2018; six years later for compounds produced in amounts between 100 and 1000 tonnes; and until 2048 for compounds produced in amounts between 1 and 100 tonnes per year. The same evaluation states that the available European CROs (Contract Research Organizations) will be unable to meet this time frame if the testing of new chemicals, pharmaceuticals, cosmetics, etc., is also taken into account. In any case, whatever the scenario, the cost is very high, both in terms of money and, more importantly, in terms of the number of vertebrates that will be needed for testing.

Protecting people and preserving the environment in order leave a better world for our children and for our children's children is a goal that is no doubt shared by everyone. Surely no one would reject the idea that the world is threatened by the abuse, both actual and potential, of chemicals and their improper disposal, and that this problem must be addressed in order to ensure protection of the planet. At the same time, Europe has a commitment to reduce the number of animals used in research: The 7th Amendment to the Cosmetics Directive is a good start, since it bans the use of animal assays for the testing of cosmetics. However, Table 1 shows that the situation is far from being perfect—first of all, because animals used in cosmetic testing amount to less than 1 % of the almost 10 million animals used annually in various kinds of research and testing The chemical industry, including the production of household chemicals, uses only 12%, whereas 55% are used in medical research, one third of these for quality control of biologicals; only 10% of research animals are used in toxicology-safety assessments (Fig. 1). Thus, the ban in the cosmetics industry will not significantly reduce the current use of laboratory animals. Moreover, the impact on the use of transgenic strains in research as well as on animals used to prepare in vitro models remains to be determined.

Table 1. Groups of animals used in experimentation per year*

Animal group	Percentage
Prosimians/monkeys/apes	0.1
Carnivores	0.4
Artio-/perissodactyla	1.3
Rabbits	2.3
Rats	26.6
Mice	54.0
Guinea pigs	3.0
Other rodents	1.0
Other mammals	0.1
Birds	4.7
Cold-blooded animals	6.6

*Data from the 3rd Commission Report on the number of animals used in the EU, 1999, released in 2003 (9,814,171 animals used).

Alternatives to the use of animals in safety tests

Currently, only three methods, substitutes for skin corrosion, hematotoxicity, and embryotoxicity, are considered as validated alternatives to animal testing and have been integrated into international guidelines. In addition, the animal acute lethal toxicity test has been further refined such that the number of animals required to conduct the test has been reduced. Nonetheless, the situation is very poor with respect to existing alternatives and is only very slowly improving, since very high-quality performances for the new testing approaches are required, much higher than those of the current animal models, which have never been correctly validated. Thus, a very interesting conflict arises: the increase in the number of animals needed to respond to REACH requirements (up to several millions!) versus the implementation of the 3Rs: the replacement refinement, and reduction of the use of animals in scientific research. The affected industries are obviously

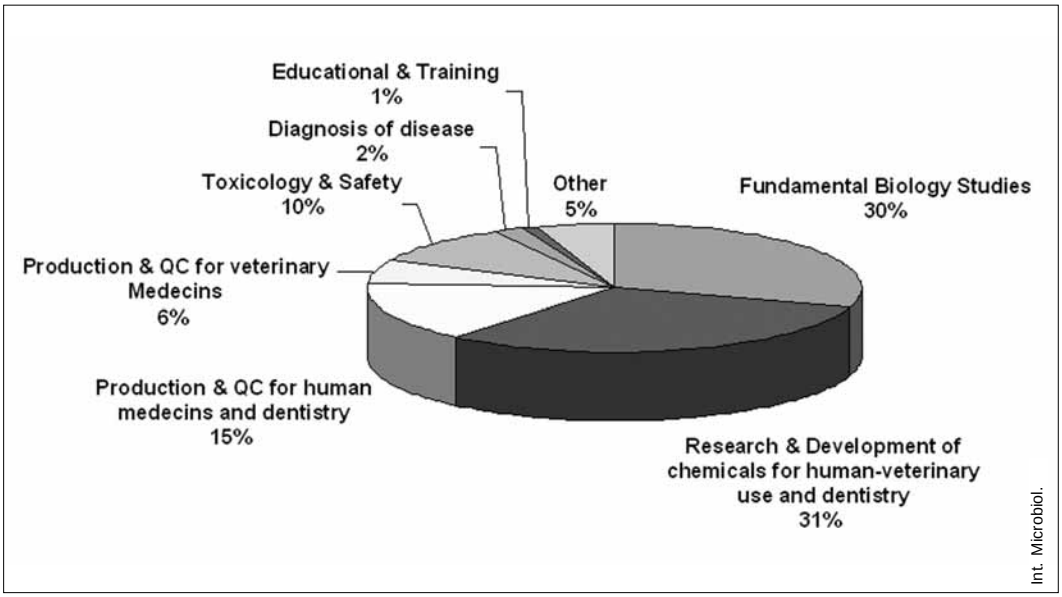

Fig. 1. Objective regarding the annual use of research animals. 3rd Commission Report on the Number of Animals Used in the EU, 1999 (9,814,171 animals used).

concerned about this situation, not only due to the costs associated with additional testing and the potential reduction in competitiveness, but also, and mainly, to the absence of validated alternatives to animal experimentation. In fact, although some assays have been accepted and entered into international guidelines, there is the feeling, among industrial researchers, that regulators and evaluators have difficulties in accepting these alternatives (and this taking into account that industries perform a great part of their research activities using in vitro methods). This situation is easily understandable since evaluators base their decisions on comparisons with existing data. Under these circumstances, it is difficult to decide whether a tested chemical is better or safer than a compound analyzed earlier by a different method. Thus, new testing approaches pose difficulties, regardless of the willingness to find alternatives to animal experimentation.

Prospects for European industries

Another major difficulty of REACH is the implications for large industrial groups versus SMEs. The latter claims that they would not be able to protect themselves against the potential of having to cease production of existing or new chemicals, in case the testing approach decided upon in order to comply with REACH regulations involved the use of not sufficiently validated predictive assays. To address such concerns, the IVTIP, the In Vitro Testing Industrial Platform [www.ivtip.org], was established by companies involved in the cosmetic, pharmaceutical, biotechnology, and chemical industries, as well as Contract Research Organizations. Its tasks include acting as a mediator between industry and EU administrators and regulators. IVTIP members meet twice a year for very informal discussions, to which both academic researchers in the field of in vitro testing and representatives from European institutions are invited. These meetings provide a good opportunity to promote multidirectional connections between industry, academics, and EU authorities, always with the aim of improving the competitiveness of European industries.

IVTIP also follows up on all European projects financed by the European Commission dealing with in vitro testing strategies, and presenting high degrees of applicability. Curiously, in the last four years, the only microbiology project addressing alternatives to animal testing that has been monitored by the IVTIP is the research of Prof. Hella Lichtenberg (University of Bonn), on the use of *Saccharomyces cerevisiae* as an alternative to the Ames test.

The potential of microbiology in safety assessment

It is likely that the interests of microbiologists and other scientists using microbes in their work are very different from those of scientists involved in safety assessment. However, accepting that I am outdated in the field of microbiology, there are two aspects, one that might have an enormous

impact on safety assessment in general and the other on the testing requirements related to REACH in particular, in which I think microbiologists have a role to play. First of all, the question of mitochondria. Any toxicologist would agree that, while the molecular mechanisms might be very different, mitochondrial abnormalities are frequently observed as part of the response to many toxic chemicals. Moreover, there is a great interest in finding test models that could predict mitochondrial dysfunction. As it is widely accepted that bacteria are related to the parasitic precursors that evolved into mitochondria, they may offer a model system for screening chemicals potentially toxic to those organelles. The second aspect has to do with membranes. Some toxic reactions, such as corrosion and irritation, have important physicochemical effects on skin and mucosa. Unfortunately, the cell wall and membranes of bacteria have a composition different from human (or eukaryotic) cell membranes. However, microbiologists have long been able to obtain bacteria free of their outer wall. Thus, it is easy to imagine a very inexpensive, fast test for corrosion and irritation based on the disruption of bacterial membranes, as determined by changes in absorbance. Just imagine how long it would take and the relative low cost of testing the 100,000 existing chemicals with such a system.

Microbial methods in safety assessment are being used, mainly for genotoxicity assessment but also in the early phases of screening, Some of these methods, including Ames II, SOS chromotest, and Umu test, have been modified to adapt to high-throughput screening requirements. This is not only because they are inexpensive, but also because they are robust and produce clear-cut responses that are easy to interpret by both researchers and governmental policy makers. Hopefully, this article will motivate microbiologists and those scientists with related interests to develop additional screening methods, so that their research activities play a larger role in the arena of safety testing.

Concluding remarks

I cannot end this discussion without adding that any new testing model with potential application for safety assessment should quickly be submitted to pre-validation and validation exercises. These involve establishing correlation models and determining intra- and inter-laboratory consistency, and have evolved with time in order to gain in efficiency and to reduce the enormous costs and amount of time involved. Interested researchers should contact the European Center for Validation of Alternative Methods (ECVAM) at the EU Joint Research Center in Italy [http://ecvam.jrc.it/index.htm], which provides instructions on how to proceed and how to find interested partners. Both ECVAM and IVTIP have been involved in recent projects granted by the European Commission whose final objective is to gain the status of pre-validated alternative prior to entry into the formal validation phase.

The final topic in this complex discussion is the impact of such research activities. It is obvious that reduction, replacement, and refinement in animal experimentation have tremendous ethical and social impact. Advancements in these areas will result in a better and more “humane” world. Unfortunately, it seems that research activities focusing on the 3Rs do not have sufficient scientific acceptance, as determined by the number of journals publishing the related results. It is somewhat surprising that the readership for a paper explaining in detail the mechanism of action of a given enzyme, using very new and sophisticated techniques, can be much higher than that of a paper reporting on a simple method that reduces animal testing, saves animal lives, and reduces the cost of research, i.e., having a direct effect on the quality of life. Perhaps even worse is that scientists not directly involved in the field of alternatives do not consider their results with respect to this very important and ethical objective.

I hope that this discussion will awaken in its readers some interest in the field of replacement, reduction and refinement, and in a further understanding of the related molecular mechanisms that may foster their development. Finally, while new in vitro screening methods for determining the safety of existing chemicals, establishing exposure levels (either to people or the environment), and labeling substances are needed, the same argument can be made regarding new cosmetics and chemicals. REACH, by forcing us to establish in vitro approaches with broad regulatory acceptance for evaluating the enormous amount of chemicals to be tested, now and in the future, will greatly contribute to protecting both humans and the world they live in.

References

1. European Commission (2003) REACH 2003 http://europa.eu.int/comm/enterprise/reach/index.htm
2. European Commission Press and Information services. Commission proposes banning animal testing for cosmetics in the EU. http://www.ivtip.org/CommDocuments/eu_cosmetics.htm
3. Holthuis E (2003) Evaluation of testing and registration costs. Workshop on REACH Impact Assessment, 21 November 2003. http://europa.eu.int/comm/enterprise/reach/docs/reach/presenta1-2003_11_21.pdf
4. Institute for Environment and Health. Annual Report 2001. Medical Research Council. http://www.le.ac.uk/ieh/pdf/AR2001.pdf