



Session 5.8

Challenges in food toxicity testing

Lecture

Current situation of alternative methods to the mouse bioassay for phycotoxins

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Although in Europe the problem of marine toxins is slightly different than in other parts of the world, the international trade requires a control for any toxin from around the world. Those toxins currently being detected by animal sacrifice methods are: hydrophilic compounds (saxitoxin and analogs), and lipophilic compounds (yessotoxins, pectenotoxins, ostreocins, maitotoxins, ciguatoxins, cyclic imines, okadaic acid analogs, azaspiracids).

The situation created in the field of marine biotoxins with current decision EU/2002/225, that requires all lipophilic toxins to be detected by the mouse bioassay, has put a great pressure on

having alternative methods readily available to replace the bioassay, and backed up by an international validation study. With the chemical diversity of marine toxins, the bioassay is not a reliable system to safely control all the lipophilic toxins.

Although there are many technical possibilities to develop alternative methods, the authors will elaborate on those that have the highest chances of success: optical biosensors, functional (biochemical based) assays, and chemical (separation, mass spectrometry) methods. Antibody-based assays will be discussed as not good candidates for this field.



Lecture

Challenges in the use of transgenic mouse models in the toxicity testing of food additives

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Introduction: The assessment of carcinogenicity potential for many types of regulated chemicals with significant human exposures currently relies on the use of two lifetime rodent bioassays. Problems with these bioassays are: numbers of animals used, long duration, high expense, histopathological complexities and potentially inconsistent results. Selected transgenic mouse models are discussed to provide a review of advantages and limitations of these methods.

Methods: The transgenic mouse models to be discussed will be: Tg.rasH2, Tg.AC and p53+/- as replacements for a mouse bioassay.

Results: A large-scale, multinational collaborative study that was co-ordinated by the Health and Environmental Sciences Institute of the International Life Sciences Institute and evalu-

ated 5 different *in vivo* and *in vitro* assays will be briefly reviewed. Twenty-one well-characterised chemicals were submitted for testing with outcomes that were predicted and others that were surprising. In addition the recent regulatory experience of the US Food and Drug Administration with these methods will be presented.

Discussion: Three regional authorities (US, Europe, Japan) regulating pharmaceutical testing for carcinogenicity potential allow the use of transgenic mouse models in substitution for a mouse bioassay. The FDA policy for assessing the potential carcinogenicity of food additives still relies upon the completion of rat and mouse bioassays. Some of the reasons for this difference in testing policy will be discussed. Possible uses of these models in food additive testing will be presented.

Lecture

Summary and recommendations of the ECVAM/DG SANCO workshop on Three Rs approaches in marine toxin testing

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Taking into account ongoing discussions on discrepancies between Council Directive 86/609/EEC on the protection of laboratory animals and the EU legislation on shellfish toxin testing, ECVAM and its Task Force on Shellfish Toxin Testing organised with DG SANCO a workshop held in January 2005 at ECVAM, which was attended by experts from national and international control laboratories and institutions as well as academia.

The objectives of the workshop were to: a) discuss the state of art of available methods and testing strategies; b) consider

immediate possibilities to reduce and refine the currently required animal tests; and c) evaluate the status of non-animal methods regarding development, validation and regulatory acceptance for monitoring purposes and/or reference methods replacing the current animal tests. The outcome of the discussion and the recommendations will be presented.



Lecture

The European Food Safety Authority's (EFSA) Animal Welfare Policy and approach of work

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The concern on the use of the animals, in particular for experimental purposes in the European Community, is laid down in the Council Directive 86/609/EEC of 24 November 1986 on the approximations of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. This Directive is currently being revised. The principles about animal welfare found in this Directive are also found in the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes. In the founding Regulation of EFSA (Regulation No. 178/2002) it is stated that the mission of EFSA includes that "The Authority shall contribute to a high level of protection of human life and health, and in this respect take account of animal health and welfare ..." (Article 22).

Methods: The EFSA Management Board at its meeting of 22 June 2004 supported EFSA's willingness to develop a proactive animal welfare policy provided that this policy would be based on sound scientific principles. The scope of the task will be restricted to vertebrates used as experimental animals. The implementation of such a policy should stimulate the development of new food and feed assessment approaches that would not only minimise the numbers of experimental animals and their suffering, but also work towards their replacement through the use of alternative techniques (replacement, reduction and refinement, i.e. the Three Rs approach).

Results and Discussion: It is recognised that the implementation of fundamental changes to improve the welfare of experimental animals in relation to EFSA's activities would take several years and can be achieved only step-by-step. The European Food Safety Authority requested its Scientific Committee to develop a stepwise approach to incorporate animal welfare approaches into EFSA's activities without compromising the quality of the safety evaluations. The Scientific Committee focuses initially on tasks which can be completed within a reasonable time frame such as:

- Development of a comprehensive overview of all current EU legislative and guidance documents that address the experimental animals and their welfare. It should also address methodologies officially accepted or in use in the EU or some countries although not formally validated;
- Identification of guidance documents and procedures, currently applied by the EFSA Panels that could have an impact on experimental animals and their welfare;
- Making an inventory of all current activities of the Panels and Scientific Committee that relate to animal welfare, e.g. implementation of the Qualified Presumption of Safety approach, voluntary data sharing;
- Proposing ways to harmonise the application of guidance and legislative elements across EFSA Panels;
- Advising on how Panels could be kept informed of the latest scientific developments related to alternative methods to animal testing, and to internationally available, alternative approaches for hazard characterisation;
- Contributing to the improvement of existing guidance documents and procedures where appropriate, in collaboration with the risk managers, in order to take account of developments in the use of alternative methods with regard to current requirements for testing and food/feed assessments;
- Advising on how to improve sharing of information with organisations active in the area of animal welfare, e.g. bodies involved in the development and validation of methodologies for safety assessment, regulatory bodies requiring animal testing; and
- Advising on how to stimulate new research activities and new approaches in the field of risk assessment which would work towards the Three Rs policy.

Longer-term achievements could include proposals for the application or evaluation of new methods for risk assessment purposes, as well as new concepts in the risk assessment process that would take better account of the Three Rs.



Lecture

Safety assessment of genetically modified foods

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The development of methods in modern biotechnology allows selected individual genes to be transferred from one organism into another and also between non-related species in a way that does not occur naturally. Foods produced using modern biotechnologies are known as genetically modified (GM) foods and widespread concerns have been expressed regarding their safety for human consumption. New challenges regarding safety assessment of GM foods are now being posed to both the food industry and food regulators. New foods are not traditionally subjected to extensive safety testing but rely on the fact that the parent varieties have a long history of safe use as food. Different approaches are required when assessing the safety of whole

foods compared to chemical or microbial contaminants. Conventional risk assessment procedures that are used to determine the safety of discrete chemical entities are not particularly useful when applied to whole foods. Animal studies for assessing the toxicological endpoints of chemicals in the diet are a major element of conventional risk assessment. However animal studies cannot be applied in the same way to whole foods. This presentation will discuss the development of the concept of substantial equivalence which is used to structure the safety assessment of GM foods and will also address the limitations of animal testing when applied to whole foods.

Lecture

The use of chemical and computational modelling combined with *in vitro* toxicity testing to assess a safety concern of chemical contaminants in food

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The finding of acrylamide in food has raised concern about potentially toxic or carcinogenic compounds formed during heat processing in the Maillard reaction. In order to anticipate similar issues, we developed an approach to model the formation and human exposure to Maillard products formed through the same mechanism. The health significance of these molecules is difficult to assess since little or no toxicological information exists. Because of the number of molecules and exposure levels involved, it appears neither feasible nor necessary to characterise them all in detail.

In the absence of toxicological data, computational toxicology was used to predict chronic toxicity of the compounds identified, and the comparison to estimated exposure levels allowed to rank the compounds according to safety concern. Data obtained until

today revealed that acrylamide is the compound of most concern and others are unlikely to raise significant safety concern.

The probability of mutagenic or carcinogenic activity of both the contaminants and potential metabolites formed was predicted in a computational approach. In order to verify results, *in vitro* mechanistic studies on primary hepatocytes were initiated to study the mechanism of toxic action, including gene expression and metabolomic profiling. Preliminary results of experimental data will be shown that confirm results of the modelling.

We demonstrate in this study that under certain conditions, in the absence of information, a preliminary evaluation of safety concern is feasible, allowing prioritisation of research needs and resources. An optimisation of animal use is also a consequence of the application of such an approach.

**Poster****Using of an *in vitro* method for determination of toxic activity in food chain products***Alexey Solyakov and Per Häggblom*

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Introduction: Mould growth in animal feeds, which form the basis of the food chain, can cause the production of mycotoxins. Intoxication by mycotoxins can cause suffering in animals, economic losses and the transfer of toxins to humans via food-stuffs. Therefore, there is a need to be able to identify feeds/foods of questionable quality. The objective of the study was to evaluate the possibility of using a cellular *in vitro* technique as a screening method for monitoring toxic components in mould-damaged silage.

Methods: A grass crop was ensiled in three ways: (1) aerobic storage, (2) aerobic storage plus a spore suspension containing *P. roqueforti* and *A. fumigatus*, and (3) anaerobic storage. Samples were taken after 45 and 90 days of ensiling, extracted, purified, applied to human neuroblastoma SH-SY5Y cells, and the general cytotoxicity was determined as described by Wenehed et al. (2003).

Results and discussion: After 45 and 90 days of ensiling, mould-damaged silage (methods 1 and 2) was more cytotoxic than control silage (method 3). The search for toxic secondary metabolites with standard chemical methods is possible but very time and labour consuming, as well as expensive. Thus the reported cell-based *in vitro* method can provide a practical and more realistic method of evaluating general toxicity in food chain products. The results of this study are important in the light of the requirements of EU legislation concerning the safety in the food chain.

References

Wenehed, V. et al. (2003). Cytotoxic response of *Aspergillus fumigatus*-produced mycotoxins. *Fd. Chem. Toxicol.* 41, 395-403.