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Overview of the test requirements in the area of food and feed safety

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Abstract
The mission of the European Food Safety Authority - EFSA - is to provide scientific opinions and technical support in all fields which have a direct or indirect impact on food and feed safety. The Authority shall also "contribute to a high level of protection of human life and health, and in this respect take account of animal health and welfare".

In the area of hazard and risk assessment, EFSA receives studies as part of the dossiers provided by applicants who applied for approval of the use of a substance. The content of such dossiers relies on legal requirements which generally include experiments on animals.

EFSA decided to adopt a pro-active animal welfare approach summarized as follows: "While recognizing that animal testing cannot be eliminated at present, EFSA could make every effort to stimulate, and participate in, the development of new food and feed assessment approaches that would minimize the use of experimental animals and would reduce, to the extent possible, the level of suffering of animals".

The paper gives an overview of the work in this area at EFSA and suggests future developments considering the 3Rs and the animal welfare.

Keywords: animal welfare, food safety, feed safety, risk assessment, threshold of toxicological concern

Introduction

The European Food Safety Authority is the keystone of European Union risk assessment regarding food and feed safety. In close collaboration with national authorities and in open consultation with stakeholders, EFSA provides independent scientific advice and clear communication on existing and emerging risks.

The mission of the European Food Safety Authority (EFSA) as laid down in its founding Regulation[1] (Regulation No. 178/2002) includes the provision of "scientific and technical support for the Community's legislation and policies in all fields which have a direct or indirect impact on food and feed safety" (Article 22). This Article also states 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…". Animal welfare considerations in the field of food and feed safety assessment notably include concerns relating to the use of laboratory animals.

In the area of hazard and risk assessment, EFSA receives documentation on animal testing as part of the dossiers provided by an applicant who seeks approval for the use of a substance. The content of such dossiers usually depends on the legal basis under which the application was made, including European Community Regulations and sometimes Guidelines. These Regulations and Guidelines generally require that studies on animals testing be performed.

In its 15th Management Board meeting[2] (22 June 2004) EFSA agreed on a pro-active animal welfare approach summarized as follow: "While recognizing that animal testing cannot be eliminated at present, EFSA could make every effort to stimulate, and participate in, the development of new food and feed assessment approaches that would minimize the use of experimental animals and would reduce to the extent possible the level of suffering of those animals that are still needed today".

The implementation of the animal welfare policy should also work towards the replacement of animal testing and, at the same time, it should guarantee a high level of human health protection.

The European Union formally recognises the welfare requirements for animals. In the area of animal testing the basis of European legislation on the welfare of animals used for scientific purposes is the Council Directive 86/609/EEC[3] of 24 November 1986. It concerns the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. Deriving from this Directive, "alternative methods" are methods that replace, reduce or refine animal tests. The Directive encourages research into the development and validation of alternative methods.

The legislation is currently being revised and several questions regarding animal welfare have been raised during the revision process. The revised version of the Directive will most probably be adopted within the coming years.

This paper gives an overview of the test requirements in the area of food and feed safety. Moreover, the Threshold of Toxicological Concern approach is presented as an example of a methodology in use at EFSA for the safety assessment of food flavouring which reduces the requirement for experimental animal studies.

Overview of the remit of activities of the EFSA Panels concerned with safety assessment dossiers

EFSA's Scientific Panels carry out risk assessment work in their respective specialized fields, as follows:

- **Food additives, flavourings, processing aids and materials in contact with food** (AFC)
- **Animal health and welfare** (AHAW)
- **Biological hazards** (BIOHAZ), including BSE-TSE-related risks hazard
- **Contaminants in the food chain** (CONTAM)
- **Additives and products or substances used in animal feed** (FEEDAP)
- **Genetically modified organisms** (GMO)
- **Dietetic products, nutrition and allergies** (NDA)
- **Plant protection products and their residues** (PPR) including **Pesticide Risk Assessment Peer Review Unit** (PRAPeR)
- **Plant health** (PLH)

Concerning assessment dossiers provided by an applicant who seeks approval for the use of a substance, EFSA works with two different models. In the first, the testing requirements are included in the annexes of the general legislation (i.e. for pesticides); in the second case, the legislation usually specifies that the substance must be assessed to ensure that its use does not present any risk to human and animal health and for the environment, whereas requirements about testing of animals are specified in separate guidance documents. The guidance documents are implemented by the Scientific Committee, Panels or by other assessment bodies and by stakeholders.

The EFSA Panels assessing dossiers to support the authorisation of defined substances are: AFC, FEEDAP, GMO, NDA, PPR. These Panels deal also with self-tasking activities of general terms that may include testing guidance.

The EFSA Panels not directly involved with assessment of authorisation dossiers but which often assess data including animal testing are: AHAW, BIOHAZ, CONTAM, and Pesticide Risk Assessment Peer Review Unit (PRAPeR).

The task of the Scientific Committee's Working Group on the Welfare of Experimental Animals is to provide the EFSA Panels with information about the latest scientific developments related to alternative methods to animal testing internationally available, alternative approaches for hazard characterisation thereby contributing to the improvement of existing guidance documents and procedures on the use of alternative methods with regard to current requirements for testing and food/feed assessments. The aim is to stimulate new research activities and new approaches in the field of risk assessment, which would work towards the Three Rs policy without compromising the present high level of human protection. The first step of the Working Group activities has been to provide an up-to-date overview of the test requirements in the area of food and feed safety.

Food additives, flavourings, processing aids and materials in contact with food (AFC) Panel

This Panel deals with questions of safety in the use of food additives, flavourings, processing aids and materials in contact with food; associated subjects concerning the safety of other substances deliberately added to food and questions related to the safety of food production processes, including irradiation.

The majority of the Panel's work relates to assessment of the safety of food additives, flavourings and food contact materials. Recent additional areas of work have included the evaluation of smoke flavourings and assessment of the safety of food supplements and certain constituents of foods intended for particular nutritional uses. In the future, the Panel will also be responsible for the safety assessment of food enzymes under Community legislation currently under development.

The Panel's safety evaluations involve a review of all available relevant scientific studies and data on toxicity, as well as estimates of human exposure from which the Panel draws conclusions regarding the safety of the substance.

Additives and products or substances used in animal feed (FEEDAP) Panel

The Panel on additives and products or substances used in animal feed deals with questions on safety for the target species, the consumer of products of animal origin, the user/worker and the environment, as well as with the efficacy of biological and chemical products/substances intended for deliberate addition/use in animal feed. Thus, the FEEDAP Panel has some additional specific testing requirements that include efficacy and tolerance studies in target species as well as, when relevant, residue depletion studies. Since no standard international guidelines exist for such studies, the protocols will depend on the type and function of the additive, the target species, considering the requirements set by the legislation and the FEEDAP Panel's recommendations.

Moreover, a distinction is made between chemical additives and additives such as micro-organisms and enzymes; for the latter ones e.g. only a limited toxicological package and no residue depletion studies are normally required.

Genetically modified organisms (GMO) Panel

This Panel is involved with questions on genetically modified organisms as defined in Directive 2001/18/EC, such as micro-organisms, plants and animals, relating to deliberate release into the environment as well as with the assessment of the risk of new GMO for food and feed as defined in Regulation (EC) 1829/2003. The safety assessment of food and feed derived from GM plants or from GM-derived product concerns: assessment of newly expressed proteins, assessment of new constituents other than protein and testing the whole GM food and feed.

In the case of GM feed with improved nutritional characteristics, various types of livestock feeding studies with target species should be conducted on a case by case basis addressing claims of nutritional benefits.

Dietary products, nutrition and allergies (NDA) Panel

This panel deals with questions related to dietetic products, human nutrition and food allergy as well as associated issues such as Novel Foods. On request, the NDA Panel provides scientific advice to the European Commission, European Parliament and Member States.

According to EU legislation, Novel Foods are defined as foods/food ingredients which have not been used for human consumption to a significant degree within the EU before 15 May 1997.

The assessment of the safety of foods including Novel Foods presents a number of scientific challenges. Conventional toxicological evaluation methods cannot be applied to foods, because they present particular difficulties not encountered with the testing of food additives and contaminants. For example, the amount of food to be incorporated in the diet for animal feeding studies without perturbing its nutritional balance makes the use of conventional uncertainty (safety) factors inappropriate for risk assessment and management for a product intended for use as a food or a major food ingredient. Furthermore, traditional toxicokinetic studies are not directly applicable to complex chemical mixtures such as foods. The use of mutagenicity and other *in vitro* tests for foods requires special techniques and cautious interpretation of the results. Therefore, other approaches for testing and assessing the safety of novel foods and novel food ingredients are still to be established. In principle, the toxicological requirements for testing of novel foods need to be considered on a case-by-case basis.

Plant protection products (PPR) Panel

The Panel addresses questions on the safety of plant protection products for the user/worker, the consumer of treated products, and the environment related to the formulation, for the active substance and for its residues.

The Panel answers scientific questions related to risk assessment of pesticide use submitted by the European Commission, the European Parliament and Member States. The Panel works closely with the Pesticide Risk Assessment Peer Review Unit (PRAPeR) which is responsible for the peer review of initial risk assessments on new or existing pesticides. The Panel deals also with self-tasking activities of general terms.

The European Commission is currently revising the data requirements for authorization of active substances and plant protection products in the framework of the Council Directive 91/414/EEC.

Overview of the remit of activities of the EFSA Panels not directly involved with safety dossier assessments

Animal health and animal welfare (AHAW) Panel

This Panel deals with questions on all aspects of animal health and animal welfare, primarily relating to food producing animals including fish.

One important aspect concerning animal testing in this Panel is related to vaccines and to diagnostic tests for animal diseases. In general, vaccines for animal diseases are tested both for safety and efficacy. Guidelines for the production and quality control of vaccines are issued according to the European Pharmacopoeia, the European Medicines Agency (EMEA) and in some rare cases by the national pharmacopoeia or control authority. EMEA and the national control authorities are responsible for the licensing and batch control of vaccines. Depending

on the type of vaccine, this often involves tests on laboratory rodents and/or target animals.

In some circumstances, EFSA can be asked to give an opinion on the application of a vaccine as an approach to control the outbreak of the disease. In this case, additional testing may be required to assess the efficacy of the vaccines. The testing of vaccines raises issues over human endpoints, depending on the vaccine, as well as the use of surrogate species for target animals, and suitable controls for the assessment of test organism viability and applied dosages.

Regarding diagnostic tests for animal diseases (e.g. Avian Influenza, Bluetongue, Newcastle disease)**,** there are requirements for the determination of the pathogenicity, the serotype or other characteristics of the organism responsible for an outbreak. The responsibility for the evaluation of diagnostic tests changes according to the different diseases (Community Reference Laboratories, National Reference Laboratories or others). In special cases, EFSA can be asked to evaluate the relevance of the diagnostic tests. In this exercise, future research may be recommended involving the use of animal testing for the evaluation of the validity of the diagnostic test.

Biological Hazard (BIOHAZ) Panel

This Panel deals with questions on biological hazards relating to food safety and food-borne disease, including food-borne zoonoses and transmissible spongiform encephalopathies, microbiology, food hygiene and associated waste management.

The Panel does not usually deal with questions which involve the utilization of animal testing.

Contaminants in the food chain (CONTAM) Panel

The Panel addresses questions on contaminants in food and feed, associated areas and undesirable substances such as natural toxicants including mycotoxins and residues on non-authorised substances not covered by other EFSA panels.

The Panel assesses all available information of relevance to an evaluation of consumer safety. In principle an evaluation includes the types of study used to support hazard identification and characterisation as part of an assessment of consumer safety, as described above for a feed or food additive. In practice, the available database is likely to vary considerably. Studies conducted in experimental animals might include regulatory toxicology studies conducted in the context of the manufacture and use of a chemical, but are frequently of less guideline-type, as well as mechanistic studies conducted in academic institutions and published in the scientific literature. Human data, particularly epidemiological studies, may also be available in some instances. Often the available data are limited, and if the Panel has concerns about consumer safety, then its opinions will include recommendations for further research.

Possible improvements in risk assessment approaches considering the 3Rs

In general, the core set of (animal) studies required for risk assessment in the different EFSA Panels are the following:

- Toxicokinetics including metabolism
- Repeated dose toxicity
- Genotoxicity including mutagenicity
- Chronic toxicity[4]
- Carcinogenicity
- Reproductive toxicity including developmental toxicity
- Other studies, where appropriate and required

These studies are usually performed following OECD Test Guidelines.

The Scientific Committee's Working Group on Welfare of Experimental Animals has discussed possible improvements in risk assessment approaches taking into consideration the 3Rs (replacement, reduction and refinement of animal tests).

There are two possible complementary approaches that can be used to implement the "3Rs", both contributing to the new conceptual framework of "Intelligent Testing Strategies" as endorsed also by the new REACH regulation:

a. To exploit at EU regulatory level the development of new tests and testing batteries carried out by International Agencies (OECD, ECVAM) and by several ongoing EU Research Projects (e.g., ReProTect, CAESAR, ACuteTox). To implement such testing batteries is an important yet complex task, requiring significant research efforts; this holds true especially when effects on complex systems (e.g., endocrine disruption, immunotoxicity, neurotoxicity) are targeted. Moreover, whereas OECD testing guidelines for chemicals generally apply to the activities of all EFSA Panels, some Panels have also additional specific testing requirements.
b. To develop novel risk assessment strategies that make the best possible use of available information; in particular, as an example, the use of the Threshold of Toxicological Concern (TTC), already in use for the safety assessment of food flavouring, has been examined as one possible tool to reduce the number of animals used in the safety assessment.

Threshold of Toxicological Concern (TTC) in safety assessment of flavouring substances

The TTC concept was developed as a generic

approach for the safety assessment of large group of chemicals or individual chemicals of unknown toxicity.

The TTC concept is based on the principle that a generic human exposure threshold value can be established below which there is no risk to human health. This approach is suitable for substances present in food at low concentrations and where the intakes are anticipated to be low. The concept proposes that such a value can be identified for many chemicals or group of chemicals, including those of unknown toxicity, based on a consideration of their chemical structures.

Under the TTC approach, chemicals are assigned (on the basis of accumulated knowledge about chemical structure and the likelihood of toxicity), to one of three structural classes – **Class 1**, presumed low toxicity (threshold value 1800 μg/person/day), **Class 2** (threshold value 540 μg/person/day), structures less innocuous than Class 1 but not containing features suggestive of toxicity, or **Class 3** (threshold value 90 μg/person/day) without clear initial presumption of safety and with a potential for significant toxicity or with the presence of reactive groups.

Human exposure threshold values have been established for each of the three structural classes by considering extensive databases of toxicity information generated in the past (Kroes R. et al, 2000 and 2004). Estimated intakes of individual flavouring substances have been compared with the relevant threshold value to indicate whether further generation of data, including toxicological data, may be required. In many instances, the intakes of flavourings are below the relevant threshold value and further data are not required. During the safety assessment, emphasis is given to the evaluation of any available genotoxicity data, since exposure to genotoxic substances may pose a risk to health, even at low intakes.

The TTC approach can be applied to many substances but it is not appropriate for substances, such as proteins, heavy metals and polyhalogenated-dibenzodioxins and related compounds, for which the TTC approach should not be used.

Useful applications of the TTC methodology are envisaged to include situations where the presence of a contaminant in food is newly discovered and for which there is no toxicological information. The TTC may also be used for setting priorities for testing among large functional groups of chemicals with similar structures and for which exposure is generally very low such as flavouring substances and substances used in food contact materials. The wider use of such a tool should provide benefits for industries, regulatory authorities and consumers because it would enable the world's limited resources for toxicity testing and safety evaluation to be focused on exposure to those chemicals which may pose a threat to human health. By eliminating the need for unnecessary toxicity testing, it would also reduce the number of animals used.

Conclusion

The task of the EFSA's Scientific Committee through its Working Group on the Welfare of the Experimental Animals is to stimulate the development of new food and feed assessment approaches that would not only minimise the numbers of experimental animals used and their potential suffering, but also work towards their possible replacement. At the same time, this policy must be consistent with the EFSA strategic priority of ensuring a level of human health protection. The Working Group will continue to discuss and propose to the different EFSA Panels development and application of new approaches or concept for chemical and microbiological risk assessment that would take better into account the welfare of experimental animals.

References

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Footnotes

1. http://eur-lex.europa.eu/LexUriServ/site/en/oj/2002/l_031/l_03120020201en00010024.pdf
2. http://www.efsa.europa.eu/EFSA/AboutEfsa/WhoWeAre/ManagementBoard/efsa_locale-1178620753812_MeetingsMB.htm
3. http://ec.europa.eu/food/fs/aw/aw_legislation/scientific/86-609-eec_en.pdf
4. Chronic toxicity studies in rats are often combined with carcinogenicity studies.