TOXICOLOGICAL STUDIES FOR THE SAFETY ASSESSMENT OF FOOD ENZYMES IN THE EU: PROTOCOLS, QUALITY REQUIREMENTS AND DATA WAIVERS

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INTRODUCTION

Toxicological studies are the core of chemical risk assessment. For the evaluation of food enzymes performed by EFSA, the toxicological data required include those relating to genotoxicity and systemic toxicity. Genotoxicity is evaluated by testing the capacity of the food enzyme to produce gene mutations (OECD TG 471) and structural and numerical chromosomal aberrations (OECD TG 487). If an *in vitro* test gives positive results, a follow-up with *in vivo* tests is required. Systemic toxicity is evaluated with a repeated-dose 90-day oral toxicity study in rodents (OECD TG 408). The outcome of the 90-day study is the identification of the no observed adverse effect level (NOAEL). The division of NOAEL with the estimated dietary exposure gives rise to a Margin of Exposure. Should an estimation of dietary exposure be unnecessary, toxicological tests can be waived. Toxicological studies can also be waived if the food enzyme originates from edible parts of non-GMO plants or animals or if the enzyme derives from microorganisms qualified for the presumption of safety status (QPS). This work presents an overview of the toxicological assessment of food enzymes performed by EFSA from 2014 to 2021.

METHODOLOGY

Scientific opinions on food enzymes published by EFSA CEF/CEP Panel from April 2014 to September 2021 were analysed. A total of 110 opinions were screened for the following criteria: i) need for toxicological studies, ii) results of the study, iii) waiving of toxicological studies and reason for such waiving and iv) outcome of the opinion. Special attention was paid to the quality of the studies and their relevance for the risk assessment and final outcome of the opinions.

RESULTS

Among the 110 published opinions on food enzymes analysed, 70 % required toxicological tests. Among the remaining 30 %, in 12 % of cases toxicological studies were nevertheless provided, and they were considered as supporting evidence for the assessment of the food

enzyme safety. In only one case were they excluded from consideration due to the lack of representativeness of the test item employed. Gene mutations were evaluated by the Ames test and chromosomal effects were evaluated by a chromosomal aberration test or a micronucleus assay – the majority of the tests were negative. Only 2 food enzymes required follow-up *in vivo* tests. In 3 cases, the substitute approach was employed for the toxicological data set. However, in one of these cases it was not considered suitable as the microbial strain used as substitute was not comparable to the one under assessment.
In 9 cases, the CEP/CEF Panel was not able to come to a conclusion on the safety of the food enzymes based on the information provided by the applicant. In 4 of those cases, this was due to an incomplete toxicological data set, or a poorly characterised batch used for the toxicological studies, or an unsuitable substitute toxicological study.

DISCUSSION

The availability of a complete set of good quality data is fundamental for a fast and thorough safety assessment of food enzymes. In this work, we analysed 110 opinions published by EFSA on food enzymes in the past 7 years with the aim of identifying whether inadequacy in protocols or technical data needed for the toxicological risk assessment could impact the final outcome of the opinions. The results showed that a poor characterisation of the test items used for the toxicological analysis, incomplete data sets and toxicological assays performed not following standardised guidelines were the reasons for nearly half of the inconclusive opinions. Scientific opinions originating from technical dossiers submitted in the past two years contain better quality data and allow a more efficient assessment. Frequent communication with stakeholders and the publication of the new guidelines on the submission of dossiers on food enzymes will help ensure further optimisation of food enzyme risk assessments by EFSA.