DEB AND AQUATIC REGULATORY TESTING
COURSE ESSAY

1. EXECUTIVE SUMMARY

The proposition of a two step approach, as derived from the DEB theory (Kooijman and Bedaux, 1996), to model the effects of xenobiotics on aquatic organisms as first, the uptake and elimination of xenobiotics and second, the effects due to the concentration in the organism, is a step towards the understanding of phenomena involved in regulatory testing. Two direct models for effects on reproduction (hazard and costs) and three indirect models (maintenance, growth, and assimilation) are used to interpret experimental data. This approach is discussed and the alternatives to a successful implementation of a mechanistic theory in regulatory testing are considered.

2. INTRODUCTION

Registration of Xenobiotics in the EU in the past years has been characterised by a higher demand on data. The typical acute and chronic studies are now nearly routine studies and establish the basis criteria to evaluate the need of what it is now known as high tier studies. These requirements are mainly for terrestrial and aquatic ecotoxicity studies; as requirements in environmental fate were increased in the late 80's when lysimetry studies were required due to the realisation of the importance to protect groundwater reservoirs.

Industry has questioned these new requirements in ecotoxicology, as the information gained is, presently, only used to confirm hypotheses. A more rewarding side to it, is that risk factors applied to high tier studies may be much lower, i.e. it is not uncommon to see that an outdoor aquatic microcosm study receives a factor of 1.

This development is certainly an expected one, if we consider that our present regulatory systems are based on the accumulation of information in order to decide with the most possible amount of information to avoid false negatives and false positives. That is, we are within the framework of a scientific oriented regulatory system and the only direction is to increase study requests (cf. Galicia and Breteler, 1999).

3. STUDY GUIDELINES

It is thus worth analysing if this development is a good one. Requiring more studies does not necessarily mean requiring essential information, or does not mean requiring that studies be interpreted with the state-of-the-art science. It is proposed here that the way acute and chronic regulatory studies are handled is very different from the way high tier studies are judged. An example is justified: an outdoor aquatic microcosm study ought to deliver information about almost anything, an acute and/or chronic study does not have to deliver information to better design a high tier study. Apart from a concentration (NOEC or ECx, or NEC) and what species are more sensitive, normally out a selection of 4 (fish, daphnia, alga, lemna), which will be furthere tested, these studies have limited use.

The type of binding/effect mechanisms, mode of action, role of the chemical structure and of the physico-chemical properties, etc. are not the main end points for these studies. Our regulatory systems do not either wish mechanistic studies, they wish certainty without having to open the black box. It is widely accepted that regulatory testing is not based on the latest developments in science. Examples can be given for any of the fields dealt with in regulatory testing. This perception was developped when the regulator's job (and the job of the regulatory staff within a chemical plant) was to tick boxes in a long form. Indeed no risk

assessment was required from the submitting companies, nor from the regulatory authorities. Presently, mainly due to societal pressure, regulators are persons who have a strong background. This has encouraged companies to hire the same type of person to be able to deal with the new requirements, and with the new regulators.

Unfortunately, 'old' guidelines have been forgotten, it is not uncommon that new guidelines are better planned and are more sound whilst older guidelines are untouched or go through minor revision. The discrepancy between new high tier and old low tier guidelines is becoming larger and larger.

4. REGULATORY TESTING

Regulatory testing has been characterised by the application of relative simple test systems, whise evaluation normally do not contribute to improving our understanding of the processes taking place but which allow a simple risk assessment.

Risk assessment schemes have experienced a recent development towards probabilistic oriented procedures. The latter, and the requirement of high tier studies (which require a better understanding of the mechanistic processes taking place in real systems), confront us with the need of parameters which will allow predictions and will allow the understanding of processes in real systems; however, the parameters obtained are based on non-mechanistic principles and thus of limited value. No need to discuss that parameters obtained dependent on the experimental design itself ought to be regarded as of limited use.

Several alternatives have been discussed and researchers feel obliged to defend their own theories or rather their own understanding of what it is necessary in the conduct of regulatory testing. It is important to notice that for a given set of regulatory rules (EU or US EPA) the usefulness of regulatory testing is highest if it fits the purpose for what is required. Industry will not see a justification in conducting testing which has a better scientific basis if the regulatory authorities will not reward this additional expense.

It follows automatically, that the impulse is not come from industry itself unless an added value is seen in the conduct of more scientifically sound studies, which is understandable; the impulse should come from the regulatory authorities if they have the long term view of re-dressing the objectives of the regulatory systems in place.

Along these lines: efforts to improve study guidelines or to provide mechanistic descriptions to regulatory testing ought to be periodically reviewed and their suitability should be evaluated. It is of paramount importance to make the difference between the application of such theories to make sense of the testing being conducted or their application to promote the scientific thinking in this community.

The use of the DEB theory in the evaluation of aquatic ecotoxicity studies has been proposed (Kooijman and Bedaux, 1996) to address the latter.

5. APPLICABILITY OF THE DEB THEORY

In order to assess the relevance of this theory the following questions must be answered:

1. Do the procedures proposed contribute to a better evaluation of the test in place?

2. Is the implementation of such procedures practical and will it lead to better study designs, or to a better use of the information obtained?
3. Do we need to modify the experimental design for a correct application of these procedures? If yes to what extent.
4. Will these requirements disqualify several current institutions perfoming these studies?
5. Are the new end points to be used in ulterior risk assessment different or improved, and do they facilitate the decisions to be made by the regulatory authorities?

It is apparent that most of these questions can be positively answered by referring to Kooijman and Bedaux (1996).

So far the application of the DEB has been proposed for the evaluation of acute and chronic tests in the aquatic ecotoxicology arena. The authors in different sections of this book point out that application in population studies is feasible (cf. also Chapter 10, Kooijman, 2000).

In the referenced book the analysis of the following studies is discussed: acute fish, acute daphnia, algal growth, and chronic tests in daphnia and in fish.

It is apparent that although the present study guidelines have some short-comings in their study designs, the authors do not seem to propose clear changes in the guidelines to fully apply the DEB models. It should be mentioned however, that a larger number of observations seem to be needed for better results (mention the page) and some adjustments may be needed.

6. IMPLEMENTATION OF IMPROVEMENTS TO TEST GUIDELINES

It can be seen that one of the main problems for the application of any improvement to present study guidelines is that the information needs to permeate to the national regulatory authorities. Moreover, very often researchers involved in regulatory testing are new to the field, and remain new to it. Apart from the fact that financial constraints impose restrictions in the time that researchers can invest in the development of better testing guidelines.

Although there were several chapters dedicated to analyse current study guidelines in a very convincing way, it could be observed that a full comparison was missing. In fact, only the last chapter dealing with algal growth mentioned statistics in detail. Also in this chapter, a first reference was made to the existing estimations of NOEC by the already accepted methods.

In other words, it would have been interesting to see a one to one comparison in the form of a table of the values obtained with the direct and indirect models with the said already used methods. It would have been very important to discuss the differences between NEC and NOEC. It would be expected that, the nearer the lower tested concentrations are to the NEC, the smaller the differences between the two approaches should be. This would support the statement made by the authors that when the NEC is not significantly different from zero, then the study was conducted in an inadequate way, or the variability could be explained by the difficulty in the analytics or due to the stability of the compound in water.

For these studies, a preliminary test is recommended and it would follow that these pre-tests were either not performed or performed in a lousy manner. An analysis of this kind would be needed to advocate for the use of NEC, as I assume that the more depurated the data for a validation are the more powerful the conclusions would be.

7. INFLUENCE OF EXPERIMENTAL DESIGN ON NEC

Static renewal systems were mentioned in one occasion. The stability of the compounds tested was also pointed out. The metabolism of compounds was also considered. Still, there are two major points that would need further discussion:

a. The presence of toxic metabolites, mentioned indirectly in the chapter dealing with algal growth.
b. Discussion of results based on stability of the test substance and then the use of flow-through systems (not for algal growth) for unstable compounds.

This is important because results obtained from static experiments may yield different results from studies conducted under flow-through conditions, i.e. the assumptions of a constant concentration of the test substance throughout the study may not hold in reality, or a variation of 10% may have a strong influence. In any case the discussion of the importance of such a variation would give a better feeling for the sensitivity of the NEC vs. NOEC. The question is here if there is supporting evidence as to the concentrations prevailing during the study.

The discussion about the three indirect models is often referred as yielding very similar results. Numerical criteria to assess which model is providing a better fit of the data would be desireable. In this case, it may be very important to analyse the mode of action of each substance so that we do not choose a model purely on statistical criteria, but rather that we know from independent information which could be the ‘right’ model. Alternatively, the analysis of studies according to the five models proposed would allow to group substances due to the resulting best fit model.

8. LIFE AFTER THE NEC

Taking the analysis furhter on, it is important to consider the effects of adopting the NEC approach. Currently a NOEC is used in combination with a PEC to calculate a given ratio, in the EU for Plant Protection Products (PPP) this is called TER (Toxicity Exposure Ratios). TER values are ususally calculated for acute studies and for chronic studies (discussion of higher tier risk assessment schemes are out of the scope of this essay). Indeed, the NOEC is also required in large multi-species outdoor aquatic microcosm studies!

It would be important to establish the limits of the NEC, can such a concept be easily adapted to and be compared to NOEC’s from high tier studies?, i.e. not only acute and chronic studies. Extended laboratory studies would be a good point to start.

Nevertheles, it would seem that we would be dealing with worse TER’s because the NEC would invariably be lower than the NOEC. If it can be demonstrated that this is related to a narrower confidence interval then the predictability of a simple TER would be improved. Thus, there should be an incentive to adopt a NEC approach e.g. through different trigger values for high tier testing because of the lower uncertainty involved.

Uncertainty is a concept, which is not frequently used, I missed it in this book too. In engineering it is very important to calculate the propagation of error. In science it is also very important but not usually reported.

If we were to give the uncertainty involved in both approaches NOEC and NEC and take it to report a TER ± the uncertainty, then a combined trigger criteria could be given, i.e. not anymore based on the TER alone but also on the certainty with which we arrive at this TER.

Industry would thus see the added value in adopting this approach. Above all, it should be made clear to the target community that this approach does not suffer from the problems mentioned throughout the text (function of selected concentration, experimental design, experimenter, etc.).

9. CONCLUSIONS

The following comments will be allocated at two study levels: acute and chronic.

Acute
If there is no change in the trigger requested for the TER, then there is little value added to the acute studies by adopting the NEC approach. For the end user it would mean, a TER of 40 or 80 makes no difference even if then we gain in insight as to the mechanisms taking place for that particular organism and that particular substance.

Chronic
Several chronic studies need to be conducted even if there are no major effects on a given organism, i.e. defined by e.g. persistence. On the other hand, higher tier studies will be trigerred if chronic end point results are unfavourable for the susbtance. It follows that a change made here will have a major impact on further testing to be required. It is clear that in this respect, the opposition to an approach that will yield lower “NOEC’s” that is, by substituting them by NEC’s will not find great support.

Really, the point here is that any change of this kind needs to also consider the impact on the risk assessement and on the risk management alternatives.

An open attitude with respect to the NEC, or any other improved assessment of single and multi-species studies, is an essential requirement to fulfil our function within the scientifc community. It seems as it is about time to implement a mechanistic description of regulatory testing to promote scientific improvement of our regulatory systems.

10. REFERENCES

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