Luigi Campanella

THE CONTRIBUTION OF CHEMISTRY TO THE REDUCTION OF THE EXPERIMENTS ON ANIMALS

The scientific community, especially chemists, are called to answer the 3R recommendation from European Union delivered in occasion of Reach and referring to experiments on animals. Here the author proposes a contribution in this direction, also looking at helping equilibrium and good health state of ecosystem, animal world being a relevant part of it.

Animals as humans are essential parts of our environment. So their welfare is essential. Unfortunately animals are still considered as second order living entities while many of us take them in their own homes and divide with them happy and sad moments of their lives. Also medicine has recently discovered that they can concur to our happiness and to our good health so that many disable young people are today cared by familiarity with animals. On this way to give up any painy treatment on animals becomes a “must”. This is reason while independently on scientific reasons, yet well present and acceptable, to propose alternative ways to experiments on animals is a duty of scientific community, on other hand so respecting the 3R (replacement, reduction, refinement) directive of EU on this subject.

Even if the title of this paper is rather specific, I want to begin from the general ethical principles that IUPAC suggests to be respected by each chemist:

- to verify that own work is ethical and in defense of the dignity and of the continuous well reputation of own profession;
- to do the best possible in order that own knowledges and technologies are used for the benefits of humanity and for the improvement of the quality of life and environment;
- to work in agreement with the principles of sustainable development and of the maintenance to the earth of its capacity to ensure full biological diversity;
- never to use products and instruments under own responsibility for illegal aims or for destructive finalities toward other persons or environment;
- always to inform the deputed authorities about any noxious use of chemicals;
- to minimise any risk that can be run by any work colleague, any citizen, any environmental site due to accidental or wanted consequences of own activity;
- to perform regular controls about the effects on health and on safety of own working activities;
- to respect national and international laws and rules about chemicals;
- to co-operate with governments and organisations to improve the contents of laws and regulations concerning safety;
- to update own knowledges about last developments on the control of health, environment, chemical risk assessment and to use them to let understand to civil society and public opinion that chemicals if well used produce benefits.

The final sentence of this ethical card says: “I (as chemist) promise solemnly to apply the finalities of the chemical science to safeguard the environment and the ecosystem, to improve the quality of life without being noxious to the surrounding world, to control, to contrast and to stop any bad use of chemistry, to diffuse knowledges about advantages and benefits that from chemical sciences can derive to public opinion”.

At this point the question of my reader could be the following: what is the correlation between this ethical card and contribution of chemistry to 3R (reduction, replacement, refinement) directive of EU about experiments on animals? The answer is at the same time easy and complex. Firstly the animals as humans are part of the ecosystem and to respect them is an ethical duty. More the high costs of experiments on animals

in terms of financial and biological resources oblige chemistry to look for alternative ways able to bring advantages to civil society. Finally the doubts about the transferability of data on animals to humans suggest to run different approaches for toxicity evaluation. Finally the living indications of tested animals are very often unacceptable with no possibility to find for them any reason.

Actual situation in Europe

Directive 86/609/EEC standardised the well state of animals in the research labs in Europe. On March 2009 the Commission of Agriculture and Rural Development of European Parliament proposed 524 amendments to the Directive of which 161 were adopted. The target was to limit experiments on animals without stopping scientific research. Tests must be subjected to ethics evaluation and applied only if alternatives are not available.

The actual situation in Europe is:

- 12 millions of animals involved in research each year;
- 10 thousands are non human primates;
- 2 third of this amount are used to evaluate safety of drugs and pharmaceuticals; 1third in medicine and veterinary.

Experiments on primates are officially forbidden in Austria, UK, Netherlands, Sweden. Practically they are not adopted in all Europe even if still considered by some committee as necessary to test drugs (for cancer, Alzheimer therapies) according the European and International guidelines. The white book on Chemistry of European Union is imperative toward Scientific Community: assesses toxicity of new chemicals. The interested fields are cosmetics, pharmacology, chemistry, food industry ecc. But it is also imperative about replacement, reduction, refinement of experiments on animals (3R recommendations). The main questions are:

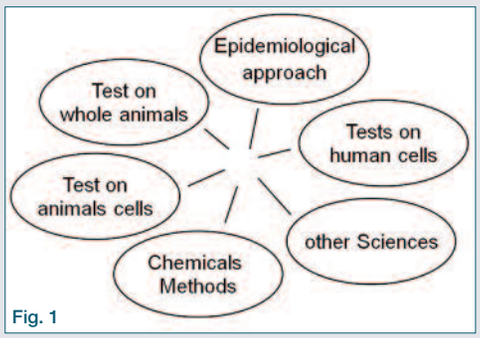

Fig. 1

are experiments on animals (from rats, cat and mice to fishes, butterflies, mussels) useful? significant? absolutely necessary? all at the same level of replaceability compared with other approaches? (Fig. 1).

Alternative methods

For each compound or substance to be tested its toxicity, stability, accumulability are related to danger risk (at $E_{xposure}$ = constant) and consequently to the possible damage produced.

Which are the alternatives to experiments on animals?

The two main ways are in vivo and in vitro tests

In vivo	Ex vivo, in vitro
Compound, product/organism	Compound, product/receptor
Whole cellular system	Single cells (free or immobilised) system

Furtherly we can adopt a biosensoristic approach to detect marker molecules or to test a biological system as target of the action of a compound. Different kinds of biosensors can be used for these studies.

Biosensors based on whole cells or cellular systems

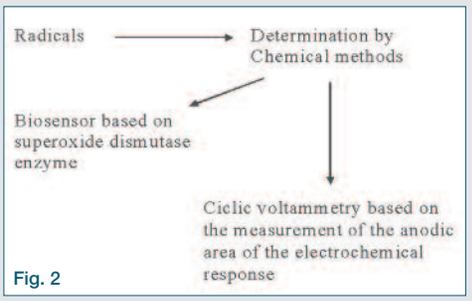

Fig. 2

Biosensors based on enzymes and receptors (new lines): both able to perform the passage from the target organism to the target site, from in vivo to in vitro tests. The couplement of biosensors with FET (field effect transistors) and electronic amplifiers results in what we call biochips, a technological development. The marker approach consists in the evaluation of the concentration of species considered as markers of toxicity. By this way radicals were recently considered. Their determination can be performed according to different methods (Fig. 2).

The biosensor is based on superoxide dismutase enzyme and on the following reaction:

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

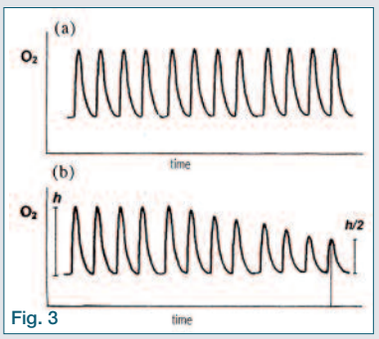

Fig. 3

that is the dismutation of superoxide into oxygen and hydrogen peroxide anodically monitored by a Clark electrode. Toxicity tests can be performed also by respirometric biosensors measuring the respirometric capacity of yeast cells. Transducer and indicator is Clark electrode able to measure oxygen consumption comparing it in presence and absence of the compound to be tested. Also inhibition of photosynthetic and respiratory activities of algae (Fig. 3) can be useful to evaluate toxicity. In this case the sensitivity is enhanced because oxygen concentration both on its increase step (under light) and on its decreasing one (in the dark) is affected by the presence of inhibiting agent. The comparison among different toxicity scales for six pharmaceutical principles carbamazepine, ofloxacine, sulfametoxazole, propranolol, diclofenac, clofibric acid clearly shows the reliability of the new approaches compared to those ones based on experiments on animals (Tab. 1).

Further Development

Three more alternative ways for toxicity evaluation can be run, 1) by inhibition of enzymatic activity (tirosinase, catalase): many enzyme activity is negatively affected by the presence of toxic compounds, 2) by a photo-

I	CBZ	OFX	SMX	PRL	DCLF	CLof
respirometric test						
I_{50}	CBZ	OFX	SMX	CLof	PRL	DCLF
$t_{1/2}$ (algal test)	OFX	SMX	CBZ	CLof	DCLF	PRL
$LD_{50\ rats}$	SMX 4100	CBZ 4025	OFX 3670	CLof 1650	PRL 1400	DCLF 1278
$LD_{50\ cats}$	OFX 5370	CBZ 3750	SMX 3662	CLof 1280	PRL 565	DCLF 390
$LD_{50\ mouses}$	SMX 5500	OFX 5400	DCLF 846	PRL 560		

Tab. 1

sensor by us set up and proposed: the basis of this photosensor is as it follows. TiO_2 is a catalyst of many photooxidative degradations of organic compounds; it behaves as a semiconductor such that the UV irradiation (3,2 eV) brings to the promotion of electrons from the valence to the conductivity band with following production of electronic holes and electrone excess respectively in the valence and in the conductivity band:

$$TiO_2 + h\nu \rightarrow TiO_2/(e^- + h^+) \rightarrow TiO_2(e_{cb}^- + H_{vb}^+)$$

The main mechanism of photodegradation consists in the production of hydroxide radicals obtained by the reaction between electronic holes and water. Such radicals react with the analytes, that are so degraded:

$$OH^- + h^+ \rightarrow OH^\bullet$$
$$OH^\bullet + RH \rightarrow R^\bullet + H_2O \rightarrow CO_2 + H_2O + \text{mineral acids}$$

So the photosensor measures the ability to be photodegraded of some organic compounds on recording its response to the stability of them, assumed as related to the response. The environmental permanence being measured by the photosensor as ratio between the delay time and the slope of increasing potential (decreasing pH) trend (Fig. 4).
The advantages of all these alternative tests compared to experiments on animals are: a) faster response times, b) less animals sacrificed or killed, c) deepening of the toxicity mechanisms, d) opportunities precious in the case of alarm situations to repeat tests with consequent increasing significance of the experimental data.

Reach

A practical application which we have been dealing with is just pressing our scientific and civil communities. We refer to technical, social, ethical aspects of Reach, European Regulation directives concerning chemicals still basing on experiments on animals. Experiments on animals are still accepted and permitted but on respecting EU directives we have to look and to work with hope and engaged efforts at the 3R recommendations.

Conclusions

Science offers many opportunities to satisfy EU directives about reduction, replacement, refinement of experiments on animals to test toxicity and possible damages to humans and environment from chemicals. Ethical and scientific interests must stimulate chemists to research about this subject.

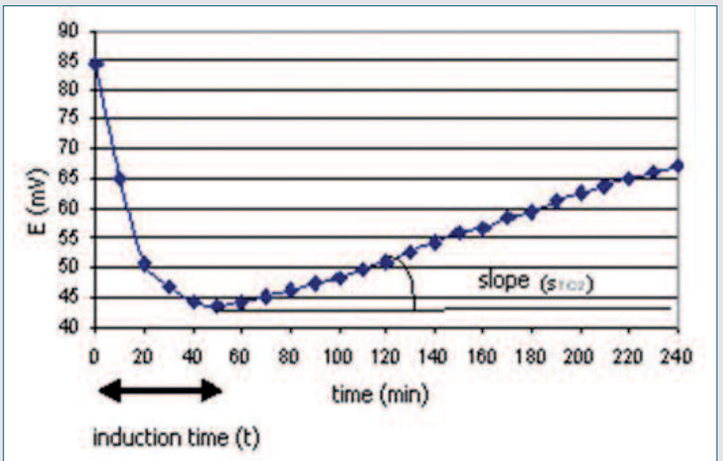

Fig. 4 - Δt = delay time, *a* = KV, V = Photodegradation rate

References

[1] M. Balls *et al., Alternative Laboratory Animal Science,* 1995, **23**, 129.
[2] M. Balls, R.H. Clothier, *Toxicology in vitro,* 1991, **5**, 535.
[3] E. Borenfreund, J. Puerner, *Toxicology Letters,* 1985, **24**, 119.
[4] W. Diembeck *et al., Food and Chemical Toxicology,* 1999, **37**, 191.
[5] Toxicology for the next millennium, R.J. Isfort, J. Lederberg (Eds.), Annals of the New York Academy of Sciences, Vol. 919, 2000.
[6] E. Marshall, *Science,* 1993, **259**, 1394.
[7] W.M.S. Russel, R.L. Burch, The principle of human experimental technique. London, Meuthen, 1959, University Federation for Animal Welfare, Special Edition, 1992.

RIASSUNTO

Il contributo della chimica alla riduzione degli esperimenti sugli animali

La comunità scientifica, in particolare quella chimica, è chiamata a rispondere alla richiesta raccomandazione dell'UE formulata a corollario del Reach in tema di sperimentazione animale: ridurre, rimpiazzare, riconsiderare. Quello presentato nell'articolo vuole essere un contributo in questa direzione, nella logica anche di contribuire all'equilibrio ed alla conservazione dell'ecosistema, del quale il mondo animale è parte rilevante.