Per aspirin ad astra...

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Summary — Taking the 110th anniversary of marketing of aspirin as starting point, the almost scary toxicological profile of aspirin is contrasted with its actual use experience. The author concludes that we are lucky that, in 1899, there was no regulatory toxicology. Adding, for the purpose of this article, a fourth R to the Three Rs, i.e. *Realism*, three reality-checks are carried out. The first one comes to the conclusion that the tools of toxicology are hardly adequate for the challenges ahead. The second one concludes that, specifically, the implementation of the EU REACH system is not feasible with these tools, mainly with regard to throughput. The third one challenges the belief that classical alternative methods, i.e. replacing animal test-based tools one by one, is actually leading to a new toxicology — it appears to change only patches of the patchwork, but not to overcome any inherent limitations other than ethical ones. The perspective lies in the Toxicology for the 21st Century initiatives, which aim to create a new approach from the scratch, by an evidence-based toxicology and a global "Human Toxicology Programme".

Key words: *alternatives, animal testing, aspirin toxicology, REACH, toxicology limitations.*

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It is a privilege to make a contribution to the double-birthday of the Three Rs and FRAME. As a "second-generation-follower" of Russell and Burch, I refrain of commenting on the history of events, where more-immediate observers can still contribute. Having only recently had the opportunity to summarise current developments, trends and visions for the field of the Three Rs, when given the honour of the being the 2008 FRAME Annual Lecturer (1), I would like to come from a somewhat different angle on this occasion. I have chosen another birthday — the 110 years of the marketing of aspirin — as the starting point for some thoughts.

The Latin phrase, *Per aspera ad astra*, by Seneca the Younger, means "A rough road leads to the stars". It is, first of all, a nice description of our journey to overcome animal testing, which brings us together to celebrate two milestone events. But what can the aspirin teach us?

I had a certain personal "enlightenment" linked to aspirin about 25 years ago, as student of biochemistry and medicine in Tübingen, Germany. As part of my practical on organic chemical synthesis, I had to synthesise aspirin. Nothing spectacular, but when I obtained a grayish powder — pure according to the crude measures we used then — I wondered whether I would dare to swallow it. I saw this "common friend", who had helped me with so many headaches, but would I believe in the safety of my own product? It would be pathetic to claim that this created my interest in toxicology — this was actually a little later, when my mentor, Albrecht Wendel, gave me the famous Lehmann lessons ("You, too, can become a toxicologist in two easy lessons, each ten years long." Arnold Lehmann, Head of the US Food and Drug Administration, *circa* 1955). I was more concerned about the contamination of the product and my analytical capability. However, it might have been interesting to go into the toxicological profile of aspirin a little deeper.

The safety data set for aspirin (2) is quite surprising: R22: "harmful if swallowed", with an LD50 = of 150 to 200mg/kg in rats; R36: "irritant to eyes"; R37: "respiratory irritant", R38 "irritant to skin"; not carcinogenic (3), but a co-carcinogen (promoter) with unclear mutagenicity (4); embryonic malformations in cat, dog, monkey, mouse, rabbit and rat (5–9). It is not very likely that any substance with such a profile would make it to clinical trials or to the market today.

Quite remarkably, by contrast, is the success story of aspirin (10–13): more than one million billion doses have been taken in its 110 years on the market. Every year, 50,000 tons are produced and 35,000 tons are consumed. More than 23,000 scientific papers on aspirin have been produced. 74% of the US population regard aspirin as the eighth wonder of the world. It is still a "cash cow", with worldwide annual sales of $840 million (35–40% of it in the USA) — almost still a blockbuster drug. The average British citizen consumes 70 aspirin tablets per year. And despite the alleged malformations, the drug is even used for pre-eclampsia, a high blood pressure problem, in pregnancy, and meta-analysis reveals no risk of malformation in humans (14).

What does this teach us? We must be happy that there was no regulatory toxicology in 1899. And this holds true for a lot of well-known drugs: paracetamol (acetaminophen) is a carcinogen in the rodent cancer bioassay…

There are various ways of interpret this observation, but it is clear that our current safety testing approach is too precautionary, if we do not allow important medicines to make it to the market. And this is certainly only one criticism we might aim at the safety assessments of today, which involves tests which require large numbers of animals, without validation of their predictive value, have low through-put in the face of thousands of substances to be tested, all at great cost (15–17). So there are many more reasons to challenge, and possibly overcome, animal-based toxicology, in addition to the ethical ones.

The creation of the Three Rs principle and the concept of humane science by Russell and Burch was the foundation of the quest for a different approach. Many have aimed to add additional Rs to the Three Rs concept, but none of them have made it to become integrated. Nevertheless, for this occasion, I would like to add a fourth R, which is *Realism*. I would like to share with you three reality checks, which made me lose three beliefs.

First reality check: The feasibility of the REACH system. I have and will continue to welcome it (18) as the largest investment into consumer product safety ever, all the more because the last-minute addition of the principal aim of developing alternative methods into Article 1 of the legislation sets a measure. It was a privilege to be involved in the origination of this legislation, and the first steps toward integrated testing strategies. However, from the beginning, it was clear that the sheer numbers of substances and tests will require a completely different approach. Unfortunately, a majority of the traditionalists have not yet perceived the need and embraced the opportunity for a new regulatory toxicology. Careful estimates of the dimensions of REACH had already indicated, five years ago, that there is no way of executing the programme within the given timelines and with the current test approaches. Originally, it was expected that about 27,000 companies would submit about 180,000 pre-registrations for about 30,000 substances. Now after the end of pre-registration in December 2008, we can see that more than 2.7 million pre-registrations have been received from about 65,000 companies for 144,000 substances. Even if some of this involves mistakes, a very substantial increase in the programme is still inevitable. There is no way that this will work without alternatives.

Second reality check: The adequacy of current toxicology. As a trained toxicologist, it takes a while to challenge the tools you have learned to use. The validation of alternative methods provided excellent training here. Although perceived by many as the promotion of alternatives, we tend to forget that, although "we put our money" only on the most promising tests, about 70% failed the validation process. And this represented a success, because we have prevented that premature test making it into use, where our safety would be at stake. However, it is unavoidable that when conducting validation, we look more and more into the point of reference, our gold standard, the animal test. And the more we apply the same scientifically rigorous thinking as we apply to alternative methods, the less golden this reference point becomes. The term toxicological ignorance has been used to describe our lack of knowledge of the hazards of most chemicals — it would be as appropriate for the lack of knowledge of the limitations of our tools. This has prompted me to call for an Evidence-based Toxicology, a thorough review of our toolbox, similar to Evidence-based Medicine in the clinical field. I tend to believe more and more that we are safe because most chemicals are no major threat to us, and we feel safe because it is largely impossible to connect exposure with the most relevant health effects, which develop only with delay and/or over time.

Third reality check: The adequateness of current alternative approaches and their integration into the toxicological toolbox. Sure, we will improve when integrating new tests; sure, we must take advantage of any opportunity to reduce animal use and suffering. However, this will not overcome the limitations of the current approach. Toxicology has developed over roughly 100 years, as a patchwork. We have added patches; we have sometimes replaced patches. We will be able to introduce more alternative patches, but this will not change the quilt. As long as we are looking for one to one substitutions of our patches, we will stay with the limited predictivity and the species differences, which limit our efforts. We need to start thinking about new points of reference for our validation studies, not the toxicology of the past (19). We have a tremendous backlog of scientific renovation to do in toxicology. No other area in the life sciences is still doing roughly the same experiments as were conducted 40 to 60 years ago.

So, it is time for revolution, as I tried to elaborate in last year's FRAME lecture (1). But there is reason for hope. We see the results of the biotech and informatics revolution, giving us new tools and approaches (20, 21). We see an increasing number of toxicologists concerned about the limitations of their art. We see political programmes like the 7th Amendment to the Cosmetics Directive in the EU(22), in response to societal demands, which can create an opportunity and a need for new approaches. And we see, with the US EPA-stimulated discussion about a toxicology for the 21st century (23), and their most recent new toxicity testing strategy, a new openness to change among the regulators. Our most recent efforts of translat-

ing Evidence-based Medicine to toxicology (24, 25) might further open this door. Finally, we will need a global Human Toxicology Programme to map the pathways of toxicology that will enable us to implement this vision.

Over the last decades, some organisations have been instrumental in paving the way to a new regulatory toxicology: FRAME, CAAT and ECVAM have played a key role here. The interplay was key in getting things moving. Their work and the individuals behind them were firmly based on Russell and Burch's pioneering thinking. Following Sir Isaac Newton's famous saying, I am aware that I am standing on the shoulders of giants, who stand on the shoulders of giants.

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