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Safety evaluation of the food enzyme phospholipase C from the genetically modified *Bacillus licheniformis* strain NZYM-VR

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Abstract

The food enzyme phospholipase C (phosphatidylcholine cholinephosphohydrolase EC 3.1.4.3) is produced with the genetically modified *Bacillus licheniformis* strain NZYM-VR by Novozymes A/S. The genetic modifications do not give rise to safety concerns. The food enzyme is free from viable cells of the production organism and its DNA. This phospholipase C is intended for use in degumming of fats and oils. The residual amounts of Total Organic Solids (TOS) are removed during washing and purification steps applied during degumming. Consequently, no dietary exposure was calculated. Genotoxicity tests did not raise a safety concern. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rats. The Panel identified a No Observed Adverse Effect Level (NOAEL) at the highest dose tested of 714 mg TOS/kg body weight (bw) per day. Similarity of the amino acid sequence to those of known allergens was searched for and no match was found. The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood is considered to be low. Based on the data provided and the removal of TOS during the degumming of fats and oils, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

Keywords: food enzyme, phospholipase C, phosphatidylcholine cholinephosphohydrolase, EC 3.1.4.3, *Bacillus licheniformis*, genetically modified microorganism, degumming

Requestor: European Commission

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Table of contents

1. Introduction

Article 3 of the Regulation (EC) No 1332/2008[1] provides definition for 'food enzyme' and 'food enzyme preparation'.

> 'Food enzyme' means a product obtained from plants, animals or micro-organisms or products thereof including a product obtained by a fermentation process using micro-organisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.
>
> 'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No 1332/2008 on food enzymes came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008[2] established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

- it does not pose a safety concern to the health of the consumer at the level of use proposed;
- there is a reasonable technological need;
- its use does not mislead the consumer.

All food enzymes currently on the European Union market and intended to remain on that market, as well as all new food enzymes, shall be subjected to a safety evaluation by the European Food Safety Authority (EFSA) and approval via an EU Community list.

The 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) lays down the administrative, technical and toxicological data required.

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background as provided by the European Commission

Only food enzymes included in the European Union (EU) Community list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7(2) of Regulation (EC) No 1332/2008 on food enzymes.

An application has been introduced by the applicant "Novozymes A/S" for the authorisation of the food enzyme phospholipase C produced by a genetically modified strain of *Bacillus licheniformis* (strain NZYM-VR).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008, the Commission has verified that the application falls within the scope of the food enzyme Regulation and contains all the elements required under Chapter II of that Regulation.

[1] Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on Food Enzymes and Amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, pp. 7–15.

[2] Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, pp. 1–6.

[3] Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 64, 11.3.2011, pp. 15–24.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessment of the following food enzyme: phospholipase C produced by a genetically modified strain of *Bacillus licheniformis* (strain NZYM-VR) in accordance with Article 29 of Regulation (EC) No 178/2002[4], and Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of the food enzyme phospholipase C from a genetically modified *Bacillus licheniformis* (strain NZYM-VR).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme phospholipase C from a genetically modified *Bacillus licheniformis* (strain NZYM-VR).

Additional information was requested from the applicant during the assessment process on 29 January 2020 and was consequently provided (see 'Documentation provided to EFSA').

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA 'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009b) as well as in the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) and following the relevant existing guidances of EFSA Scientific Committees.

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) has been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance to the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016).

3. Assessment

IUBMB nomenclature:	Phospholipase C
Systematic name:	phosphatidylcholine cholinephosphohydrolase
Synonyms:	lipophosphodiesterase I; lecithinase C; lipophosphodiesterase C
IUBMB No:	EC 3.1.4.3
CAS No:	9001-86-9
EINECS No:	232-638-2

Phospholipase C catalyses the hydrolysis of the phosphoester bond linking glycerol and phosphate moieties at the sn-3 position of glycerophospholipids. The enzyme is intended to be used in degumming of fats and oils.

3.1. Source of the food enzyme

The phospholipase C is produced with a genetically modified bacterium *Bacillus licheniformis* strain NZYM-VR, which is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), with deposition number [redacted].[5]

The whole genome sequence of the production strain was screened for antimicrobial resistance genes [redacted] *Bacillus*

[4] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

[5] Technical dossier/1st submission/Annex 4-A3.

licheniformis strains have in general a reduced susceptibility [redacted] (Agersø et al., 2018), suggesting that [redacted] may be constitutive in this species.

3.1.1. Characteristics of the parental and recipient microorganisms

The parental strain [redacted] was identified [redacted] *B. licheniformis* type strain. [redacted][5]. The recipient strain [redacted][6]. The genetic modifications were introduced [redacted][7] [redacted][8] [redacted][9] [redacted].

3.1.2. Characteristics of introduced sequences

The DNA sequence for the introduced phospholipase C [redacted][9].

3.1.3. Description of the genetic modification process

The phospholipase C production strain NZYM-VR was constructed from recipient strain [redacted]

[6] Technical dossier/1st submission/Annex 4, p. 11-19/Annex 4-B1-B21.
[7] Technical dossier/1st submission/Annex 4 p. 13/Annex 4-B35-B40 and B44-B50.
[8] Technical dossier/1st submission/Annex 4 p. 13/Annex 4-B41-B43.
[9] Technical dossier/1st submission/Annex 4 p. 19-20.

[redacted][10]

[redacted][11]

3.1.4. Safety aspects of the genetic modification

The technical dossier contains all necessary information on the recipient microorganism, the donor organism and the genetic modification process.

The production strain *B. licheniformis* NZYM-VR differs from the recipient strain [redacted] only in its capacity to produce phospholipase C. [redacted][12]

No issues of concern arising from the genetic modifications were identified by the Panel.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[13], with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[14]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, fed-batch fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration leaving a supernatant containing the food enzyme. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained while most of the low molecular weight material passes the filtration membrane and is discarded.[15] The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.[16]

The Panel considered that sufficient information has been provided on the manufacturing process and the quality assurance system implemented by the applicant to exclude issues of concern.

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The phospholipase C is a single polypeptide chain of [redacted] amino acids.[17] The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be [redacted] kDa.[17] The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of three batches of the food enzyme used for commercialisation and the batch used for the toxicological studies consistently showed one major protein band migrating in the expected position for the phospholipase and a number of other bands of minor staining intensity.[18] The food enzyme was analysed for the presence of α-amylase, amyloglucosidase, lipase, peroxidase and protease activities. None were detected.[19]

The in-house method used to determine the phospholipase C activity follows the rate at which the phospholipase hydrolyses a phospholipid to release diglyceride and phosphocholine at pH 7.5 and at 30°C. The released phosphocholine is titrated with bromothymol and the change in absorbance at 600 nm is

[10] Technical dossier/1st submission/Annex 4 p. 23-25/Annex 4-B1.
[11] Technical dossier/1st submission/Annex 4-D1.
[12] Technical dossier/1st submission/Annex 4 p. 26/Annex 4-D2.
[13] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of food additives. OJ L 226, 25.6.2004, pp. 3–21.
[14] Technical dossier/1st submission/Annex 5.
[15] Technical dossier/1st submission/p. 44–50.
[16] Technical dossier/1st submission/Annex 6.
[17] Technical dossier/1st submission/p. 30/Annex 1.
[18] Technical dossier/1st submission/p. 32.
[19] Technical dossier/1st submission/p. 37/Annexes: 3.02, 3.03, 3.04, 3.05, 3.06.

followed as a direct measurement of enzyme activity. The activity is determined relative to an enzyme standard. The result is given in PLC(S)/g.[20]

The phospholipase C exhibits more than 80% of maximum activity between pH 7 and 9 with an optimum at pH 8.0 (at 30°C). The optimum temperature for activity is about 65°C (at pH 7.0). Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures. Under the assay conditions used, the phospholipase C activity was stable up to 60°C. Thereafter, activity decreased rapidly showing no residual activity at 80°C.[21]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and a further batch used for the toxicological tests (Table 1). The average total organic solids (TOS) of the three food enzyme batches for commercialisation is 8.5% and the average enzyme activity/TOS ratio is 167.8 PLC(S)/mg TOS.[22]

Table 1: Compositional data provided for the food enzyme

Parameter	Unit	Batches			
		1	2	3	4[(c)]
Phospholipase C activity	PLC(S)/g[(b)]	12,900	11,900	17,400	7,010
Protein	%	6.5	6.0	6.4	5.1
Ash	%	0.5	0.5	0.6	1.5
Water	%	91.6	90.2	91.2	88.0
Total Organic Solids (TOS)[(a)]	%	7.9	9.3	8.2	10.5
Phospholipase C activity/mg TOS	PLC(S)/mg TOS	163.3	128.0	212.2	66.8

(a): TOS calculated as 100% – % water – % ash.
(b): PLC(S)/g: phospholipase C unit/g (see Section 3.3.1).
(c): Batch used in toxicological studies.

3.3.3. Purity

The lead content in three commercial batches and in the batch used for toxicological studies was below 0.5 mg/kg[23] which complies with the specification for lead (≤ 5 mg/kg) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006). In addition, the levels of cadmium, mercury and arsenic were below the limits of detection of the employed methodologies.[24,25]

The food enzyme complies with the microbiological criteria as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006), which stipulate that *Escherichia coli* and *Salmonella* species are absent in 25 g of sample and total coliforms should not exceed 30 colony-forming unit per gram.[26] No antimicrobial activity was detected in any of these batches (FAO/WHO, 2006).[25]

The Panel considered that the information provided on the purity of the food enzyme is sufficient.

3.3.4. Viable cells and DNA of the production strain

The absence of the production strain in the food enzyme was demonstrated in three independent batches analysed in triplicate. [redacted] No colonies were produced. A positive control including the production strain in combination with the product was run in parallel with each batch to ensure that no growth inhibition

[20] Technical dossier/1st submission/p. 35/Annex 3.01.
[21] Technical dossier/1st submission/p. 36-37/Annex 9.
[22] Technical dossier/1st submission/p. 31/Annex 10.
[23] LOD: Pb = 0.5 mg/kg.
[24] LOD: Cd = 0.05 mg/kg, Hg = 0.05 mg/kg, As = 0.3 mg/kg.
[25] Technical dossier/1st submission/p. 33/Annex 10.
[26] Technical dossier/1st submission/p. 34/Annex 10.

was caused by the product. A reference sample included the production strain with no addition of product was also run confirming the viability of the strain.[27]

The absence of recombinant DNA in the food enzyme was demonstrated by polymerase chain reaction (PCR) analysis of three independent batches in triplicate. No DNA was detected [redacted].[28]

3.4. Toxicological data

A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* micronucleus assay and a repeated dose 90-day oral toxicity study in rats has been provided. The test item (see Batch 4, Table 1) which was used for all of the reported toxicological studies has much lower specific activity compared to the batches used for commercialisation. Since the focus of the toxicological studies of food enzymes is the assessment of the non-protein components of TOS rather than the enzyme protein, batch 4 is considered a suitable test material.

3.4.1. Genotoxicity

3.4.2. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP).[29] Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* (WP2 uvrA (pKM101)) were used in the presence or absence of metabolic activation, applying the 'treat and plate' assay. Two separate experiments were carried out in triplicate.

In the first experiment, seven different concentrations of the food enzyme TOS were used (5, 16, 50, 160, 500, 1,600 and 5,000 μg TOS/plate), while in the second experiment, a narrower range of concentrations was applied (160, 300, 625, 1,250, 2,500 and 5,000 μg TOS/plate). No evidence of cytotoxicity was observed. Upon treatment with the food enzyme, there was no significant increase in revertant colony numbers above the control values in any strain with or without metabolic activation in either experiment.

The Panel concluded that the food enzyme did not induce gene mutations under the test conditions employed in this study.

3.4.2.1. *In vitro* micronucleus assay

The *in vitro* micronucleus test was carried out according to OECD Draft Guideline 487 (OECD, 2014) and following GLP.[30] A single experiment was performed in duplicate cultures of human peripheral whole blood lymphocytes. Cells were exposed to the test substance for 3 h in the presence or absence of the metabolic activation (S9-mix) and harvested 21 h after the beginning of treatment. Additionally, a continuous 24-h treatment without S9-mix was included with harvesting 24-h after removal of the test substance. The food enzyme was tested at 1,000, 3,000 and 5,000 μg TOS/mL in the absence of metabolic activation and 3,000, 4,000 and 5,000 μg TOS/mL in the presence of metabolic activation in the short exposure assay and at 100, 500 and 2,000 μg TOS/mL in the 24-h exposure assay. The maximum concentration tested in the 24-h exposure assay was determined by evidence of cytotoxicity (56% based on the replication index) seen with 2,000 μg TOS/mL. The frequency of bi-nucleated cells with micronuclei (MNBN) did not differ significantly from the concurrent controls at all concentrations tested.

The Panel concluded that the food enzyme phospholipase C did not induce an increase in the frequency of MNBNs in cultured human peripheral blood lymphocytes, under the test conditions employed in this study.

[27] Technical dossier/1st submission/Annex 4-E1.
[28] Technical dossier/1st submission/Annex 4-E2.
[29] Technical dossier/1st submission/Annex 7.01.
[30] Technical dossier/1st submission/Annex 7.02.

3.4.3. Repeated dose 90-day oral toxicity study in rodents

The repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP.[31] Groups of 10 male and 10 female Han Wistar (RccHan, WIST strain) rats received by gavage the food enzyme in amounts corresponding to 110, 275 or 714 mg TOS/kg body weight (bw) per day selected on the basis of a preliminary 14-day study. Controls received the vehicle (water).

One female from low-dose group was found dead on day 44. The cause of death could not be clarified due to cannibalism. One control female escaped from its cage and subsequently died manifesting convulsions on day 68. Necropsy and histologic examination confirmed that the death was caused by the accidental trauma (haemorrhages within the heart, muscle and/or skin/subcutis). Neither deaths are considered related to treatment.

Amongst the functional battery of observation, the only statistically significant differences seen were intermittent differences in low beam scores for high-dose females compared to the control group. These findings were not consistent and therefore they were considered incidental.

Haematological examination revealed minor but statistically significant differences in some erythrocyte parameters. In males, all dose groups showed higher red cell distribution widths than controls, but there was no evidence of dose response. In high-dose females, there was a reduction in red cell counts and an increase in cell haemoglobin concentration. These changes were considered by the Panel not to be of toxicological significance, as the differences to the control were small, without dose response relationship or evidence of similar trends in the opposite sex.

Clinical chemistry examination revealed a significantly elevated blood glucose concentration in low- and high-dose females. Furthermore, high-dose females had a slightly and statistically significant elevated creatine concentration. All differences from control values which attained statistical significance, were minor, confined to one sex or were not dose-related and, consequently, were considered by the Panel to represent normal biological variation.

There was a small but statistically significant increase in spleen weight in females at all the doses tested. In light of no evidence of a dose dependency and lack of accompanying histopathological findings, this finding was considered not to be of toxicological significance.

No other statistically significant differences to controls were observed.

The Panel identified the no observed adverse effect level (NOAEL) of 714 mg TOS/kg bw per day, the highest dose tested.

3.4.4. Allergenicity

The allergenicity assessment considers only the food enzyme and not any carrier or other excipient which may be used in the final formulation.

The potential allergenicity of the phospholipase C from the genetically modified *B. licheniformis* strain NZYM-VR was assessed by comparing its amino acid sequence with those of known allergens according to the scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms (EFSA GMO Panel, 2017). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, no match was found.[32]

No information is available on oral or respiratory sensitisation and elicitation reactions to this phospholipase C. In addition, no allergic reactions upon dietary exposure to any phospholipase have been reported in the literature.

Quantifying the risk for allergenicity is not possible in view of the individual susceptibility to food allergens. Allergenicity can be ruled out only if the proteins are fully removed. In the fats and oils processing for degumming vegetable oils, experimental data showed a significant removal (> 99%) of protein. However, traces of protein could be present in the final fats and oils.

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011[33]) are used as raw materials ([redacted]) in the media

[31] Technical dossier/1st submission/Annex 7.03.

[32] Technical dossier/1st submission/Annex 8.

[33] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

fed to the microorganisms. However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the microbial biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that potentially allergenic residues of these foods employed as protein sources are not expected to be present.

The Panel considered that under the intended condition of use, the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood of such reactions to occur is considered to be low.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

Phospholipase C is intended to be used in degumming of fats and oils at an intended use level up to 1000 PLC(S)/kg oil[34] (equivalent to 6 mg TOS/kg oil).

When added to crude oil, phospholipase C hydrolyses the phospholipids naturally present in crude oil to form diacylglycerol and phosphate esters. This conversion helps to reduce the amount of gum phospholipids. The resulting products together with the phospholipase migrate into the aqueous phase and are subsequently removed as water-based sludge. This process results in higher oil yields, cleaner final products and better stability and processability of the oils.

Experimental data have been provided showing the removal (> 99%) of phospholipase A and C in the course of fats and oils processing for degumming the vegetable oils (Documentation provided to EFSA No◦3). In addition, taking into account the phase separation after degumming, the washing and purification steps applied to obtain refined oils and fats, i.e. bleaching and deodorisation, the Panel considers that the amount of TOS in the final fats and oils will be removed to a similar degree (Annex B in EFSA CEF Panel, 2016).

3.5.2. Dietary exposure estimation

The Panel considered the evidence provided as sufficient to conclude that the presence of residual amounts of TOS after processing is negligible (Section 3.5.1). Consequently, a dietary exposure to the food enzyme-TOS was not calculated.

4. Conclusions

Based on the data provided and the removal of TOS during the degumming of fats and oils, the Panel considers that this food enzyme phospholipase C from the genetically modified *B. licheniformis* strain NZYM-VR does not give rise to safety concerns under the intended conditions of use.

The CEP Panel considers the food enzyme free from viable cells of the production organism and recombinant DNA.

5. Documentation as provided to EFSA

1) Phospholipase C produced by a genetically modified strain of *Bacillus licheniformis* (strain NZYM-VR). July 2019. Submitted by Novozymes.
2) Additional information. February 2020. Submitted by Novozymes.
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[34] Technical dossier/p. 53.

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
CFU	colony forming units
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LOD	limit of detection
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TOS	total organic solids
WHO	World Health Organization