

SCIENTIFIC OPINION

Scientific Opinion on the safety evaluation of a time-temperature indicator system, based on *Carnobacterium maltaromaticum* and acid fuchsin for use in food contact materials¹

EFSA Panel on Food Contact Materials, Enzymes,
Flavourings and Processing Aids (CEF)^{2,3}

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ABSTRACT

This scientific opinion of the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids deals with the safety evaluation of a system based on *Carnobacterium maltaromaticum* and a colour change indicator, acid fuchsin (CAS number 3244-88-0, FCM Substance No 01033), used as a time-temperature indicator on the packaging of chilled food. The micro-organism, the indicator and a nutritive medium gel are incorporated in a multilayer plastic sachet glued onto the outer layer of the food package. All the substances constituting the system, with the exception of acid fuchsin, are authorised as food additives, food colorants or novel food ingredients, or are an enzymatic digest of edible protein sources and yeast edible extract. Specific migration of acid fuchsin was estimated to be less than 7×10^{-9} mg/kg food. Acid fuchsin elicited a positive response in a bacterial gene mutation assay and a negative response in an *in vitro* micronucleus assay. Given the lack of *in vivo* studies, the genotoxicity potential of acid fuchsin cannot be ruled out. However, the Panel noted that the layer of the plastic sachet in contact with food contact articles behaves as a barrier which prevents any release of its content (including acid fuchsin), and that the sachet is stuck onto the outer layer of the packaging, hence is not in contact with the food. Thus no exposure to the substances constituting the system from the consumption of the packed food is expected under the intended conditions of use. Therefore, the Panel concluded that the substances of the intelligent system, *C. maltaromaticum* and acid fuchsin, do not raise a safety concern for the consumer when used in a plastic sachet which prevents any migration from the system into food and which is stuck onto the outer layer of the packaging of chilled food.

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KEY WORDS

Time-temperature indicator system; *Carnobacterium maltaromaticum*; Acid fuchsin; FCM Substance No 01033; Food contact materials; Active and intelligent materials; Safety evaluation.

SUMMARY

According to the Commission Regulation (EC) No 450/2009⁴ of the Commission of European Communities of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food, substances responsible for the active or intelligent function need first to be evaluated by EFSA before their inclusion into a positive Community list. The procedure of the evaluation and the tasks of EFSA are described in the Regulation (EC) No. 1935/2004⁵ of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food.

In the context of this evaluation procedure following a request from the Direction Générale de la Concurrence, de la Consommation et de la Répression des Fraudes, France, the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) was asked to deliver an opinion on an intelligent system based on *Carnobacterium maltaromaticum* and acid fuchsin used as a time-temperature indicator on packaging of chilled food. The application was submitted by the applicant, Cryolog S.A., France.

The intelligent system is based on a non-pathogenic micro-organism *C. maltaromaticum* which is used together with a colour change indicator (acid fuchsin) and a nutritive medium gel. The micro-organism, the indicator and the gel are incorporated in a multilayer plastic sachet which is glued onto the outer layer of the food package.

All the substances constituting the intelligent system, with the exception of acid fuchsin, are authorised as food additives, food colorants or novel food ingredient, or are an enzymatic digest of edible protein sources and yeast edible extract.

Specific migration of acid fuchsin was estimated by migration modelling under an exaggerated scenario to be less than 7×10^{-9} mg/kg food. Therefore no migration is to be expected under the intended conditions of use.

The strain of *C. maltaromaticum* used in the intelligent system produces a biogenic amine (tyramine). However no migration of the microbiological components is to be expected and therefore no exposure of tyramine from the consumption of the packed food is expected under the intended conditions of use.

Two *in vitro* genotoxicity tests were applied to assess the genotoxicity of acid fuchsin. In the bacterial reversion test, the indicator elicited a positive response in experiments with exogenous metabolic activation. Given the lack of *in vivo* studies and the available limited data, the genotoxicity potential of acid fuchsin cannot be ruled out.

However, the Panel noted that the layer of the plastic sachet in contact with food contact articles behaves as a barrier which prevents any release of its content (including acid fuchsin) and that the sachet is stuck onto the outer layer of the packaging, hence is not in contact with the food. Therefore, no exposure to the substances constituting the system from the consumption of the packed food is expected.

Therefore, although the genotoxicity potential of the colour change indicator acid fuchsin cannot be ruled out, the Panel concluded that the substances of the intelligent system, *C. maltaromaticum* and acid fuchsin, do not raise a safety concern for the consumer when used in a plastic sachet which prevents any migration from the system into food and which is stuck onto the outer layer of the packaging of chilled food.

⁴ Commission Regulation (EC) No 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food. OJ L 135, 30.5.2009, p. 3–11

⁵ Regulation (EC) No 1935/2004 of the European parliament and of the council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. OJ L 338, 13.11.2004, p. 4–17

TABLE OF CONTENTS

Abstract	1
Summary	3
Table of contents	4
Background as provided by the legislation	5
Terms of reference as provided by the applicant.....	5
Assessment	6
1. Introduction	6
2. General information.....	6
3. Data available in the dossier used for this evaluation.....	7
4. Evaluation.....	7
4.1. Non-toxicological data.....	7
4.2. Microbiological data	8
4.3. Toxicological data.....	8
Conclusions	9
Documentation provided to EFSA	9
References	9
Abbreviations	10

BACKGROUND AS PROVIDED BY THE LEGISLATION

Regulation (EC) No 450/2009 of the Commission of European Communities is a specific measure that lays down specific rules for active and intelligent materials and articles intended for contact with foodstuffs in addition to the general requirements established in Regulation (EC) No 1935/2004 of the European Parliament and of the Council on materials and articles intended to come into contact with food. Active materials and articles are intended to extend the shelf-life or to maintain or improve the condition of packaged food; they are designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food.

The substance(s) responsible for the active and/or intelligent function of the material should be included in a positive list by the Commission following a safety evaluation by EFSA according to the procedure described in the above mentioned regulations.

According to this procedure the industry submits applications to the Member States competent Authorities which transmit the applications to EFSA for their evaluation. The application is supported by a technical dossier submitted by the industry following the EFSA “guidelines on submission of a dossier for safety evaluation by the EFSA of active or intelligent substances present in active and intelligent materials and articles intended to come into contact with food” (EFSA, 2009).

In this case, EFSA received an application from the Direction Générale de la Concurrence, de la Consommation et de la Répression des Fraudes, France, requesting the evaluation of a time-temperature indicator system based on *Carnobacterium maltaromaticum* and a colour change indicator, acid fuchsin.

TERMS OF REFERENCE AS PROVIDED BY THE APPLICANT

EFSA is required to carry out an assessment on the risk originating from the migration into food of substances from the intelligent system based on *Carnobacterium maltaromaticum* and a colour change indicator (acid fuchsin) used as time-temperature indicators on food contact materials and deliver a scientific opinion, according to the Regulation (EC) No 1935/2004 of the European Parliament and of the Council on materials and articles intended to come into contact with food.

The opinion of EFSA will be considered by the Commission for adoption of a Community list of authorised substances where according to the Regulation (EC) No 450/2009 there will be specified:

- (a) the identity of the substance(s);
- (b) the function of the substance(s);
- (c) the reference number;
- (d) if necessary, the conditions of use of the substance(s) or component;
- (e) if necessary, restrictions and/or specifications of use of the substance(s);
- (f) if necessary, conditions of use of the material or article to which the substance or component is added or into which it is incorporated.

ASSESSMENT

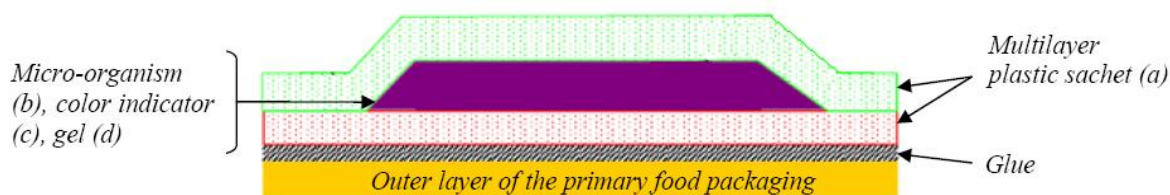
1. Introduction

The European Food Safety Authority was asked by the Direction Générale de la Concurrence, de la Consommation et de la Répression des Fraudes, France, to evaluate the safety of a time-temperature indicator system based on *Carnobacterium maltaromaticum* and a colour change indicator, acid fuchsin (CAS number 3244-88-0, FCM Substance No 01033). The request has been registered in the EFSA's register of questions under the number EFSA-Q-2011-00120. The dossier was submitted by the applicant Cryolog S.A., France.

2. General information

According to the applicant, the intelligent system is a time-temperature indicator. It is based on a non-pathogenic micro-organism *C. maltaromaticum* which is used together with a colour change indicator, acid fuchsin, and a gel made of a nutritive medium. Depending on the time-temperature profile experienced by the system, *C. maltaromaticum* grows using the carbon source of the medium and releases substances that cause a change of pH and consecutively a change of the colour from green to red. This change is used to monitor whether the cold chain has been maintained.

All constituents are contained within a multilayer plastic sachet which is glued onto the outer layer of the food package, as shown in the scheme below. The sachet is intended to act as a barrier to prevent any migration of the contents from the sachet. The intended applications are for all types of chilled foods stored at temperatures between 2 °C and 12 °C for a few hours up to eight days.



- (a) multilayer plastic sachet that contains the micro-organisms (b), the indicator (c) and the gel (d).
- (b) non-pathogenic lactic acid bacterium, *C. maltaromaticum*.
- (c) colour change indicator which contains a coloured acid-basic indicator acid fuchsin (which change its colour from colourless in basic to red in acidic environment) and a green food colour.
- (d) gel made of a nutritive medium for growth and metabolism of *C. maltaromaticum*. It is composed of an enzymatic digest of edible protein sources used as growth medium together with yeast edible extract and other substances that are evaluated and authorised as food colorant, as food additives or as novel food ingredients.

The intelligent system as such has not been evaluated by the SCF or EFSA in the past. However, with the exception of acid fuchsin, the substances constituting the intelligent system have been evaluated and authorised as food colorant (Directive 94/36/EC) or as food additives (Directive 95/2/EC⁶) or as novel food ingredients (Regulation (EC) No 258/97).

⁶ EUROPEAN PARLIAMENT AND COUNCIL DIRECTIVE No 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. OJ L 61, 18.3.1995, p. 1-63

3. Data available in the dossier used for this evaluation

The studies submitted for evaluation followed the EFSA “guidelines on submission of a dossier for safety evaluation by the EFSA of active or intelligent substances present in active and intelligent materials and articles intended to come into contact with food” (EC, 2009).

Non-toxicity data:

- Data on identity
- Data on manufacturing process
- Data on function, intended use and authorisation
- Data on composition and migration

Microbiology data:

- Phenotypic identification of *C. maltaromaticum* CNCM I-3298
- Molecular characterisation of *C. maltaromaticum* CNCM I-3298
- Biochemical characterisation of *C. maltaromaticum* CNCM I-3298
- Bibliographic study of the genus *Carnobacterium* and of *C. maltaromaticum*

Toxicity data:

- Bacterial gene mutation assay on the whole content of the intelligent system and related migrants
- *In vitro* micronucleus assay on the whole content of the intelligent system and related migrants
- Cell viability test (resazurin) on cultured human cells, for the whole content of the intelligent system and related migrants
- Bacterial gene mutation assay on acid fuchsin
- *In vitro* micronucleus assay on acid fuchsin

4. Evaluation

4.1. Non-toxicological data

The micro-organism, the colour indicator and the gel are placed in a plastic sachet which is stuck onto the outer layer of the food package using a hot-melt adhesive. The sachet and its contents are therefore not in contact with the food.

Overall migration from the intelligent system glued on a polyethylene film of 20 µm thickness was performed in 3 % acetic acid, 50 % ethanol and 95 % ethanol for 10 days at 40 °C, and in isoctane for 2 days at 20 °C. Overall migration was up to 2.6 mg per item, which is attributed to the migration from the glue and the multilayer plastic sachet.

Specific migration of acid fuchsin was not determined experimentally but was estimated by migration modelling under an exaggerated scenario for 5 days at 40 °C (under which the chilled foods would be spoiled and the time temperature indicator would be triggered). Migration was calculated to be less than 7×10^{-9} mg/kg food. Therefore no migration is to be expected under realistic use conditions.

The Panel concluded that the plastic sachet behaves as a barrier which prevents any migration of its content. In addition, the sachet is stuck onto the food package and is not in contact with the food.

Therefore, the Panel concluded that there will be no exposure to the contents of the sachet when it is used under the intended conditions of use.

4.2. Microbiological data

The bacterium used in the intelligent system is a strain of *Carnobacterium maltaromaticum*, recorded in the collection of Institute Pasteur (Paris-FR) under the number CNCM I-3298. Both phenotypic and molecular (16SrDNA sequence) identifications were done.

C. maltaromaticum has been evaluated by EFSA (EFSA Panel on Biological Hazards (BIOHAZ), 2012) for including in the qualified presumption of safety (QPS) list of biological agents intentionally added to food and feed. The BIOHAZ Panel concluded that a QPS recommendation cannot be given because it is a fish pathogen and because potential virulence factors were described in one strain, different from the strain used in the intelligent system.

The strain used in the intelligent system produces a biogenic amine (tyramine), at a concentration of 47 mg/kg of the growth substrate, after growth from 10^5 to 10^8 cfu/g at 20 °C. Tyramine is commonly found in several types of foods and may have some adverse effects on human health with a threshold of 600 mg per person per meal (50 mg for person under some medical treatments) (EFSA Panel on Biological Hazards (BIOHAZ), 2011).

Given that no migration of the microbiological components is to be expected, no exposure to tyramine from the consumption of the packed food is expected under the intended conditions of use. Therefore the Panel concluded that the use of *C. maltaromaticum* in the intelligent system does not raise a safety concern for consumer.

4.3. Toxicological data

All the components of the intelligent system, with the exception of the colour change indicator acid fuchsin, are authorised as food additives, food colorants or novel food ingredients, or are derived from the yeast edible extract and the enzymatic digest of edible protein sources. Therefore, no toxicological data are needed on these components.

Two genotoxicity assays were performed using the whole content of the active system and the migrants in ethanol and isooctane as test material. The assays consisted of a reverse mutation test in *S.typhimurium* TA1535, TA1537, TA98 and TA100 and in *E.coli* WP2 uvrA pKM101, with and without exogenous metabolic activation by rat liver S9, and an *in vitro* micronucleus test on the metabolically proficient human cell line HepG2. Both studies provided negative results, which have however an ancillary role in this evaluation as the dosage and identity of tested materials remain undefined.

The genotoxicity of the colour change indicator acid fuchsin was tested using a reverse mutation test in *S.typhimurium* TA1535, TA1537, TA98 and TA100 and in *E.coli* WP2 uvrA pKM101, with and without exogenous metabolic activation by rat liver S9, and an *in vitro* micronucleus test on the metabolically proficient human cell line HepG2. In the bacterial reversion test, the dye elicited a positive response in the strain TA98, inducing a dose-related increase of revertant colonies in experiments with exogenous metabolic activation. No clastogenic/aneugenic activity was observed in the micronucleus test in HepG2 cells. Given the lack of *in vivo* studies and the available limited data, the genotoxicity potential of the colour indicator acid fuchsin cannot be ruled out.

However, the Panel noted that the layer of the plastic sachet in contact with food contact articles behaves as a barrier which prevents any release of its content (including acid fuchsin) and that the sachet is stuck onto the outer layer of the packaging, hence is not in contact with the food. Therefore, no exposure to the substances constituting the system from the consumption of the packed food is expected, and the use of the colour indicator acid fuchsin in the active system does not raise a safety concern for consumer.

CONCLUSIONS

After having considered the above-mentioned data and although the genotoxicity potential of the colour change indicator acid fuchsin cannot be ruled out, the Panel concluded that the substances of the intelligent system, *C. maltaromaticum* and acid fuchsin, do not raise a safety concern for the consumer when used in a plastic sachet which prevents any migration from the system into food and which is stuck onto the outer layer of the packaging of chilled food.

DOCUMENTATION PROVIDED TO EFSA

1. Time temperature indicator (TTI). February 2011. Submitted by Cryolog S.A., France.
2. Time temperature indicator (TTI). December 2012. Submitted by Cryolog S.A., France.

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ABBREVIATIONS

CAS	Chemical Abstracts Service
CEF	Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CFU	Colony forming unit
EC	European Commission
EFSA	European Food Safety Authority
FCM	Food Contact Materials
QPS	Qualified presumption of safety